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Histopathological Studies on Twelve Benthic Invertebrates from the Middle Atlantic Outer Continental Shelf

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HISTOPATHOLOGICAL STUDIES ON TWELVE BENTHIC INVERTEBRATES FROM THE MIDDLE ATLANTIC OUTER CONTINENTAL SHELF

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CHAPTER 10

HISTOPATHOLOGICAL STUDIES

Craig Ruddell

INTRODUCTION

A number of investigators have shown that acute and chronic pollution of the marine environment with crude or waste oils resulted in the appearance of histologically demonstrable lesions in fish and shellfish (Blanton and Robinson 1973; Barry 1974; Barry and Yevich 1975; Gardner 1975; Gardner et al. 1975; Yevich 1975a, b). This work suggested that profound physiological changes and dysfunctions may have occurred in animals subjected to these compounds. The work presented below was prompted by these studies and was undertaken in an effort to define the normal or baseline histology of a number of common benthic marine invertebrates found in the vicinity of the Middle Atlantic oil drilling lease areas. It is expected that this work will be useful in evaluating the effects of environmental contamination associated with future gas and oil production in these areas.

The material presented below will review some of the significant findings of the histopathological effort; more specifically, this report will focus on (1) the distribution of the organisms chosen for study, (2) details relating to the production of gametes by these organisms, and (3) the symbionts associated with these organisms.

A histological atlas depicting the major organ systems, parasites, and pathologies of these invertebrates will be presented at a later time.

METHODS AND MATERIALS

Overview

A number of benthic marine invertebrates were collected from the dredge and trawl stations during each of the four cruises (see Chapter 2 for locations and general framework). Portions of these animals were then prepared for histological examination. Sections of these animals were examined in an effort to determine baseline histological data. Details of these procedures are presented below.

Organisms Chosen for Analysis

Twelve benthic marine invertebrates were chosen for histopathological analysis. These organisms were selected because: (1) they were representative of the fauna found in a given area; (2) they could be captured by the gear available to us; (3) they occurred in numbers sufficient to satisfy the needs not only of the histopathology effort but also of the chemists; (4) they could

be captured and brought to the surface in a relatively untraumatized condition; (5) they represented diverse feeding types; and (6) some of the organisms, i.e. *Placopecten megallanicus*, represented species of commercial importance. The organisms chosen for analysis included:

Molluscs:	*Astarte undata*, *A. castanea*, and *Placopecten magellanicus*
Shrimp:	*Dichelopandalus leptoceras*, *Pontophilus brevirostris*, and *Crangon septemspinosa*
Crabs:	*Cancer irroratus* and *C. borealis*
Echinoderms:	*Echinarachnius parma*, *Asterias forbesi*, *A. vulgaris* and *Astropecten americanus*

Processing of Samples on Shipboard

Organs and Tissues Sampled

For routine work, the smaller crustacea, specifically the shrimp and small crabs, were fixed whole without any attempt to dissect out discrete organ systems. Similarly, the echinoderms and small molluscs were reduced or dissected to yield only broad anatomical units such as the arms or disk area in the echinoderms or slabs of gut, mantle, and foot in the smaller molluscs. The larger crabs and the giant scallop, *P. magellanicus*, were dissected to provide portions of gill, digestive diverticula, stomach (crabs only), gonad, muscle, heart, mantle (scallops only), or kidney (scallops only). Fine dissections of other animals were occasionally performed when it was desired to obtain material for plastic embedding (see below).

Fixation. Samples to be processed in the "routine" manner were preserved in Dietrich's fluid (9000 ml distilled water, 4500 ml 95% ethanol, 1500 ml 40% formalin, 300 ml glacial acetic acid). This fixative was recommended by Barozcz and Yevich (1975) as the fixative of choice for field work and shipboard use because animals can be stored in this fixative for several months without undue hardening of tissues. In addition, Dietrich's fixative is easily prepared, penetrates tissues quickly, remains usable for long periods, even when maintained at room temperature, is inexpensive to prepare, and does not leave explosive or toxic residues on spilling. (Many of the classical histological fixatives do leave dangerous residues.) During the first (fall) cruise, as recommended by Barozcz and Yevich (1975), samples were placed in perforated "zip-lock" plastic bags, appropriately labeled, and immersed in large containers of fixative. This procedure proved to be somewhat tedious and wasteful of fixative, and resulted in a number of poorly preserved specimens. On all subsequent cruises, therefore, specimens were placed in small, 100 ml polyethylene screw-top bottles which had been filled with fixative one week prior to the cruise.

Although Dietrich's fluid was an adequate histological fixative, it did not preserve the fine structural elements of tissues and appeared to wash out glycogen from tissues. In order to appreciate the finer morphological details, we preserved selected portions of the animals referred to above in aqueous fixatives employing a variety of aldehydes as the primary fixative species. These fixatives included a phosphate-buffered, acrolein-formaldehyde mixture (PAF) and a two-step seawater acrolein-glutaraldehyde (SAG) formulation. PAF consisted of: 90 ml distilled water, 10 ml formalin (40% formaldehyde), 1.2 g $NaH_2PO_4 \cdot H_2O$, 2.1 g Na_2HPO_4, and 24 drops of acrolein (0.41 ml) delivered from a Pasteur pipette. Small portions of tissue, generally less than 5 mm in diameter, were placed in PAF, transported back to the laboratory, and embedded in glycol methacrylate (see below) for routine examination at the light microscope level. The SAG fixation procedure consisted of placing small portions of tissue, less than 3 mm in diameter, in cold seawater-acrolein (10 drops acrolein from a Pasteur pipette per 100 ml sea water) for approximately 10 minutes, and then transferring the tissue to a seawater glutaraldehyde solution (5 ml 50% glutaraldehyde per 100 ml sea water). Tissues were maintained in the seawater-glutaraldehyde solution for 18-24 hours and then transferred to a phosphate-buffered holding solution (pH 7.2; 90 ml distilled water, 0.4 g $NaH_2PO_4 \cdot H_2O$ and 0.65 g Na_2HPO_4). Tissues preserved in SAG were later osmicated in the laboratory and embedded in resins suited to transmission electron microscopy. Both fixatives were designed by the author - PAF, in this laboratory, and SAG several years ago (Ruddell 1971a) for use in examining the fine structure of oyster tissues.

Numbers of Animals Sampled

Five to 10 organisms per species were sampled at a given station on each cruise.

Data Collection

Each animal collected on shipboard was assigned a "specimen number". In addition, whenever organs or organ systems were dissected out, these tissues were assigned a "sub-number" corresponding to or coding for a given organ (see Table 10-2, below). As each animal was fixed, data relating to the size, sex, and/or anatomical anomolies of the animal were entered on a histopathology field form. Ancillary data, including the date of collection, portion of animal sampled, station number, and gear type were also entered on the field form.

Mensuration. In order to obtain an estimate of the relative size of the animals collected, the following measurements were taken (in mm):

Bivalve molluscs, shell length, umbo to edge of shell;
Shrimp, total length, tip of the rostrum to end of telson;
Crabs, carapace width;
Sand dollars, diameter;
Sea stars, distance between the tips of any two non-adjacent arms.

Laboratory Processing

Preparation of Tissues for Sectioning

Material Preserved in Dietrich's Fixative. On arrival at the laboratory, material preserved in Dietrich's fixative was trimmed and then washed in tap water overnight or decalcified if tissues were encased with a calcified exoskeleton (all echinoderms and shrimp were routinely decalcified) placing them in several changes of 0.1 N HCl for 12-18 hours. Tissues were dehydrated and cleared on a "Technicon"-brand Automatic Tissue Processor, employing Technicon's dehydration and clearing agents, S-29 and UC-670, infiltrated with paraffin under vacuum and embedded.

Material Preserved in Aldehyde Mixtures. Tissues preserved in phosphate-buffered acrolein-formalin (PAF) were dehydrated in pure methanol, infiltrated with a variant of Ruddell's (1971b) glycol methacrylate monomer mixture, and polymerized under an incandescent lamp. Tissues preserved in seawater-acrolein-glutaraldehyde were osmicated and embedded in Durcupan ACM, an araldite base resin mixture designed for use with the electron microscope, according to standard procedures (Hyatt 1970).

Sectioning

Paraffin Blocks. Material preserved in Dietrich's fixative was sectioned at 5 μm with a steel knife on a standard rotary microtome. The difficulties in obtaining high quality sections of many of the organisms chosen for analysis cannot be over-emphasized. These organisms often combined lavish quantities of sand with a chitinous or refractory matrix; crustacean gills, ova and eggs of all the animals, and crab sperm plugs would often not infiltrate with paraffin. Another source of frustration, especially among the shrimp, was that even after obtaining sections of acceptable quality, many important organs and tissues would be absent from the sections, passed over by the knife. Blocks of these animals had to be resectioned and restained - perhaps several times - until the plane of sectioning incorporated or intersected a desired organ.

We found that the single most important factor in obtaining high quality sections was a sharp, well-honed steel knife. Because of the abrasive nature of the material from the OCS, it was also absolutely essential to have sharp knives in abundant quantity. The purchase of the "Temtool" microtome knife sharpener solved this problem.

Plastic Blocks. Glycol methacrylate blocks were sectioned on a standard rotary microtome using steel knives sharpened on the "Temtool" knife sharpener. Sections were cut at 2-4 μm and stored in small boxes.

Araldite resin blocks were sectioned on an LKB ultramicrotome using glass knives.

Staining

Paraffin Sections. After paraffin sections had been fixed to glass

slides with the aid of Haupt's gelatin fixative (without phenol; Humason 1972), they were dried and stained on the "Technicon". Slides from material collected on the first two cruises were stained with Harris' hematoxylin and eosin. However, it became evident that tissues stained with these solutions were overwhelmed with hematoxylin. Nuclei, AMP's (acid mucopolysaccharide), collagen, and many cytoplasmic elements were colored in shades of purple or blue making it difficult to evaluate a given section. We, therefore, developed a routine, automated staining procedure which differentially stained AMP's, nucleic acids, and the "proteinaceous" components of tissues. This procedure was predicated on the use of the basic dye, Astrablue, to stain AMPs (Bloom and Kelley 1960), Mayer's hematoxylin to stain nucleic acids, and eosin to stain proteins. This procedure (automated) is given below:

I. Stains used in automated procedure.

A. Mayer's hematoxylin
B. Astrablue
C. Eosin Y

II. Composition of stock and working solutions.

A. Mayer's hematoxylin. (recipe from "Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology" - AFIP - 1968, p. 33) - working solution.

Distilled water	1000.0 ml
Ammonium or potassium alum	50.0 g
Hematoxylin crystals	1.0 g
Sodium iodate	0.2 g
Citric acid	1.0 g
Chloral hydrate	50.0 g

Dissolve the alum in the water, without heat. When the alum has completely dissolved, add the hematoxylin and allow to dissolve completely. Then add the sodium iodate, citric acid, and the chloral hydrate, and shake or stir until all the components are in complete solution. The final color of the stain is reddish-violet.

B. Astrablue. (Astrablau FM; Chroma Gesellschaft, Stuttgart - Untertürkheim, purchased from Roboz Surgical Instrument Co., Inc., 810 18th Street, N.W., Washington, D. C. 20006) - working solution.

Astrablue	0.210 g
Distilled water	1000 ml
Glacial acetic acid	3 ml

Dissolve stain completely in water; then add acetic acid.

C. Eosin Y. (C.I. # 45380; recipe from AFIP, 1968, pp. 35-36).

1. 1% stock.

Eosin Y, water soluble	10.0 g
Distilled water	200.0 ml
Dissolve and add:	
Alcohol, 95%	800.0 ml

2. Working Solution.

Eosin stock	200.0 ml
Distilled water	95 ml
Alcohol, 95%	505 ml
	800 ml

Just before use, add 0.5 ml of glacial acetic acid to each 100 ml of stain and stir (for 800 ml = 4 ml acid).

The scheduling and timing of the automated "Technicon" was developed by Susan Fox, Laboratory Specialist. Her method follows.

III. Schedule for "Technicon"

A. Bottom Row:

Beaker #	Chemical	Time (min.)
1	Xylene	10
2	Xylene	5
3	100% ETOH	5
4	95% ETOH	3
5	70% ETOH	3
6	Distilled water	3
7	Astrablue	7
8	Tap water	2
9	Distilled water	3
10	Distilled water	3
11	Mayer's hematoxylin	8
12	Tap water	5

B. Top Row:

Beaker #	Chemical	Time (min.)
1	0.2% Na bicarbonate	1
2	Distilled water	5
3	Distilled water	5
4	Distilled water	5*
5	Eosin	3
6	95% ETOH	2
7	95% ETOH	2
8	100% ETOH	2
9	100% ETOH	2
10	Xylene	2
11	Xylene	3
12	Xylene	store til coverslipped

*Note: A 20-minute wash after Mayer's is recommended to prevent fading.

IV. Maintenance of solutions.

A. Water. Fresh water should be used each day. After each rack of slides, change the water beakers immediately following Astrablue and Mayer's hematoxylin stains.

B. Alcohols. Fresh alcohol should be used each day. Watch first beaker of 100% ETOH for xylene contamination; xylene may carry over to 95% ETOH if 100% ETOH becomes saturated.

C. Sodium bicarbonate. Make fresh daily. About 1.5 grams per 750 ml of distilled water.

D. Stains. Store Mayer's hematoxylin and Astrablue in jars overnight to prevent evaporation. Periodically check acidity of Eosin. Reuse until unsatisfactory. Filter when necessary.

The automated procedure presented above was easily set up and maintained. Tissues stained with this staining sequence were clearly and brilliantly colored in shades of azure blue (AMP's), purple (nucleic acids), and red (proteins). The various morphological components of organs and tissues were clearly defined.

Glycol Methacrylate Sections. Glycol methacrylate (GMA) sections were stained with a variety of histological stains and histochemical substrates. Several of the stains used on GMA sections emphasized the histological aspects of cells and tissues; others emphasized cytological aspects. The histochemical procedures used on GMA sections included P.A.S. for vic-hydroxyl groups, acid-Feulgen-Schiff's for DNA; Astrablue for AMP's, alizarin Red S for calcium and acid ferrocyanide for iron (Fe^{+3}) (cf. Pearse 1968 or Lillie 1965).

Araldite Sections. Thick (1 μm) sections were stained with toluidine blue 0 at high pH and thin sections with Reynolds' lead citrate and uranyl actetate (cf. Hyatt 1970).

Storage of Sections and Blocks

During the first year approximately 6253 slides were prepared from 3477 paraffin blocks. These slides were labeled with the sample and sample sub-number, genus and species of the sample, the date, and the stains employed to stain the tissues on the slide. Slides were stored in slide storage files and blocks in block storage files in numerical order.

Data Recording

Field Data

As indicated above, basic field data pertaining specifically to the histopathology effort were entered aboard ship on histopathology field forms. This information was incorporated into sample headers (Table 10-1) which provided identification for information developed during tissue examination.

Table 10-1. Histopathology Sample Header Format.

Card Column	Information
1-6	Trawl (type)
7-11	Specimen no. (= slide no.)
12-16	Specimen subnumber
17-21	Tissue type
22-31	VIMS ten-digit identification code
32-36	Size (mm)
56-61	Station date
63-65	Station time (GMT)
73-74	Station no.
75-77	Sample no.

Laboratory Data

Data generated during examination of stained sections were entered on data reporting forms designed in this laboratory. The format employed and information recorded is shown in Table 10-2. This data reporting scheme is easy to use and interpret. It can be used not only as a means to store histological or histopathological information but also as a worksheet from which data can be easily abstracted, or entered, at the work bench. Each major data category is announced by an easily recognized alphabetized code letter or letters allowing one to rapidly scan and evaluate computer printouts. This reporting format is open-ended in the sense that it assumes nothing about the physiological state of an organism and that details concerning the nature of a symbiont or lesion may be incorporated into the data base as they are encountered or identified. One is not expected to know the identity or range of symbiont or lesion types that may occur in a given animal. Lastly, this format allows the investigator to incorporate detailed information on a given organism, symbiont, or lesion in an appendix.

With regard to details on gonads and gonadal maturation, an attempt has been made to make the reporting of the details of the degree of gonadal maturation as simple and straightforward as possible and yet leave room for interpretation. This format does not require that one identify or quantitate all stages of gamete maturation nor compute mitotic or meiotic indices. An approach of this nature would be not only time consuming but also, considering the nature of the sampling scheme, quite redundant. This scheme only requires an honest evaluation of gonadal histology; it is not necessary to know the entire gonadal cycle of an OCS organism before applying it.

RESULTS

Crustacea

Dichelopandalus leptoceras (Crustacea, Decapoda, Natantia, Pandalidae)

The pandalid shrimp, *D. leptoceras* has a moderately wide distribution,

Table 10- 2 . Histopathological Data Reporting Format.

Card Column	Information
1-3	BLM cruise designation (01B, 02W, etc.)
4-8	Station and sample number
9-14	Specimen number
15-16	Specimen subnumber
17	Fixative - coded; 1 = Dietrich's
18	Type embedment - coded; 1 = paraffin
19	Stains - coded; 1 = Harris hematoxylin-eosin; 2 = Astrablue, Mayer's hematoxylin, eosin
20	Sex of animal; M = male, F = female, H = hermaphrodite, X = unknown
(21-28)	Degree of gonadal maturation:
21	Early or primordial or stage 1 gonad, no primary gonocytes, Ø = none, 1 = present
22	Stage 2 gonad; presence of primary gonocytes, oocytes previtellogenic, Ø = none, 1 = present
23	Stage 3 gonad; male gonad post-meiotic, sperm present; female gonad, ova vitellogenic or postvitellogenic; Ø = none, 1 = present, 2 = postvitellogenic ova only
24	Relative abundance of stage 3 cells; Ø = none, 1 = very few, 2 = moderate numbers, 3 = many
25	Stage 4 gonad; gonads appear "spawned out" or resting. Ø = none, 1 = probably stage 4 gonad but could be confused with stage 1, 2, or early 3, 2 = most definitely stage 4 gonad, 3 = only a portion of gonad in what appears to be stage 4, 4 = stage 4 gonad portions of which have been invaded by blood cells (amebocytes) or appear atrophied.
26	"Mixed" gonad; stages 1, 2, or 3 and 4, Ø = none, 1 = present

Table 10- 2. (continued)

Card Column	Information
27	Presence of spermatophores; Ø = none, 1 = present
28	Presence of embryos (2 n); Ø = none, 1 = early embryo, less than 5 nuclei in a given cross-section, 2 = late embryo
(29-47)	Parasites - (symbionts) - commensals)
29-32	Easily recognized 4 letter abbreviation announcing a given symbiont; i.e., XKNN = unknown, MICR = prokaryotic organisms, FUNG = fungus, mold, ALGA = algae, DINO = dinoflagellates, TREM = trematode, CEST = cestode, NEMA = nematode, ACAN = acanthocephalan, CRUS = crustacean, etc.
33-34	A simple 2 space number-letter reference code (AA, AB, -- 1A, 1B, -- 1,1,1,2---) which will direct one to a detailed description of or reference to the symbiont announced directly above in an appendicized computer print-out.
35	Nature of symbiont; 1 = parasitic; 2 = commensal; 3 = commensal, but large numbers or size may indicate physiological impairment of host; 4 = symbiont dead.
36	Relative numbers of symbiont; 1 = few; 2 = moderate numbers; 3 = many
37	Ecto- or endosymbiont; 1 = ecto-; 2 = endo-; 3 = both
38-39	Site of infestation; 01 = muscle, 02 = gill, 03 = kidney, kidney-like organ, 04 = gonad, 05 = digestive diverticula, 06 = stomach, 07 = intestine, 08 = connective tissues, 09 = blood cells, 10 = test, exoskeleton, 11 = gonad and gills, 12 = variety of tissues, 13 = heart.
40	Extra- or intracellular; 1 = intra-, 2 = extra-, 3 = both, 4 = cannot determine

Table 10- 2 . (concluded)

Card Column	Information
41-44	4 letter abbreviation announcing the response of host to symbiont: NONE, FIBR = fibrosis, INFL = infiltration by blood cells, LEUC = leucocytosis, INFM = inflammation, MELZ = melanization, MELC = melanized cyst, MELS = small melanized spot or focus, ENCP = encapsulation; DEGN = obvious degenerative change, PHAG = phagocytosis, HYPT = hypertrophy, HYPL = hyperplasia, etc.
45-46	Simple 2 space number-letter reference code relating to a detailed description in appendicized computer print out.
47	Degree host response to symbiont; Ø = none, 1 = slight, 2 = moderate, 3 = severe.
(48-58	Lesions - not obviously related to presence of symbionts)
48	Type lesion; Ø = none, 1 = local, 2 = systemic
49-50	Lesion site - same code as Columns 38-39.
51-54	4 letter abbreviation announcing response of animal to lesion - same code as Columns 41-44.
55-56	Simple 2 space number-letter reference code relating to a detailed description in appendicized computer print out.
57	Degree of response; 1 = slight, 2 = moderate, 3 = severe.
58	Numbers of lesions; 1 = few, 2 = moderate numbers, 3 = many.
59-	OPEN

having been reported from as far north as Canada and as far south as New Jersey (Smith 1881; Rathbun 1905, 1929; Scattergood 1952; Wigley 1960; Couture and Trudel 1968; also cf. Chapter 6 above). *D. leptoceras* would appear to be a somewhat atypical pandalid in that it is not restricted to cold, deep water and is not a protandric hermaphrodite. Both Rathbun (1905) and Wigley (1960) reported that this shrimp ranged widely in depth in waters off New England (Rathbun: 12.6-774 m; Wigley: 36-342 m). Wigley (op. cit.) also indicated that *D. leptoceras* was not stenothermic, having been taken from both cold (5°C) and warm (19.4°C) water. Scattergood (1952), on the basis of length-frequency data for both male and female *D. leptoceras* taken off the Maine coast, surmised that this shrimp was probably not hermaphroditic. The data presented below and in Chapter 6 confirm these observations.

Distribution and Length-Frequency Data. *D. leptoceras* was found at all benthic trawl stations at depths ranging from 21-410 m (cf. Chapter 6) and at temperatures ranging from 6-15.5°C (cf. Chapter 3). Length-frequency data (Table 10-3) on shrimp collected during the first year suggest that *D. leptoceras* (1) in all probability did not live for more than 2+ years; (2) was a migratory shrimp; in fact, the data indicated that some shrimp may have been prompted to migrate twice during their lifetime. The first of these migrations was undertaken during the fall and winter months by 0-year shrimp living in the deeper waters of the continental shelf (stations E1, F1, A1, and I1; depths > 65 m) and was directed toward the shallower waters (stations D1, N3 and B1; depths < 65 m but > 26 m) of the shelf where the shrimp may have over-wintered. The second migration occurred during the spring and summer months: 1-year shrimp migrated from the over-wintering areas (D1, N3, B1) to the outer continental shelf, and thence to the continental slope (i.e., J1). The conclusions on the migratory behavior of *D. leptoceras* were based on the following observations: (1) both 0- and 1-year shrimp were found in the deeper waters (> 65 m in depth) of the shelf stations during spring and summer, (2) no shrimp were found at these stations during the winter, and (3) no small shrimp were found at Station J1 (the continental slope). The length-frequency data presented in Table 10-3 also indicated that *D. leptoceras* spawned or extruded eggs during the colder months.

Gonads and Gonadal Maturation. The gonads of *D. leptoceras* were categorized with respect to sex, degree of sexual maturation, and the presence of eggs. The data (Table 10-4) indicated that (1) *D. leptoceras* spawned or extruded eggs principally during the winter, (2) *D. leptoceras* had only one brood per year, (3) gonadal development began in shrimp as small as 21 mm (total length), (4) in the winter months both mid-shelf and continental slope populations of *D. leptoceras* extruded eggs, (5) females outnumbered males 2.6:1, and (6) females were slightly larger than males. Based on length-frequency data for shrimp taken at stations D1, N3, and B1, during the fall and winter, it was calculated that during the first year of life, female shrimps attain a length of approximately 42.4 mm and males, 38.5 mm. By the end of the second year, based on the limited data now available, females attained a maximum length of approximately 100 mm and males 87 mm.

Symbionts. Three parasitic symbionts were found in *D. leptoceras*

I. A bopyrid isopod. These parasites were not common. Of 802 *D. leptoceras* taken from Station J1 in the fall of 1975, only 13 shrimp (1.6%) were parasitized

Table 10-3. Length-frequency data, by location and season, for *Dichelopandalus leptoceras*. For convenience in presentation and interpretation, data have been grouped according to the three primary habitats referred to in the text: over-wintering stations D1, N3, and B1 (depths <65 m); shelf stations (E1, F1, I1, and A1) exceeding 65 m in depth; and the continental slope, J1. F, fall (Oct-Nov 1975); W, winter (Feb 1976); Sp, spring (June 1976); Su, summer (Aug 1976). Many of the fall and winter shrimp were taken from collections made by Kraeuter.

Total length in 5 mm intervals \ Stations	D,N,B				E,F,A,I				J			
Season	F	W	Sp	Su	F	W[1]	Sp	Su	F	W[2]	Sp	Su
16 - 20	2		5		1							
21 - 25	6	1	6		10		6					
26 - 30	23	6	8	1	51		3					
31 - 35	44	18		3	82		5	3				
36 - 40	38	56		8	25			3				
41 - 45	24	46		3	17		1	14				
46 - 50	10	7	4	1	7		5	7	2			1
51 - 55	3	2			1		2		3			
56 - 60		1		1			2	1	6			5
61 - 65									4			1
66 - 70									8			2
71 - 75									7		2	3
76 - 80									9		1	3
81 - 85									4		3	1
86 - 90									5		2	
91 - 95									2		1	1
96 - 100											1	
101 - 105												1

[1]No shrimp captured in these areas during winter.
[2]Otter trawl lost at J1, no shrimp taken.

Table 10-4. Length-frequency distribution of female and male *Dichelopandalus leptoceras* arranged according to season, sex, and degree of sexual maturity. Headings: F, W, Sp, Su, fall, winter, spring, summer; 2, 3, and E refer to degree of sexual maturity--2 = gonadal stage 2 (gonads relatively immature, ova previtellogenic, and male gametes premeiotic), 3 = gonadal stage 3 (gonads mature), E = fertile eggs on abdomen.

Total length in 5 mm intervals \ Sex:	Female												Male							
Season:	F			W			Sp			Su			F		W		Sp		Su	
Gonadal maturity:	2	3	E	2	3	E	2	3	E	2	3	E	2	3	2	3	2	3	2	3
16 - 20																				
21 - 25							3										1	1	1	
26 - 30						1	3										3			
31 - 35	4			1		1				1			2		1	2	1	1		
36 - 40	2	1			1	5				5				1		2			1	
41 - 45	2	2			2	6		1		8				2		1			3	1
46 - 50		3					3	2		3	2					1		1	1	
51 - 55		2			1															
56 - 60			1	1			1			1	3									2
61 - 65																				1
66 - 70											1									
71 - 75								1			3									
76 - 80											1							1		
81 - 85							1	1										1		1
86 - 90							1	1												
91 - 95								1			1									
96 - 100								1												
101 - 105											1									

II. A trematode metacercaria. Two shrimp out of a sample of 28 from Station J1 harbored metacercaria; only one metacercaria per shrimp was observed. None of the shrimp taken from the continental shelf were infected with metacercaria.

III. Black spot gill disease. Small black spots or cysts were commonly found on the gill lamellae of *D. leptoceras*. Histologically, the cysts were seen to consist of melanized, hypertrophied gill filaments which were walled-off or segregated from normal gill tissue by a melanized plug which incorporated masses of dead or senile-appearing connective tissue cells. Morphologically, with respect to degree of lamellar involvement and size of cyst, there was a considerable degree of variation. The larger cysts often contained numbers of ciliates, each with a recognizable macro- and micronucleus. The ciliates were round or ovoid, were morphologically unremarkable, and measured 18-33 μm in length. Ciliates similar in appearance and size to those observed in cysts were seen attached to gill filaments in shrimp with or without black spots. Sawyer (National Marine Fisheries Lab., Oxford, Md.) examined sections of ciliate-filled cysts and identified the ciliates as *Synophrya hypertrophica*, a parasitic apostome ciliate which has been shown to cause hypertrophy and blackening of gill filaments in crabs and in a penaeid shrimp (Chatton and Lwoff 1935; Johnson and Bradbury 1976). This parasite has a wide host affinity and seems to prefer high salinity waters (Johnson and Bradbury 1976). Chatton and Lwoff (op. cit.) indicated that the maturation and development of *Synophrya* in the gills of crustacea were firmly integrated with the moulting cycle of the host, the parasites reaching maturity before or during ecdysis. These researchers also found that during ecdysis the host was purged of the cysts.

The results of our work on this parasite (Table 10-5) show that the occurrence of black spot cysts reached a peak in fall and winter and that cysts were not observed in 0-year shrimp taken during the spring and summer. These observations suggested that the incidence of black spot disease may have been directly related to the frequency or periodicity of ecdysis or moulting in *D. leptoceras* and that the incidence of this disease may be employed to estimate or measure the frequency of moulting in a given population of *D. leptoceras*. In this regard, it was interesting to note that the few shrimp taken from the outer continental shelf areas A1 and I1 during the spring and summer had a rather high incidence of black spot gill disease (Table 10-6). This could be interpreted to mean that areas A1 and I1 were marginal or poor habitats for *D. leptoceras* or that shrimp in these areas were simply migrating through, and that ecdysis in these shrimp was suppressed.

A number of other researchers have noted the presence of black spots on the gills of pandalids. Uzmann and Haynes (1968) noted black spots on the gills of *D. leptoceras* taken from waters off southern Nova Scotia, New England, and Long Island. Infection rates varied between 52 and 96% The authors ascribed the black spots to a fungus - a chytridlike phycomycete. Apollonio and

Table 10-5. Length-frequency distribution of *Dichelopandalus leptoceras* arranged according to the presence (+) or absence (-) of black spots on gills, by season, and area. Shrimp have been grouped according to the frequency of black spot disease with season and length.

Total length in 5 mm intervals	Incidence Groups:	I (Stations D,N,B,E,F)								II (Stations A,I)				III (Station J)			
	Season:	F		W		Sp		Su		Sp		Su		Sp		Su	
	Black Spots:	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
16 - 20							5										
21 - 25							12		1								
26 - 30		1	1		2		11		1								
31 - 35		5	3	4	2	2	3		3				1				
36 - 40		4	2	4	7			1	9			2					
41 - 45		6	2	3	6		1		11			5	2				
46 - 50		3		1			7	1	4	2	1	3					1
51 - 55		2			1	2											
56 - 60		1		1					1	3			1			3	2
61 - 65																1	
66 - 70																2	
71 - 75														2		2	1
76 - 80														1		2	1
81 - 85														2	1		1
86 - 90														2			
91 - 95														1		1	
96 - 100															1		
101 - 105																	1

Table 10-6. Percent *Dichelopandalus leptoceras* with black spot disease by area and season.

Stations (grouped)	Season			
	Fall	Winter	Spring	Summer
D1,N3,B1,E1,F1	75.3	42.0	9.3	6.3
A1, I1	--	--	83.3	71.4
J1	--	--	80.0	61.1

Dunton (1969) found that populations of *Pandalus borealis* in the Gulf of Maine were infested with black spots; the infecting organism was believed to be the apostome ciliate *Gymnodinioides*. Rinaldo and Yevich (1974) examined very large numbers of *P. borealis* from the Gulf of Maine and Greenland and were apparently unable to confirm or find the etiological agent claimed by Apollonio and Dunton (1969).

Crangon septemspinosa (Crustacea, Decapoda, Natantia, Crangonidae)

The sand shrimp, *C. septemspinosa*, is a common inhabitant of estuaries and nearshore waters from Newfoundland to eastern Florida (Squires 1965; Williams 1965). The obvious importance of *C. septemspinosa* in the trophic spectrum has prompted numerous studies on the growth, feeding, and physiology of this shrimp (cf. Haefner 1976, for references).

Distribution and Length-Frequency Data. Concentrations of *C. septemspinosa* were found at stations C2, D1, N3, E1, B1, and I1 (Table 10-7; see Chapter 6 also). However, E1 and I1 may have been somewhat marginal for the sand shrimp as they were only found at I1 during one season (spring), and shrimp taken from these areas were smaller than shrimp from the other stations. This length-frequency data supported one other generalization: there appeared to be a seasonal progression in size of shrimp from spring to winter.

Gonads and Gonadal Maturation. Studies of the gonads of the sand shrimp showed (Table 10-8) that eggs were extruded over long periods (in fact, egg-bearing females were observed during all four seasons), shrimp had more than one brood per year, and males were more common (on all stations) during the winter than any other season. It was also interesting to note that the degree of maturation of the ovaries of "two-brood" egg-bearing shrimp was correlated with the degree of maturation of the embryos that the shrimp carried on their swimmerets (Table 10-9). These data indicated, as mentioned above, that the sand shrimp may carry more than one brood per year, but also that the extrusion of the second brood was timed to coincide with the release of the mature embryos from the swimmerets. Haefner (1972) suggested on the basis of length-frequency data that *C. septemspinosa* may bear more than one brood per year.

Table 10-7. Length-frequency distributions of *Crangon septemspinosa* by station and season. Shrimp were collected at I1 during the spring only. The low numbers of shrimp collected during the fall is not a seasonal trend but simply a collection artifact.

Length in 5 mm intervals	Stations						Seasons			
	C2	D1	N3	B1	E1	I1	F	W	Sp	Su
11 - 15	2								2	
16 - 20		7	9	1	5			7	11	4
21 - 25	4	4	3	10	4	1	1	8	7	10
26 - 30	1	3	19	2	9	3		9	8	20
31 - 35	3	4	4	10	1	1		15	6	2
36 - 40	3	2	2					6		1
41 - 45	1			2				3		

Table 10-8. *Crangon septemspinosa* categorized according to sex, degree of sexual maturation, and season. Percent males by season for all areas has also been included. The one shrimp taken on the fall (1975) cruise was an egg-bearing female. Headings: 2, gonadal stage 2 (females with previtellogenic primary oocytes; males with primary or secondary spermatocytes); 3, gonadal stage 3 (females with postvitellogenic oocytes; males, sperm present).

Sex :	Female									Male		
Stage of gonadal maturation :	2			3			Embryos (Eggs)			2&3		
Season :	W	Sp	Su	W	Sp	Su	W	Sp	Su	W	Sp	Su
No. individuals per category :	2	0	4	10	5	3	10	21	20	19	3	6
Percent male by season, all stations = W : 45 Sp : 10 Su : 18												

Table 10-9. The relationship between the degree of ovarian maturation and the degree of maturation of the embryos appended to the swimmerets of "two brood" *Crangon septemspinosa* is demonstrated (see text).

Stage of gonadal maturation :	Stage 2		Stage 3	
Degree of maturation of embryo :	Early	Advanced	Early	Advanced
No. individuals :	7	6	0	13

Symbionts.

I. Gill ciliates. The gills of the sand shrimp were infested with a ciliate, which in paraffin sections, resembled the ciliates found on the gills of *Dichelopandalus leptoceras* (see above) and the other Crustacea sampled during the first year. Although these ciliates appeared to be "commensal" in nature and did not evoke any obvious responses from the shrimp, the large numbers of ciliates found on the gills of several shrimp may have caused some physiological impairment in the host.

II. Trematode metacercaria. *C. septemspinosa* was also parasitized by trematode metacercaria. These were found encysted in the body musculature. The infection rate was low (2%) and the parasite did not elicit an obvious response from the host.

Pontophilus brevirostris (Crustacea, Decapoda, Natantia, Crangonidae)

P. brevirostris is a deep-water shrimp related to *Crangon*. Virtually nothing is known about this shrimp.

Distribution and Length-Frequency Data. This shrimp was collected at stations F1, A1, and J1 in waters deeper than 80 m. Only one shrimp was collected during the first (fall) cruise. The length-frequency data presented

in Table 10-10 implied that this shrimp may have been migratory (note the absence of shrimp at Station J1 during spring or summer) and probably did not live more than one year.

Table 10-10. Relationship between length, season, and station for the shrimp, *Pontophilus brevirostris*.

Total length in 5 mm intervals \ Station:	F1			A1			J1		
Season:	W	Sp	Su	W	Sp	Su	W	Sp	Su
16 - 20	3		1	2	5				
21 - 25	4	2	8	3	7				
26 - 30	2	6			7	2	2		
31 - 35		2		4	4		7		
36 - 40					2		1		

Gonads and Gonadal Maturation. Histological examination of the gonads permitted the following conclusions: (1) *P. brevirostris* spawned or extruded eggs in the spring and summer (Table 10-11); (2) females were slightly larger than males; (3) females outnumbered males. Interestingly, a greater proportion of males was found at Station A1 (33% male) than at either F1 or J1 (10 and 11% male, respectively). The apparent concentration of males at A1 was found to be statistically significant. When the combined data (male vs. female) from areas F1 and J1 were compared with the data from A1 by means of a Chi-square test (χ^2_c), the null hypothesis (that the proportion of males to females was equal regardless of area) was rejected at < 5%; (4) like *Crangon septemspinosa*, *P. brevirostris* produced at least two broods per year.

Symbionts.

I. Gill ciliates. This shrimp was infested, like the other shrimp in this study, with gill ciliates.

II. Trematode metacercaria. One shrimp of the 74 examined histologically (1.4%) was parasitized with a single trematode metacercaria.

Cancer irroratus (Crustacea, Decapoda, Brachyura, Cancridae)

The rock crab is found only in the Western Atlantic from Labrador to South Carolina (Rathbun 1930; Rees 1963; Williams 1965; Wilder 1966). It is commonly found in depths ranging from less than 1 m to 550 m. Despite the commercial importance of this crab in more northern waters little is known of its life history, physiology, or pathobiology.

Table 10-11. Length-frequency table indicating the relationship between degree of gonadal maturation, sex, and location of *Pontophilus brevirostris*.

Station	Length in 5 mm intervals / Sex: / Gonadal Stage: / Season:	Females									Males					
		2			3			Embryos			2			3		
		W	Sp	Su	W	Sp	Su	W	Sp	Su	W	Sp	Su	W	Sp	Su
A1	16 - 20					2			1					1	2	
	21 - 25	1							4					2	1	
	26 - 30					1	2		1						4	
	31 - 35	1			2	2			2							
	36 - 40					2										
F1	16 - 20	1														
	21 - 25	1		3		2				2	1					
	26 - 30				1	6										
	31 - 35					2										
	36 - 40															
J1	16 - 20															
	21 - 25															
	26 - 30	1														
	31 - 35	6														
	36 - 40	1														

Distribution and Length-Frequency Data. The rock crab was found at all benthic trawl stations (see Chapter 6) and, in company with *Dichelopandalus leptoceras* appeared to be one of the few cosmopolites of the Middle Atlantic continental shelf area. Concentrations of juvenile rock crabs (<50 mm carapace width) were found only in waters less than 70 m deep (Table 10-12). Large numbers of adults (>50 mm) were found only at Station D1. The data implied that whereas *C. irroratus* may have been cosmopolitan in its distribution on the continental shelf, it was relatively rare in deeper waters.

Table 10-12. Numbers of juvenile (≤ 50 mm in width) and adult (> 50 mm) *Cancer irroratus* per station; stations arranged from C2 to J1, according to depth, C2 being the most shallow station (see Chapter 2). The dichotomization between juvenile and adult crabs was made on the basis of data presented in Table 10-13.

Numbers	Stations								
	C2	D1	N3	B1	E1	I1	F1	A1	J1
Nos. Juveniles	14	23	15	14	15	1	4	0	2
Nos. Adults	1	12	2	1	0	1	5	1	1
Total	15	35	17	15	15	2	9	1	3

Gonads and Gonadal Maturation. Few mature crabs and a plethora of small crabs were examined histologically. Still, the data (Table 10-13) did indicate that mature gametes were generally evident in crabs greater than 50 mm in width.

Symbionts and Lesions. Rock crabs had several interesting symbionts and lesions:

I. Gill ciliates. Again, these ciliates resembled the *Synophrya*-like ciliates found in the gills of the other crustacea examined in this study. These ciliates were found almost exclusively in crabs less than 40 mm in length.

II. Gill bacteria. Crab gills were occasionally coated with "fuzzy" basophilic mats of filamentous epiphytic bacteria.

III. Small copepods. Small copepods were also found wedged among gill lamellae.

IV. *Hematodinium*. *Hematodinium* sp., a parasitic dinoflagellate of crab vascular spaces, was found in the blood of one rock crab collected at Station N3 during the fall of 1976. We have also found *Hematodinium* in the blood of several *Cancer borealis* (see below). This parasite has been previously observed in the blood of *Carcinus*, *Portunus*, and *Callinectes* (see Newman and Johnson 1975). Maclane (National Marine Fisheries Laboratory, Oxford, Md.;

Table 10-13. The relationship between the size (width) of *Cancer irroratus* and the degree of gonadal maturation.

Width in 10 mm intervals \ Sex:	Undifferentiated	Female		Male	
Gonadal Stage:		2	3	2	3
1 - 10	33				
11 - 20	38				
21 - 30	6				
31 - 40	3	1			
41 - 50	6		1		
51 - 60	2		1	1	2
61 - 70			2		
71 - 80			2		1
81 - 90	2		2		1
91 - 100	1		2		
101 - 110					2
111 - 120					1
121 - 130			1		

personal communication, 1976) has also recently discovered *Hematodinium* in the blood of one rock crab taken from the New York Bight area. She reported that the incidence of this disease in rock crabs was very low (< ½%). This report (and Maclane's) constitute a new host record for *Hematodinium*.

From the point of view of the pathobiologist, *Hematodinium* is an extremely interesting parasite. Histologically, this organism closely resembled crab blood cells. In fact, the only obvious histological parameters that differentiated *Hematodinium* from crab blood cells were (1) the distinctive nucleus of this organism--nuclei seemed to be frozen into a prophase or early metaphase configuration and (2) the rare coalescence of these cells into multinucleate giant-type cells.

Although *Hematodinium* usually occurred in enormous numbers in crab blood, it did not seem to have any obvious effect on the organs and tissues of the infected crabs. There appeared to be fewer numbers of blood cells in vascular spaces, also noted in *Callinectes* by Newman and Johnson (1975), but this may have been a reflection of an attenuation or dilution effect by *Hematodinium*. The pathogenicity of this organism when present in low or moderate numbers would be expected to be minimal. In overwhelming numbers, the organism would of course cause physiological impairment, which under stressful conditions, could result in death of the host.

V. Melanized foci and cysts. The gills of many crabs were found to contain small (< 70 µm in diameter) melanized foci and

cysts; no viable organisms were apparent in the cysts.

VI. Microsporidian-like bodies. Small, round to ovoid bodies, reminiscent of microsporidians, were found on several occasions in blood cells and in the connective tissue and epithelial cells of the gill. These bodies have not been identified to date. Sawyer (personal communication, 1976) has also found these entities in rock crabs from the New York Bight area. The distribution and prevalence of some of these entities in the rock crab was interesting. It has already been mentioned that the gill ciliates were confined to small crabs. The bacterial "fuzz", copepods, and melanized foci were confined, with several exceptions, to the larger crabs from stations C2 and D1 (Table 10-14). The presence or prevalence of the filamentous bacteria on crab gills, like the black spots on the gills of *Dichelopandalus*, would seem to be closely correlated to the moulting cycle of the crabs; bacteria would be found only on crabs with long intermoult periods.

Cancer borealis (Crustacea, Decapoda, Brachyura, Cancridae)

Although *C. borealis*, the northern crab, looks like, and is often found in the same general locale as its close relative, *C. irroratus*, there appears to be little mixing of the two species, at least in inshore waters (Perry 1966). The work presented below implies that this may be true also for offshore populations of the two crabs.

Distribution and Basic Length-Frequency Data. Northern crabs were concentrated at stations F1, I1, A1, and J1; one specimen was caught at D1 (Table 10-15). The larger crabs appeared to be concentrated at J1.

Table 10-15. Numbers of juvenile (< 50 mm in width) and adult (> 50 mm) *Cancer borealis*; stations arranged by depth. Since very few mature *C. borealis* were taken at the benthic trawl stations, the criterion for separation of juvenile and adult *C. irroratus* was employed.

Numbers	Stations								
	C2	D1	N3	B1	E1	I1	F1	A1	J1
Nos. Juveniles	0	0	0	0	0	13	14	5	19
Nos. Adults	0	1	0	0	0	2	0	0	17
Total	0	1	0	0	0	15	14	5	36

Gonads and Gonadal Maturation. The few sexually mature crabs noted were all males (2 from J1, approximately 65 mm in width, 1 from D1, 125 mm; and 1 from I1, 135 mm). One sexually immature (stage 2 gonad) female was taken from Station I1. The absence of mature female crabs at the benthic trawl

Table 10-14. Relationship between the size of *Cancer irroratus* and the presence of various gill commensals and lesions.

Width in 10 mm intervals	Nos. crabs, all stations	Nos. crabs with gill ciliates	% +	Nos. crabs with gill bacteria	% +	Nos. crabs with copepods	% +	Nos. crabs with melanotic foci	% +
1 - 10	33	17	51	0	0	0	0	0	0
11 - 20	38	11	29	0	0	0	0	1	3
21 - 30	6	1	17	0	0	0	0	1	17
31 - 40	4	2	50	0	0	0	0	0	0
41 - 50	7	0	0	0	0	0	0	0	0
51 - 60	6	0	0	0	0	0	0	0	0
61 - 70	2	0	0	0	0	0	0	1	50
71 - 80	4	0	0	1	25	2	50	2	50
81 - 90	5	0	0	0	0	0	0	0	0
91 - 100	3	0	0	2	67	0	0	0	0
101 - 110	2	0	0	0	0	0	0	1	50
111 - 120	1	0	0	1	100	1	100	1	100
121 - 130	1	0	0	0	0	0	0	0	0

stations was surprising; however, Carpenter and Haefner (VIMS, personal communication, 1976) report that they have never observed egg-bearing female *C. borealis* taken off the Virginia coast.

Symbionts. In comparison with the rock crab, the northern crab was relatively "clean". Few ciliates were noted among juveniles; melanized foci were noted in only two crabs (approximately 45 mm in width), and only one crab had gill copepods. However, two crabs from Station J1 were parasitized by *Hematodinium*. The infection rate for all crabs collected during the first year was only 3%. The infection rate for all moderately large crabs ($\geq$ 40 mm) on these stations was 7%.

Molluscs

Astarte castanea (Mollusca, Lamellibranchia, Heterodonta, Astartidae)

Astarte spp. are small clams of no commercial importance. The common name for *Astarte*, "blood clams", relates to the hemoglobin-containing respiratory protein of *Astarte*.

Distribution. *A. castanea* was found at stations C2, N3, E1, and B1.

Gonads and Gonadal Maturity. Male and female clams occurred in equal numbers; both sexes were equal in length (Table 10-16). *A. castanea* began producing mature gametes when 15-16 mm in length. Production of gametes was not seasonal or cyclic as mature, apparently viable gametes were found in abundance in these animals throughout the year.

Table 10-16. Length-frequency data for male, female, and undifferentiated *Astarte castanea*.

Length in 5 mm intervals	Sex		
	Undifferentiated	Female	Male
6 - 10	2		
11 - 15	3		1
16 - 20		2	6
21 - 25		20	13
26 - 30		6	6
31 - 35			1
Totals	5	28	27

Symbionts.

I. Sporocysts of digeneic trematodes. Fifteen percent of *A. castanea* were parasitized with branching trematode sporocysts

resembling the sporocysts of Bucephalid trematodes (see Cheng and Burton 1965). Branching sporocysts were found ramifying throughout many of the soft tissues of the clam. The parasite penetrated and spread throughout gonadal tissues, vascular spaces, and in heavily infected individuals, gill tissue. In heavily parasitized clams, gonadal tissues were greatly reduced. Sporocysts did not invade heart, kidney, muscle, or digestive diverticula. The host response to the sporocyst was minimal. This parasite resembled both morphologically and in organ preference *Bucephalus cuculus*, a trematode parasite of oysters which causes the "parasitic castration" of its host (see Cheng and Burton 1965, for review).

II. Cytoplasmic inclusion bodies. DNA-positive cytoplasmic inclusion bodies, approximately 14 μm in diameter, were found in the cells of the digestive diverticular areas of 9% of the *A. castanea* examined. The inclusions were well-preserved by both phosphate-buffered aldehyde mixtures and Dietrich's fluid. Even in "heavily infected" individuals the inclusions were never common; in a given section 6 to 8 might be counted. They were basophilic and stained for DNA (acid-Feulgen; methyl green-pyronin), We have also observed digestive diverticular inclusion bodies in *A. undata* and *Placopecten* (see below). Harshbarger (Registry of Tumors for Lower Animals, Smithsonian Institute; personal communication, 1976) has recently found inclusion bodies in the digestive diverticular cells of clams and oysters and has observed with the electron microscope organisms resembling both rickettsia and mycoplasmas in the inclusions bodies.

III. Nuclear inclusions. Structures resembling hypertrophied nucleoli or virus-inclusion bodies were seen in the nuclei of the digestive diverticular cells in 20% of male *A. castanea* taken from Station C2 during the spring and summer. These entities were acidophilic and often quite large (up to 6.6 μm in diameter). Nothing is known about the genesis or etiology of this "disease", or if, in fact, whether this structure is a manifestation of a disease process.

IV. Ciliates of the digestive diverticula. Ciliates were seen nestling among the cells of the digestive diverticula in GMA sections of both *Astarte* spp. and *Placopecten magellanicus;* these ciliates were not obvious in paraffin sections of Dietrich's-fixed material. The ciliates were round to oval in cross-section, were approximately 15 μm in diameter, and typically possessed a number of small 1-4 μm diameter-DNA-positive "micronuclei". The organism did not possess a discrete macronucleus. A moderately large (2-3 μm) vacuole-like structure was seen in the cytoplasm of the ciliate. Histochemically, the vacuole was determined to contain acid mucopolysaccharides. The cilia of this organism were flattened down on the pellicle and were never observed in an extended position. It was evident that these organisms were able to reproduce themselves; it was not uncommon to observe ciliates in the process of dividing or budding off from one another. These ciliates were never observed in large numbers in the digestive diverticula of the host, and they never seemed to elicit pathological reactions.

Astarte undata (Mollusca, Lamellibranchia, Heterodonta, Astartidae)

Distribution. This clam was found in deeper waters than its congener, *A. castanea;* it was taken at stations E1, F1, J1, I1, A1, and B1.

Gonads and Gonadal Maturity. Like *A. castanea*, the gonads of *A. undata* did not appear to exhibit seasonal or cyclic activity. Males and females occurred in equal numbers (Table 10-17); females appeared to be somewhat larger ($\bar{X}$ = 23.9 mm F; 21.8 mm M), but more data will be required to substantiate this statistically.

Table 10-17. Length-frequency data for male, female, and undifferentiated *Astarte undata*.

Length in 5 mm intervals	Sex		
	Undifferentiated	Female	Male
6 - 10	7		1
11 - 15	14	3	5
16 - 20		3	3
21 - 25		8	6
26 - 30		5	8
31 - 35		4	1
Totals	21	23	24

Symbionts. Unlike its congener *A. castanea*, *A. undata* was not infested with trematode sporocysts, neither were nuclear inclusion bodies found in the cells of the digestive diverticula. 5.4% of the clams, however, were infected with the DNA-positive cytoplasmic inclusions noted in the digestive diverticular cells of *A. castanea*. Ciliates were also found in GMA sections of digestive diverticula (see above).

Placopecten magellanicus (Mollusca, Lamellibranchia, Anisomyaria, Pectinidae)

The giant scallop inhabits the colder waters off the Atlantic coast from Labrador to Cape Hatteras (Abbott 1954; Culliney 1974) and is the object of a regional fishery in both eastern Canada and New England.

Biologically, these scallops are interesting, not only because they are mobile and possess "eyes", but also because of their longevity--they may live 10-12 years (Stevenson and Dickie 1954; Merrill et al. 1966); because they may serve as a refuge for small red hake (Wigley and Theroux 1971; Musick 1974); and because they are parasitized by green algae (Naidu 1971; Stevenson and South 1974).

Distribution. Giant scallops were taken at stations N3, E1, F1, I1, A1, and B1.

Gonads and Gonadal Maturity. The giant scallop, in contrast to many of the other Pectinidae, was not hermaphroditic; males and females occurred in equal numbers. Sexually immature scallops ranged in size between 21 and 110 mm, and sexually mature scallops between 61 and 147 mm (Table 10-18). The degree of sexual maturity of the giant scallop gonad was found to exhibit seasonal variations which seemed to be cyclic. These seasonal variations (Table 10-19) permitted the conclusion that scallops had an annual gonadal cycle in which spawning occurred in the spring and summer months.

Symbionts. Giant scallops taken from the stations indicated above had 3 symbionts. These organisms proved particularly difficult to observe in material fixed in Dietrich's fluid and embedded in paraffin; for the most part, they were manifest only in material preserved in phosphate-buffered aldehyde mixtures and embedded in glycol methacrylate. The following symbionts were observed:

I. DNA-positive cytoplasmic inclusions. DNA-positive cytoplasmic inclusions were seen in the cells of the digestive diverticula. These inclusions resembled those observed in the *Astarte*.

II. Ciliates. Ciliates resembling those seen in the digestive diverticula of *Astarte* were also seen in the digestive diverticula of the giant scallop.

III. Spores bearing a marked resemblance to the spores of the gregarine sporozoan, *Nematopsis*, (see Sprague 1970) were observed in the epithelial cells of the intestine. Although these spores were often very common, they did not seem to elicit any overt pathological reaction.

Echinoderms

Echinarachnius parma (Echinodermata, Eleutherozoa, Echinoidea)

Distribution. *E. parma*, the common sand dollar of the North American east coast, was found in abundance at depths of less than 70 m (stations C2, D1, N3, E1, and B1; see also Chapter 6).

Gonads and Gonadal Maturity. Males and females occurred in equal numbers (Table 10-20). There was no apparent difference in the size of male and female sand dollars. The sex of sand dollars could be differentiated histologically (stage 2 gonad) in animals as small as 16 mm (Table 10-21). Fully mature gametes were noted in sand dollars larger than 27 mm in diameter. Reproductive activity in *E. parma* was cyclic: in males, fully "ripe" gonads filled with sperm were found most frequently in fall; in females, ripe, yolk-filled eggs (stage 3 gonads) were observed only in animals taken during the spring, summer, and fall. In both sexes, stage 2, immature (no sperm or vitellogenic eggs) gonads were almost never, with the exception of one small sand dollar, seen in animals taken in the fall (Table 10-21). These data signified that gamete production in *E. parma* from the OCS study areas was a lengthy process, beginning in the winter and terminating in the late summer or fall, and *E. parma* spawned during the late summer and fall.

Table 10-18. Relationship between the length, sex, and degree of gonadal maturation of *Placopecten magellanicus*. 0, follicular development not apparent; 1-4 refers to gonadal stages outlined in Methods.

Length in 10 mm intervals \ Sex: / Gonadal Maturation:	Undifferentiated			Female		Male	
	0	1	2	2	3&4	2	3&4
21 - 30	6	2				1	
31 - 40	9						
41 - 50						1	
51 - 60			1				2
61 - 70		2		1	3	1	3
71 - 80		1		1	3		7
81 - 90			2		8		4
91 - 100		1			4		3
101 - 110			1		3		2
111 - 120					2		
121 - 130					1		
131 - 140							
141 - 150							1

Table 10-19. The seasonal change in the degree of gonadal maturation of the giant scallop, *Placopecten magellanicus*. Data represent combined sexes. Numbers are % relative frequency.

Stages	Season			
	Fall	Winter	Spring	Summer
Early Stage 3	36	25	0	37
Late Stage 3	64	75	100	5
Stage 4	0	0	0	58
No. scallops examined	11	4	12	19

Table 10-20. The relationship between length, sex, and degree of gonadal maturation in the sand dollar, *Echinarachnius parma*. 0, no follicular development; 1-4, gonadal stages discussed in Methods.

Diameter in 5 mm intervals / Sex:	Undifferentiated		Female			Male		
Gonad Stage:	0	1	2	3	4	2	3	4
6 - 10	4	6						
11 - 15	3	5						
16 - 20		2	2					
21 - 25		1	1			3		
26 - 30		1	2			2	1	
31 - 35			1	1		4	4	1
36 - 40		2	10	2		4	10	1
41 - 45		2	8	3	2	1	12	1
46 - 50		1	5	3	2	1	5	
51 - 55			2	1			2	
56 - 60					1			

Table 10-21. Relative frequency of *Echinarachnius parma* by season, of a given gonadal stage. Early Stage 3: few mature gametes evident; Late Stage 3: many mature gametes observed in sections.

Gonad Stage / Sex:	Female				Male			
Season:	F	W	Sp	Su	F	W	Sp	Su
Stage 2	3	51	30	16	0	33	47	20
Early Stage 3	60	0	20	20	0	50	25	25
Late Stage 3	60	0	0	40	64	18	14	4
Stage 4	60	20	0	20	67	0	33	0

Symbionts. Approximately 8% of the sand dollars examined had commensal peritrich ciliates attached to the test. In sections, these oval-shaped ciliates measured 40 x 66 µm in diameter and possessed an elongate, horseshoe-shaped macronucleus. Peritrich ciliates similar to the one observed on *E. parma* were also noted in *Asterias forbesi* and *A. vulgaris* (see below) but not in *Astropecten americanus*.

Astropecten americanus (Echinodermata, Eleutherozoa, Asteroidea)

Distribution and Size. This sea star was found at stations E1, F1, J1, I1, and A1. Individuals from stations J1 and E1 were smaller than those from stations F1, I1, or A1 (Table 10-22). This indicated that stations E1 and J1 may have been marginal habitats for *A. americanus*.

Table 10-22. Length-frequency data for all *Astropecten americanus* taken at stations E1, F1, J1, I1, and A1 during the first year.

Length in 10 mm intervals	Station				
	E1	F1	J1	I1	A1
1 - 10			3		
11 - 20		1	4		
21 - 30	10		9		
31 - 40	9	1	5	1	
41 - 50	5	2			1
51 - 60				4	5
61 - 70	1	5		6	3
71 - 80		5		2	7
81 - 90		5		1	7
91 - 100					1
101 - 110					1

Gonads and Gonadal Maturity. Males and females occurred in equal numbers, and the sexes were equivalent in length (Table 10-23). *A. americanus* began producing mature gametes at 39 mm in diameter. The gonads of the sea stars taken from the "marginal" stations E1 and J1 were undeveloped or rudimentary; none of the individuals taken from Station J1 had advanced beyond a stage 1, primordial gonad, and only 14% of the individuals from Station E1 had advanced beyond this stage. *A. americanus* from the "optimal" stations average 67% beyond stage 1. These results were not unexpected since individuals at stations E1 and J1 were smaller than those from the other stations. Lastly, it was noted that reproductive activity in *A. americanus* may have been cyclic. This conclusion was predicated on the following observations: in considering the ratio of stage 2 sexually immature individuals to stage 3 mature individuals it was noted that proportionally more immature animals were taken during the winter than at any other season (Table 10-24) and that proportionally more stage 3 specimens were taken during the spring, summer, and fall. When the numbers of immature and mature animals taken during the winter was compared

Table 10-23. Relationship between the size of *Astropecten americanus*, sex, and gonadal maturity.

Diameter in 10 mm intervals / Sex: / Gonad Stage:	Undifferentiated	Female		Male	
		2	3	2	3
1 - 10	3				
11 - 20	5				
21 - 30	18			1	
31 - 40	14				1
41 - 50	5		2		1
51 - 60	3	1	1		4
61 - 70	4	1	6		3
71 - 80	2	3	3	3	3
81 - 90	4	1	5	1	2
91 - 100					1
101 - 110	1				1

No. Stage 2&3 females: 22
No. Stage 2&3 males: 21

Table 10-24. The numbers of Stage 2 and Stage 3 gonads by season (proportion expressed as a percentage) of Stage 2 to Stage 3 *Astropecten americanus*.

Season	Gonadal Maturation		% Stage 2
	Stage 2	Stage 3	
Fall	0	6	0
Winter	9	6	60
Spring	1	11	8
Summer	1	10	9

statistically by means of a Chi-square test (χ^2_c) with individuals taken during the spring, summer, and fall (combined data), the null hypothesis that there was no seasonality in the proportion of immature to mature animals was rejected at a very high level (< 0.5%). It was concluded that *A. americanus* spawned during the warmer months of the year.

Symbionts. Only one symbiotic form was noted histologically in *A. americanus*. A number of elongate, rather nondescript ciliates, approximately 40 μm in length, were noted in the gonad of one male sea star. Large numbers of blood cells were clustered around the ciliates. This ciliate was, in all likelihood, *Orchitophyra stellarum*, an Astomatid ciliate parasite of the gonads of *Asterias rubens*, *A. forbesi*, and *A. vulgaris* (Cépède 1907; Smith 1936; Galtsoff and Loosanoff 1939; Vevers 1951). It has been hypothesized that infection of asteroids with this organism may result in the parasitic castration of the host. The infection rate of sexually mature (stage 3) *A. americanus* was 3%.

Asterias forbesi (Echinodermata, Eleutherozoa, Asteroidea)

A. forbesi is the common "starfish" of inshore waters which has plagued the oyster growers of the New England coast for so many years.

Distribution. Our collections were composed of individuals taken from stations C2, D1, N3, E1, F1, and B1; 85% of our samples (45 of 53 animals) were taken from stations C2 and D1. *A. forbesi* would seem to concentrate at the inshore stations.

Gonads and Gonadal Maturity. The data suggest that *A. forbesi* became sexually mature - began manufacturing ripe gametes - at approximately 65 mm in diameter (Table 10-25). The data suggested also that *A. forbesi* from the continental shelf area exhibited cyclic reproductive activity (Table 10-26), with *A. forbesi* spawning during the spring and summer. The data are still too fragmentary to permit any firm conclusions.

Symbionts. Approximately 85% of the *A. forbesi* were found to have commensal peritrich ciliates attached to its body surfaces. The ciliates resembled those described above for the sand dollar.

Asterias vulgaris (Echinodermata, Eleutherozoa, Asteroidea)

Distribution. *A. vulgaris* was collected from stations D1, N3, E1, F1, I1, A1, and B1; only 1 specimen was taken from A1.

Gonads and Gonadal Maturation. *A. vulgaris* began producing mature gametes at approximately 35 mm in diameter.

Symbionts. A commensal peritrich ciliate was found on the external body surfaces of approximately 26% of the *A. vulgaris* collected. This ciliate resembled the ciliates observed on the surfaces of sand dollars and *A. forbesi*. In addition, the parasitic Astomatid ciliate, *Orchitophyra stellarum*, was observed in the gonads of two male sea stars; both were 72 mm in diameter. The infestation rate among mature sea stars was rather high: 9%.

Table 10-25. Relationship between sex, degree of gonadal maturation, and length for *Asterias forbesi*. 0, no gonadal tissue observed; 1, 2, and 3 refer to gonadal stages described in Methods.

Length in 10 mm intervals \ Sex:	Undifferentiated			Female & Male	
Gonad Stage:	0	1	2	2	3
11 - 20	2				
21 - 30	7	2			
31 - 40	9			1	
41 - 50	5			3	
51 - 60	3		1		
61 - 70	3			2	2
71 - 80	1			1	2
81 - 90	1			1	
91 - 100	1			1	1
101 - 110				1	1
111 - 120					
121 - 130					
131 - 140					
141 - 150					2

Table 10-26. Numbers of *Asterias forbesi* with immature, Stage 2 gonads and mature Stage 3 gonads related to the season in which they were taken. Male and female are combined.

Season	Gonadal Stage	
	2	3
Fall & winter	10	2
Spring & summer	0	6

DISCUSSION

The histopathological study of 12 benthic continental shelf invertebrates presented above focused on three aspects or facets of their biology: (1) the distribution and range of the animals; (2) their reproduction and maturation; and (3) the symbionts associated with these animals. Although these facets have been treated, in many cases, as separate and distinct sets of data, it must be understood that they are, in fact, inseparable, each parameter or data point being merely a reflection of an organism's past and current physiological state. Hopefully, as work progresses, the diverse types of data generated will combine to yield a meaningful statement on a given organism's physiological state.

Basic ecological relationships between a wide range of benthic invertebrates including those organisms chosen for histopathological analysis and their physical environment have already been presented and discussed previously (see Chapter 6); these results demonstrated that a certain area or station will be found to possess a characteristic faunal assemblage and that, conversely, certain organisms have distinct habitat preferences. The data presented in this chapter reinforced this conclusion and, in addition, indicated that (1) the habitat preference of some of the continental shelf animals appeared to change with both the season and size of the animal, and (2) some areas or stations were only marginally suited to the growth and reproduction of a given species.

That an organism should have exhibited a change in habitat preference may have been a consequence of a migratory drive or tendency in the organism. Evidence of migratory tendencies was obvious to a greater or lesser degree in all the shrimp studied and in *Cancer borealis*. The evidence suggesting migratory behavior in *Crangon septemspinosa*, *Pontophilus brevirostris*, and *C. borealis* was somewhat circumstantial. (1) During the winter, relatively large numbers of male *C. septemspinosa* were taken from our trawl stations; males were not common during other seasons. (2) The few sexually mature *C. borealis* taken at the trawl stations were all males. One of these males was taken during the fall at the mid-shelf station D1. (3) During the winter, numbers of large *P. brevirostris* were caught at Station J1. No *P. brevirostris* were caught at this station during the spring and summer (see Results). Evidence indicating migratory behavior was more clear-cut for *Dichelopandalus leptoceras* (see Results). This shrimp apparently migrated not once but twice during its lifetime. It is hypothesized that with the approach of winter, 0- or early 1-year shrimp, which until this time had ranged widely over the continental shelf, including stations E1 and F1 (depth at F1, 84-85 m), fell back to or congregated in waters of less than 65 m in depth but greater than 26 m (stations D1, N3, B1). While over-wintering in these waters young *D. leptoceras* became sexually mature and spawned. Sometime during late winter or spring, after the hatching of the eggs, the shrimp migrated into the deeper waters of the outer continental shelf and thence to the continental slope where, during the winter following, they spawned once more.

It is evident that more data will be required before one can declaim, with any degree of certainty, on the exact migratory patterns of those crustacea from the continental shelf. It would not be surprising, however, to learn that these animals have pronounced migrations or extended migratory paths; it has been calculated that *D. leptoceras* may, in fact, migrate as

much as 60 km during one season.

It was evident, after considering both the numbers and length-frequency relationships of various animals, that some environments, even though supporting or permitting limited growth of a given species, were marginal habitats for that species. This concept was illustrated most clearly by the sea stars and in particular by *Astropecten americanus*. *A. americanus* was taken at stations El, Fl, Il, Al, and Jl, representing depths ranging from 66 m (El) to 410 m (Jl). Animals taken from stations El and Jl, the shallowest and deepest stations respectively, at which *A. americanus* was taken were smaller than those taken from stations Fl, Il, and Al, representing a rather narrow span of depths (77-91 m). Not only were the animals from El and Jl small, but they also had a low proportion of sexually mature individuals, all of which were males.

Thus, in the event that it may be necessary to evaluate the impact of man's activities on the continental shelf, it will be important to bear in mind that some of the "indicator" organisms may have migrated from areas far removed from the site of impact and that some of the animals under consideration as indicators may have already been subjected to stress as they may have been living in marginal situations.

A knowledge of the sexual and reproductive biology of an animal should be considered a sine qua non in baseline histopathological studies: whether an organism can survive on a short-term basis in an impacted area is not a true measure of an organism's ability to cope with that environment. That organism must be able to reproduce itself so that its progeny will continue to colonize that environment.

Studies on the gonads and gonadal maturity of selected benthic invertebrates reported in this chapter show that many of the animals under consideration spawned or extruded gametes during a particular season or seasons; that is, reproductive activity was cyclic or seasonal. The exceptions to this statement were *Crangon septemspinosa* and *Astarte* spp. (see below) and the *Cancer* crabs and *Asterias vulgaris* for which reliable data were not available. Thus, *Dichelopandalus leptoceras*, *Pontophilus brevirostris*, *Placopecten magellanicus*, *Echinarachnius parma*, *Astropecten americanus*, and *Asterias forbesi* were found to exhibit cyclic reproductive activity. *D. leptoceras* spawned during the winter months and the remainder of the animals from this list during the warmer months of the year (see Results). The reproductive activity of *C. septemspinosa* and *Astarte* spp. did not seem to be restricted to any one season or seasons. Great numbers of viable gametes were found in these animals the year around. These observations on spawning periodicity, or lack of it in many cases, confirm the findings of other researchers on these species or closely related animals: *C. septemspinosa* (Cowles 1930; Price 1962; Haefner 1976); pandalid shrimp (Berkeley 1930; Pike 1952; Butler 1964); *P. magellanicus* (Posgay and Norman 1958); *E. parma* (Cocanour 1969); sea stars (Hyman 1955; Boolootian 1966). Saleuddin (1964) reported that *Astarte sulcata* taken from the coast of Scotland exhibited marked reproductive seasonality conforming to the classic spring-summer waxing and fall-winter waning pattern of other lamellibranch gonadal cycles. It was impossible to show that *Astarte* from the Middle Atlantic continental shelf area conformed to this pattern. The gonads of larger *A. castanea* and *A. undata* were always filled with viable-looking mature gametes. Gonads that appeared to be spawned-out, resting, or wasted were never observed. It was possible, of course, that gonads of these *Astarte* did undergo cyclic changes but that these changes occurred between sampling periods.

Many of the observations presented above on the distribution and spawning times of the shrimp studied histologically were supported by the data on the distribution of crustacean larval forms developed by the zooplankton/neuston studies (Chapter 4). These observations and my expectations, based on gonad histological analysis, of larval distribution of these shrimp are compared in Table 10-27. This table indicates that there was substantial agreement between the data with respect to shrimp distribution and spawning times. It should be noted that there was, in some instances, no agreement between the data sets--indicated by a '2' or '3' in the table. This lack of agreement may have been the result of a number of factors including (1) a shoreward drift of larvae, or (2) an extended larval period. A shoreward drift of slope waters in the Middle Atlantic Bight area has been proposed by Iselin (1939; 1940), Bumpus (1965) and Wright (1976); see Chapter 3 also. Even if evidence for a shoreward component of slope and shelf waters was non-existent, it would be necessary to evoke "shoreward drift" in order to account for the distribution of larval and adult shrimp as reflected in Table 10-27.

Symbiotic organisms were found on or in all 12 of the animals selected for histopathological analysis. Several of these organisms were obviously commensal forms; that is, these symbionts lived with the host and not at the expense of the host. The commensal forms included the peritrich ciliates found attached to the test of most echinoderm species and the filamentous epiphytic bacteria found on the gills of *Cancer irroratus*. The ciliates noted on the gills of *C. septemspinosa*, *P. brevirostris*, and juvenile *C. irroratus*, which are assumed to be non-parasitic exuviotrophic apostome ciliates (cf. Grimes 1976), might also be placed in this category. In the absence of drastic traumatic episodes and under "normal" conditions, these commensals would not be expected to harm or cause physiological impairment of the host.

The status or type of host-symbiont interaction of many of the other symbiotic forms was less clearly defined although it is probable that the status of these organisms will be clarified as more data become available and as soon as some of them can be identified. For instance, little is known about the biology or status of (1) the several symbionts infesting the digestive diverticula and intestine of the lamellibranchs collected for this study and (2) the gill copepods of *C. irroratus*. Observations on the symbionts of the lamellibranch gut indicated that although these organisms were undoubtedly parasitic, that is, they lived at the expense of the host, they did not elicit a host response or result in the destruction of host tissue. These parasites, then, appeared to be well adapted to their host and quite benign.

Several of the symbionts were clearly neither commensals nor benign parasites. These organisms included, (1) the branching trematode sporocysts infecting *Astarte castanea*, (2) the trematode metacercaria found in the shrimp, (3) *Orchitophyra*, the ciliate which parasitized the gonads of the sea stars, (4) the apostome ciliate *Synophrya hypertrophica*, the causative organism of gill black spot disease in *Dichelopandalus leptoceras*, and (5) the parasitic dinoflagellate, *Hematodinium*, which was found in the haemal spaces of the *Cancer* crabs. Any of these organisms, under suitable circumstances, might effect either severe physiological impairment or the death of the host, or in the case of the gonadal parasites, the trematode sporocysts in *A. castanea* and *Orchitophyra*, a drastic reduction in the numbers of viable gametes produced by the host.

In evaluating the present or potential harm that a given symbiont might cause, one must keep in mind that in habitats where the host is well adapted,

Table 10-27. Comparison between the seasonal distribution of three larval shrimp at the C-J transect and the expected seasonal distribution of larval shrimp based on histological studies of post-larval shrimp gonads and eggs from the same transect. A dash (-) indicates that no shrimp larvae were found and none expected; 1, larvae found and expected (complete agreement); 2, larvae not expected but found; 3, larvae expected but not found.

Area \ Species:	*Crangon septemspinosa*				*Pontophilus brevirostris*				*Dichelopandalus leptoceras*			
Season:	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp	Su
C	1	1	1	1	-	-	-	-	-	-	-	-
D	1	1	1	1	-	-	-	-	-	1	1	-
N	3	1	1	1	-	-	2	-	-	1	1	-
E	1	1	1	1	-	2	-	2	-	2	2	-
F	-	-	-	-	1	2	1	1	-	2	-	-
J	-	-	-	-	-	-	-	-	-	1	3	-

an accommodation or balance between the host population and its symbionts will exist. However, in the face of chemical or physical insult, or perhaps as a result of degenerative processes associated with senescence, even the most accommodating or benign symbiont may turn on its host; examples from human and comparative medicine are legion.

Continued study of the symbionts of the benthic invertebrates selected for analysis will undoubtedly result in a more thorough knowledge of the hosts themselves. In this regard, Sawyer (National Marine Fisheries Lab., Oxford, Md., personal communication, 1976) and I have determined that the numbers and types of symbiotic organisms on the gills of *C. irroratus* and *D. leptoceras* seem to be largely determined by the frequency of moulting in these animals; the longer the intermoult period, the more organisms settle in and on the gills.

The hydrocarbon and trace metal data were examined for possible correlations with the histopathology data. Although statistical tests were not performed, no obvious correlations were found to exist between the hydrocarbon and histopathology data. However, two interesting trends were observed with respect to several of the biologically important trace metals (Fe, Zn) and the histopathology data. First, zinc levels in both *Asterias forbesi* and *A. vulgaris* appeared to reach a maximum value, for a given station, during the spring (Table 10-28; see Chapter 8, also). Since the synthesis and accumulation of gonadal products in both species may also reach a peak during the spring, it is suggested that a significant portion of the zinc in these animals may be associated with gonadal processes and products. In this regard, it was interesting to note that the highest zinc levels in the sea star, *Sclerasterias tanneri* (see Chapter 8), were noted in animals collected during the winter and spring. Second, iron levels in *Astarte undata* appeared to be higher than those in *Astarte castanea* (Table 10-29) although differences may be explained by station location. The iron levels in the *Astarte* clams may reflect the availability of iron in the environment, but it may also be true that the differences in the iron levels between these clams may be attributed to differences in basic metabolic and physiological processes. For instance, *Astarte* clams contain hemoglobin (Waxman 1975). Since it is quite possible that much of the iron in *Astarte* may be incorporated into hemoglobin, iron levels in *Astarte* may reflect levels of respiratory enzyme and ultimately oxygen levels or other environmental factors. In addition, iron in the *Astarte* clams may be associated with the ability of *Astarte* to ward off parasites. *Astarte undata* - the "high-iron" blood clam - was not infested with the trematode sporocysts or the nuclear inclusion bodies which were routinely found in its congener, *A. castanea*.

The discussion of the trends in the trace metal data presented above serves to introduce an extremely important concept, namely, that trace metal data may, in conjunction with histopathology data, be used to define physiological states in megabenthic organisms. In this regard, Martin (1973) has shown that iron levels in the gills of *Cancer irroratus* increase during the intermoult cycle, reaching maximum values prior to moulting. It is suggested that iron levels in the gills of *C. irroratus* and perhaps *C. borealis* can be employed, therefore, in a routine fashion as an aid in determining details of the moulting pattern in these crabs.

Ruddell (1971c) and Ruddell and Rains (1975) have indicated that zinc and copper in oysters may be employed to mediate environmental insult. A

Table 10-28. Zinc levels for *Asterias forbesi* and *A. vulgaris* in ppm dry weight; data excerpted from Chapter 8.

Species	Station	Season			
		Fall	Winter	Spring	Summer
A. forbesi	C	927	123	1830	*
A. vulgaris	B	393	650	1200	268
	E	154	428	690	*

* No data.

Table 10-29. Iron levels for *Astarte castanea* and *A. undata* in ppm dry weight; data excerpted from Chapter 8.

Species	Station	Season			
		Fall	Winter	Spring	Summer
A. castanea	C2	186	177	*	136
A. undata	A1	516	*	*	*
	I1	708	*	377	534

* No data.

very large portion of these metals appeared to be localized in blood cells; histochemical studies indicated that zinc and copper were released from blood cells as a result of trauma. Further, it was shown that oysters (*Crassostrea gigas*) living under non-optimal conditions had higher numbers of blood cells and greater levels of zinc and copper than those taken from good environments. Thus, levels of zinc and copper in oysters could be considered as indicators of environmental quality or suitability or conversely, as indicators of an oyster's "health".

The report presented above constitutes an important first step in the understanding of the parasites and pathologies of deep-water animals from the Middle Atlantic Bight area. This data should also provide the first strong historical account of several measurable parameters that may be used for comparative studies by investigators involved in similar work on other portions of the continental shelf of North America.

Summary of Significant Findings

1. Many or all of the five crustaceans examined may be migratory. However, precise details of migratory patterns cannot be furnished at this time.

2. Some areas or stations may be marginally suited to the growth and reproduction of some species.

3. In evaluating the impact of man's activities on the continental shelf, it will be important to keep in mind that some of the "indicator" organisms may have migrated from areas far removed from the site of impact and some of the animals under consideration as indicators may have been already subjected to stress since they may have been living in marginal situations.

4. Many of the invertebrates selected for analysis spawned or extruded gametes during a particular season or seasons; reproductive activity was cyclic. The exceptions to this were the *Astarte* clams, *Crangon septemspinosa*, and possibly *Asterias vulgaris*. Reliable data for *A. vulgaris* was not available.

5. There appeared to be a close correlation between the spatial and temporal distribution of shrimp larvae collected by Grant and Smyth (see Chapter 4) and the hypothesized spatial and temporal distribution of larvae based on histological analysis.

6. A knowledge of the sexual and reproductive biology of indicator organisms should be considered a sine qua non in baseline histopathological studies.

7. Symbiotic organisms were found on or in all animals studied. Some of these organisms were obviously commensal forms and some clearly parasitic.

8. In evaluating the present or potential harm that a given symbiont may cause, one must keep in mind that in habitats where the host is well adapted, an accommodation or balance between the host and its symbionts will exist. However, in the face of environmental insult or as a result of processes associated with senescence even the most benign symbiont may become pathogenic.

9. Continued study of the symbionts may result in a more thorough knowledge of the host themselves.

10. No correlations could be made with hydrocarbon data.

11. Several trends or associations were noted between trace metal and histopathology data.

12. It was suggested that trace metal analyses of megabenthic organisms may, in conjunction with histopathological analyses, furnish valuable information on the physiological states of these organisms.

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