MFR PAPER 1342

Thomas K. Sawyer and Sharon A. MacLean are with the Oxford Laboratory, Northeast Fisheries Center, National Marine Fisheries Service, NOAA, Oxford, MD 21654.

Some Protozoan Diseases of Decapod Crustaceans

THOMAS K. SAWYER and SHARON A. MacLEAN

Decapod crustaceans of commercial value such as the blue crab, *Callinectes sapidus,* and the American lobster, *Homarus americanus,* have received considerable attention in recent years because of their economic value. Studies on environmental and nutritional factors which affect commercial yields invariably call attention to the role of diseases in the success or failure of the industry. The development of new and sophisticated research techniques has stimulated many investigators to redescribe disease entities at the cellular and molecular level, and to propose major revisions for poorly understood microorganisms. Thus, the health of fish and shellfish has become a matter of concern to a growing constituency.

In this paper, we will attempt to condense the present status of several important protozoan parasites of decapod crustaceans into a brief summary of species which are deserving of continued investigation. We also include a limited discussion of the role of sessile free-living peritrich and suctorian ciliates as fouling organisms of several crustacean species. It is not our intent to provide a bibliographic overview of the species of protozoans that are included here, but rather to summarize some of our current research activities, including our findings with decapods which are not of significant commercial value.

The discovery of "gray crab" disease in the blue crab, *C. sapidus,* by Sprague and Beckett (1966) probably represents the most interesting and remarkable crustacean host-parasite relationship to be recognized in recent years. The authors first observed aberrant cells in the hemolymph of affected crabs and suggested that the extraordinary cells possibly were infected by a virus. This was suggested because, in addition to a typical nucleus, a DNA-rich body was always in juxtaposition to the unremarkable nucleus.

Shortly after publication of the first report, the authors (Sprague and Beckett, 1968) noted that a free-living amoeba, *Paramoeba eilhardi* Schaudinn, 1896, was described to accommodate a new genus of Amoebida that was characterized by an enigmatic secondary nucleus or "Nebenkorper." Cooperative studies subsequently were initiated in which *P. eilhardi* and the blue crab organism were cultured in vitro under identical conditions, and the latter species was cultured further in a complex series of media (Sprague et al., 1969). The apparent inability of the blue crab organism to survive under any of the test conditions suggested that perhaps it was a new and different species. The name *Paramoeba perniciosa* (Sprague et al., 1969) subsequently was established to characterize the new species of *Paramoeba* (Fig. 1). Shortly thereafter, Page (1970) created *P. aesturina* and *P. pemaquidensis* to accommodate two new free-living species which he discovered in Maine. The genus *Paramoeba* now shares with *Naegleria* and *Acanthamoeba* the distinction of having been proposed originally to accommodate free-living amoeba, and found subsequently to include species capable of causing death in naturally infected hosts—the latter two genera affecting only vertebrates. The mode of infection by *P. perniciosa* and the origin and function of the "Nepenkorper" remain to be discovered. Broader treatment of research and more extensive literature citations on *Paramoeba* may be found in publications by Sawyer et al. (1970) and Johnson (1977). Published accounts of *Paramoeba* infection in decapods recently have established the rock crab, *Cancer irroratus,* and the lobster, *Homarus americanus,* as additional host animals (Sawyer, 1976).

A parasitic dinoflagellate, *Hematodinium perezi,* also may be considered an enigmatic parasite of decapods. Originally identified from hemolymph in two species of crabs, *Carcinus maenas* and *Portunus depurator,* it eventually was placed in the dinoflagellate family Blastodidiidae by Chatton and Poisson (1930) who described its principal diagnostic features. Although the original investigators made particular mention of the small numbers of diseased crabs in the collections, Newman and Johnson (1975) found up to 30 percent of *C. sapidus* to be infected in collections made in the fall of the year. More recently, *H. perezi* was found in *C. irroratus,* the Jonah crab, *Cancer borealis,* and the lady crab, *Ovalipes ocellatus,* all taken in near- or offshore waters of the northeastern Atlantic Ocean (MacLean and Ruddell, 1978). The dinoflagellate parasitizes infected hosts by an unknown mode of entry; the dinospore stage of *H. perezi* remains to be discovered and described. The correct identification of the dinoflagellate in tissue sections of decapods is critical because the histological appearance may resemble a

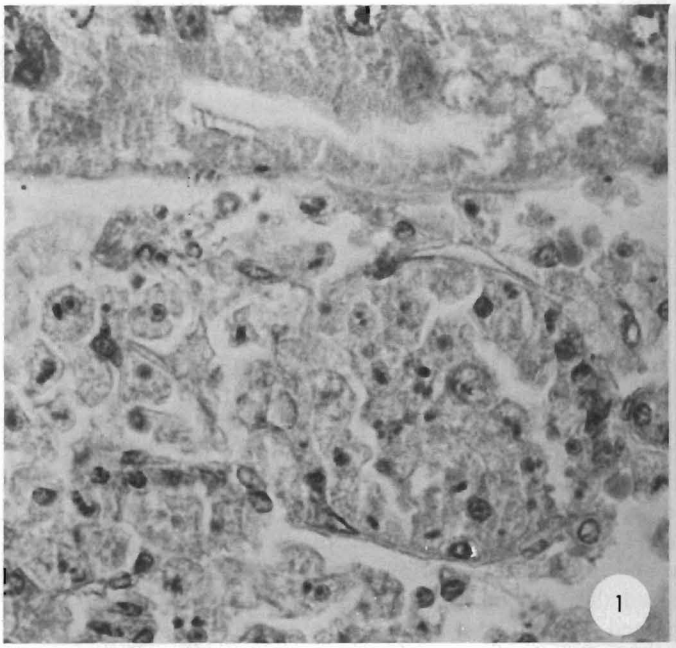

Figure 1.—*Paramoeba perniciosa* in hemal spaces of the hepatopancreas of *Cancer irroratus*. Harris hematoxylin-eosin; 640×.

Figure 2.—Stalked ciliate (*Acineta* sp.) attached to gill epicuticle of *Cancer irroratus*. Harris hematoxylin-eosin; 640×.

possible neoplastic condition (Newman, 1970). Continued research on *H. perezi* is necessary to clarify the status of all life cycle stages and the conclusive systematic position of the parasite. The organism may be well-suited for studies which employ immunological and in vitro culture techniques.

Unidentified gregarines have been found in the hindgut of *C. irroratus*. The parasites have been observed only in stained tissue sections, but should be dissected in situ from affected tissues of infected animals. An excellent account of gregarines in decapod species is given by Ball (1951) who carefully described and illustrated several new species. We are indebted to Phyllis Johnson for suggesting the use of the PAS procedure for the recognition of parasites in histological sections. After such staining, the parasites appear bright purple-red and are not likely to be confused with other parasites or tissue elements. This procedure provided excellent visualization of a large gregarine, possibly *Porospora gigantea*, in the hindgut of American lobsters. The junction between the protomerite and deutomerite and the details in interphase nuclei were exceptionally clear in longitudinal or saggital sections of paired syngyns. The life cycle of *P. gigantea* involves mussels during asexual phases of development, and surveys for the presence of growth stages of the gregarine in naturally infected hosts deserve continued effort. Our observations on gregarines have been made only during histological surveys of selected tissues, and little is known of the prevalence of the parasites in the decapod hosts. Several authors have suggested that heavy infestations of adult gregarines may interfere with the normal functioning of the digestive tract. Our limited histological studies have not shown any obvious effects on the health of parasitized individuals, but further research, possibly with experimentally infected animals, is worthy of consideration.

Infections of decapods by members of the Cnidospora are encountered in natural populations of decapods and are especially amenable to study by electron microscopy. We have observed a possible microsporidan infection in *C. irroratus*, but material so far collected has not been suitable for definitive identification of the parasite. Exceptionally small, spherical parasites have been found in gill epidermal cells and in large nephrocytes; in one specimen, several large plasmodiumlike structures filled with small nuclei were found in the shaft of a single gill. We cannot provide a meaningful discussion of possible cnidosporan parasites of decapods at present because our survey results are only preliminary. A recent publication by Sindermann (1977) will serve as a useful starting point for investigators who are specifically interested in this important group of parasites. For the present, it should be sufficient to call attention to the fact that the taxonomy and systematics of this group are undergoing constant revision, especially as new ultrastructural observations are reported. Sindermann (1977) provides references to several microsporidan infections (*Nosema, Pleistophora*), and one possible haplosporidan *(Minchinia?)* in his recent book. There is an obvious need for extensive studies concerning this group.

Peritrich and suctorian ciliates are well-recognized epibionts on body surfaces of most aquatic animals. Simi-

larly, nonstalked ciliates identified as *Anophrys, Synophrya, Hyalophysa,* and others which occur as endoparasites in decapods have been described or redescribed in recent publications. We will discuss the stalked forms that have been observed in remarkably large numbers on gills of crabs and lobsters, and then summarize our knowledge of the parasitic forms. Histologic sections of gills of approximately 600 *C. irroratus* and 200 *H. americanus* have been examined to date, and all of the animals have been collected in the vicinity of Sandy Hook Bay, N.J., and Ambrose Light in the New York shipping channel. Undetermined species of suctorians, *Ephelota* and *Acineta,* and peritrichs which resemble *Orbopercularia* are gill epibionts throughout all seasons of the year. Occasionally, we have counted up to 300 peritrichs or 500 suctorians *(Acineta)* on one histological section of crab gill, and somewhat smaller numbers of lobster gills. Also, without exception, the higher counts are obtained from animals taken from the bay stations. We have not yet attempted to make precise identifications because fairly lengthy procedures are required to obtain silver-impregnated specimens, and, as with the Cnidospora, ciliate taxonomy and systematics are undergoing extensive revisions. Recent descriptions of many ciliate species incorporate transmission and scanning electron microscope techniques in addition to the more classical silver-impregnation techniques. Consequently, modern approaches to ciliate taxonomy often are beyond the capability of many laboratories. Our report of the occurrence of a suctorian, *Ephelota* sp. (Sawyer et al., 1976) on crustacean gills was limited to a generic identification because most species within the genus need redescription. In our study, gill surfaces were found to be a new substrate for the suctorian, and we suggested that even in an area of recognized high pollution an adequate food web was present to support a predatory species. Peritrichs feed on bacteria, while suctorians feed on protozoa, algae, diatoms, and other larger microorganisms. Although convincing data yet has not been published, it has been suggested that large numbers of sessile ciliates may influence the respiratory activity of infested hosts (Fig. 2).

The occurrence of parasitic ciliates in tissues and blood spaces of decapods has been of interest since the late 1800's. Grimes (1976), Johnson and Bradbury (1976), and Sindermann (1977) have provided descriptive accounts of the parasitic species and the effects on their hosts in a variety of decapods. Until recently, we were concerned about the obvious absence of such species in decapod crustaceans examined in our studies. Johnson and Bradbury (1976) reported that apostome ciliates occurred frequently in specimens that were collected from offshore waters and rarely in those collected close to shore and in estuaries. The authors emphasized the fact that nearshore waters ranged in salinity from 15 to 35 ppt, while offshore waters ranged from 35 to 36 ppt. Most of our animals were from nearshore stations, and parasitic ciliates rarely were noted. These observations agree with those of Johnson and Bradbury (1976). The possibility that salinity may be a barrier to the spread of certain parasitic ciliate infections should be studied further in the laboratory where salinity may be varied. We are continuing our studies on the distribution and numerical abundance of ciliate epibionts in areas distant from our present sampling areas in order to obtain further data. Preliminary observations suggest that water depth may be limiting ciliate abundance, due in part to the scarcity of suitable food organisms with increasing water depth. Research on the role of ciliate species in the marine environment and in crustacean health is just beginning to receive attention by North American investigators. More research is needed for a better understanding of the roles of these organisms in nutrient cycling and the pathogenesis of disease.

The identification of ciliate genera and species is limiting for most investigators who do not have adequate research facilities and technical assistance. Meaningful research findings on ciliate-decapod interactions should not remain in laboratory files simply because their specific identification is beyond the capability of the investigator. Such observations should be published and supported by quality line drawings or photomicrographs. Excellent publications on ciliate systematics have been published by Borror (1973) and Corliss (1974, 1975) and should be consulted as problems of identification occur. Sprague and Couch (1971) have published a comprehensive bibliography on protozoan parasites of decapods which should be reviewed before it is decided to publish observations on ciliate species whose identity is beyond the capability of the investigator.

In summary, it is becoming apparent that many protozoans of decapod crustaceans which have been described primarily in very old literature are being rediscovered. Many original descriptions of genera and species were made prior to the advent of electron microscopy, phase contrast microscopy, and specialized staining procedures such as the Feulgen reaction, and are in need of careful redescription. Renewed interest in parasites such as *Paramoeba, Hematodinium, Pleistophora,* and others is providing valuable new information to investigators in a wide variety of research disciplines. Recent years have testified to the importance of accurate descriptions of numerous parasitic species, especially when previously unexplained mortalities occurred in natural and laboratory-reared stocks. Until recently, most comprehensive accounts of parasites and parasitic diseases of decapod crustaceans dealt only with those animals which are of commercial value. Studies of noncommercially important crustaceans presently are receiving increased interest because we are becoming concerned with man-induced impacts on previously unstressed environments, and because previously underutilized animals are becoming commercially valuable.

LITERATURE CITED

Ball, G. H. 1951. Gregarines from Bermuda marine crustaceans. Univ. Calif. Publ. Zool. 47:351-367.

Borror, A. C. 1973. Marine flora and fauna of the northeastern United States. Protozoa: Ciliophora. U.S. Dep. Commer., NOAA Tech. Rep. NMFS CIRC.-378, 62 p.

Chatton, E., and R. Poisson. 1930. Sur l'existence, dans le sang des crabes, de péridiniens parasites: *Hematodinium perezi* n.g., n. sp. *(Syndinidae)*. C. R. Seances Soc. Biol. Fil. 105:553-557.

Corliss, J. O. 1974. The changing world of ciliate systematics: historical analysis of past efforts and a newly proposed phylogenetic scheme of classification for the protistan phylym Ciliophora. Syst. Zool. 23:91-138.

———. 1975. Taxonomic characterization of the suprafamilial groups in a revision of recently proposed schemes of classification for the phylum Ciliophora. Trans. Am. Microsc. Soc. 94:224-267.

Grimes, B. H. 1976. Notes on the distribution of *Hyalophysa* and *Gymnodinioides* on crustacean hosts in coastal North Carolina and a description of *Hyalophysa trageri* sp. n. J. Protozool. 23:246-251.

Johnson, C. A., III, and P. C. Bradbury. 1976. Observations on the occurrence of the parasitic ciliate *Synophrya* in decapods in coastal waters off the southeastern United States. J. Protozool. 23:252-256.

Johnson, P. T. 1977. Paramoebiasis in the blue crab, *Callinectes sapidus*. J. Invertebr. Pathol. 29:308-320.

MacLean, S. A., and C. L. Ruddell. 1978. Three new crustacean hosts for the parasitic dinoflagellate *Hematodinium perezi* (Dinoflagellata: Syndinidae). J. Parasitol. 64:158-160.

Newman, M. W. 1970. A possible neoplastic blood disease of blue crabs. (Abstr.) Bibl. Haematol. 36:648.

———, and C. A. Johnson. 1975. A disease of blue crabs *(Callinectes sapidus)* caused by a parasitic dinoflagellate, *Hematodinium* sp. J. Parasitol. 61:554-557.

Page, F. C. 1970. Two new species of *Paramoeba* from Maine. J. Protozool. 17:421-427.

Sawyer, T. K. 1976. Two new crustacean hosts for the parasitic amoeba, *Paramoeba perniciosa*. (Abstr.) Trans. Am. Microsc. Soc. 95:271.

———, R. Cox, and M. Higginbottom. 1970. Hemocyte values in healthy blue crabs, *Callinectes sapidus*, and crabs infected with the amoeba, *Paramoeba perniciosa*. J. Invertebr. Pathol. 15:440-446.

———, S. A. MacLean, and J. Ziskowski. 1976. A report on *Ephelota* sp. (Ciliata, Suctorida) as an epibiont on the gills of decapod crustaceans. Trans. Am. Microsc. Soc. 95:712-717.

Sindermann, C. J. (editor). 1977. Disease diagnosis and control in North American marine aquaculture. Elsevier Scientific Publishing Co., N.Y., 329 p.

Sprague, V., and R. L. Beckett. 1966. A disease of blue crabs *(Callinectes sapidus)* in Maryland and Virginia. J. Invertebr. Pathol. 8:287-289.

———, and ———. 1968. The nature of the etiological agent of "gray crab" disease. J. Invertebr. Pathol. 11:503.

———, ———, and T. K. Sawyer. 1969. A new species of *Paramoeba* (Amoebida, Paramoebidae) parasitic in the crab *Callinectes sapidus*. J. Invertebr. Pathol. 14:167-174.

———, and J. Couch. 1971. An annotated list of protozoan parasites, hyperparasites, and commensals of decapod crustacea. J. Protozool. 18:526-537.

MFR Paper 1342. From Marine Fisheries Review, *Vol. 40, No. 10, October 1978. Copies of this paper, in limited numbers, are available from D822, User Services Branch, Environmental Science Information Center, NOAA, Rockville, MD 20852. Copies of* Marine Fisheries Review *are available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402 for $1.10 each.*