PARASITES OF BENTHIC AMPHIPODS: DINOFLAGELLATES (DUBOSCQUODINIDA: SYNDINIDAE)

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ABSTRACT

During a 2½-yr survey, 13 species of benthic amphipods collected from the continental shelf of the northeastern United States were found infected by dinoflagellates. Prevalences ranged from <1% to 67%, depending on amphipod species, time, and place of collection. The parasites are assigned to the order Duboscquodinida, family Syndinidae, based on similar life histories and a similar kind of mitosis ("mitose syndinienne"). Two types of organisms were involved, both apparently more closely related to *Hematodinium* Chatton and Poisson than to other described syndinids. Morphology and development of the parasites and host-parasite interactions are discussed. A cytochemical method used to determine presence or absence of basic nuclear proteins was strongly positive for basic proteins in spores and prespores but negative in most other stages. A few spores in four infections possessed a distinct flagellum, but in the absence of living material, shape of spores and whether they were biflagellate could not be determined. With three possible exceptions in the group of 303 infections studied, the syndinids were not recognized as foreign by their hosts, and in joint infections of syndinids and fungi, only the fungi were being attacked by host hemocytes. High prevalences in certain of the amphipod species suggest that the syndinids might be population regulators in these species.

This paper is one of three that describe and discuss the more common parasites found in populations of benthic amphipods of the continental shelf of the northeastern United States. The other papers concern microsporidans and ciliates (Johnson 1985, 1986).

Because my observations on the parasites discussed in this paper were based on examination of histological sections, I could not determine whether spores were typical "dinospores". However, agreement with other developmental stages of well-studied species of syndinids from copepods and an amphipod, and the nuclear type, indicates that the parasites of benthic amphipods are related to species currently placed in the Syndinidae, order Duboscquodinida (sensu Chatton 1952 and Cachon 1964). Previously described syndinids occur intracellularly in radiolarians and in copepod and shrimp eggs (Chatton 1952; Stickney 1978) and extracellularly in the hemocoel of copepods, an amphipod, and portunid and cancrid crabs (Chatton and Poisson 1931; Chatton 1952; Manier et al. 1971; Newman and Johnson 1975; MacLean and Ruddell 1978).

The relationship of the Duboscquodinida to free-living dinoflagellates is in doubt (Cachon 1964; Ris and Kubai 1974; Siebert and West 1974; Hollande 1975; Loeblich 1976; Herzog et al. 1984). Lacking a definitive consensus, the parasitic protists discussed here are provisionally referred to the Dinoflagellata.

The data presented and discussed in this paper show that species of syndinids are probably ubiquitous hemocoelic parasites of benthic and epibenthic amphipods, and may be population regulators in some species.

METHODS

The data are based on material collected during monitoring surveys carried out over a 2½-yr period by the Northeast Fisheries Center, National Marine Fisheries Service. The 35 stations where benthic amphipods were collected during the surveys are shown in Figure 1. Amphipods were sampled during 11 cruises, but not all stations were visited on each cruise, so that stations were sampled from 1 to 10 times each during the survey. A Smith-McIntyre[2] grab and occasionally an epibenthic sled or scallop dredge were used to obtain the samples. The 11 stations indicated by solid circles on Figure 1 had the most consistent and numerous populations of amphipods, and were sampled at least five times each. They yielded the majority of data presented here.

Amphipods were preserved in 10% seawater Formalin. Up to 30 individuals of each species pres-

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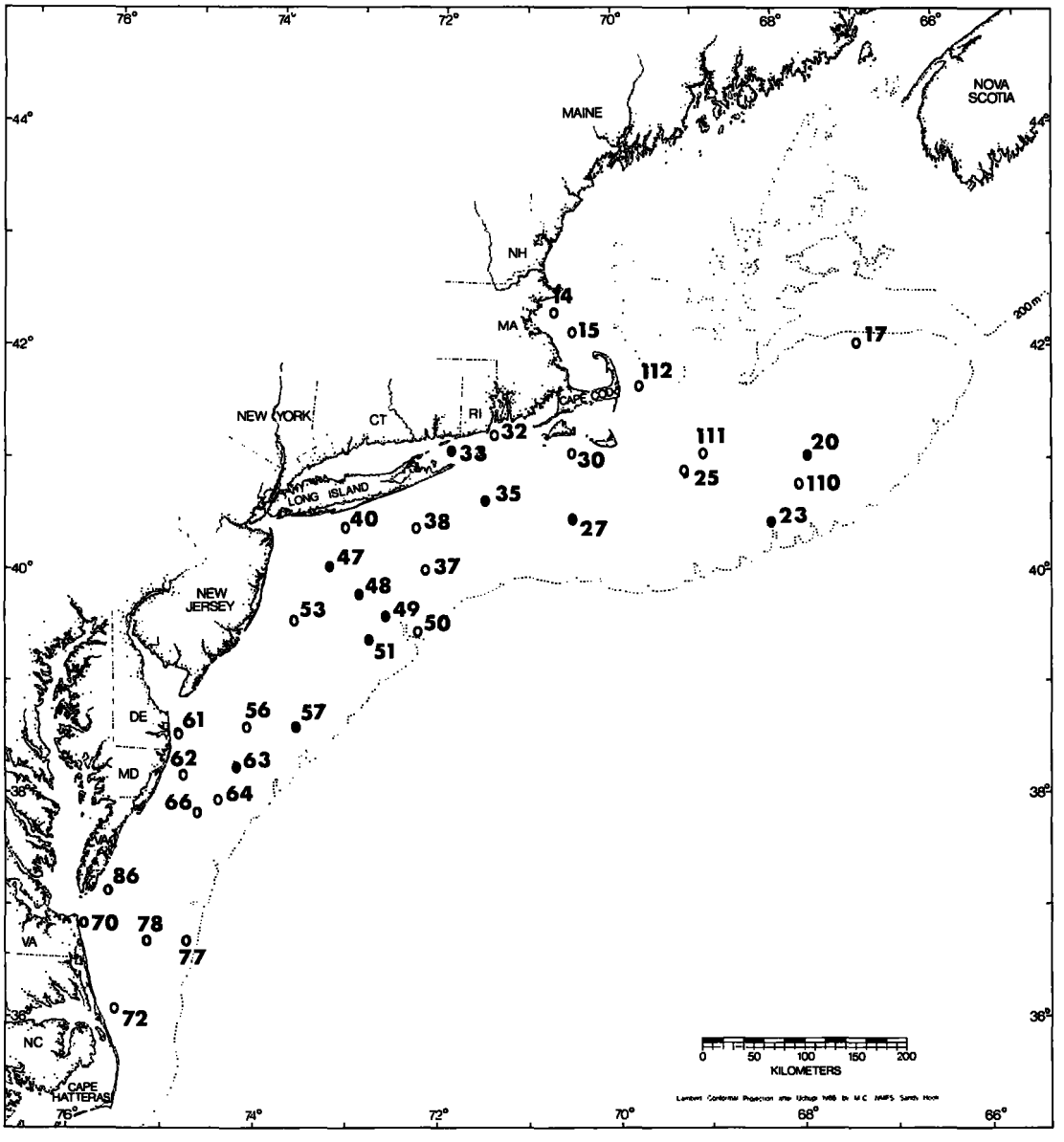


FIGURE 1.—Benthic stations where gammaridean amphipods were sampled during the survey.

ent in a sample, and sometimes more, depending on numbers present, were prepared for histological study. Details of collecting procedures and histological preparation of the amphipods are given by Johnson (1985). Sections were cut at 6 μm. Staining methods included Harris' hematoxylin and eosin (H&E), the Feulgen reaction, and Alfert and Geschwind's (1953) fast-green method for demonstration of basic nuclear proteins. Harris' hematoxylin and eosin is specified because this combination stains nuclei of the parasites purple during certain stages. Other hematoxylin solutions, used with eosin, will not necessarily impart the same distinct purple color. Unless otherwise indicated, references to staining properties of the organisms are to H&E-stained specimens.

OBSERVATIONS

Thirteen amphipod species were infected with syn-

dinids (Table 1). The organisms occupied the hemocoel and morphologically were most like *Hematodinium perezi* Chatton and Poisson, which was described from European portunid crabs. There were two distinct types, based on morphology and development. There is not enough information about the life history stages of *Hematodinium* to warrant assigning either or both types to that genus, and they are identified casually in this paper as "Type AA" and "Type AV" (Table 2). The Type AA forms were similar in all the amphipod species they infected, but there was variation in forms assigned to Type AV, and probably more than one species was involved.

Host and Geographic Distribution

Juvenile and mature amphipods of both sexes were attacked. Only Type AA was found in *Ampelisca agassizi* (Judd), *Byblis serrata* Smith, and

TABLE 1.—Amphipod species infected with Type AA and Type AV parasites.

Species of amphipod	Type of parasite	Prevalence positive stations (%)	Prevalence all stations (%)
Ampelisca agassizi (Judd)	AA	7 (101/1468)	4 (101/2403)
Byblis serrata Smith	AA	14 (24/170)	8 (24/316)
Harpinia propinqua Sars	AA[1]	18 (3/17)	3 (3/116)
Ampelisca vadorum Mills	AV	41 (74/181)	17 (74/448)
Ampelisca verrilli Mills	AV	18 (7/38)	15 (7/48)
Casco bigelowi (Blake)	AV	67 (6/9)	10 (6/60)
Leptocheirus pinguis (Stimpson)	AV	4 (7/163)	0.8 (7/913)
Melita dentata (Krøyer) *s. lat.*	AV	8 (1/12)	2 (1/44)
Monoculodes edwardsi Holmes	AV	27 (25/93)	23 (25/110)
Protohaustorius wigleyi Bousfield	AV	20 (1/5)	0.9 (1/110)
Phoxocephalus holbolli Krøyer	AV	27 (10/37)	14 (10/73)
Rhepoxynius epistomus (Shoemaker)	AA and AV	20 (7/35)	3 (7/249)
Unciola species (probably all *U. irrorata* Say and *U. inermis* Shoemaker)	AA and AV	9 (37/404)	3 (37/1365)

[1]Parasites in two of the infections may not be either Type AA or Type AV.

TABLE 2.—Main characteristics of Type AA and Type AV.

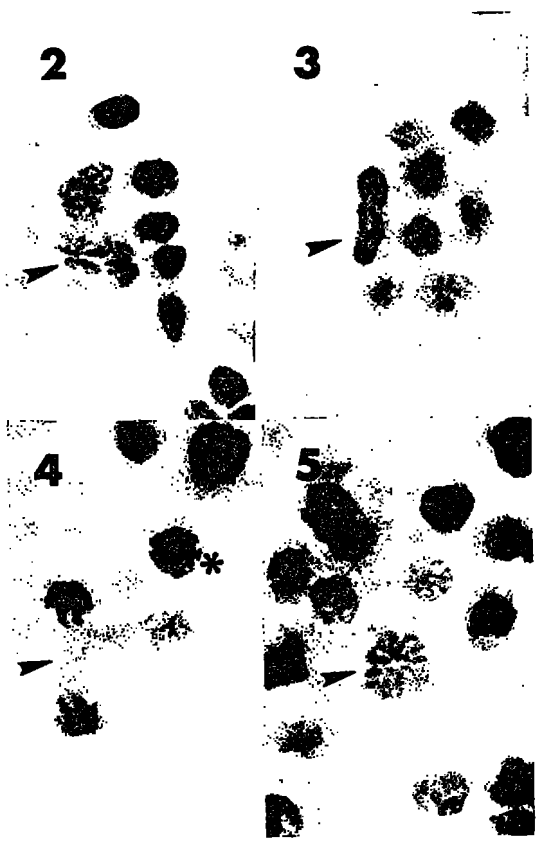
Stage	Characteristic	Type AA	Type AV
I	Nuclear diameter	<3 to >5 μm	2.5 to 3 μm
	Nuclear color	Blue or purple	Purple
	Chromosomes	Usually condensed	Not condensed
	Plasmodia	Present, small	Present, small
	Single cells	Common	Absent or uncommon
	Cytoplasm	Scanty	Abundant, faintly fibrous
IA	Dense bodies	Not present	Present, <2 μm in diameter
	Nuclear diameter	—	2.5 to 4 μm
	Nuclear color	—	Purple
	Plasmodia	—	Present, small
	Single cells	—	Present
II	Nuclear diameter	4 to 5.5 μm	3 to 4 μm
	Nuclear color	Purple	Purple
	Chromosomes	Indistinct, partly condensed	Distinct, partly condensed
	Plasmodia	Uncommon, small	Very rare
	Cytoplasm	Vacuolate	Homogeneous
III	Nuclear diameter (spore)	2.5 to 3 μm	<2 μm
	Nuclear color (spore)	Deep blue	Deep blue
	Chromosomes (spore)	Always condensed	Always condensed
	Cytoplasm (spore)	Scanty	Scanty
	Plasmodia	Absent	Present
	Nuclear diameter (plasmodia)	—	3.5 μm
	Nuclear color (plasmodia)	—	Purple

Harpinia propinqua Sars. Both Types AA and AV occurred in *Rhepoxynius epistomus* (Shoemaker) and *Unciola* species (*U. irrorata* Say and *U. inermis* Shoemaker), and only Type AV occurred in the remaining species (Table 1). Both types of syndinids were present in *Unciola* species taken in a single sample at station 35, but individual specimens were parasitized by only one type. There are not enough data to indicate whether or not incidence varies by time of year in any of the amphipod species infected with these parasites. Infected amphipods were not found at the most northern and southern of the stations, but these stations were sampled fewer times than most of the "positive" stations (i.e., stations where amphipods with syndinid infections occurred). There were 18 positive stations. Only Type AA was found at stations 23, 37, and 50. Only Type AV occurred at stations 33, 40, 56, and 62. Both types were represented at stations 20, 27, 35, 38, 47, 48, 49, 51, 57, 63, and 64.

Whether one or both types occurred at a single station depended variously on which amphipod species were present, and on unknown factors. Two species of *Ampelisca*, *A. vadorum* Mills and *A. agassizi*, were common at inshore station 33. Prevalence of Type AV in *A. vadorum* was 35% (56/158). However, Type AA did not occur at station 33 although a favored host, *A. agassizi*, was abundant there. In contrast, only Type AA was found at station 23, no doubt because of 2,811 amphipods collected there, only 23 were not *A. agassizi*.

Development and Morphology

All forms were similar in that extensive plasmodia were never present and chromosomes were condensed in the interphase nuclei of the spores. There were four, possibly five, chromosomes. There was no metaphase plate. At telophase the apices of the two sets of chromosomes were touching (see Figure 3), and at all stages of mitosis the chromosomes of each group were juxtaposed basally (where they presumably were attached to the nuclear membrane) and spread out apically to varying degrees, like the spokes of a parasol (Figs. 2-4). These events are typical of "mitose syndinienne" (Chatton 1921). Syndinid chromosomes are V-shaped, so that each has two arms. In tissue sections the V shape was best seen in cells that had lysed, leaving only the chromosomes (Fig. 5). During telophase there were often only four (sometimes five?) visible arms of chromosomes in each daughter nucleus. If sectioning artifact was not responsible for the small number



FIGURES 2-3.—Mitosis in Type AV parasites in *Ampelisca vadorum* (arrowheads). Interphase parasites of Figure 3 are stage II.
FIGURE 4.—Mitosis in a Type AA parasite in *Ampelisca agassizi* (arrowhead). Chromosomes form a rosette in the interphase nucleus to the right (asterisk).
FIGURE 5.—Chromosomes in a lysed Type AA parasite from *Byblis serrata* (arrowhead). The V shape of the chromosomes is evident. Figures 2-5, × 1500.

of visible arms, the cells might have been haploid.

Before spore formation, chromatin disposition in nuclei was variable, depending on the type of parasite and the stage of development. Resting nuclei with unfolded chromosomes were granular or vesicular, and sometimes rimmed with chromatin (see Figures 8, 17). In nuclei with partially unfolded chromosomes, clumps of chromatin often were arranged so that they created a dashed or dotted line in the position that would be occupied by a completely condensed chromosome (see Figure 9). When seen in a polar view, chromosomes or chromatin clumps formed rosettes (Figs. 4, 5). Morphology of the persistent chromosomes of spores was variable and will be described later.

Staining characteristics of nuclei differed depending on the stage. Except for spores, prespores, and some cells in early Type AA infections, nuclei tended to be purple, not blue, with both chromatin and the matrix staining similarly in some cases. When nuclei at these stages were in mitosis, the chromosomes were little, if at all, bluer than chromatin in resting cells, although sometimes chromatin was more deeply staining in the dividing cells. Types of chromosomes that stained with fast green by the Alfert and Geschwind method (indicating presence of basic proteins on the chromosomes) would stain blue in H&E preparations. Chromatin and chromosomes that did not stain with fast green in the Alfert and Geschwind method would stain purple with H&E.

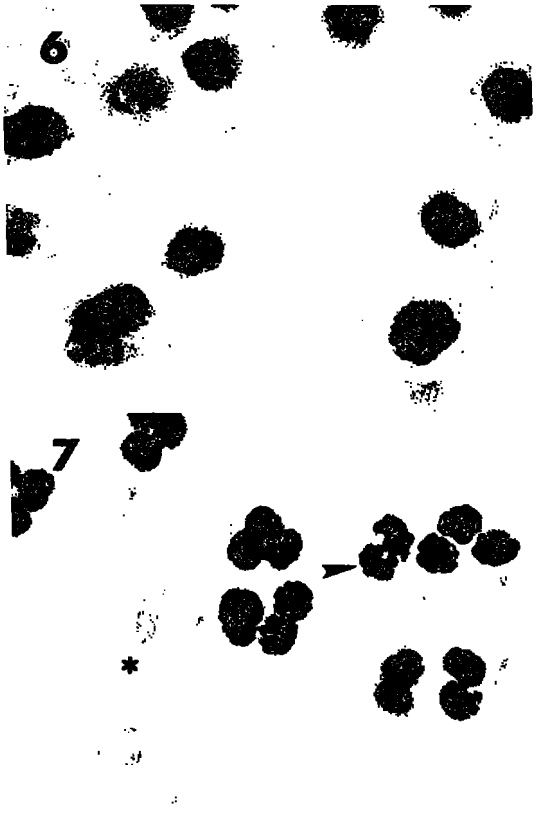
A comparison of Types AA and AV, by developmental stage, is given in Table 2. Infections consisting of few parasites were considered to be the earliest ones and are here designated stage I infections. Stage II infections consisted of more numerous and generally larger organisms, and stage III infections consisted of prespores and spores that usually filled the hemocoel.

Type AA

Most Type AA infections were in *Ampelisca agassizi* (Table 1). Type AA chromosomes of all developmental stages were usually thicker than those of Type AV (compare Figures 2 and 4), and the organisms and their nuclei were larger (Table 2). Stage I organisms were scattered through the hemocoel, never numerous, and variable in morphology and staining characteristics. The one common attribute was scanty and poorly staining cytoplasm. Chromosomes were usually distinct. The most usual stage I infection consisted of scattered single cells and small plasmodia with nuclei that measured 3 to 4 μm and had rather distinct chromosomes or chromatin clumps that stained a clear blue. Mitotic figures were not frequent, but were more common than in the other stages. A few cells in a late stage I (or very early stage II) infection probably were polyploid. They had many rather long, tangled chromosomes that sometimes formed partially separated groups within the nuclear area. The nuclei of these cells measured more than 7 μm in the greater dimension.

Stage II organisms were more numerous and distinguished by having voluminous vacuolate or foamy cytoplasm (Fig. 6). Chromosomes and chromatin clumps were often obscured because the nuclear matrix stained almost as strong a purple as the chromatin. The nuclear matrix did not stain in the Feulgen reaction. Plasmodia were uncommon, always small, and sometimes consisted of short chains of joined cells. Mitosis was rarely seen in stage II and stage III, and probably was closely synchronized, which would reduce the probability of finding mitotic figures in fixed material. As the spore stage was approached, nuclei became smaller and bluer, and chromatin clumps and chromosomes gained clear outlines, because the matrix no longer stained.

By the time of spore formation (stage III), organisms filled the hemocoel, and infected amphipods in H&E-stained sections could be distinguished with the naked eye because of their overall dark-blue color. Spore nuclei were spherical, and chromosomes were condensed but tightly packed and impossible to count (Fig. 7). In one infection, synchronized nuclear division had apparently just taken place, and daughter cells had not yet separated, so that bi- and



FIGURES 6-7.—Type AA parasites in *Ampelisca agassizi*. 6: Stage II. Nuclei do not have distinct clumps of chromatin and the cytoplasm is vacuolate. 7: Stage III (spores) (arrowhead). An unidentified fungus was also infecting the amphipod (asterisk). Figures 6-7, × 1500.

quadrinucleate plasmodia were common. Cytoplasm of spores was scant. Sometimes spores were shaped like teardrops but generally they had amorphous outlines. A flagellum was visible on a few spores in an individual of *Unciola* species.

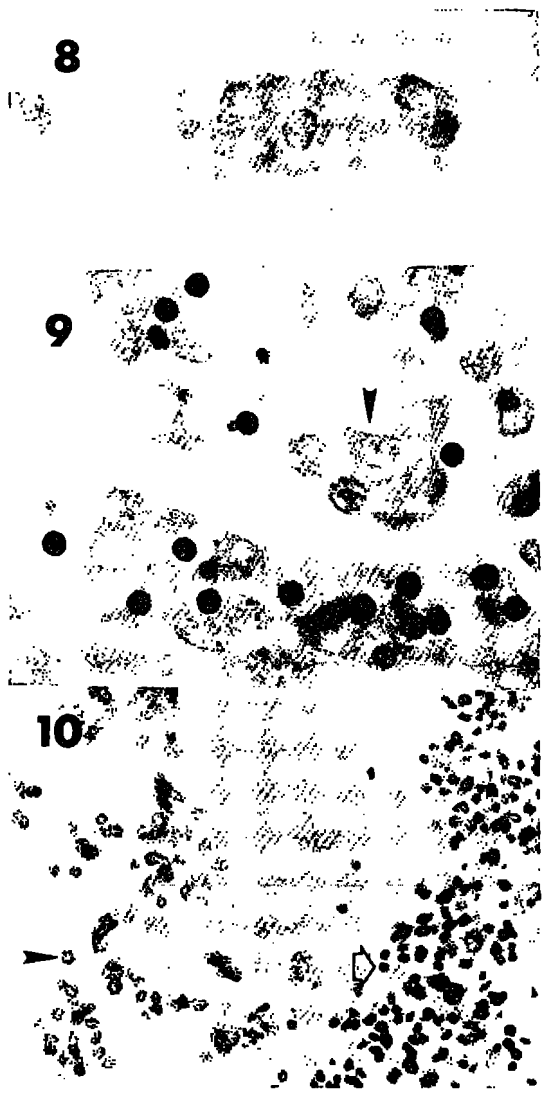
Type AV

This description is based on the organisms that infected *Ampelisca vadorum*. Stage I consisted of scarce and scattered small plasmodia, typically each with 2 to 10 nuclei. Their cytoplasm was faintly fibrous. Chromatin and the nuclear matrix were always purplish and nuclei were often rimmed with chromatin (Fig. 8). The nuclear matrix was not Feulgen positive, and chromatin did not stain strongly by this method. Slightly more advanced infections, with more parasites, had irregularly shaped single cells as well as plasmodia. The single organisms were often elongate, their nuclei were as above, and their cytoplasm was faintly stained.

Stage IA, which I presume follows stage I, and which did not occur in Type AA, had moderate numbers of small plasmodia and single cells. Chromatin patterns were rather distinct in most nuclei, particularly in the larger ones. Chromatin stained purple. Stage IA was distinguished by the presence of small, densely staining bodies. They were usually spherical but sometimes oval, and were usually surrounded by thin rims of cytoplasm. The bodies were associated with the plasmodia (Fig. 9) and also scattered through the hemocoel. They were intensely Feulgen positive and stained bright green by the Alfert and Geschwind method. The dense bodies were never extremely abundant and were present only in the company of many stage IA cells.

Chromosomes of stage II cells were partially condensed, and chromosomes and chromatin clumps were distinct because there was minimal staining in the nuclear matrix, unlike Type AA parasites in stage II. The cytoplasm was usually densely and homogeneously stained (Fig. 3). Cells were often very numerous and closely packed, but were not plasmodial. Occasionally there were a few dense bodies like those associated with stage IA.

Occasional stage III infections were not as heavy as some stage II infections. There was apparently an abrupt transition from stage II cells to stage III prespores and spores. In one infection, a mass of spores with distinct deep-blue chromosomes occupied a circumscribed area in the hemocoel, and larger single cells with condensed chromosomes that stained purple, and were probably very late stage II, occupied the remainder of the hemocoel (Fig. 10).



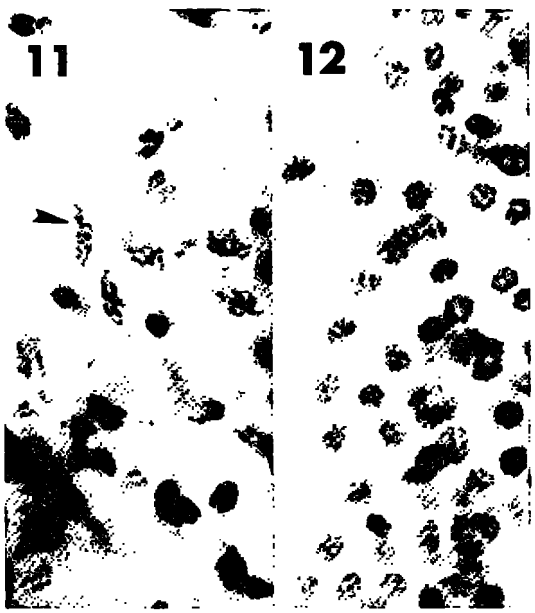
FIGURES 8-10.—Type AV parasites in *Ampelisca vadorum*. 8: Stage I. Several nuclei in the plasmodia are rimmed with chromatin. 9: Stage IA. Plasmodia with associated spherical dense bodies. Nuclei are pale and chromosomes are partially unfolded in some nuclei (arrowhead). 10: Late Stage II (larger, pale nuclei to the left—arrowhead) and Stage III (smaller, deeply staining nuclei to the right—open arrow). A demonstration of synchronized division of the parasite. Larger host nuclei are also present. Figures 8-9, × 1500; Figure 10, × 600.

Presumably, the mass of spores resulted from synchronized but circumscribed division of a part of the population of the larger cell type. The roughly spherical nuclei of the spores in this infection were <2 μm in diameter; nuclei of the larger cells were slightly >3 μm in diameter.

Cells presumed to represent spores had either elongate or spherical nuclei (Figs. 11, 12). The two types did not occur together. Mitosis took place in very small cells, and possibly cells with spherical nuclei were prespores. They might also have been spores that had not yet acquired their final form, because cells of an intermediate shape also occurred. Chromosomes of the spherical nuclei were short; those of elongate nuclei were longer, somewhat more slender, and beaded. Because the cytoplasm was usually indistinct or invisible, outlines of spores were also indistinct. It is probable that spores often ruptured during fixation, resulting in loss of all cell components except the chromosomes, as shown in Figure 11.

A probable polyploid cell was present in one early stage III infection, and there were small plasmodia in all stage III infections (as in Figure 17). Nuclei in plasmodia had purple-staining chromatin and did not stain by the Alfert and Geschwind method, unlike chromosomes of the spores. The relationship of the small plasmodia to spore formation was not obvious.

Numbers of Type AV-infected individuals of species other than *A. vadorum* and *M. edwardsi* were small, and all stages of development were not usually represented. Stage IA infections, as well as some or all the other stages, were seen in *Ampelisca verrilli* Mills, *Leptocheirus pinguis* (Stimpson), *Casco bigelowi* (Blake), and *Unciola* species. Stage IA infections of *A. verrilli* and *C. bigelowi* differed from those of *A. vadorum* because the small dense bodies were often irregularly shaped or composed of two or three contiguous particles rather than being single and spherical or oval. In one of two stage III infections in *L. pinguis*, spores had almost spherical chromosomes (Fig. 13). In the other, chromosomes were indistinct because they were closely packed, but were longer than in the first infection and apparently beaded. All stages of infection were represented in *Unciola* species. Spore nuclei were round or oval and a flagellum was visible on a few spores in two infections. The final divisions were just taking place in one of these infections, and many cells were still binucleate. Most of the single spores had rounded outlines, but spores with a visible flagellum were oval.

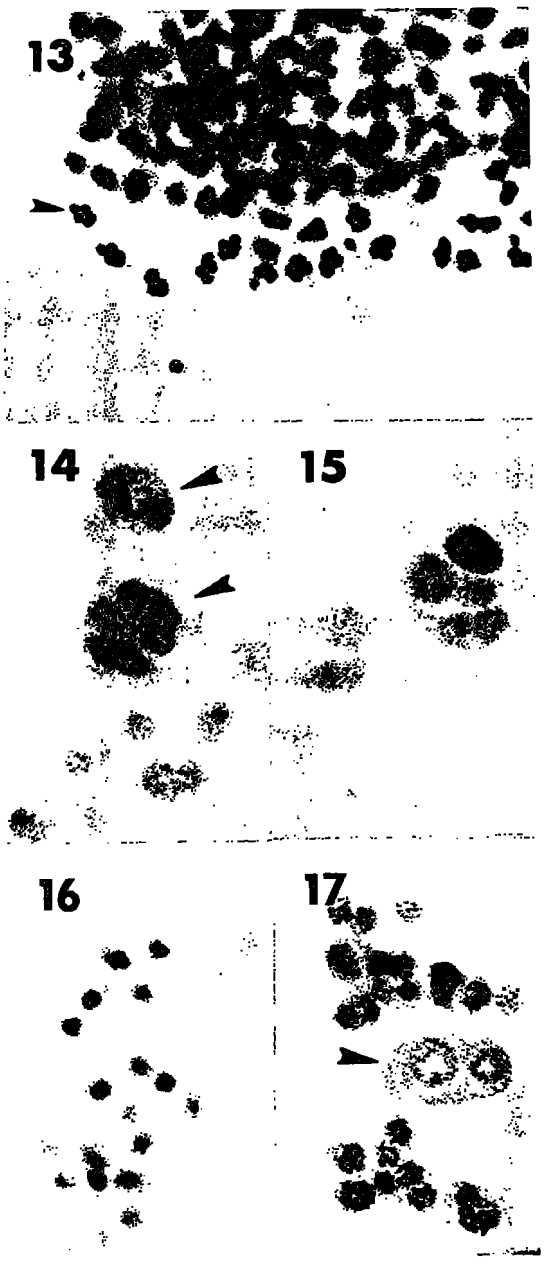


FIGURES 11-12.—Type AV, Stage III (spores), in *Ampelisca vadorum*. 11: Elongate spores. Note the beaded appearance of the chromosomes in one spore (arrowhead). 12: Spherical spores. Figures 11-12, × 1500.

Monoculodes edwardsi had the highest overall prevalence of Type AV (Table 1). The 25 infections encompassed all stages except Stage IA. There were polyploid cells in stage II infections. Their nuclei were sometimes over 6 μm in diameter, often had chromatin separated into several areas (Fig. 14), and their chromosomes were seldom completely condensed, except in mitotic cells. Polyploid cells in mitosis had at least three sets of chromosomes. Outlines of both the interphase nucleus and the entire cell were often highly irregular. Plasmodia that presumably resulted from nuclear division of the polyploid cells often had nuclei of two or more sizes (Fig. 15), suggesting that all chromosome sets did not divide at the same time, or that the genetic material was not distributed equally at the time of division, so that a single plasmodium might have contained haploid, diploid, and polyploid nuclei. Nuclei of Type AV spores in *M. edwardsi* were about 1 μm in diameter (Fig. 16). A single flagellum (not pictured) was visible on some spores in the infection presented in Figure 16. As typical of Type AV, plasmodia were present in all stage III infections (Fig. 17).

Host Response

Reactions against the syndinid parasites were extremely rare. One Type AV-infected specimen each of *Melita dentata* (Krøyer) *s. lat.* and *Unciola* species had scattered, melanized, amorphous nodules in the hemocoel, but the nodules could not be definitely associated with the syndinid infections. In one specimen of *L. pinguis*, hemocytes were associated



FIGURES 13-17.—Type AV parasites. 13: Stage III (spores) in *Leptocheirus pinguis*. The chromosomes are spherical (arrowhead). 14: Stage II in *Monoculodes edwardsi*. Two of the parasites are polyploid (arrowheads). Note separate groups of chromosomes or chromatin clumps in both these parasites. 15: Plasmodium resulting from nuclear division of a polyploid parasite in *M. edwardsi*. Note differently sized nuclei. 16: Stage III (spores) in *M. edwardsi*. There were flagellated spores in this infection. 17: Prespores, some dividing, in *M. edwardsi*. A plasmodium, with rimmed nuclei, is also present (arrowhead). Figures 13-17, × 1500.

with Type AV organisms, and karyorrhexis had occurred in unidentified cells in the area. With the possible exception of the Type AV infection in *L. pinguis*, the syndinids were not being attacked by hemocytes at the time of fixation.

There was another sign that the syndinid parasites successfully evaded detection by their hosts. Two specimens of *A. agassizi*, both collected at station 47 but at different times, were infected jointly and heavily with Type AA and an unidentified fungus (Fig. 7). Of the more than 7,000 examined microscopically, these were the only two amphipods that had systemic fungal infections. Fungi were being phagocytized by hemocytes and fixed phagocytes, and other groups of fungi were being transformed into melanized nodules. (Probably the latter fungi had originally been phagocytized and killed by hemocytes that did not survive the process themselves.) Although hemocytes and fixed phagocytes were actively destroying fungi, there was no indication that the accompanying syndinids were recognized as foreign.

Numbers of hemocytes apparently decreased during syndinid infection, but even in heavy infections some hemocytes remained and were still functional as shown by their ability to phagocytize the fungi discussed above. It is probable that the two successful fungal infections in syndinid-infected amphipods resulted in part from the fungi multiplying more rapidly than they could be phagocytized and degraded by the few remaining hemocytes and the fixed phagocytes associated with the heart.

The syndinid parasites did not castrate their hosts. Whether death ensues from every infection with these parasites is not known. However, the general lack of discernible host response makes it unlikely that amphipods could successfully combat the parasites.

DISCUSSION

Like species of *Syndinium* described from copepods, Types AA and AV have a small number of chromosomes which are permanently condensed in spores and partially condensed in certain other stages; plasmodia (small and multiple in the case of Types AA and AV) are present during some developmental stages; and spore formation takes place in the hemocoel of the host. However, species of *Syndinium* in copepods differ from Types AA and AV in that they develop from a plasmodium that is first applied to the wall of the gut and then expands to fill the entire hemocoel. The massive plasmodium then fragments to form individual dinospores. By

the time of sporulation, the host is castrated (Chatton 1910, 1920). Types AA and AV resemble *Hematodinium*, not *Syndinium*, in that apparently none of these organisms develop from a primary plasmodium associated with the gut, but instead they multiply from a few single cells and small plasmodia in the general hemocoel and never form a single massive plasmodium. Further, these parasites do not castrate their hosts (Newman and Johnson 1975; MacLean and Ruddell 1978; P. T. Johnson, unpubl. data).

Syndinium gammari, like Types AA and AV, is perhaps more closely related to *Hematodinium* than to *Syndinium*. *Syndinium gammari* was assigned to *Syndinium* by Manier et al. (1971) on the assumption that a massive plasmodium was present during development. However, none of the infections studied by these authors had either a primary plasmodium associated with the gut or a later and massive plasmodium throughout the hemocoel. The first stage of *S. gammari* observed consisted of small irregular plasmodia up to 15 μm in diameter, which Manier and coworkers assumed resulted from the splitting-up of a large plasmodium. The small plasmodia then divided to form "diplococcal" forms, and these divided to give round, single organisms which transformed into spores measuring 7-8 μm by 3-3.5 μm. In the later stages of division, typical "dinomitosis" and "dinokaryons" were present. Considering the course of development in the apparently related parasites of benthic amphipods, Types AA and AV, it is possible that *S. gammari* does not have a primary plasmodium associated with the gut wall and does not develop an extensive plasmodium in the hemocoel. If early stages of *S. gammari* consist of a few single cells or small plasmodia, these could have escaped notice because the parasites were observed after their removal from the host amphipod, either alive or in fixed and stained smears (Manier et al. 1971). Scattered organisms could more easily be missed by this technique than by inspection of paraffin-embedded and sectioned whole amphipods.

Chromosomes of *Solenodinium globiforme* and three species of *Syndinium*, all parasites of radiolarians, stain with fast green in the Alfert and Geschwind method for demonstration of basic nuclear proteins (Ris and Kubai 1974; Hollande 1975). Ris and Kubai remarked that chromosomes of the *Syndinium* species they studied also stained brightly in the Feulgen reaction. Although not definitely stated by the above authors, apparently chromosomes of all developmental stages of the above parasites stained equally with fast green. Chromosomes of these species tend to remain condensed through the entire developmental cycle. On the other hand, Hollande (1975) found that trophont nuclei of the duboscquodinids *Amoebophrya ceratii* and *Duboscquella melo* do not stain by the Alfert and Geschwind method. He pointed out that chromosomes are not condensed in the trophont nuclei of these forms and that he did not investigate staining properties of the condensed chromosomes of spores. Hollande did find that a portion of the nucleolus of *A. ceratii* stains with fast green in the Alfert and Geschwind method. Like the syndinid parasites of radiolarians, chromosomes of Type AA and AV spores stain brightly in both Alfert and Geschwind's technique and the Feulgen reaction. However, Feulgen staining is less intense in stages I and II nuclei and these nuclei do not stain at all with fast green.

Eukaryotes have a greater quantity of histone in rapidly dividing cells than in quiescent ones (DuPraw 1968; Wu et al. 1982), and nonhistone basic nuclear proteins—although scarce at all times—are much more abundant in log-phase than in stationary-phase cultures of the free-living dinoflagellates *Gyrodinium cohnii* and *Peridinium trochoideum* (Rizzo and Noodén 1974). It would be interesting to determine the relative amounts of basic nuclear proteins through the developmental cycle of syndinids and other duboscquodinids, and to determine whether basic proteins of the amphipod parasites increase when cells are dividing rapidly; and whether these proteins are masked by other substances (acidic proteins?) in stages where both chromatin and nuclear matrix stain purple with H&E and do not stain in the Alfert and Geschwind method.

Probably fixation and paraffin embedment not only damaged flagella and were responsible for apparent lack of flagella on most spores of Types AA and AV, but also distorted spores of these parasites. Cachon (1964) cautioned that because spores of parasitic dinoflagellates become distorted or ruptured both on fixation and when physical conditions are not proper, their shapes must be determined in living material.

Origin and function of the small dense bodies present in Type AV, stage IA infections were not evident. These bodies might represent necrotic nuclei like those seen in *Syndinium* infections (Jepps 1936-37), discarded chromatin resulting from reduction divisions, or, perhaps, nuclei of microspores (Cachon 1964).

Numbers of *Gammarus locusta* (Linn.) infected with *Syndinium gammari* in the Étang de Thau, France, varied from few to all members of a popula-

tion (Manier et al. 1971). The infected amphipods these authors examined were apparently unaffected by the parasite. However, before one could determine the mortality rate due to syndinid infection, it would be necessary to examine moribund and dead amphipods found in the field for presence of syndinids, as well as to follow progress of infection in the laboratory. Syndinids appear to be unaffected by host defense mechanisms. Spores of syndinids that parasitize the hemocoel must exit through breaks in the exoskeleton or gut. Because hemocytes are in short supply by time of sporulation and other host resources can be expected to be depleted, host defense mechanisms probably would not be sufficient to prevent death by infection with other microorganisms that would enter through the breaks or death by leakage of body fluids. Assuming, on the basis of evidence presented in this paper, that amphipods are unable to contain syndinid infections and that most infections would therefore progress to the spore stage, syndinid infection could serve as a population regulator in heavily parasitized species. *Monoculodes edwardsi* and *Ampelisca vadorum*, which had overall prevalences of syndinid infection of 23% and 17% respectively, are examples of species that might be affected in this manner.

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LITERATURE CITED

ALFERT, M., AND I. I. GESCHWIND.
1953. A selective staining method for the basic proteins of cell nuclei. Proc. Nat. Acad. Sci. USA 39:991-999.

CACHON, J.
1964. Contribution à l'étude des Péridiniens parasites. Ann. Sci. Nat. Zool. Fr., Sér. 12, 6:1-158.

CHATTON, E.
1910. Sur l'existence de Dinoflagellés parasites coelomiques. Les *Syndinium* chez les Copépodes pélagiques. C. R. Acad. Sci. Paris, 151:654-656.

CHATTON, E.
1920. Les Péridiniens parasites. Morphologie, reproduction, éthologie. Arch. Zool. Exp. Gén. 59:1-475.

CHATTON, E.
1921. Sur un mécanisme cinétique nouveau: la mitose syndinienne chez les Péridiniens parasites plasmodiaux. C. R. Acad. Sci. Paris, Sér. D, 173:859-862.

CHATTON, E.
1952. Classe des Dinoflagellés ou Péridiniens. *In* P. P. Grasse (editor), Traité de Zoologie, Vol. 1, p. 309-390. Masson et Cie, Paris.

CHATTON, E., AND R. POISSON.
1931. Sur l'existence, dans le sang des Crabes de Péridiniens parasites: *Hematodinium perezi* n.g., n. sp. (Syndinidae). C. R. Soc. Biol. 105:553-557.

DU PRAW, E. J.
1968. Cell and molecular biology. Academic Press, N.Y.

HERZOG, M., S. VON BOLETZKY, AND M.-O. SOYER.
1984. Ultrastructural and biochemical nuclear aspects of eukaryote classification: independent evolution of the dinoflagellates as a sister group of the actual eukaryotes? Origins Life 13:205-215.

HOLLANDE, A.
1975. Étude comparée de la mitose syndinienne et de celle des Péridiniens libres et des Hypermastigines infrastructure et cycle évolutif des Syndinides parasites de Radiolaires. Protistologica 10:413-451.

JEPPS, M. W.
1936-37. On the protozoan parasites of *Calanus finmarchicus* in the Clyde Sea area. Q. J. Microsc. Sci. 79:589-662.

JOHNSON, P. T.
1985. Parasites of benthic amphipods: microsporidans of *Ampelisca agassizi* (Judd) and some other gammarideans. Fish. Bull., U.S. 83:497-505.
1986. Parasites of benthic amphipods: ciliates. Fish. Bull., U.S. 84:204-209.

LOEBLICH, A. R., III.
1976. Dinoflagellate evolution: speculation and evidence. J. Protozool. 23:13-28.

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.

MANIER, J.-F., A. FIZE, AND H. GRIZEL.
1971. *Syndinium gammari* n. sp. péridinien Duboscquodinida Syndinidae, parasite de *Gammarus locusta* (Lin.) Crustacé Amphipode. Protistologica 7:213-219.

NEWMAN, M. W., AND C. A. JOHNSON.
1975. A disease of blue crabs (*Callinectes sapidus*) caused by a parasitic dinoflagellate, *Hematodinium* sp. J. Parasitol. 61:554-557.

RIS, H., AND D. F. KUBAI.
1974. An unusual mitotic mechanism in the parasitic protozoan *Syndinium* sp. J. Cell Biol. 60:702-720.

RIZZO, P. J., AND L. D. NOODÉN.
1974. Isolation and partial characterization of dinoflagellate chromatin. Biochim. Biophys. Acta 349:402-414.

SIEBERT, A. E., AND J. A. WEST.
1974. The fine structure of the parasitic dinoflagellate *Haplozoon axiothellae*. Protoplasma 81:17-35.

STICKNEY, A. P.
1978. A previously unreported peridinian parasite in the eggs of the northern shrimp, *Pandalus borealis*. J. Invertebr. Pathol. 32:212-215.

WU, R. S., S. TSAI, AND W. M. BONNER.
1982. Patterns of histone variant synthesis can distinguish G_0 from G_1 cells. Cell 31:367-374.