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Preliminary Results on the Seasonality and Life Cycle of the Parasitic Dinoflagellate Causing Bitter Crab Disease in Alaskan Tanner Crabs (*Chionoecetes bairdi*)

W. D. EATON,*[,1] D. C. LOVE,* C. BOTELHO,† T. R. MEYERS,† K. IMAMURA,† AND T. KOENEMAN†

*University of Alaska Fairbanks, Juneau Center for Fisheries and Ocean Sciences, 11120 Glacier Highway, Juneau, Alaska 99801; †Alaska Department of Fish and Game, F.R.E.D. and Commercial Fisheries Division, Juneau, Alaska 99802-2000

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Tanner crabs (*Chionoecetes bairdi*) from the Sullivan Island area of southeast Alaska were sampled for 1 year to determine the prevalence and intensity of the parasitic dinoflagellate which causes bitter crab disease (BCD). The prevalence and intensity of infection were the greatest in the summer, declined in the fall and winter, and increased again in the spring. A possible relationship between softer, newer shells and higher levels of parasitism was also observed. In vivo transmission studies in the laboratory suggested there are several morphologically different forms of the vegetative cell of the BCD dinoflagellate which occur prior to sporulation of the parasite. In addition, it appears that both the two spore types produced by the parasite are infectious by injection and that there is no ploidy difference between the two spore types and the vegetative cell, suggesting that the two spore types may not represent separate sexes.

KEY WORDS: *Hematodinium; Chionoecetes bairdi;* parasitic dinoflagellate; bitter crab disease; Sullivan Island.

INTRODUCTION

The Tanner crab (*Chionoecetes bairdi* and *C. opilio*) fishery in Alaska is a multimillion dollar industry which, as a result of the decline of king crab populations, represents the primary crab fishery remaining in Alaska. In southeast Alaska this industry nets an annual worth of about $4 million dollars per year.

Since 1985, an increasing number of Tanner crabs from southeast Alaska have been found to be infected with a parasitic dinoflagellate similar to *Hematodinium perezi,* known to infect several species of crabs on the east coast of the United States (Newman and Johnson, 1975; MacLean and Ruddell, 1978). This dinoflagellate causes a syndrome known as bitter crab disease (BCD) in the Tanner crabs (Meyers et al., 1987).

Crabs affected with the syndrome have a pink carapace, chalky-textured meat which has a distinctly bitter aspirin-like flavor, and milky hemolymph which contains the dinoflagellate protozoan. Such animals are nonmarketable. The parasite is found in three forms: a nonmotile vegetative stage and two motile dinospores. The vegetative stage is the replicative form and appears to divide into tremendous numbers within the crab. During this process, most of the normal hemocytes and much of the tissues are replaced with the parasite. The bitter flavor may come from extracellular products released by the parasite, which are visible under light microscopy as a very foamy cytoplasm and electron microscopy as droplets exuding from the surface of the vegetative cells. The two spore types consist of a larger slow moving biflagellated dinospore, or macrospore, and a fast moving, biflagellated dinospore, or microspore. Observations to date suggest that only one spore type appears to be present in a single crab at any time (Meyers et al., 1987).

Some areas in southeast Alaska have a

[1] Current address and to whom correspondence should be addressed at Malaspina College, Biology Department, 900 Fifth St., Nanaimo, British Columbia, Canada V9R 5S5.

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prevalence of BCD as high as 95% in the Tanner crabs. In 1985, a high prevalence of BCD was found in crabs from the Sullivan Island area in the upper Lynn Canal. In 1986, 1987, and 1988 more intensive sampling was conducted and the disease was found in numerous locations in southeast Alaska. During this sampling period some areas negative for the disease in 1986 were positive for BCD in 1987 and 1988, and some regions positive for BCD in 1986 had a higher prevalence in 1988 (Meyers, 1990).

Appropriate management decisions concerning BCD and the Tanner crab industry can only be made after the biology and pathogenicity of the parasite are understood. The purpose of this study was to determine if a seasonal increase in prevalence and intensity of infection occurs in crabs. In addition, attempts were made to elucidate the life cycle of the dinoflagellate.

MATERIALS AND METHODS

Seasonality

Tanner crabs were collected from random locations within the original Sullivan Island sampling grid (58° 51′ N. latitude, 135° 21′ E. longitude) in June, August, and October of 1988 and in February, March, and May of 1989. Crabs were captured in 2 × 2-m commercial crab pots, submerged for 12–24 hr. The sex, carapace width, and shell condition (newly molted or old shell) of each captured crab were determined and a hemolymph sample was taken. A smear from the hemolymph sample was made on a glass slide, air dried, and stained with Diff-Quik stain (Dade Diagnostics, Inc., Aguada, Puerto Rico). The smears were then examined for both prevalence and intensity of the dinoflagellate causing BCD.

Prevalence was recorded as the percentage of crabs infected with the dinoflagellate while intensity was recorded as follows: 1+, the parasites comprise up to 10% of the visible cells in the stained hemolymph smear; 2+, the parasites comprise 11–40% of the visible cells; 3+, the parasites comprise 41–70% of the visible cells; 4+, the parasites comprise >70% of the visible cells; and 5+, prespores or spores of the dinoflagellate were present.

Life Cycle

Tanner crabs were collected from Auke Bay, Alaska, near Juneau, and held in the laboratory at the Juneau Center For Fisheries and Ocean Sciences. Hemolymph samples were collected from the crabs and examined for the presence of the macro- or microspores of the dinoflagellate. After a crab was found to contain one of the spore types, a wet mount examination was made of the hemolymph and 50 fields of view were examined to assure that only one spore type and no vegetative stages were present in that animal. The hemolymph from the infected crab was then used as the source for the spore types.

After both spore types or vegetative stages were collected, groups of five uninfected Tanner crabs were injected with 5×10^4 parasites from the macrospore, microspore, both spores, or the vegetative stage samples. The crabs were maintained in isolated tanks of ambient seawater. A group of uninfected crabs was also maintained in ambient seawater to serve as a sentinal group of control crabs.

Hemolymph was collected from the different crabs every 3 days for 1 month and every 2 weeks thereafter. Stained hemolymph smears were examined for evidence of the parasite. Different forms of the parasite were compared according to the amount of foamy cytoplasm present in relation to the total size of the cell, the relative density and staining characteristics of the nucleus, and whether the parasite was present as single cells or multinucleate forms called plasmodia.

Samples of vegetative cells, large spores, and small spores were examined for the total mass of DNA by two different procedures to determine if the vegetative cells were diploid and if the spore types were haploid. The DNA mass was determined by

the potassium acetate precipitation method described by Davis et al. (1980) and the ethidium bromide spectrofluorometry method described by Bentle et al. (1981). In each instance, the dinoflagellate cells were pelleted at 2000*g* for 20 min, washed with STE buffer, and pelleted again. The samples were then resuspended in a lysing buffer (0.2 M Tris–Cl, *p*H 8.5, 0.1 M EDTA, 0.1 M NaCl, 1% SDS, 100 μg/ml proteinase K) and the two procedures were completed.

RESULTS

Seasonality

The prevalence and intensity of the BCD dinoflagellate in Sullivan Island Tanner crabs were the highest in the summer months of June and August of 1988, then decreased in October 1988 and February 1989, and finally began to increase in March and May of 1989 (Table 1). When a χ^2 analysis (Zar, 1974) was performed on this data, it was found that there was a statistically significant relationship between month and prevalence and intensity of infection ($P < 0.001$). These data correlate well with preliminary samples collected from Tanner crabs in the Sullivan Island area in 1987 by the Alaska Department of Fish and Game (Table 1; Meyers et al., 1990) which showed that 37% (1.9+ intensity) and 61% (1.8+ intensity) of the crabs examined in March and October of 1987, respectively, were infected by the parasite.

TABLE 1
SEASONALITY OF INFECTION OF BITTER CRAB DISEASE IN ALASKAN TANNER CRABS COLLECTED FROM THE SULLIVAN ISLAND AREA, NORTHERN LYNN CANAL, ALASKA

Month crabs collected	Percentage infected	Number positive	Mean intensity[a]
March 1987[b]	37	17/46	1.9 ± 0.3[c]
October 1987[b]	61	42/69	1.8 ± 0.4
June 1988	84	280/333	2.8 ± 0.4
August 1988	99	99/100	3 ± 0.2
October 1988	55	56/102	2.2 ± 0.3
February 1989	46	46/100	2 ± 0.2
March 1989	53	74/138	2.2 ± 0.4
May 1989	60	57/95	2.4 ± 0.2

[a] Mean intensity was calculated from all BCD-infected crabs collected during a particular month.
[b] Samples conducted by the Alaska Department of Fish and Game.
[c] Standard deviation.

No relationship was found among carapace width, sex of crab, and infection rates. However, a relationship was discovered between shell condition and parasitism (Table 2). From June 1988 through February 1989, 610 crabs collected had new or softer shells and 63 crabs collected had older or harder shells. Data on the shell condition of crabs collected from other months were not available. About 81% (494/610) of the crabs with newer, softer shells were infected with the dinoflagellate, while only 24% (15/63) of the crabs with older, harder shells were infected. When a χ^2 analysis was performed on the data, the relationship between newer shells and an increased incidence of infection proved statistically significant ($P < 0.001$). At the time of submission of this work, Meyers et al. (1990) had also submitted results which corroborate the apparent relationship between shell condition and parasitism.

Life Cycle

Although Meyers et al. (1987) described both the large and small spore types as oval (15.2 × 11.4 μm, $n = 8$; 12.0 × 4.4 μm, $n = 5$; respectively), we found the earliest motile form of the different spore types to be circular. Following removal from the crabs, both the macrospore and the microspore types were first observed as round, motile cells with two flagella and were 12.3 and 7.9 μm in diameter ($n = 100$), respectively. Six hours after removal from the crabs the motile spore types had elongated to 13.3 × 11.4 μm and 9.5 × 7.6 μm ($n = 100$), respectively. After 12 hr, the motile spore types had elongated to 17.1 × 9.9 μm and 11.8 × 4.8 μm ($n = 100$), respectively (Fig. 1).

Several different forms of the parasite were observed in crabs during the injection study, suggesting a continuum from small, dense, slow replicating forms to large, dif-

TABLE 2
THE RELATIONSHIP BETWEEN THE NUMBER OF TANNER CRABS HAVING A NEW SHELL OR OLD SHELL AND THE INCIDENCE OF INFECTION WITH THE PARASITIC DINOFLAGELLATE, FROM SULLIVAN ISLAND, ALASKA

	June 1988	August 1988	October 1988	February 1988	Total
New shell	91% (272/297)	99% (92/93)	62% (56/91)	49% (63/129)	81% (494/610)
Old shell	22% (8/36)	100% (7/7)	0% (0/11)	0% (0/9)	24% (15/63)

fuse, rapidly replicating forms to prespores and spores. None of the control crabs developed an infection. The earliest forms of the parasite (type 1 cells; Fig. 2) were small, round, vegetative cells (6–11 μm in diameter) that occurred individually or in plasmodia composed of two to eight cells, containing very little cytoplasm (no more than a 2-μm region of the total cell) and a dense basophilic nucleus (suggesting slow cell division). Only a few of these forms were observed in crabs injected with both spores from 25 to 63 days postinjection. These were small cells with almost no cytoplasm and were present as individual cells. From day 77 to day 99, type 1 cells were present at 1+ levels and were generally larger with some measurable cytoplasm and were occasionally present in plasmodia of two to eight cells (Table 3).

Later stages of the parasite (type 2 cells; Fig. 2) found in all injection groups were larger vegetative cells (12–20 μm in diameter) with an eccentric, eosinophilic, less dense nucleus (suggesting more rapid cell division) and extensive amounts of foamy cytoplasm (a 4- to 8-μm region of the total cell). These forms were found individually or in plasmodia containing up to 30 cells and were seen in crabs injected with the different spores from about 100 to 370 days postinjection (Table 3). A few of these forms of the parasite were present in crabs 7 days following injection with the vegetative stage. However, this probably represented residual cells remaining after inoculation rather than replicating parasites.

The type 2 cells appeared to develop into two types of prespores (type 3 and 4 cells; Fig. 2), the earliest of which were present as plasmodia, with pleomorphic, eosinophilic, diffuse nuclei containing extensive foamy cytoplasm which, immediately before sporulation, developed into smaller cells in plasmodia with little or no foamy cytoplasm, and extremely dense basophilic nuclei. The parasite developed into spores in two of the five crabs with prespore infections, while the other three crabs with prespore infections died before the parasite developed into spores (Table 3). The crab injected originally with both spore types contained both the macro- and the microspore in the hemolymph and the crab injected with the microspore contained the macrospore in the hemolymph. The different spore type morphologies were previously described by Meyers et al. (1987).

Estimates of the mass of DNA in the different cell types showed there to be no more than 8 μg DNA/10^6 cells difference in DNA mass between the vegetative cells and the two spore types. In addition, the results of the potassium acetate and ethidium bromide procedures used to estimate the mass of DNA were similar as there was no more than a 7 μg DNA/10^6 cells difference when a single sample was examined by the two procedures (Table 4). Although there was some variation between procedures, there appears to be a similar mass of DNA in the two spore types and the vegetative cell, suggesting a similarity in ploidy.

DISCUSSION

A seasonal occurrence of *H. perezi* was reported by Newman and Johnson (1975) in the parasitic dinoflagellate in blue crabs on the east coast of the United States from late spring to early winter, but not during winter

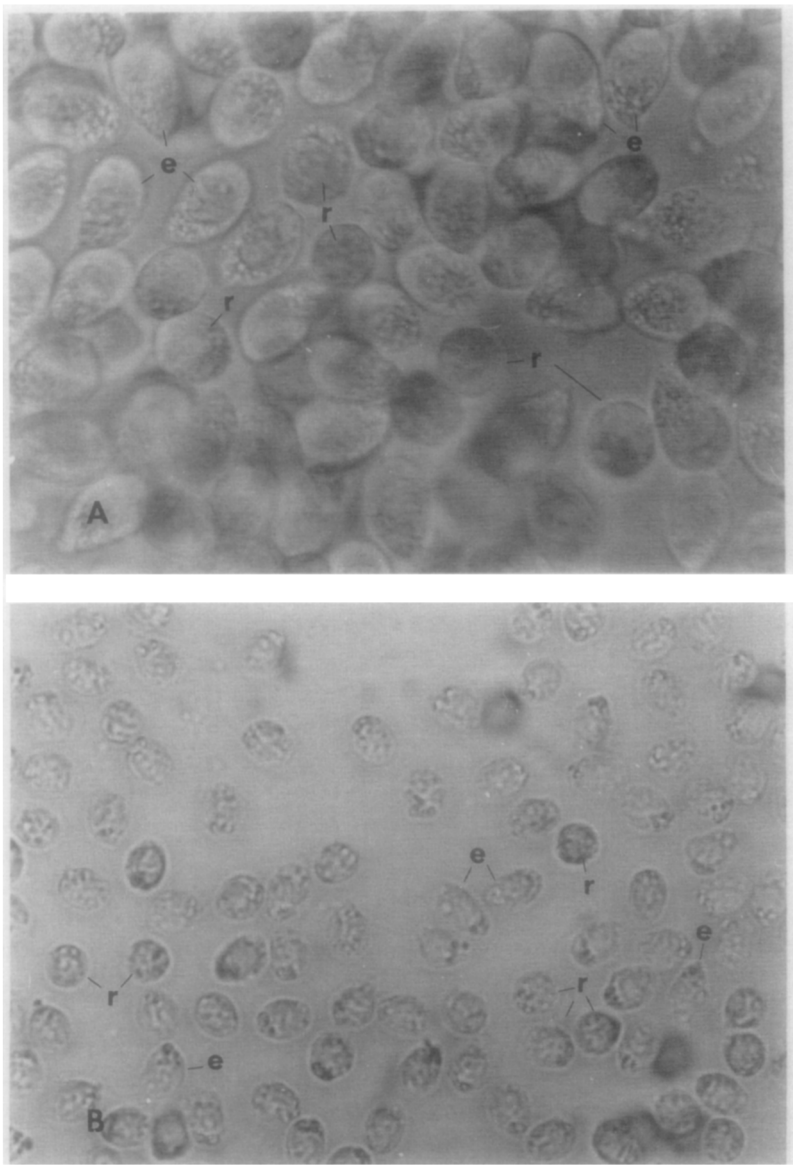

FIG. 1. Different forms of the macrospore and microspore produced by the parasitic dinoflagellate causing bitter crab disease. (A) Macrospores in naturally infected Tanner crab hemolymph (r, round form of the spore; e, elongate form of the spore; 3000×). (B) Microspores in naturally infected Tanner crab hemolymph (r, rounded form of the spore; e, elongate form of the spore; 3000×).

to early spring. In contrast, MacLean and Ruddell (1978) found the same parasite in different species of cancer and rock crabs year round on the east coast of the United States.

The results of the year-long sampling from the Sullivan Island area confirm the suggestion by Meyers et al. (1987) that a seasonal increase occurs in prevalence and intensity of infection with the BCD dinoflagellate in Tanner crabs in at least the northern part of the Lynn Canal in southeast Alaska during the summer months in a manner somewhat analogous to that reported by Newman and Johnson (1975). It is not clear if this apparent relationship is

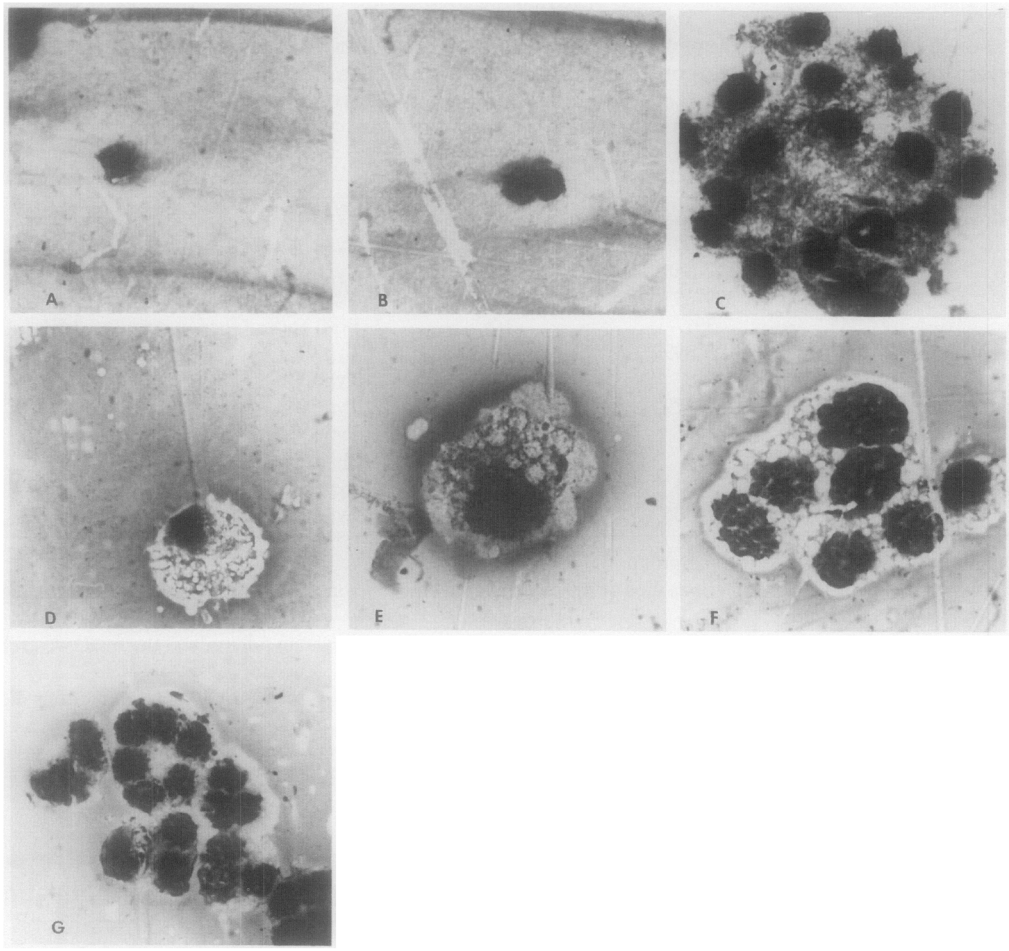

FIG. 2. Different forms of the bitter crab disease dinoflagellate found in Alaskan Tanner crabs (*Chionoecetes bairdi*) following injection of the crabs with the parasite (1650×). (A) earliest type 1 cell with almost no cytoplasm and found as individual cells, (B) later type 1 cell that is slightly larger, with some measurable cytoplasm and occasionally found as plasmodia; (C–E) various forms of the type 2 cell; (F) type 3 cell, the earliest prespore seen; (G) cell type 4, the later prespore.

due to regulation of the life cycle of the parasite by some environmental factors (such as salinity, temperature, photoperiod, etc.) or if significant mortalities have occurred in infected crabs in the summer so that by the fall and winter mostly uninfected or low level infected crabs remain. To address this question, a study is now being conducted in an attempt to determine the mortality rates experienced by infected Tanner crabs in the wild.

The relationship between shell hardness and infectivity is an interesting one. The results from the current study and that of Meyers et al. (1990) indicate that the more recently molted crabs with a newer, softer shell have a much higher prevalence of infection than crabs with older, harder shells. The Tanner crabs in southeast Alaska appear to molt from early April to early June, with a peak in late April or early May and continue for several months (K. Imamura and T. Koeneman, pers. commun.).

After molting, the crab epidermis, gills, and posterior and anterior intestinal epithelium are soft and not yet covered with a protective layer and thus are more accessable and susceptible to invasion by micro-

TABLE 3
FORMS OF THE PARASITIC DINOFLAGELLATE OBSERVED, TIME OF DEVELOPMENT, AND INTENSITY OF INFECTION FOLLOWING INJECTION OF TANNER CRABS WITH THE SPORE TYPE, BOTH SPORE TYPES, OR THE VEGETATIVE STAGE OF THE PARASITE

Injection group[a]	Days postinjection	Number infected	Cell type	Intensity of infection
Both spore types[b]	25–63	4/5	Type 1	Few
	77–99	4/5	Type 1	1+
	112–365	2/3	Type 2	1–3+
	379–393	1/2	Types 3 and 4	4+
	399	1/2	Both spore types	5+
Macrospore[c]	126–399	2/4	Type 2	2–3+
	419	2/2	Type 3	4+
Microspore[d]	140–379	2/5	Type 2	2–3+
	399	2/2	Type 4	4+
	419	1/2	Macrospore	5+
Vegetative stage[e]	7–63	5/5	Type 2	Few
	77–305	1/2	Type 2	2–3+

[a] Five crabs were injected in each group.
[b] Two crabs with 2+ BCD infections died at 200 days postinjection and one crab with a 3+ BCD infection died at 305 days postinjection.
[c] One crab died immediately after injection, two crabs died due to water system failure, and the final two crabs died with 4+ BCD prespore infections.
[d] One crab with a 3+ BCD infection died at 263 days postinjection and two crabs with 3+ BCD infections died at 305 days postinjection.
[e] Two crabs with 2+ BCD infections died at 168 days postinjection, two crabs with 3+ BCD infections died due to a water system failure, and one crab with a 3+ infection died at 319 days.

bial pathogens. Spores of the parasitic dinoflagellate have been observed within crabs from the Sullivan Island area in the mid- to late summer (T. R. Meyers and W. D. Eaton, unpubl.) and from the Auke Bay area as early as mid-May (D. C. Love and W. D. Eaton, unpubl.). Thus the period of overlap of dinoflagellate sporulation and Tanner crab molting is minimal.

Although some transmission of the parasite may occur as the spores enter the crab during molting, there must also be another mechanism of infection. Meyers et al. (1990) suggests that transmission of the vegetative stages may occur at this time. It may also be that during molting, crabs are somewhat stressed and presumably immunosuppresed so that low level infections may be exacerbated into the disease state during this time. In any case, the question of parasitism and shell condition needs further examination.

The results from this study suggest that the parasite appears to metamorphose from a small, slowly replicating cell with a dense, basophilic nucleus into a larger cell with extensive foamy cytoplasm and a diffuse eosinophilic nucleus that is rapidly undergoing cell division (as suggested by its eosinophilic nature), then into the pleomorphic

TABLE 4
ESTIMATES OF THE AMOUNT OF DNA IN PRESPORES, SPORES, AND VEGETATIVE STAGES OF THE PARASITIC DINOFLAGELLATE CAUSING BITTER CRAB DISEASE IN TANNER CRABS

Stage of the parasite	Amount of DNA	
	Potassium acetate precipitation[a]	Ethidium bromide fluorescence[b]
Vegetative	30 μg DNA[c]	34 μg DNA
Macrospore	35 μg DNA	42 μg DNA
Microspore	29 μg DNA	37 μg DNA

[a] Potassium acetate precipitation procedure for estimation of the mass of DNA in cells.
[b] Ethidium bromide fluorescence procedure for estimation of the mass of DNA in cells.
[c] Amounts are reported as μg of DNA/10^6 cells.

early prespore to the smaller, denser prespore, and finally to the two spore types, which initially are round, motile, biflagellated forms that elongate into the final spore shapes. This work is being repeated using larger numbers of animals and the waterborne route of infection.

The data from this work also suggest that both spore types are infectious by injection and that each can individually result in spore formation as the final step in the life cycle of the parasite. Although it is unknown at this time whether the spore types and vegetative stages are diploid, haploid, or possibly aneuploid, it appears that the mass of the DNA in all three forms of the parasite are similar enough to suggest that the ploidy of all three forms is similar. Thus, the two spore types may not, in fact, represent two different sexes, both of which would be required for a successful infection.

Although some genera (such as *Merodinium* and *Coccidinium*) of parasitic dinoflagellates are thought to produce a large (macrospore) and small (microspore) spore, other genera (such as *Cochlosyndinium*) are thought to produce only one size spore (Chatton, 1952). If two different spore types are produced by a single species of dinoflagellate, but are not necessary for a successful infection to occur, this raises some interesting questions as to the selective advantage of a single species of dinoflagellate producing two morphologically different, diploid spore types, both of which are infectious. It may be that we are seeing in the Tanner crabs two different but related species of dinoflagellates or two different forms of the same species, both of which are capable of developing into spores. A more in-depth comparison of the DNA of the two spore types is necessary to address this problem.

It is not certain at this time if the parasite from Alaskan Tanner crabs is the same as that found in crabs from the east coast of the United States. Although the uninucleate and plasmodial masses of cells described by MacLean and Ruddell (1978) and Newman and Johnson (1975) seem similar to those described by Meyers et al. (1987) and from the current study, there are still some differences observed. No dinospores have been observed in crabs infected with *Hematodinium* from the east coast, while dinospores have been found in infected Tanner crabs from Alaska. The plasmodia seen from the eastern infections are much smaller than those observed in crabs from Alaska. In addition, Newman and Johnson (1975) describe the presence of highly motile vermiform multinucleate bodies up to 64 μm in diameter containing up to 12 nuclei in some of the eastern crabs. This form of the parasite has not been observed in either the naturally infected Tanner crabs from the wild or the artificially infected crabs in the laboratory. A side by side comparison of the morphology and DNA homology of both the east coast and west coast parasites is necessary in order to determine if these are the same or different dinoflagellates.

It is quite evident that there is much more to be learned about the seasonality and life cycle of this dinoflagellate. The life cycle of the parasite presents a number of interesting biological questions which are being addressed in future studies on BCD. The question of seasonality of infection is still a concern. If a true seasonality is associated with the infection, then the Tanner crab fishing season can be moved to a time when the prevalence and intensity of infection are the lowest, so that there is a greater chance that those animals harvested will be marketable.

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