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Seasonal prevalence and intensity of Bitter Crab dinoflagellate infection and host mortality in Alaskan Tanner crabs *Chionoecetes bairdi* from Auke Bay, Alaska, USA

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ABSTRACT: Since the 1984–1985 Tanner crab *Chionoecetes bairdi* season in southeastern Alaska, USA, an increasing number of crabs infected with a dinoflagellate parasite similar to *Hematodinium perezi* have been identified in the commercial catches. This parasite causes a fatal disease known as Bitter Crab Disease (BCD) with peak mortality occurring during August and September. Average intensity and prevalence of this disease, as well as dinospore releases in Auke Bay Tanner crabs increased from May until August and September in both 1989 and 1990 falling again to zero by mid-winter. Prevalence of BCD infection and host mortality was not significantly different between years. Knowledge of the seasonal prevalence and intensity of BCD parasitism and associated host mortality would assist management in conserving local Tanner crab stocks.

INTRODUCTION

Since the 1984–1985 Tanner crab *Chionoecetes bairdi* season in southeastern Alaska, USA, an increasing number of crabs infected with a dinoflagellate parasite have been identified in the commercial catches (Meyers et al. 1987). This parasite is morphologically similar to *Hematodinium perezi* found in blue crabs *Callinectes sapidus* from the east coast of the United States and causes a similar disease (Newman & Johnson 1975). The syndrome caused by the Tanner crab dinoflagellate is known as Bitter Crab Disease (BCD). Tanner crabs and snow crabs *Chionoecetes opilio* from the Bering Sea also hosted this dinoflagellate, although prevalence of infection was lower than in southeastern Alaska (Meyers et al. 1987, 1990). The potential biological and economic impact of this fatal disease could threaten these highly valued fisheries.

Heavily infected crabs have a pink abdomen and carapace, white lines along the underside of each merus and milky hemolymph filled with the BCD parasites. The BCD parasite resides for several months within the Tanner crabs' hemal sinuses and tissues, causing considerable tissue destruction. Skeletal muscles of infected crabs have an astringent, aspirin-like aftertaste making them unmarketable. During July through September, the resident vegetative forms in the hemocoel undergo multiplicative mitosis prior to becoming 1 or both of 2 different sizes of motile dinospores which presumably exit the crab via the gills through the exhalent openings of the branchial cham-

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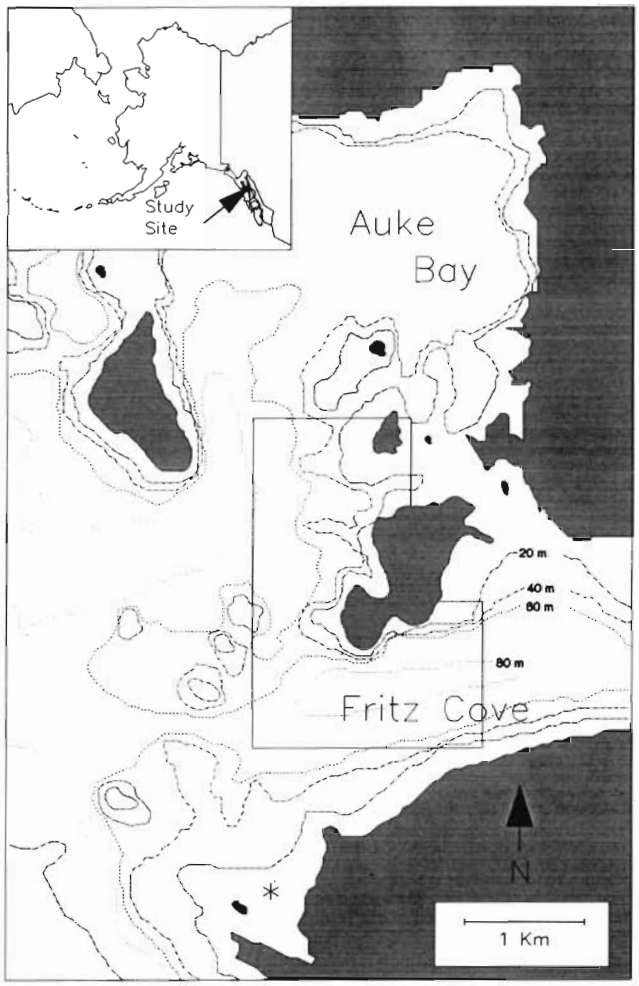

Fig. 1. Area in Auke Bay and Fritz Cove, southeastern Alaska, USA, where Tanner crabs *Chionoecetes bairdi* were randomly sampled from June 1989 to September 1990. *: area where newly molted juvenile and female crabs were gathered during April 1990

ber (Love 1992). Death of the host results from organ and/or respiratory dysfunction (Meyers et al. 1987). Preliminary results on the prevalence and intensity of infection near Sullivan Island in southeastern Alaska suggested low levels of infection in wild crabs during the fall and winter months, although prevalence below 37 % was not observed and sequential monthly samples were not collected (Eaton et al. 1991).

Knowledge of the seasonal prevalence and intensity of BCD in southeastern Alaskan Tanner crab populations would augment our understanding of the epidemiology of this disease, enable management to selectively permit harvest of lightly infected, possibly marketable crabs, and protect local disease-depleted stocks from possible overharvesting. This project was designed to determine the seasonal variations in prevalence and intensity of BCD in a relatively undisturbed population of Tanner crabs which does not support a commercial harvest. This study also addresses the relationship between crab mortality and average intensity of infection.

MATERIALS AND METHODS

Tanner crabs were collected monthly from random locations north and west of Spuhn Island in Auke Bay and Fritz Cove, ca 20 km NW of Juneau, Alaska from June 1989 through September 1990 (Fig. 1). Crabs could not be collected with pots in January or April because of mechanical problems with the research vessel but were sampled using SCUBA in April from 10 m when crabs had moved to shallower depths to molt. Crabs were captured by submerging 2 m × 2 m conical commercial Tanner crab pots for 12 to 36 h in waters 90 m in depth. The minimum sample size needed to detect at least 1 infected crab in populations of 1 000 000 crabs or more at a minimal disease prevalence of 5 %, at the 95 % confidence level, was determined to be 60 crabs (Ossiander & Wedermeyer 1970). All crabs were individually tagged with Floy* 2 mm fingerling disk tags (Floy Tag and Manufacturing, Inc. Seattle, WA 98105, USA) affixed to the dorsal surface of the carapace with Quick-Gel* glue.

Hemolymph was drawn from each captured crab at the intersegmental membranes at the base of the right coxa using a disposable 1 cc syringe. A drop of hemolymph was used immediately after collection to make smears on glass slides, which were dried and stained using Diff-Quik* histological stain (Baxter Scientific, Inc.). Using light microscopy, monthly prevalence was recorded as presence or absence of *Hematodinium*-like dinoflagellates in the hemolymph smears while monthly intensity of infection was recorded in a system derived from that of Meyers et al. (1987), using a numerical code ranging from 0 to 4. A value of 0 meant that no parasites were found in 10 fields of view (400×), 1 denoted that 1 to 10 % of all the cells present in an average of 10 fields of view were parasites, 2 denoted 11 to 40 %, 3 meant 41 to 70 % and 4 meant that more than 70 % of all cells were *Hematodinium* sp. parasites. Following the initial screening, crabs were placed in aquaria and fed weekly a mixture of frozen squid and mussels for an observation period of 60 d. At the end of this observation period a final hemolymph sample was collected and levels of infection were again determined. Hemolymph samples and gill

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squashes were taken from all moribund and postmortem crabs to determine the final intensity of infection and presence of *Hematodinium* sp. dinospores. Bacterial and ciliate infections could also be identified from the hemolymph and gill squashes. Prevalence and average intensity of infection, spore releases and mortality were compared between seasons (summer-fall and winter-spring) and between years (1989 and 1990) using Mann-Whitney non-parametric tests (Zar 1974). Summer-fall season was defined as June through September and winter-spring as October through May.

RESULTS

Prevalence and average intensity of BCD in Auke Bay Tanner crabs increased during the summer months and decreased to 0 by mid-winter (Fig. 2). Percent prevalence of BCD increased from 75 % in June, to 96 % in August 1989, decreased to 0 by December and increased again until August of the following year. Prevalence of disease was significantly greater during the June through September period (i.e. summer and fall months) than during the October through May period (i.e. winter and spring) of both 1989 and 1990 ($U = 46$, $0.001 < p < 0.005$). Percent prevalence during August of 1990 was 56 %, ca half that observed in August of 1989 at 96 %. Prevalence of infection in 1989 and 1990 was not significantly different in 1989 from that in 1990 ($U = 25.5$, $p > 0.2$).

Table 1. *Chionoecetes bairdi*. Percent of Tanner crabs captured monthly from Auke Bay, held in seawater aquaria, and observed releasing large and small type *Hematodinium* dinospores within a 60 d observation period

Month	Sample size	Percentages Small spore	Large spore	Both spores	Total percent spores released[a]
1989					
Jul/Aug	67	31.3	16.4	4.5	61.1
Sept	84	22.7	3.4	6.8	41.0
Oct	61	0.0	0.0	0.0	6.5
Nov	81	0.0	0.0	0.0	0.0
Dec	28	0.0	0.0	0.0	0.0
1990					
Feb	71	0.0	0.0	0.0	4.2
Mar	113	0.0	0.0	0.0	0.9
Apr	79	0.0	0.0	0.0	0.0
May	100	0.0	1.0	0.0	1.0
Jun	84	9.5	2.4	0.0	17.9
Jul	108	3.7	1.8	0.9	9.3
Aug	88	17.1	1.1	0.0	24.9
Sep	56	1.7	5.1	0.0	11.9

[a] Total percent spores released includes those crabs observed sporulating and post-mortem crabs having dinospores in gill squashes

Average intensity of BCD infection in Tanner crab from Auke Bay paralleled prevalence, rising until August, decreasing to 0 by December and increasing steadily again from February until August of the following year. Average intensity of infection was significantly greater from June through September for both 1989 and 1990 than from October through May ($U = 49$, $p = 0.001$). Average intensity of infection was not significantly different between years ($U = 26$, $p > 0.2$). Samples for all months except December 1989 and September 1990 included at least 60 specimens, allowing disease prevalence of 5 % or greater to be detected. Sample sizes in December 1989 and September 1990 were 28 and 56 respectively, large enough to detect disease prevalence at the 10 % level.

Releases of BCD dinospores occurred during the summer months of 1989 and 1990 (Table 1). In 1989, small and large dinospores exited host crabs during July through September. In 1990, release of dinospores from infected crabs was observed as early as May and continued through September, when the study was terminated. Peak production of large and small dinospores occurred in August and September of both years. Tanner crabs collected during June 1989 were released immediately following the initial hemo-

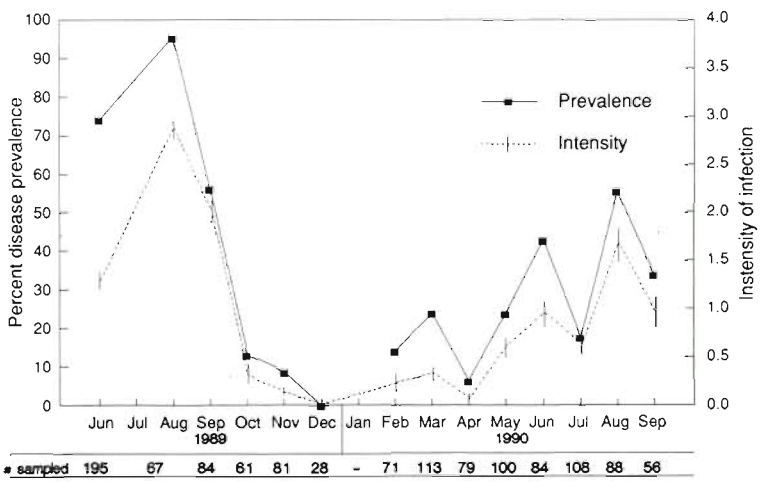

Fig. 2. *Chionoecetes bairdi*. Prevalence and intensity of Bitter Crab Disease (BCD) in Tanner crabs collected in Auke Bay from June 1989 until September 1990

Table 2. *Chionoecetes bairdi*. Causes of mortality in Tanner crabs collected from Auke Bay and held for 60 d in seawater aquaria

Month	Sample size	Percent mortalities				Total % mortality after 60 d
		Hematodinium spore release[a]	Bacteria/ ciliates[b]	Cannibalism	Unknown cause	
1989						
July/Aug	67	61.1	16.4	0.0	19.5	97.0
Sep	84	41.0	9.1	0.0	5.9	56.0
Oct	61	6.5	0.0	0.0	4.5	11.0
Nov	81	0.0	0.0	0.0	23.0	23.0
Dec	28	0.0	0.0	0.0	0.0	0.0
1990						
Feb	71	4.2	0.0	2.8	11.0	18.0
Mar	113	0.9	1.3	0.0	35.8	38.0
Apr	79	0.0	4.0	0.0	10.0	14.0
May	100	1.0	2.4	0.0	37.6	41.0
Jun	84	17.9	1.0	0.0	35.1	54.0
Jul	108	9.3	0.0	0.0	1.7	11.0
Aug	88	24.9	4.4	0.0	28.7	58.0
Sep	56	11.9	1.7	0.0	7.4	21.0

[a] *Hematodinium* spore releases include those crabs observed directly releasing dinospores and those with dinospores in the gill squashes upon post-mortem examination

[b] Those BCD-infected crabs which had numerous bacteria or ciliates (identified as *Paranophrys* spp.) in hemolymph smears or gill squashes as observed post-mortem

Table 3. *Chionoecetes bairdi*. Mortality of Auke Bay Tanner crabs infected with the BCD dinoflagellate before and after a 60 d observation period and mortality of all crabs in a given sample

Month	Sample size	Initial no. of infected crabs	No. of infected crabs after 60 d	Percent mortality of infected crabs during 60 d	Total % mortality after 60 d
1989					
Jul/Aug	67	64	0	100	97
Sep	84	46	1	98	56
Oct	61	7	4	43	11
Nov	81	6	6	0	23
Dec	28	0	[a]		
1990					
Feb	71	8	6	25	18
Mar	113	23	7	70	38
Apr	79	0	5	[b]	14
May	100	8	7	13	41
Jun	84	18	13	28	54
Jul	108	19	10	47	11
Aug	88	49	8	84	58
Sep	56	19	8	58	21

[a] No infected crabs were collected during December 1989 and no crabs were collected in January 1990

[b] Parasites were not evident in the initial hemolymph samples but were identified in the final hemolymph sample. All crabs in the April sample had either recently molted or molted within the 60 d observation period

lymph sample, and were not observed releasing dinospores. Most crabs during both years released either small or large spore types, however, on occasion, both dinospore types were produced within the same crab. Total percent of dinospores released during 1989 (61.1 %), was higher than observed during 1990 (24.9 %). Release of BCD dinospores was significantly greater during the summer and fall months than during the winter to spring ($U = 36$, $p < 0.005$) but did not vary significantly between years ($U' = 20.5$, $p > 0.2$).

Mortalities were more frequent during the late spring and summer infection and sporulation period (Table 2) and among heavily infected crabs (Table 3). Crabs heavily infected with the BCD parasite were visibly lethargic and often also infected with bacteria or the ciliate *Paranophrys* spp. As with infection rates and dinospore releases, mortality rates increased steadily until August of both 1989 and 1990. However, total mortality between seasons ($U = 33.5$, $0.1 < p < 0.2$) and between years ($U = 20.5$, $p > 0.2$) was not significantly different. Mortality of crabs during the 60 d observation period reached 97 % in August, 1989 and 58 % in August of 1990 (Table 2). Mortalities generally increased throughout the year in 1990. Mean monthly total mortality for the 6 mo sampled during 1989 equaled 37.4 %. Mean monthly mortality from February to September 1990 equaled 31.9 %, with an average for the entire study of 34.0 %. Mortality of infected Tanner crabs was 100 % in August of 1989, not significantly higher than the 84 % mortality observed in 1990.

Mortality rates of infected crabs increased through August of both 1989 and 1990 (Table 3). Percent mortality equalled about 100 % in late July and August and 98 % in September of 1989, reaching 84 % in August 1990. These percentages are not significantly different ($U = 18$, $p > 0.2$), despite larger sample sizes and fewer infected crabs captured during 1990. Mortality of infected crabs was significantly different ($U = 32$, $0.02 < p < 0.05$) for June to September for both 1989 and 1990 than October through May. Of uninfected crabs collected in September 1989, 75 % lived until February 1990 and 45 % of these crabs lived until September 1990; suggesting that crabs experienced little stress during the shorter 60 d holding period (Table 4).

Table 4. *Chionoecetes bairdi*. Survival of uninfected Tanner crabs held in aquaria from September 1989 until September 1990

	1989		1990		
	Sep	Nov	Feb	May	Sep
Survivals					
No.	60	50	45	41	27
Percent	100	83	75	68	45

DISCUSSION

A long latency period punctuated by shorter, intense periods of spore releases and host mortality seems typical of *Hematodinium* sp. parasitism. *Hematodinium* spp. from the east coast of the United States parasitized blue crabs *Callinectes sapidus* in all but the late winter to early spring months (Newman & Johnson 1975). Likewise, monthly samples from Auke Bay Tanner crabs indicated that parasitism was significantly higher in the summer decreasing to undetectable levels during mid-winter. Tanner crabs heavily infected with BCD parasites were most abundant during August and September in Auke Bay confirming what had been suggested by a preliminary study undertaken in the Sullivan Island area, southeastern Alaska (Eaton et al. 1991).

The lower estimates of prevalence and intensity in Auke Bay during April and July 1990 may have been a result of atypical sample composition. The April sample, collected from 10 m of water via SCUBA, consisted of smaller, immature male crabs and primiparous females. All of these younger crabs were newly molted individuals which may have been spatially segregated from the larger crabs captured from deeper waters, and possibly less exposed to the dinoflagellate. Although ontogenetic spatial segregation has not been documented for southeastern Alaskan *Chionoecetes bairdi*, *C. opilio* in the Sea of Japan undergo ontogenetic migrations which result in spatial segregation of adults and juveniles (Kon 1982). Meyers et al. (1987) reported that BCD infections occurred in all sizes of Tanner crabs collected from the Sullivan Island area. Size-specific differential infectivity studies of BCD have not been conducted and are needed. The July sample contained a higher proportion of terminal molt crabs (80 %) than occurred on average in samples taken during the remainder of the sampling period (55 %). These older, postmolt crabs typically have a lower prevalence of BCD (Meyers et al. 1987)

No interannual variations in disease prevalence or intensity occurred between 1989 and 1990. Prevalence and intensity of BCD infection was not significantly different in 1989 from 1990 based on Mann-Whitney *U* non-parametric rank test (Zar 1974). Tanner crab mortality rates in 1990 at the end of the 60 d observation period were also not significantly lower. Decreasing catch rates and lower average intensity of infection were indicated for second-year samples taken near Sullivan Island, Alaska (Meyers et al. 1987). The epizootiology of other protozoan parasites seems to vary

with time, location and host population. *Haplosporidium nelsoni* was implicated in Delaware Bay and Chesapeake Bay oyster *Crassostrea virginica* epizootics during 1957 and 1958. In a subsequent 12 yr study of the epizootiology of *H. nelsoni* in lower Chesapeake Bay (USA), disease prevalence and host mortalities were consistently higher during September becoming low in December and January. A smaller peak in prevalence and mortality occurred during February and March (Andrews & Frierman 1974). Twenty-year studies initiated in 1958 of *H. nelsoni* prevalence and mortality in Delaware Bay oysters were not as consistent. Seasonal cycles were similar to Chesapeake Bay, with highest prevalence in winter and late spring but mortality rates were consistently higher and exhibited a cyclic pattern with peaks every 6 to 8 yr (Ford & Haskins 1982). While Bitter Crab Disease prevalence and subsequent mortality may not be significantly different for 1989 and 1990, multi-year studies are needed to accurately assess long-term BCD epizootiology.

Bitter Crab Disease appears to be fatal to all Tanner crabs which become infected. Among crabs captured in Auke Bay, mortality rates increased until August and September when spore releases, disease prevalence and intensity were highest. All infected crabs held for extended periods beyond 60 d eventually died following late summer spore release of the BCD dinospores. It may be possible that the moribund condition of the host stimulated sporulation and subsequent discharge of dinospores. In general, mortality during the late fall, winter and spring was not due to *Hematodinium* sp. sporulation, which was highest in late summer. Autopsied, heavily infected crabs often had large numbers of bacteria in the hemolymph smears and gill squashes post-mortem. These crabs had been lethargic and inappetent; the additional stress of bacterial infection could have speeded the host's death. Large numbers of ciliates were also evident in hemolymph wet mounts but may represent opportunistic infestations of weakened, infected crabs. The pathogenicity of these bacteria and ciliates remains unknown. The hemocytes of blue crabs are important for defense against disease agents and for clotting (Johnson 1980). As BCD progressed in infected Tanner crabs, fewer hemocytes and more parasites were observed in subsequent hemolymph samples, potentially leaving the crab immunosupressed and vulnerable to any pathogen able to penetrate the cuticle. Organs including the heart, hepatopancreas, and antennal glands of heavily infected crabs were also damaged, contributing to the unthriftiness of the host (Love 1992).

Tanner crab mortality due to parasite sporulation and heavy bacterial and ciliate infections may explain the decreasing prevalence of BCD seen during the fall and winter. Since the parasite seems highly virulent, killing most infected Tanner crabs, it is unlikely the development of immunity plays any role in decreasing prevalence of BCD in the fall and winter months. None of 10 BCD-injected crabs held in seawater aquaria from July 1989 to July 1990 developed immunity to the disease. All eventually developed heavy infections and died following the late summer 1990 spore releases (Love 1992). The source or route of infection during the spring, which eventually results in an increase in prevalence and intensity of BCD, is still unknown. Many questions still remain. Molting in Tanner crabs in southeastern Alaska is generally believed to occur in late March to early April (C. Bothelo, Alaska Dept of Fish and Game, pers. comm.). Meyers et al. (1990) suggested that BCD-dinospores may attach to uninfected Tanner crabs in late summer, entering the host through breaks in the cuticle shortly after molting the following spring. If newly molted crabs are more likely to be infected, why does sporulation not occur in the spring? Like the free-living dinoflagellates, do the BCD dinospores serve as disseminatory stages which eventually form overwintering hypnocysts, becoming infective the following spring? Perhaps the spore stage is simply an evolutionary remnant of a once free-living life cycle?

In recent years the southeastern Alaska Tanner crab fishery has occurred during the first 2 wk of February, when Tanner crabs aggregate in shallower waters prior to molting and mating, and are easier to catch. Although February is the time of optimum meat yield and condition for most stocks, unmarketable BCD-infected crabs are also captured (Imamura & Bothelo 1989). Although several processors now collect, cook and grind 'sick' crabs thus presumably killing the parasite, culling and disposal of infected crabs on the fishing grounds occurs and the risk of spreading the disease to other areas remains. Knowledge of disease-related mortality of fished stocks allows management to more accurately plan harvest levels and highlights the importance of such epizootilogical studies.

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