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Aspects of the Epidemiology of Bitter Crab Disease (*Hematodinium* sp.) in Snow Crabs, *Chionoecetes opilio* from Newfoundland, Canada

Aspects de l'épidémiologie de la maladie du crabe amer (*Hematodinium* sp.) chez le crabe des neiges (*Chionoecetes opilio*) à Terre-Neuve, au Canada.

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ABSTRACT

The parasitic dinoflagellate *Hematodinium* sp. causes a condition known as bitter crab disease (BCD) in snow crabs, *Chionoecetes opilio*, and Tanner crabs, *C. bairdi*. As the name of the condition implies, crabs infected with BCD are unmarketable due to their bitter flavor. We surveyed the distribution of BCD in three regions within the snow crab fishery of Newfoundland from 1997 to 2003. Over time, the disease has become firmly established in Conception and Bonavista bays and persists at low levels in the Avalon fishing grounds. An epizootic occurred within Bonavista and Conception bays in 1999 and persisted in Conception Bay in 2000 reaching prevalences of over 2% to 9% in trapped and trawled male crabs and from 19% to 26% in trawled and trapped female crabs, respectively. The hydrography of this bay may have contributed to the epizootic as infections were centered within the deeper confines or near the inshore terminus of the bay. Infections were highest in females and small males, i.e., the unfished and pre-recruit portions of the fishery. In a mortality study, all of the overtly infected crabs died and 50% of the experimentally inoculated crabs died. Patterns in the molting cycle and prevalence of infection indicate that transmission occurs during the post-molt condition, and that overt infections probably develop two to four months after infection, lasting three to four months thereafter. Analysis of various abiotic factors uncovered a significant positive association between prevalence, depth and mud/sand substrates; the nature of this relationship was not apparent but may be related to diet or alternate hosts. Lastly, given the startling rise in BCD in snow crabs in Newfoundland, we recommend that fishery management programs employ non-selective gear to monitor for *Hematodinium* infections in female and juvenile crabs because these under-sampled members of the population may forewarn of impending recruitment declines that might otherwise remain unexplained.

RÉSUMÉ

Le dinoflagellé parasite *Hematodinium* sp. cause chez les crabes des neiges *Chionoecetes opilio* et *C. bairdi* une condition connue sous le nom de maladie du crabe amer. Comme le nom de la condition le laisse entendre, les crabes atteints sont invendables à cause du goût amer de leur chair. Nous avons étudié la distribution de la maladie dans trois régions où le crabe des neiges a été pêché à Terre-Neuve de 1997 à 2003. Au fil du temps, la maladie s'est solidement établie dans les baies de la Conception et de Bonavista et perdure à des niveaux faibles dans les pêcheries d'Avalon. Une épizootie s'est déclarée dans les baies de la Conception et de Bonavista en 1999, et a perduré dans la baie de la Conception en 2000, atteignant des taux de prévalence de plus de 2 % à 9 % chez les mâles capturés respectivement au casier et au chalut et de 19 % à 26 % chez les femelles capturées respectivement au casier et au chalut. Les caractéristiques hydrographiques de cette baie peuvent avoir contribué à cette épizootie car les cas d'infection étaient concentrés dans les profondeurs ou près du fond de la baie. Les taux d'infection les plus élevés ont été relevés chez les femelles et les petits mâles, soit la composante non pêchée et la composante des prérecrues. Lors d'une étude du taux de mortalité, tous les crabes souffrant d'une infection patente et 50 % des crabes contaminés artificiellement sont morts. Les patrons du cycle de mue et la prévalence de la maladie indiquent qu'elle est transmise après la mue et que les infections patentes se manifestent probablement de deux à quatre mois après l'infection initiale, et durent ensuite de trois à quatre mois. L'analyse de divers facteurs abiotiques a mis à jour une association positive marquée entre la prévalence de la maladie, la profondeur et les substrats de vase/de sable; la nature de cette relation n'était pas évidente, mais elle peut être liée au régime alimentaire ou à d'autres hôtes. En dernier lieu, étant donné la progression alarmante de cette maladie chez le crabe des neiges à Terre-Neuve, nous recommandons que des engins non sélectifs soient utilisés pour les programmes de gestion des pêches afin de pouvoir déceler les cas d'infection chez les femelles et les juvéniles, car ces éléments sous-échantillonnés de la population peuvent être des indicateurs de déclins imminents du recrutement, qui pourraient autrement demeurer inexpliqués.

INTRODUCTION

The snow crab (*Chionoecetes opilio*) supports the largest commercial fishery in Newfoundland. In 2003, landings for this fishery were in excess of 58,000 mt with an ex-vessel value of approximately $300 million (Can.). Bitter crab disease (BCD) was first observed in snow crabs off Newfoundland in 1990 where it was reported at very low levels (0.037%) (Taylor & Khan 1995). The disease is now centered primarily in the productive fisheries of the inshore northern and eastern bays within the Northwest Atlantic Fishing Organization's (NAFO) Division 3L (Taylor & Khan 1995, Pestal et al. 2003), and in some deep-water offshore areas of NAFO Divisions 2J and 3K (Dawe 2002), but there is significant concern that it may be spreading to other locations. Recent surveys in Conception Bay on the east coast of Newfoundland have revealed that the prevalence of disease has increased markedly since 1996, reaching 4.25% in 1998 (Pestal et al. 2003).

Bitter crab disease is caused by an unusual parasitic dinoflagellate, *Hematodinium* sp. (Meyers et al. 1987). The bitter flavor of the meat of infected crabs is thought to arise from physiological changes to the host from long-term, chronic infections by the protozoan (Meyers et al. 1987, Eaton et al. 1991). Unfortunately, a single infected crab can ruin an entire batch during bulk processing of crab meat or sections (Taylor, pers. obs.). BCD has been reported in *C. bairdi* and *C. opilio* from southeast Alaska (Meyers et al. 1987, 1990), in *C. opilio* from the Bering Sea (Meyers et al. 1996), and in *C. tanneri* from British Columbia (Bower et al. 2003). At present, it is not clear if the same species of *Hematodinium* occurs in Alaska, British Columbia and Newfoundland, but this is an important issue that should be resolved by studies on the molecular biology of the parasite.

Outbreaks of *Hematodinium* spp. have damaged many crustacean fisheries. In Alaska, the Tanner crab (*C. bairdi*) fishery has suffered localized declines due to *Hematodinium* sp. (Meyers et al. 1987, 1990, Eaton et al. 1991). Focal outbreaks have occurred in enclosed embayments and fjords with shallow sills (Meyers et al. 1987, 1990, 1996). Similarly, in the coastal bays of Virginia and Maryland, the blue crab (*Callinectes sapidus*) fishery has suffered annual mortalities in high salinity waters, but the larger, riverine fishery has not been affected (Messick 1994; Messick & Shields 2000). In Scotland, the Norway lobster (*Nephrops norvegicus*) fishery lost an estimated £2-4 million in an outbreak of a *Hematodinium*-like parasite (Field et al. 1992), and the parasite has remained in that fishery at sustained levels (35-50%) through several years (Field & Appleton 1995, Stentiford et al. 2001). In France, the velvet crab (*Necora puber*) fishery suffered a catastrophic decline (>96%) due to *H. perezi* (Wilhelm & Miahle 1996), and stocks of the edible crab (*Cancer pagurus*) were also heavily infected (Latrouite et al. 1988, Stentiford et al. 2002). Thus, the presence of the dinoflagellate in any fishery appears to be a significant cause for concern.

Mortality studies with Tanner crabs, Norway lobsters and blue crabs infected with *Hematodinium* spp. indicate that infections are generally fatal to the host (Meyers et al. 1987, Eaton et al. 1991, Field et al. 1992, Shields & Squyars 2000). Naturally-infected adult Tanner crabs survived from 20 to 158 days in the laboratory (Meyers et al. 1987), but in other trials infected crabs survived for over 400 days (Love et al. 1993). Naturally-infected adult Norway lobsters had mortality rates 2 to 4 times higher than uninfected

lobsters, with most of the deaths occurring early in the course of the experiment (Field et al. 1992). Experimentally-infected blue crabs experienced 87% mortality over 40 days, but in a few cases hosts were refractory to infection (Shields & Squyars 2000). There is some evidence that low temperatures and low salinities limit the proliferation of the parasite in blue crabs (Messick et al. 1999), but the parasite in snow crabs thrives at low temperatures and would likely not be subjected to low salinities.

Host factors show some distinct correlations with infection. Juvenile snow and blue crabs and Norway lobsters have a significantly higher prevalence of *Hematodinium* spp. infections than adults (Messick 1994, Field et al. 1998, Messick & Shields 2000, Stentiford et al. 2001, Pestal et al. 2003). Infections in Tanner crabs from southeast Alaska show few differences between sexes (Meyers et al. 1987, 1990), presumably because the high prevalences mask more subtle host factors, but these studies used commercial traps and did not sample small crabs. Such differences in prevalence may reflect either the intense fishing pressure on the males, a predilection of the disease for the smaller size classes of hosts, or increased mortalities in larger crabs.

In 1993, we began incorporating the prevalence of BCD into annual assessments of snow crab stocks in three survey areas off the northeast coast of Newfoundland. By 1997, monitoring of BCD was fully incorporated into the assessments. Here we report on data collected from 1997 to 2003. The primary goal of the monitoring program was to assess the importance of the disease to Newfoundland's snow crab fishery. The objectives were (1) to document the distribution and prevalence of BCD in snow crabs from three hydrographically different regions (Conception Bay, Bonavista Bay, and Avalon), (2) to assess the biotic and abiotic factors that affected the distribution and abundance of the disease, and (3) to analyze differences in trawl and pot surveys that may have affected estimates of prevalence in snow crab populations. In addition, to more fully understand the effect of the disease in snow crabs, we examined the mortality rate of overtly infected crabs in comparison with healthy ones.

MATERIALS AND METHODS

Epizootiological survey

The study sites were Conception Bay, Bonavista Bay, and an area 3 to 45 nm off the northeast Avalon Peninsula (Fig. 1). These sites, which are all situated on the northeast coast of Newfoundland, each have features that make them distinct from each other. Bonavista Bay, the most northerly, is a partially enclosed deep-water bay which has a steep sill at its mouth. The bottom type consists of thick mud on the commercial crab grounds. Commercial crab fishing is usually concentrated in areas where water depth exceeds 270m. Conception Bay is also partially enclosed, but has a more diverse bottom type on the fishing grounds, ranging from sand and gravel at the mouth to thick mud in the inner deep basin. Commercial crab fishing is conducted from 130m to 250m. The Avalon study area off the Avalon Peninsula is in open ocean with a bottom type consisting of sand and gravel. Commercial fishing depths are confined here, averaging 190m. The bottom temperature in this study site is colder than in the others as its relative shallowness exposes it to the full effect of the Labrador Current.

The surveys took place during the DFO annual stock assessment research cruises. Sampling at Avalon took place in May-June, at Bonavista Bay in August, and at Conception Bay in September-October. Briefly, stations were chosen randomly and stratified by depth. Crabs were sampled at each station with either a trawl, or a fleet of traps, or both as in Pestal et al. (2003). A lined No. 36 shrimp bottom trawl with footgear modified to maximize snow crab capture and retention along with SCANMAR sensors was towed for 10 minutes at a speed of 2.5 knots. Traps were deployed in "fleets" of standard Japanese conical traps, five of which had 13.1 cm mesh and three of which had 3.1 cm mesh. The number of standard Japanese traps per "fleet" varied from 4-8 over the study period due to other research goals in some years which required using traps of varying mesh sizes. Further details regarding baiting of traps and the shipboard sampling of crabs are given in Pestal et al. (2003). The primary substrate types was determined from each trawl as mud, sand, gravel, shell, rock, other.

Sex, carapace width, shell condition, maturity status (for females) and macroscopic signs of bitter crab disease were noted for crabs that were examined. Shell condition was determined using criteria described by Taylor et al. (1989). Briefly, shell categories are Shell 1 (soft, recent molt), Shell 2 (recently hard, molted within last year), Shell 3 (fouled, not molted within 2 years) and Shell 6 (intermediate, not molted within last year). For diagnosis of BCD, the primary macroscopic sign of infection with *Hematodinium* is a distinct color change to the carapace that gives the crabs a cooked appearance (Pestal et al. 2003). These heavily infected crabs also have an opaque, solid white ventrum, listless or lethargic behavior, and milky, discolored hemolymph (Meyers et al. 1990, Taylor and Khan 1995). In some cases crabs suspected of having infection were further diagnosed by removal of the carapace and examination of the heart. Histological analysis confirmed that cream-colored hearts were indicative of infection compared to the translucent, beige color of normal healthy hearts, and in no cases were other hemolymph parasites present (Taylor & Shields unpubl. data).

Mortality study

In controlled laboratory experiments, the mortality rates of experimentally and naturally infected hosts were examined. Due to space limitations and collection bias, these experiments were undertaken with relatively small numbers of male and female crabs. Crabs were held in 600 L aquaria with flow-through running seawater at 4º C. Treatments consisted of an experimental group of naturally infected crabs, an experimental group of crabs injected with known aliquots of *Hematodinium* sp. (see below), and a control group of uninfected crabs injected with the support buffer used for preparations of aliquots. Mortality was censused at weekly intervals and analyzed via survival analysis (Cox & Oakes 1984).

For inoculations with *Hematodinium* sp., hemolymph was drawn from infected crabs and pooled into support buffer, a physiological saline consisting of NaCl, 27.99 g/l, KCl 0.95 g/l, $CaCl_2$ 2.014 g/l, $MgSO_4$ 2.465 g/l, Na_2SO_4 0.554 g/l, HEPES 1.92 g/l, adjusted to pH 7.8, with added glucose (1.0 mg/ml) after Appleton and Vickerman (1998). Amoeboid cells of *Hematodinium* sp. were counted in a hemacytometer (5 replicates) and aliquoted in buffer to give an estimated dose of 1.0×10^5 parasites which was injected into uninfected crabs.

At biweekly intervals, hemolymph samples were drawn from experimentally inoculated and control crabs as in Pestal et al. (2003). Briefly, hemolymph samples were drawn into individual 3-ml syringes with a 23-ga. needle preloaded with 1 ml ice-cold, 10% formalin in filtered seawater at a proportion of 1:2 to 1:5 hemolymph to fixative. The fixed hemolymph was gently shaken and refrigerated until processed. In the laboratory, aliquots of fixed hemolymph were placed on poly-l-lysine-coated slides, allowed to settle for 45 s, post-fixed in Bouin's solution for 24 hr, and transferred to 70% ethanol for holding. Smears were hydrated, stained with Jenner-Giemsa for 10-20 min (Presnell & Schreibmann 1997), dehydrated through an acetone series, cleared in a xylene series, and mounted in cytoseal. Stained smears were examined with a light microscope at 400x and classified as "infected" when at least one clearly identifiable *Hematodinium* cell of any stage was found (Meyers et al. 1987, Taylor & Khan 1995).

Statistical analysis

Logistic regression and Chi-square were used to analyze possible differences in host and environmental factors in relation to infection. The proportional hazards model (Kaplan-Meier) with the Weibull distribution was used to examine survival data and the Tarone-Ware log-rank test was used to examine differences between survivorship curves (Wilkinson 1997). Size classes represent the 5 mm increments (i.e., 90mm class includes 90mm to 94mm crabs).

Snow crabs sampled by trawling in Conception Bay from 1997 to 2000 (n=15,803) were used to assess the effects of biotic and abiotic variables in relation to the prevalence of BCD. Crabs sampled from Bonavista Bay and the Avalon fishing grounds were not used in this analysis because BCD prevalence was significantly lower in these areas, and we wished to avoid possible confounding of the results by a location effect. Prevalence is the number of infected crabs divided by the total number sampled.

Logistic regression was used to model the effects of biotic and abiotic variables on the probability of a crab being infected with BCD. The logistic regression model took the following form:

$$\ln(p/1\text{-}p) = \alpha + \Sigma\beta i Xi$$

where p = probability of a given crab being infected with BCD; (p/1-p) = odds of a given crab being infected with BCD; α = constant; β = vector of regression parameters; and X = vector of independent variables. Interpretation of the fitted model is based on the odds ratio which is the odds of a crab being infected with BCD at one level of an independent variable divided by the odds of a crab being infected with BCD at another (lower) level of that independent variable (with all other independent variables held constant). An odds ratio greater than 1.0 indicates that the odds of a crab being infected with BCD are a positive function of the independent variable whereas an odds ratio less than 1.0 indicates that the odds of a crab being infected are a negative function of the independent variable. Odds ratios further from 1.0 indicate a stronger association between the two variables (Agresti 1996).

Biotic variables tested for effects on the probability of a crab being infected by BCD were carapace width, sex, maturity stage (for females), and shell condition. Abiotic factors tested were substrate type (classified as either mud/sand, shells/gravel, or

rock/boulder) and depth (classified as either <200m, 200m - 249m, or >250m). Statistical significance was set at $\alpha = 0.05$.

RESULTS

Temporal and spatial distribution of disease

Over the 7-year period of study, 217,136 snow crabs were visually examined for overt infections of bitter crab disease (BCD) (Table 1). Of these, 172,743 crabs were caught in traps and 44,393 were caught in trawls. Traps showed a significant bias toward large juvenile and adult male crabs whereas trawl samples showed less bias between sexes. Samples were comprised of 70,644 crabs from Avalon, 68,153 crabs from Bonavista Bay, and 78,339 crabs from Conception Bay. Due to logistical constraints, collections periods did not coincide seasonally between regions. Sampling at Avalon took place in May-June, at Bonavista Bay in August, and at Conception Bay in September-October. Further, in Conception Bay, the 2001 trawl data were inadvertently collected with a larger mesh gear than that used normally; these data were thus excluded from the analysis due to poor sample sizes and significant sample bias. Due to logistical constraints on ship and crew time, seasonal differences in prevalence within and between regions could not be examined.

The prevalence of *Hematodinium* infections varied markedly between regions with the Avalon fishing grounds showing consistently lower prevalences (<1.00%) in both trawl and trap surveys than the other regions (Fig. 2). The prevalences of infection in Bonavista Bay were intermediate (1.00 – 4.93%), generally higher than those at Avalon, but almost an order of magnitude lower than those in Conception Bay (2.00 – 26.67%). However, some of the differences in prevalence may have been due to seasonal variation in the development of overt infections.

During 1999 and 2000, snow crabs experienced an epizootic of *Hematodinium* sp. within Bonavista and Conception bays. In 1999, the prevalence in Bonavista Bay reached 4.93% and 2.96% in females collected by trap and trawl, respectively. Males also showed increased prevalences at 1.16% and 1.12%, trap and trawl, respectively. Similarly high prevalences were observed from crabs in Conception Bay (i.e., 9.52% and 2.75% in females and males from trawls). Moreover, in 2000, the prevalence of infection in Conception Bay reached dramatic new highs of 26.67% and 18.24% in females collected by trap and trawl, respectively, with males also showing high prevalences (2.68% and 8.04%, trap and trawl, respectively). However, by 2000, the prevalences of *Hematodinium* sp. had returned to low levels in Bonavista Bay and remained low in Avalon. From 2001 and 2002, the trap data indicated that the epizootic in Conception Bay had subsided, but in 2003, the prevalence of infection had risen to 2.88% and 6.95% in trawled and trapped male crabs, and 4.67% and 6.95% in trawled and trapped female crabs, respectively. The prevalences remained comparatively low in Avalon and Bonavista Bay during this period, but again, these regions were sampled in different time periods.

The spatial distribution of *Hematodinium*-infected crabs was examined within the three regions over time. No patterns in prevalence were evident for Avalon and Bonavista Bay; the prevalence of diseased crabs was low and infections were widely dispersed

throughout each region. However, from 1997 to 2003, infections in Conception Bay were located at depths greater than 100 m and typically near the inland terminus of the bay (Fig 3). Prevalence was significantly lower at depths <200 m than at depths >200 m and depths >250 m (0.40%, 2.12%, 2.19%, respectivly, $\chi^2 = 74.81$, d.f. = 2, $p<0.001$).

The prevalence of infection was generally higher in trawl surveys compared to trap surveys, primarily because the former caught more juvenile and female crabs which are known to have a higher prevalence than adult males (Pestal et al. 2003). Trap surveys were biased toward large adult males, whereas trawl surveys showed less bias. There was sufficient data to make 28 temporal comparisons (annually by location and sex) between trap and trawl surveys. In 17 of 28 cases, prevalences were higher in trawls than comparible trap surveys. Prevalences were lower in trawls than traps in 7 of 28 cases. In 4 comparisons, prevalence did not differ between method of capture. In the 7 trawl surveys showing lower prevalences, nearly all were trap samples that had relatively small sample sizes of females skewed by the presence of relatively more infected animals. In general, prevalences were 2.4 times higher in trawl surveys than in trap surveys, but the overall variance was high.

Biotic and Abiotic Factors

To determine whether size of crab influenced prevalence of bitter crab disease, we examined size-frequency distributions of infected and uninfected crabs collected by trawl from Conception Bay for 1997 to 2000 (Fig. 4). The prevalence of infection was highest in small (<70 mm CW) male and female crabs with most infections found in crabs less than 50mm in carapace width. Prevalences peaked at around 20% for 40mm crabs of both sexes and were consistently over 10% in the 25 through 40mm size classes. This relationship was consistent for other years. In all cases, large males had substantially lower prevalences than small males and females (see also below). In addition, immature females had a significantly higher prevalence than mature females (15.299% vs. 2.925%, respectively, $\chi^2 = 94.15$, d.f. = 1, $p<0.001$, n = 3229), even though their size distributions overlapped significantly.

The prevalence of infection differed significantly across shell types in Conception Bay from 1997 through 2000. Prevalences were 1.08% for shell 1 (recently molted; one infected animals), 7.30% for shell 2 (molted within last year; 837 infected animals), 0.06% for shell 3 (not molted within 2 years; one infected animal), and 0.50% for shell 6 (molted within two years; 12 animals). That is, 98.35% of the infections occurred in crabs in the shell 2 condition. This pattern was consistent between years and sites. Due to this strong relationship between shell type and prevalence of BCD, and the resulting lack of infected animals with shell types 1, 3 and 6, shell type was not included as a variable in subsequent logistic regressions.

Preliminary analyses of the effects of biotic factors on the probability of infection with BCD revealed significant interactions between sex, maturity, and carapace width ($\chi^2 = 767.43$, df = 3; $p < 0.001$) indicating that the effects of carapace width and maturity stage on probability of BCD infection differed between male and female crabs. Therefore, logistic regression models testing the effects of carapace width and maturity stage on the probability of infection were fit separately for male and female crabs (Tables 2 and 3). Predicted probabilities of infection for males, immature females, and mature

females showed marked contrasts in relation to size (Fig 5). Carapace width was significantly negatively correlated with the probability of infection for male crabs (odds ratio for a 1mm increase in carapace width = 0.967). However, carapace width was significantly positively correlated with the probability of infection for female crabs (odds ratio for a 1mm increase in carapace width = 1.02). For female crabs there was also a significant effect of maturity stage on probability of BCD infection: the odds of an immature female being infected were 5.6 times the odds of a mature female being infected.

Results of logistic regression suggested that two abiotic variables influence the probability of infection (Table 4). To account for possible relationships between abiotic variables and those biotic variables found to influence the probability of BCD infection, sex/maturity stage and carapace width were included in the logistic regression model. Depth strata showed a significant effect on the probability of infection with higher prevalences occurring at depths >200m (prevalences of 1.44% vs. 3.93% vs. 5.96% for <200m, 200-250m, >250m depths, respectively). Substrate type showed a significant effect on the probability of infection after controlling for the effects of depth, sex, maturity stage, and carapace width. For crabs of any given sex, maturity stage, and carapace width, the odds of an individual sampled from mud/sand substrate being infected with BCD were 1.88 times (weak trend, $p=0.057$) the odds of an individual sampled from rock/boulder substrate being infected, and 2.12 times (highly significant, $p<0.001$) the odds of an individual sampled from gravel substrate being infected. There was no difference ($p = 0.729$) in the odds of infection between crabs sampled from rock/boulder substrate and those sampled from shell/gravel substrate. Similarly, crabs from mud/sand bottoms showed significantly higher prevalences than those from gravel or rock/boulder bottoms via contigency table analysis (5.793% vs. 3.935% vs. 1.484%, respectively, $\chi^2 = 32.319$, df= 2, $p< 0.001$, n = 15,803); and this relationship was consistent between sexes and maturity status.

Mortality

The mortality rate for naturally infected crabs differed from those experimentally inoculated or in the control group. However, the mortality study had a small sample size and was exploratory in nature. The median time to death for naturally (overtly) infected snow crabs was 61.8 $\pm$ 7.9 (se) days. Naturally infected crabs (n = 12) began dying after two weeks and all of them were dead by day 99 (Fig. 6). The median time to death for experimentally inoculated crabs was 91.4 + 17.0 (se) days. Inoculated crabs (n=18) experienced 50% mortality by day 99, with all of the moribund crabs developing infections of *Hematodinium* sp., and negligible mortality thereafter to day 127 when the experiment was terminated. Because the controls exhibited negligible mortalities, the median time to death for the uninfected controls could not be calculated. One of the uninfected controls (n=8) died on day 54, but no other mortalities occurred in the control treatment. Uninfected control crabs experienced significantly less mortality than naturally infected hosts (log rank test, $p<0.001$, Chi-square = 15.671 with 2 df), but not inoculated hosts, albeit the trend was almost significant for the latter (log-rank, Chi-square = 3.261 with 1 df, $p = 0.071$). Inoculated crabs experienced significantly less

mortality than naturally infected hosts (log-rank, Chi-square = 7.844 with 1 df, $p < 0.005$). None of the control crabs developed infections.

DISCUSSION

We have documented a disturbing increase of bitter crab disease (BCD) in snow crabs in Newfoundland. Once very rare, *Hematodinium* sp. is now firmly established in the northern bays and has undergone at least one small outbreak in Bonavista and Conception bays in 1999, and one larger epizootic in Conception Bay in 2000. Previous surveys of BCD in Conception Bay have also shown a marked increase in prevalence over time. In the 1992-1993 assessment, prevalences in trapped males were extremely low (0.037%) (Taylor and Khan 1995), but in the 1998 fishery assessment, they had increased more than tenfold (0.57%) and were even higher in trawled males (1.60%) (Pestal et al. 2003). The outbreak in 2000 reached a prevalence of over 8% in trawled males and over 26% in trapped females. Given that prevalences were based on macroscopic diagnosis of infection, and that Pestal et al. (2003) indicated that macroscopic infections represent approximately 50% of the actual prevalence during October 1998, then the actual prevalence approached 18% in trapped males and 50% in trawled females. These are extraordinary levels hitherto unreported in Newfoundland. Even higher prevalences of BCD have been reported from focal outbreaks in the Tanner crab fishery from the southeastern fjords Alaska, where direct losses were estimated annually at more than $250,000 in 1987 (Meyers et al. 1987). In Newfoundland, the foci of BCD are primarily located within the northern bays which represent approximately 10-12% of the fishery (Anonymous 2002). Whereas direct losses are difficult to estimate for this region, our data show that the disease has a more subtle, indirect effect on the fishery because it is most prevalent in under-sized males and females. Further, all macroscopically infected crabs will die from the infection. Therefore, in Newfoundland the disease has its greatest on the pre-recruit and unfished segments of the population. At present, the disease has not been reported from off other Maritime provinces, but given its rapid spread, other snow crab stocks may be at considerable risk.

Outbreaks of *Hematodinium* species often occur in constricted areas or areas with entrained water masses such as lagoons, embayments or fjords with shallow sills (Meyers et al. 1987, 1990, Latrouite et al. 1988, Eaton et al. 1991, Field et al. 1992, Wilhem & Miahle 1996, Field et al. 1998, Messick & Shields 2000). Such associations with physiography and hydrography were first noted for the rhizocephalan *Briarosaccus callosus* in king crabs (Sloan 1984, Hawkes et al. 1985) and have since been noted for nemertean infestations on king crabs as well (Kuris et al. 1991). In the fjords off Alaska, prevalences of BCD reached extremely high levels in Tanner crabs (Meyers et al. 1987, 1990), while open ocean habitats had prevalences in snow crabs that ranged from very low to moderate (Meyers et al. 1996). Similarly, in Newfoundland, the accumulation of high prevalences and an epizootic outbreak occurred within the relatively constricted confines of Conception Bay, low to moderate prevalences occurred within the less confined Bonavista Bay, and extremely low prevalences occurred within the open ocean environment at the Avalon fishing grounds. Offshore occurrences of BCD in snow crabs also appear associated with deep-water muddy-bottomed “gullys” or basins (Dawe 2002). Our data combined with the hydrographic affinities of the disease, suggests that once the

parasite successfully colonizes hosts within a confined embayment, it will rapidly become enzootic, possibly serving as a locus for the further spread of infections.

The large bias in prevalence between trawl and trap surveys has distinct management implications for monitoring the disease. Similar biases have been noted for BCD in snow crabs from Newfoundland (Pestal et al. 2003) and for *Hematodinium* sp. in velvet crabs, *Necora puber* off Brittany, France (Wilhelm & Miahle 1996). In both cases, trawl samples had significantly higher prevalences presumably because of non-selective sampling of infected and uninfected crabs. However, trawl samples also had a lower minimum size of retention than traps, and at least for snow crabs, prevalences were higher in smaller crabs than in larger crabs. Considering the size of the snow and Tanner crab fisheries, and the relative number of studies on *Hematodinium* infections in these hosts, it is surprising that differences in gear bias have not been explored for these crustaceans. Further, the disease is most prevalent in the female and pre-recruit male crabs which are not removed by the fishery and which are not typically monitored by management agencies. Therefore, we recommend that future monitoring for BCD in snow and Tanner crab populations employ less-selective or non-selective gear such as trawls or small-mesh traps because prevalences in these under-sampled members of the population may forewarn of an impending epizootic that could be used to predict recruitment failure or impacts on future recruitment to the fishery.

Several other host and environmental factors were clearly associated with BCD in snow crabs from Conception Bay. Host size, sex and molt status (shell type) have been previously associated with infections in Tanner crabs (Meyers et al. 1990, Eaton et al. 1991, Love et al. 1993). However, the disease dynamics in Tanner crabs from Alaska are different from those in snow crabs from Newfoundland. For example, molting of the host appears important to transmission or the onset of the late stages of the disease in both host species. In snow crabs from Newfoundland, anecdotal observations based on macroscopically-diagnosed BCD infections indicate that the disease is more prevalent in October, well after the end of the commercial fishing season (August 31), and well after the summer peak in molting (Taylor unpubl. data). Recently molted snow crabs (shell 1) progress to the shell 2 stage over the course of two to three months and shell 2 crabs are indicative of a molt within the last 6-12 months (Taylor et al. 1989). Thus, given that 98.35% of macroscopically overt infections occur within crabs in the shell 2 condition, and that infections are rare (1.5%) in shell 3 and 6 animals, snow crabs must obtain the infection while in the postmolt condition. In contrast, infected Tanner crabs molt in early spring, before sporulation occurs in infected crabs, and experimental infections take up to 3 mo for infections to be detected, leading Meyers et al. (1990) to speculate that infections may take 15 to 18 months to develop, and that crabs obtain infections during their molt to the *preceeding* instar. However, in Newfoundland, infected crabs are present in the fishery from the spring through fall as are recently molted animals. Infected crabs in shell stage 2 have been caught in the spring during the Avalon research cruises (Taylor and Khan 1995), and these animals likely serve to transmit the disease to the recently molted individuals. Further, infections in snow crabs in shell 3 and shell 6 conditions (no molt within 1 to 2 years) are exceedingly rare (1.5%), indicating that few hosts survive infections for more than one year. However, in Tanner crabs, 3% to 7% of infections occur in "old shell" crabs (viz. shell 3 and shell 6 conditions) (Eaton et al. 1991) indicating that either longer infections occur in Tanner crabs, or transmission may

have an alternate mode such as diet, cannibalism, or possibly sexual contact (Meyers et al. 1996). While snow crabs do cannibalize each other (Lovrich & Sainte-Marie 1997, Squires & Dawe 2003), and cannibalism may transmit *Hematodinium* sp. to blue crabs (Sheppard et al. 2003), the extremely low prevalences in the shell 3 and shell 6 animals suggest that it is not a contributory factor in the transmission of the parasite. Thus, for snow crabs in Newfoundland, the parasite is mostly likely transmitted to crabs during molting or while in the post-molt condition. Macroscopically overt infections appear to develop over the course of two to four months or longer but rarely persist longer than one year. Mortalities likely occur three to four months after the infections become macroscopically apparent. Similarly, in the Norway lobster, infections appear to be acquired at molting and develop to patency over three to four months (Stentiford et al. 2001).

The relationship between prevalence and substrate type, mud/sand, has not been previously recognized. Dirt, detritus, bivalves and amphipods are often large components of the snow crab diet (Wieczoreck & Hooper 1995, Squires and Dawe 2003). Perhaps transmissive stages are ingested by crabs feeding on bivalves, which can efficiently filter large quantities of water, hence dinospores. Alternatively, amphipods have *Hematodinium*-like infections (Johnson 1986) and have been posited as potential alternate hosts for crabs (Shields 1994). Several amphipod species co-occur in trawls of snow crabs, but it is not known whether they harbor infections. Lastly, Pestal et al. (2003) showed that depth was related to prevalence of infection with deeper sites (>250 m) having higher prevalences. Further, in our logistic regression of trawl data, shallow depths (<200 m) showed much lower prevalences than those >200m, with the highest prevalences occurring at >250m. Few other studies have assessed depth and substrate type as potential contributory factors in *Hematodinium* infections. Our data indicate that abiotic factors other than temperature, salinity, and hydrography are also important in the spread of the parasite.

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Table 1. Annual sample sizes for trapped and trawled crabs by sex and region. The Avalon region was sampled in May-June, Bonavista Bay in August, and Conception Bay in September-October.

Region, Method	Sex	1997	1998	1999	2000	2001	2002	2003	Totals
Avalon, Trap									
	Female	183	88	467	78	60	218	309	1403
	Male	1739	6172	8024	6638	12489	10428	12441	57931
Avalon, Trawl									
	Female	389	1237	1499	556		335	892	4908
	Male	575	1440	1898	732		554	1203	6402
Bonavista Bay, Trap									
	Female	70	320	1644	1573	1428	540	861	6436
	Male	3036	8248	6630	8874	6960	9834	7023	50605
Bonavista Bay, Trawl									
	Female	121	627	574	1660		589	265	3836
	Male	191	829	890	2895		2143	328	7276
Conception Bay, Trap									
	Female	312	19	45	15	36	24	23	474
	Male	8458	5421	7826	7690	8911	8682	8906	55894
Conception Bay, Trawl									
	Female	81	660	1282	1206		172	793	4194
	Male	935	4047	4394	3198		1700	3503	17777
Totals		16090	29108	35173	35115	29884	35219	36547	217136

Table 2. Estimates for the logistic regression testing the effects of carapace width and maturity stage on probability of infection by bitter crab disease for female snow crabs sampled from Conception Bay, pooled from 1997-2000. Only crabs in shell 2 (molted within the year) were included.

Parameter	df	Estimate	SE	t-ratio	p	Odds Ratio
Intercept	1	-4.158	0.385	-10.793	<0.001	
Carapace Width	1	0.019	0.006	3.280	<0.001	1.02[a]
Maturity Stage (Immature vs. Mature)	1	1.715	0.237	7.234	<0.001	5.559

n = 382 infected, 2,513 uninfected

Model chi-square = 69.884; df=2; $p < 0.001$

a Odds ratio for 1mm increase. Odds ratio for Xmm increase = $e^{X(0.019)}$.

Table 3. Estimates for the logistic regression testing the effects carapace width and maturity stage on probability of infection by bitter crab disease for male snow crabs sampled from Conception Bay, pooled from 1997-2000. Only crabs in shell 2 (molted within the year) were included. Maturity was not included for males as chelar measurements to determine maturity status were only recorded for subsets of males in the samples.

Parameter	df	Estimate	SE	t-ratio	p	Odds Ratio
Intercept	1	-0.943	0.128	-7.346	<0.001	
Carapace Width	1	-0.034	0.002	-14.207	<0.001	0.967[a]

n = 455 infected, 7972 uninfected

Model chi-square = 228.484, df=1, $p < 0.001$

a Odds ratio for 1mm increase. Odds ratio for Xmm increase = $e^{X(-0.034)}$.

Table 4. Estimates for the logistic regression testing the effects of biotic and abiotic variables on probability of infection by bitter crab disease for snow crabs sampled from Conception Bay, pooled from 1997-2000.

Parameter	df	Estimate	SE	t-ratio	p	Odds Ratio
Intercept	1	-2.248	0.152	-14.783	0.001	
Substrate Type						
Mud/sand vs. Rock/boulder	1	0.629	0.330	1.906	0.057	1.876
Mud/sand vs. Gravel	1	0.750	0.133	5.625	<0.001	2.117
Gravel vs. Rock/boulder	1	0.121	0.349	0.347	0.729	1.129
Depth						
<200m vs. >250m	1	-1.611	0.327	-4.919	<0.001	0.200
200-250m vs. >250m	1	-0.084	0.080	-1.053	0.292	0.920
<200m vs. 200-250m	1	-1.527	0.326	-4.690	<0.001	0.217
Sex/maturity						
Mature Females vs. Males	1	2.247	0.248	9.043	<0.001	9.457
Immature Females vs. Males	1	0.871	0.144	6.057	<0.001	2.389
Mature Females vs. Immature Females	1	-0.653	2.151	-0.304	0.761	0.521
Carapace Width						
Females vs. Males	1	-0.037	0.002	-14.822	<0.001	0.964
Mature Females vs. Immature Females	1	-0.013	0.039	-0.338	0.735	0.987
n = 837 infected, 10,485 uninfected						
Model chi-square = 544.049, df=8, $p < 0.001$						

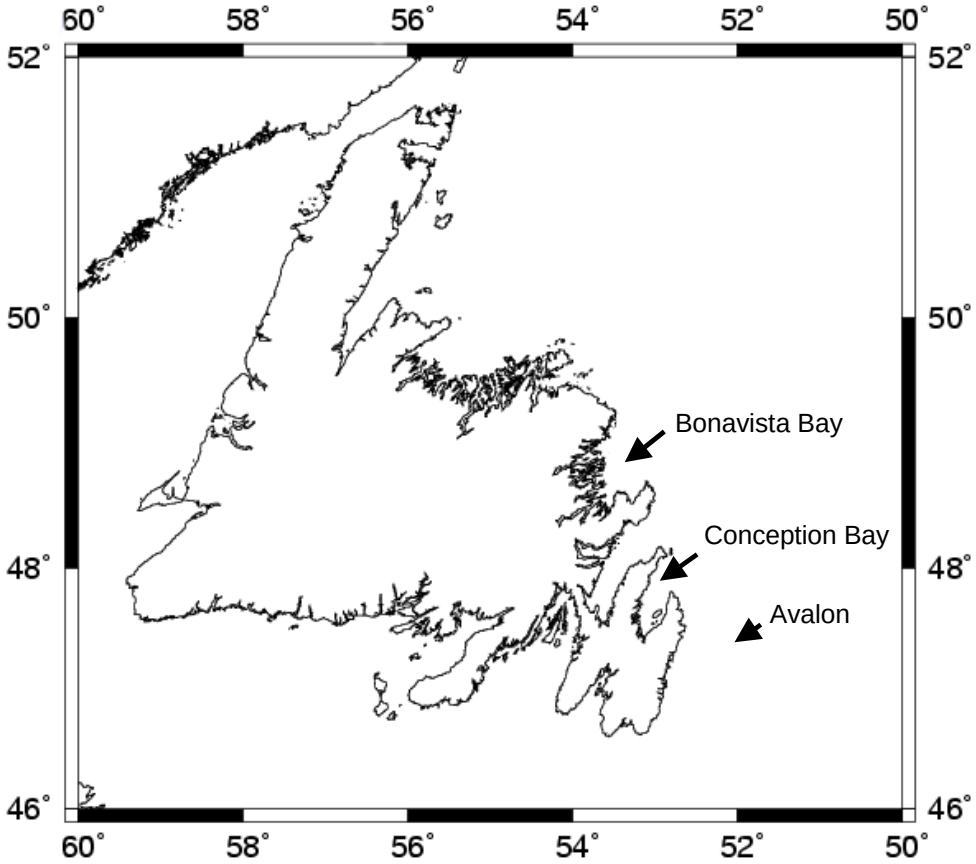

Figure 1. Location of study sites off Newfoundland.

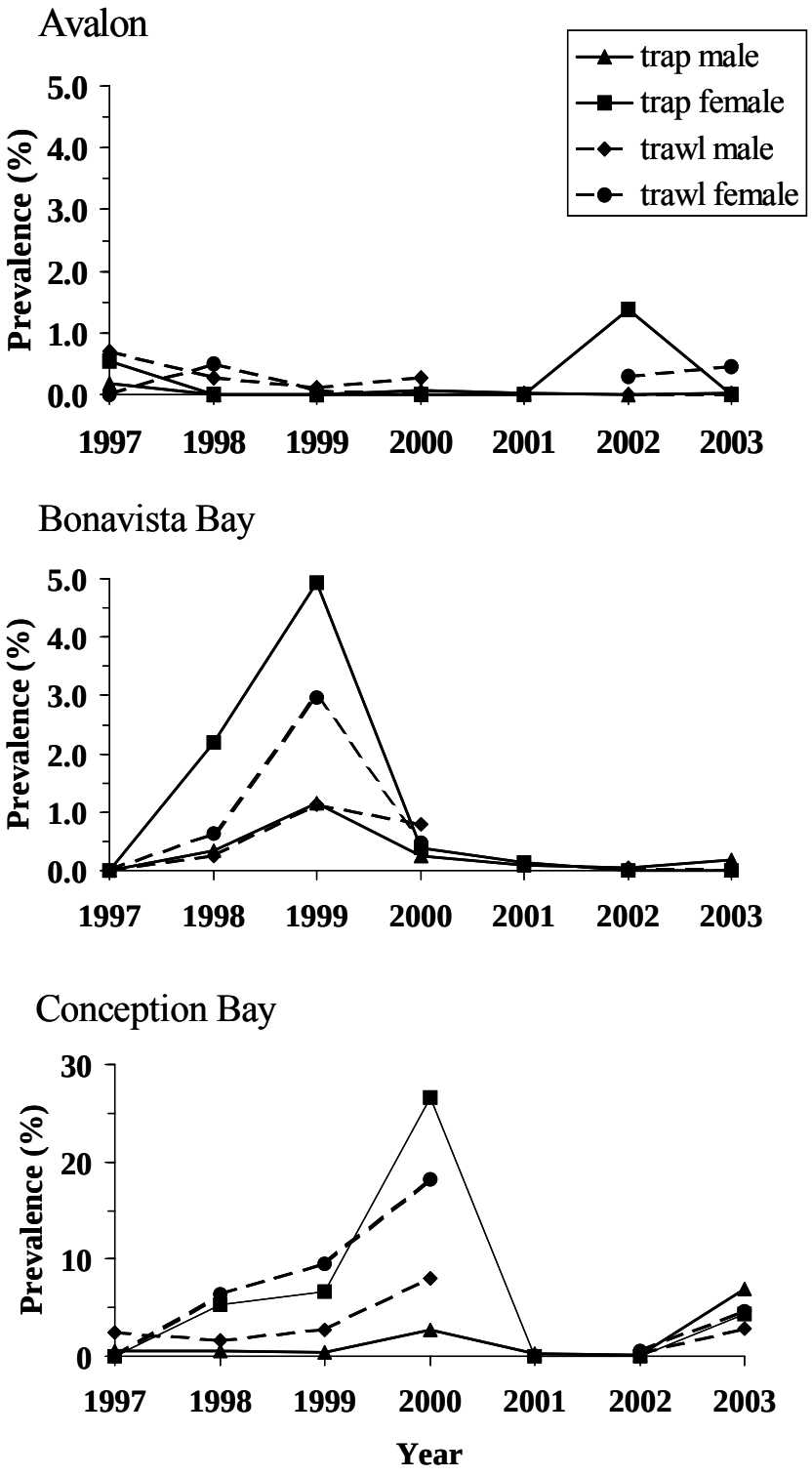

Figure 2. Annual prevalences of bitter crab disease in snow crabs from (A) Avalon, (B) Bonavista Bay, and (C) Conception Bay. Trap (solid lines) and trawl (dashed lines) data are separated by sex (male= triangle or diamond, female = box or circle). Note change in scale of Y axis between graphs.

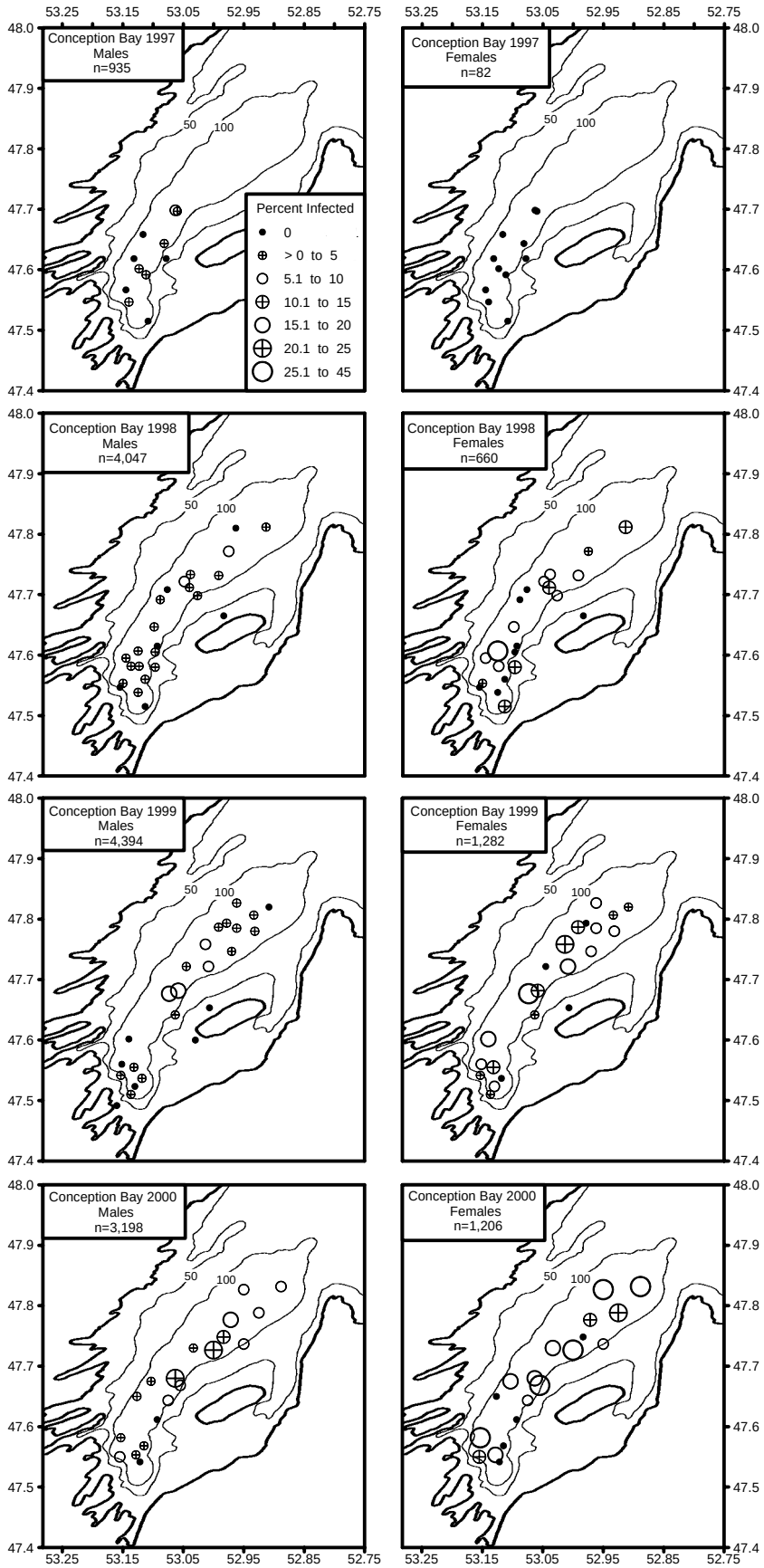

Figure 3. Spatial distribution of bitter crab disease in Conception Bay over time. Prevalence is shown by the size and fill of each circle. Note the clustering in the deeper waters in the bay.

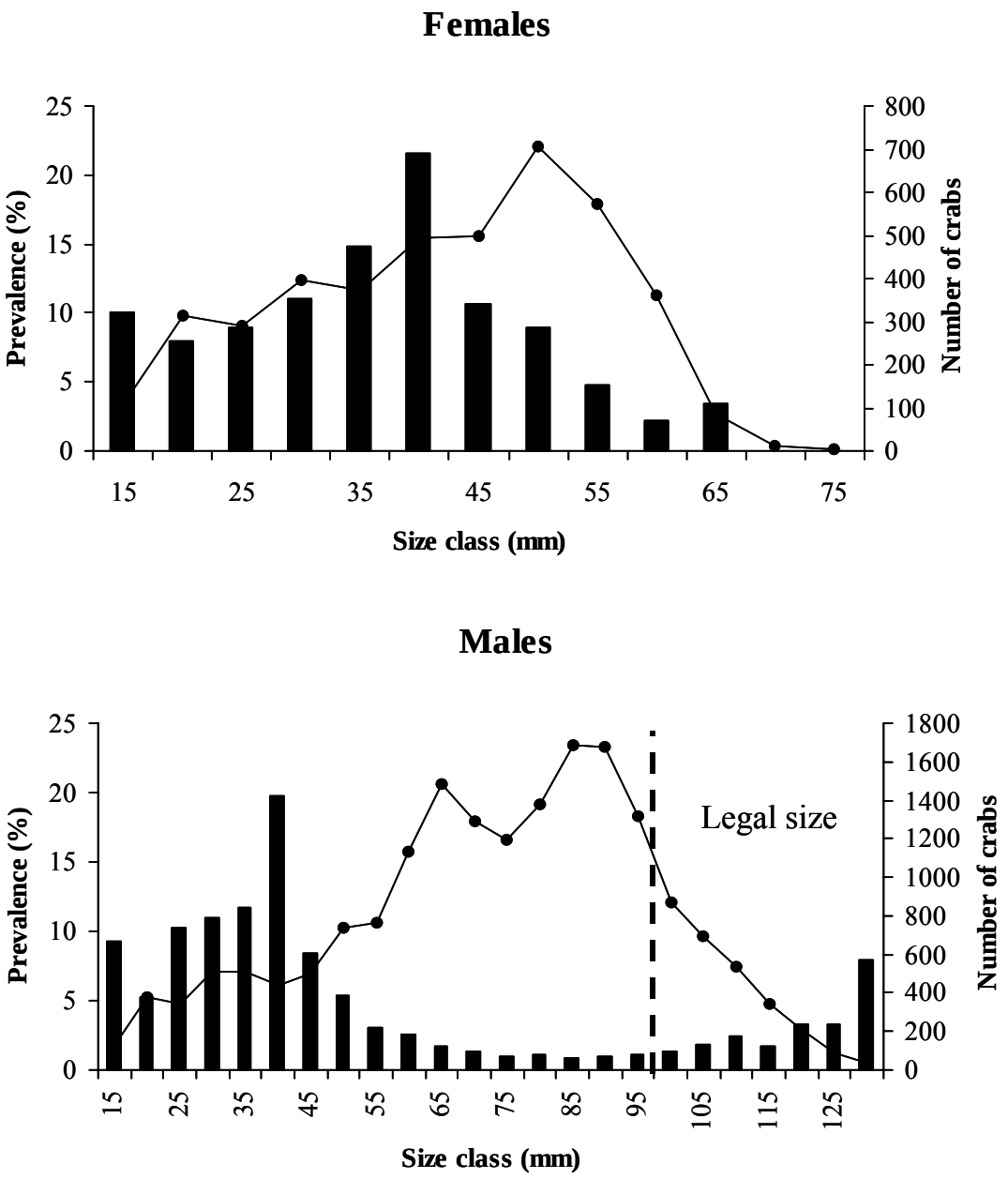

Figure 4**.** Size-frequency distributions in 5 mm classes for male and female crabs from trawl data for Conception Bay, 1997-2003. Solid bars = prevalence (%) of bitter crab disease, solid line = number of animals in size class. Note change in scale of Y axis between graphs.

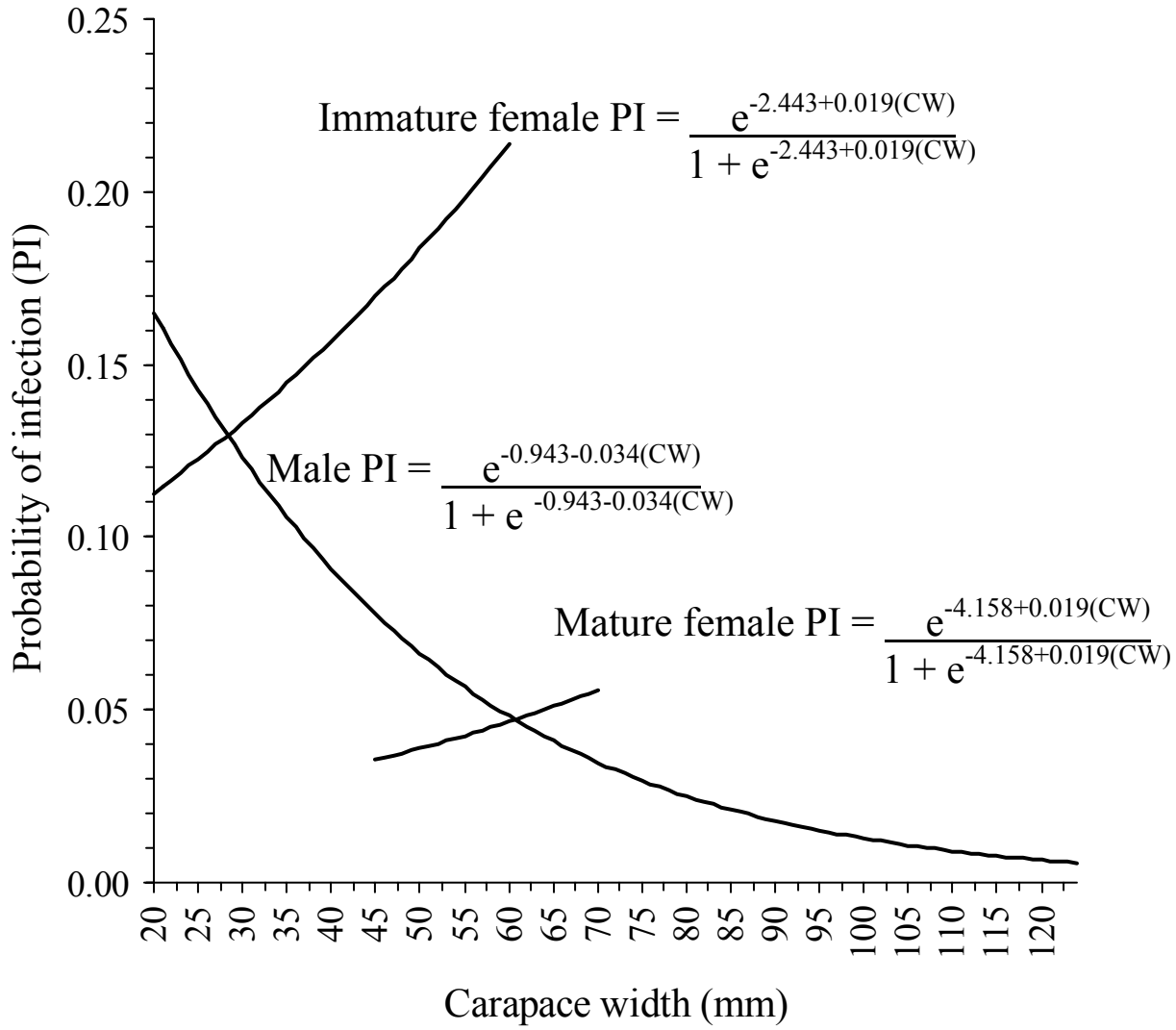

Figure 5. Estimated probability of infection with bitter crab disease by carapace width for male, mature female, and immature female snow crabs sampled from Conception Bay.

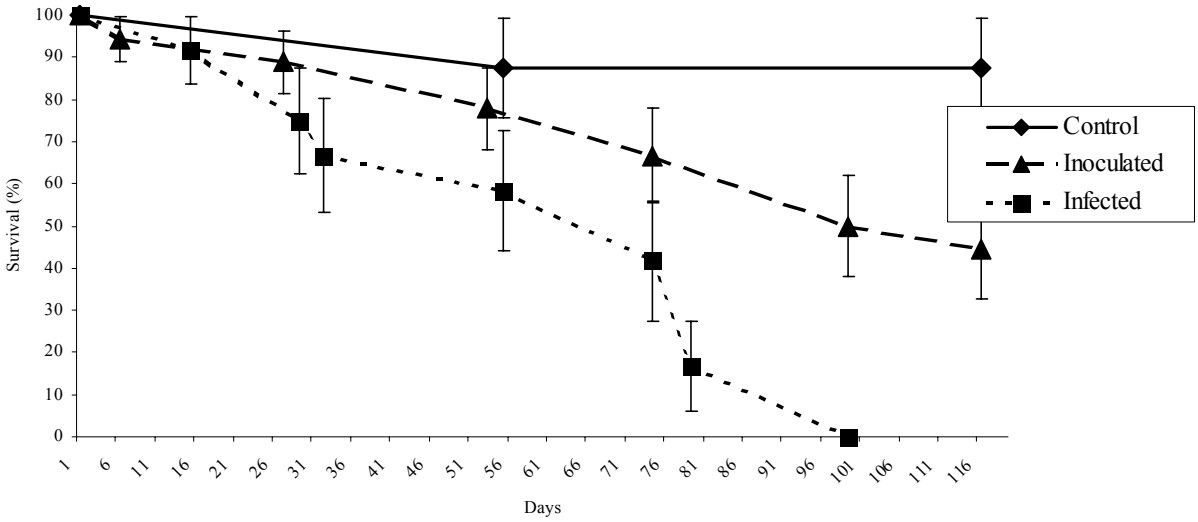

Figure 6. Kaplan-Meier survival analysis of uninfected, overtly infected and inoculated crabs over time. The infected crabs had a significantly lower survival than uninfected or inoculated crabs (log rank test, $p<0.001$, Chi-square = 15.671 with 2 df).