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Trends in prevalence of Bitter Crab Disease (*Hematodinium spp.*) in snow crab (*Chionecetes opilio*) at Newfoundland and Labrador

E. G. Dawe, H. J. Drew and R. T. Warren

Department of Fisheries and Oceans
Science, Oceans and Environment Branch
P. O. Box 5667
St. John's, NF A1C 5X1

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Abstract

This paper describes the spatial distribution and prevalence of Bitter Crab Disease (BCD) in snow crab throughout the Newfoundland and southern Labrador Continental Shelf (NAFO Divisions 2J3KLNO) during 1996-2000. The disease, caused by hemo-parasitic infection by a dinoflagellate of the genus *Hematodinium*, occurs predominately in recently-molted (new-shelled) crabs of both sexes and is fatal to the snow crab host. Estimation of prevalence of this parasitism was based on macroscopic identification of chronic cases during annual fall bottom trawl surveys. BCD was overall most prevalent within the center of the broad snow crab distribution (Div. 3K) and was rare in southern-most areas of the Grand Bank (Div. 3NO). Spatial and annual changes in both distribution and prevalence of BCD were considerable. Prevalence was highest in mature females and in intermediate-sized males, but there was annual and spatial variation in size ranges most affected. Inverse relationships were apparent between catch rates and prevalence of BCD, suggesting that this parasitism may represent a substantial source of natural mortality. There is considerable uncertainty regarding the extent to which observations from the trawl-caught samples represent true prevalence in the wild.

Résumé

Ce document décrit la répartition spatiale et la prévalence de la maladie du crabe amer (MCA) chez le crabe des neiges du plateau continental de Terre-Neuve et du sud du Labrador (divisions 2J3KLNO de l'OPANO) pour les années 1996 à 2000. Touchant surtout des crabes des neiges mâles ou femelles ayant récemment complété la mue (venant de former une nouvelle carapace), cette maladie létale est causée par un dinoflagellé parasite du genre *Hematodinium* qui infecte le sang des crabes. La prévalence de ce parasitisme a été évaluée par identification macroscopique de cas chroniques parmi les crabes capturés au cours de relevés d'automne annuels au chalut de fond. La prévalence de la MCA était généralement la plus élevée au centre de l'aire de répartition du crabe des neiges (dans la division 3K), tandis que la maladie était rare dans les secteurs du Grand Banc situés les plus au sud (division 3NO). La répartition et la prévalence de la MCA présentaient des variations spatiales et annuelles considérables. Les femelles matures et les mâles de taille intermédiaire présentaient la plus forte prévalence, mais les classes de taille les plus touchées variaient dans l'espace et d'une année à l'autre. Le taux de capture et la prévalence de la MCA étaient inversement reliés, ce qui laisse croire que cette maladie parasitaire constitue une importante cause de mortalité naturelle. Il y a cependant une grande incertitude quant à la mesure dans laquelle les observations faites sur les échantillons prélevés au chalut sont représentatives de la prévalence réelle dans le milieu naturel.

Introduction

Parasitic dinoflagellates of the genus *Hematodinium* have been found in the hemolymph of a variety of crustaceans (MacLean and Ruddell 1978; Newman and Johnson 1975; Meyers et al. 1987). Infection of spider crabs of the genus *Chionoecetes* in Alaska by this parasite has been termed Bitter Crab Syndrome or Bitter Crab Disease (BCD) because meats from heavily-parasitized crabs are bitter-tasting and unmarketable, although harmless to humans. BCD is 100% fatal to its host (Meyers et al. 1987). Infection is recognizable externally in heavily-parasitized crabs by abnormal pink or orange coloration of the dorsal carapace and joints of the walking legs, as well as an opaque white 'cooked' appearance of the ventral carapace. Often, there are white opaque streaks along the translucent mid-ventral merus leg section. Internally, opaque hemolymph is evident (Meyers et al. 1990).

BCD was first reported in southeast Alaskan tanner crab (*C. bairdi*) as early as 1974, but was not attributed to infection by *Hematodinium spp.* until 1986 (Meyers et al. 1987). It has subsequently been found in snow crab (*C. opilio*) from the Bering Sea (Meyers 1990), where its prevalence tends to increase with latitude (Morando et al. 2000). Alaskan studies of spatial distribution and seasonality of BCD have been primarily based on *C. bairdi* (Eaton et al.1991, Love et al 1993, Meyers et al. 1987, 1990).

The duration of the parasite's life cycle as well as the mode of transmission are unclear. Meyers et al. (1990) noted that the dinoflagellate's life cycle may be annual with transmission occurring by direct penetration of motile spores through cracks in the new soft cuticle. Alternatively the parasite's life cycle could be about 16 months if disease transmission is through cannibalism. Both mechanisms of transmission may be associated with molting (Meyers et al. 1990).

Taylor and Khan (1995) documented the first known occurrence of BCD in Newfoundland snow crab (*C. opilio*) in 1992 and they identified the causative agent as *Hematodinium sp.*. They found, based on sampling in three localized fishing areas during various months, that incidence was very low, at about 0.1% during 1992-1993.

Spatial, annual, and seasonal trends in prevalence of this disease in snow crab are unknown in Newfoundland and Labrador. Also, possible relationships with crab density, host sex or body size and water depth are virtually unknown. This is unfortunate because an understanding of such trends and relationships may indicate mechanisms for transmission of the disease and factors that regulate its prevalence.

In this paper we describe the spatial distribution and prevalence of BCD in snow crab throughout the range of snow crab distribution along the northeast Newfoundland and southern Labrador continental shelf. Annual changes in spatial distribution of BCD are described based on 1996-2000 fall bottom trawl surveys. Further details on effects of year, density, and host sex and body size are provided. The possible effects of this disease on natural mortality levels and recruitment are also considered.

Methods

Sampling.

Snow crab samples were acquired from 1996-2000 fall multi-species stratified random bottom trawl surveys, which extended throughout NAFO Divisions 2J3KLNO (Fig. 1). These surveys utilized the Campelen 1800 survey trawl, a shrimp trawl with mesh size of 44-80 mm and a nylon codend liner of 12.7 mm mesh. The trawl has a wingspread of about 16 m and a footrope equipped with rock-hopper gear. It was fished in standard tows of 15 min duration, at a speed of 3.0 knots, over a distance of 0.75 nm. These multi-species surveys with the Campelen trawl began in 1995, but BCD was not routinely monitored in that year.

Snow crab catches were sorted by sex and either fully sampled or subsampled, especially in the case of very large catches. More than 77,000 crabs were sampled across all years (Table 1), representing 74% of all crabs caught. All crabs sampled were measured in carapace width (CW, mm). Chela height (CH, 0.1 mm) was estimated for males and maturity was determined for females. Shell condition was assigned one of three categories: (1) new-shelled - these crab had molted in spring of the current year; legal-sized males (>94 mm CW) have a low meat yield throughout most of the fishing season, and are generally not retained in the current fishery until fall; mature females are carrying their first egg clutch. (2) intermediate-shelled – these crab last molted in the previous year and legal-sized males are fully recruited to the fishery throughout the current fishing season. (3) old-shelled – legal-sized males of this category have been available to the fishery for at least 2 years.

Occurrence of advanced stages of BCD was noted based on macroscopic examination. In cases of uncertain BCD classification, crabs were dissected and classified based on observation of the hemolymph. Cloudy or milky hemolymph confirmed infection by *Hematodinium* sp.. Specimens displaying clear external characteristics of BCD were randomly selected and dissected to examine the hemolymph and so validate the macroscopic observations. All those specimens displayed milky hemolymph, confirming chronic infection.

Treatment and analysis of data.

Most trends in prevalence of BCD are described based on males only, because only males are of economic value and males have a broader size range than females. However the most general trends are compared between the sexes to determine applicability to the entire population.

To examine size composition of males, carapace widths were grouped into 3 mm intervals and adjusted up to total population abundance. Each size interval was partitioned by claw type by applying a model which separates two ‘clouds’ of chela height on carapace width data ($CW = 0.0806CH^{1.1999}$). In this way, each male was classified as either large-clawed or small-clawed. Males develop enlarged chelae when they undergo a final molt, which may occur at any size larger than about 40 mm CW. Therefore only males with small chelae will continue to molt and subsequently recruit to the fishery. Males may achieve maximum sizes of about 140 mm CW. Females become sexually mature and cease molting within a size range of about 40-75 mm CW.

To examine the effect of size on prevalence of BCD, males were grouped into consecutive size classes, some of which approximate molt classes based on growth per molt data (Moriyasu et al. 1987, Taylor and Hoenig 1990, and Hoenig et al. 1994). Five size groups were established: legal-sized crabs (≥95 mm CW); Sub-legal 1, those which would achieve legal size after one molt (76-94 mm CW); Sub-legal 2, those which would achieve legal size after two molts (60-75 mm CW). All other males were partitioned between a category of Sub-legal 3 males (41-59 mm CW) and smallest males (<41 mm CW). These latter two groups represent groupings of convenience and do not approximate molt classes.

Results

Prevalence and Distribution of Bitter Crab Disease.

The incidence of BCD was low across the entire fall survey area and all five years (Table 1). Only 1.2 % (1302 specimens) of all crabs caught were found to be heavily infected. BCD was consistently about twice as prevalent in females as in males. Annual prevalence was similar among four of the five years, ranging 0.7-1.1 % for sexes pooled in all years except 1997. Overall prevalence in 1997 was 2.6 % (Table 1), with 1.9 % of males and 4.4 % of females recognized as chronically infected.

BCD was broadly distributed throughout most of the range of male snow crab distribution (NAFO Div. 2J3KL, Fig. 1-2), but was rarely encountered in the southern portion (NAFO Div.3NO) (Fig. 2). It was most prevalent in NAFO Div. 3K during 1996-1998, whereas it became most prevalent in northern-most Div. 2J in 1999 and virtually absent from all more southern divisions. It shifted south again in 2000, to a spatial distribution similar to that of 1998.

BCD occurred predominately in new-shelled crabs that had molted in the immediately-preceding spring (Fig.3). Whereas only 69-71% of all males examined during 1997-2000 were new-shelled, consistently much higher percentages of those with BCD were found to be new-shelled (91-96%). Relatively high prevalence in intermediate-shelled males during 1996 (25%), the first year of monitoring BCD, was likely related to inexperience of some observers and subjectivity of shell categories. Only four males across all five survey years were categorized as old-shelled, likely reflecting misclassification of carapace condition.

BCD was recognized, within the new-shelled crabs, in all male size groups and both female maturity categories (Fig. 4). However, in new-shelled males, it was most prevalent in the intermediate size groups, usually the 41-59 mm CW group. Highest prevalence for this size group was 16% in Div. 2J in 1999. Prevalence in 41-59 mm CW males within Div. 3K was generally stable over the three years 1996-1998, ranging only 6.9-8.4%, before disappearing in 1999. It increased in all size groups in 2000 in both Div. 3K and 3L but remained below 1998 levels. Meanwhile, it decreased in Div. 2J in 2000 to approximate 1998 levels within the two smallest size groups. BCD was most uncommon in largest, legal-sized males (>94 mm CW), with a maximum prevalence of about 0.8% in Div. 3K in 1997 and 1998. Trends in prevalence in new-shelled females generally reflected the trends in males (Fig. 4). Prevalence was usually highest in mature females, as was true for the approximately comparably-sized males of 41-59 mm and 60-75 mm CW. The highest prevalence in mature females was about 10% in Div. 3K in 1997 and in Div. 2J in 1999.

No consistent annual trends were apparent among areas. Prevalence increased in small males (<41 mm CW) and immature females in all three northern areas (Div. 2J3KL) from 1996 to 1997, but decreased again in 1998 (Fig. 4). Prevalence in Div. 3K and Div. 3L, across all male sizes and both female maturity classes, generally peaked in 1997, virtually disappeared by 1999, and increased again in 2000. In contrast, it generally declined regularly in the most northern area (Div. 2J) over the three years 1996-1998 before increasing greatly in 1999 and subsequently declining again in 2000.

Relationships with density and abundance.

To investigate possible relationships between prevalence and density we focused on Div. 3K, where prevalence was highest, and compared prevalence with catch rate across 3-mm CW groups of new-shelled males, by year (Fig.5). Clearly, CW-specific catch rate was inversely related with prevalence in each year. Lowest catch rates and highest BCD prevalence was consistently at intermediate sizes. Peak prevalence by 3-mm group approached 18% in 1996, 16% in 1997 and 1998, and 9% in 2000. Characteristic of this association was a virtual absence of BCD at smallest sizes (smaller than about 24-30 mm CW) and at largest sizes (>114 mm CW) in most years. The annual variability in the sizes at which this inverse association developed suggests a causative relationship. For example, sharpest increase in BCD prevalence and decrease in catch rate coincided at about 33 mm CW in 1997, 39 mm CW in 1998, and 24 mm CW in 2000 (Fig. 5).

Survey catch rates were compared with prevalence of BCD across years for each of the five size groups of new-shelled males, to further explore the relationship between BCD prevalence and density (Fig.6). Negative relationships were again common. This was especially evident in Div. 2J, for all size groups except the largest (legal-sized, >94 mm CW). In Div. 3K and 3L a negative association was also suggested for the two smallest size groups, but it also appeared that direct relationships may exist at extreme levels of catch rate. Very high BCD prevalence frequently occurred one year later than very high catch rates, particularly in larger size groups, and very low catch rates frequently coincided with very low prevalence, particularly in smaller size groups.

Discussion

General distribution of BCD.

This study showed that BCD was found predominately in recently-molted (new-shelled) crabs, supporting the belief that infection is somehow associated with molting. Eaton et al. (1991) found that about 81% of new-shelled and 24% of older-shelled tanner crabs (*C. bairdi*) were infected from June 1988 to Feb. 1989 in southeast Alaska. An annual dinoflagellate life cycle in the snow crab host is implied off Newfoundland and Labrador. Cannibalism has been hypothesized as one likely mode of transmission, but would not be consistent with the predominance of BCD to recently-molted crabs. Results of this study are much more supportive of a more direct mode of transmission, by motile stages from sediments through the soft new cuticle. It is also possible, however that this parasitism may occur mostly in newly-molted crabs because stresses of molting and poor condition of post-molt crabs may render them particularly susceptible.

It is recognized that infection in general, and in crabs that had not recently molted in particular, may be underestimated in this study, based on external observation only. Recognition of external characteristics of BCD may be reduced in older-shelled crabs with relatively thick, opaque, and heavily-fouled carapaces. This possibility is consistent with the relatively higher incidence reported in older-shelled Alaskan tanner crabs, based on microscopic examination of hemolymph smears (Eaton et al. 1991), than in this study.

The factors that may regulate the spatial distribution of BCD are unclear. It is possible that spatial distribution is regulated by ocean circulation features. This is consistent with the concentration of BCD within the center of the broad crab distribution in most years, and the consistent virtual absence of BCD in the southern-most slope region of Div. 3NO. Areas of highest prevalence in southeast Alaskan tanner crabs have been associated with embayments, arms, and sills, which may serve to retain the infected population, and the causative parasite, within a very localized area (Meyers et al. 1990). Annual changes in spatial distribution of BCD in Newfoundland and southern Labrador *C. opilio*, particularly the pronounced shift to the north in 1999 and back south in 2000, may be related to changes in circulation patterns, but also may be due to shifts in crab density.

BCD has clearly been most prevalent in intermediate-sized crabs, but there has been considerable spatial and annual variability in this size range. This may reflect the overall distribution of intermediate-sized crabs at intermediate depths (Dawe and Colbourne, in press). Annual shifts in the size range most affected may reflect annual shifts in the distribution of size groups, or it may reflect annual changes in distribution of the dinoflagellate. Relationships of the distribution of the dinoflagellate with such abiotic factors as depth, sediment type, temperature or salinity are unknown. Eaton et al (1991) found, from June 1988 to Feb. 1989, that prevalence in southeast Alaskan tanner crabs was not related to sex or carapace width.

Possible effects on mortality and recruitment.

The inverse relationship between BCD prevalence and catch rate of intermediate-sized males implies a substantial effect on mortality. These low catch rates of intermediate-sized crabs could also reflect a size-related catchability effect. However spatial and annual shifts in this size relationship imply mortality due to BCD. The apparent inverse relationships between annual catch rates and BCD prevalence further suggest a direct effect of BCD mortality on density, especially for Div. 2J, and smallest size groups. Based on these relationships it is hypothesized that BCD has an adverse effect on survival of prerecruit males that may subsequently affect recruitment. BCD also appears to impose a substantial mortality on mature females. It is possible that prevalence is density dependent in that thresholds exist such that proliferation occurs above some upper threshold and dissipation occurs below some lower threshold. The disappearance of BCD from Div. 3K in 1998 and its increase in Div. 2J in 1999 represent examples of such possible density dependence.

Although prevalence may be high at very specific carapace widths, the overall low levels do not seem consistent with this hypothesized heavy mortality. However true prevalence may be under-represented by these observations. Prevalence may be higher than indicated within the trawl catches because trawls may not efficiently catch diseased crabs that are lethargic and selectively passed over by the trawl footgear. Furthermore, molting is frequent at small sizes (Sainte-Marie et al. 1995) and so the cumulative effect of this mortality across successive instars of a year class may be substantial.

It is also possible that the annual Oct-Dec trawl surveys may be late relative to the seasonal peak in prevalence, such that considerable mortality may have already occurred each year before the survey. Eaton et al (1991) reported that BCD in Southeast Alaskan tanner crab was highest in summer (June and August) and decreased in October, in 1988. Meyers et al (1987) also found in the same area a similar seasonal increase in prevalence and intensity of infection. This is similar to seasonality of this parasitic infection in blue crab (*Callinectes sapidus*). Love et al (1993) found highest prevalence in southeast Alaskan tanner crab in August of both 1989 (96%) and 1990; prevalence and intensity were both very low throughout Oct.-Apr, before increasing in May; prevalence was particularly low during Oct-Dec, decreasing from about 11-12% in Oct to 0% in Dec. 1989.

The crude method of diagnosis in our surveys likely also contributes to under-estimation of true prevalence. Macroscopic examination detects only chronic infections. Eaton et al. (1991) and all others (Love 1993, Meyers et al. 1987,1990) used hemolymph smears for diagnosis of prevalence in Southeast Alaskan tanner crabs.

The prevalence of BCD across the Newfoundland and Labrador Shelf prior to 1996 is unknown. It is known that the parasitism existed in 1995 because it was detected from an inshore 1995 Div. 3K trap survey, at low levels and in smallest crabs only (Dawe et al 2000). It was not detected at all during the first year of that survey in 1994. Other observations support the existence of BCD in northern areas in 1995 including the broad spatial distribution of BCD in Div 2J3KL in 1996, the relatively high prevalence in 1996, and regular decline during 1996-1998 in Div. 2J.

Although there are no data on incidence of BCD over the broad survey area prior to 1996, it is suspected that its prevalence was much lower in the early than late 1990's. If this is so, then it suggests that increased prevalence since 1995 may be related to density or the environment. Density of intermediate-sized crabs was particularly high during the mid-1990's, leading to strong recruitment in the late 1990's (Dawe et al. 2000). Also, the period 1995-2000 was an unusually warm period (Colbourne, 2001) that may have promoted establishment of BCD within the host population. However, the relationship of BCD prevalence in snow crabs with bottom temperature is presently unknown.

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Table 1. Numbers of crabs examined for BCD and total numbers caught, with percent infected, by year and sex.

Sample/Sex	Category	Year					Years Pooled
		1996	1997	1998	1999	2000	
Examined:							
Males	All	15525	13596	12821	7031	9830	**58803**
	BCD	120	284	135	41	102	**682**
Females	All	5999	4166	2293	2078	4331	**18867**
	BCD	91	185	35	25	84	**420**
Sexes Pooled:							
	All	**21524**	**17762**	**15114**	**9109**	**14161**	**77670**
	BCD	**211**	**469**	**170**	**66**	**186**	**102**
Caught:							
Males	All	23087	16630	15869	7392	13869	**76846**
	BCD	142	320	149	41	134	**788**
	% BCD	0.6	1.9	0.9	0.6	1.0	**1.0**
Females	All	9547	5642	2851	2165	7372	**27577**
	BCD	105	248	40	25	96	**514**
	% BCD	1.1	4.4	1.4	1.2	1.3	**1.9**
Sexes Pooled:							
	All	**32633**	**22271**	**18719**	**9557**	**21241**	**104422**
	BCD	**247**	**569**	**189**	**66**	**230**	**1302**
	% BCD	**0.8**	**2.6**	**1.0**	**0.7**	**1.1**	**1.2**

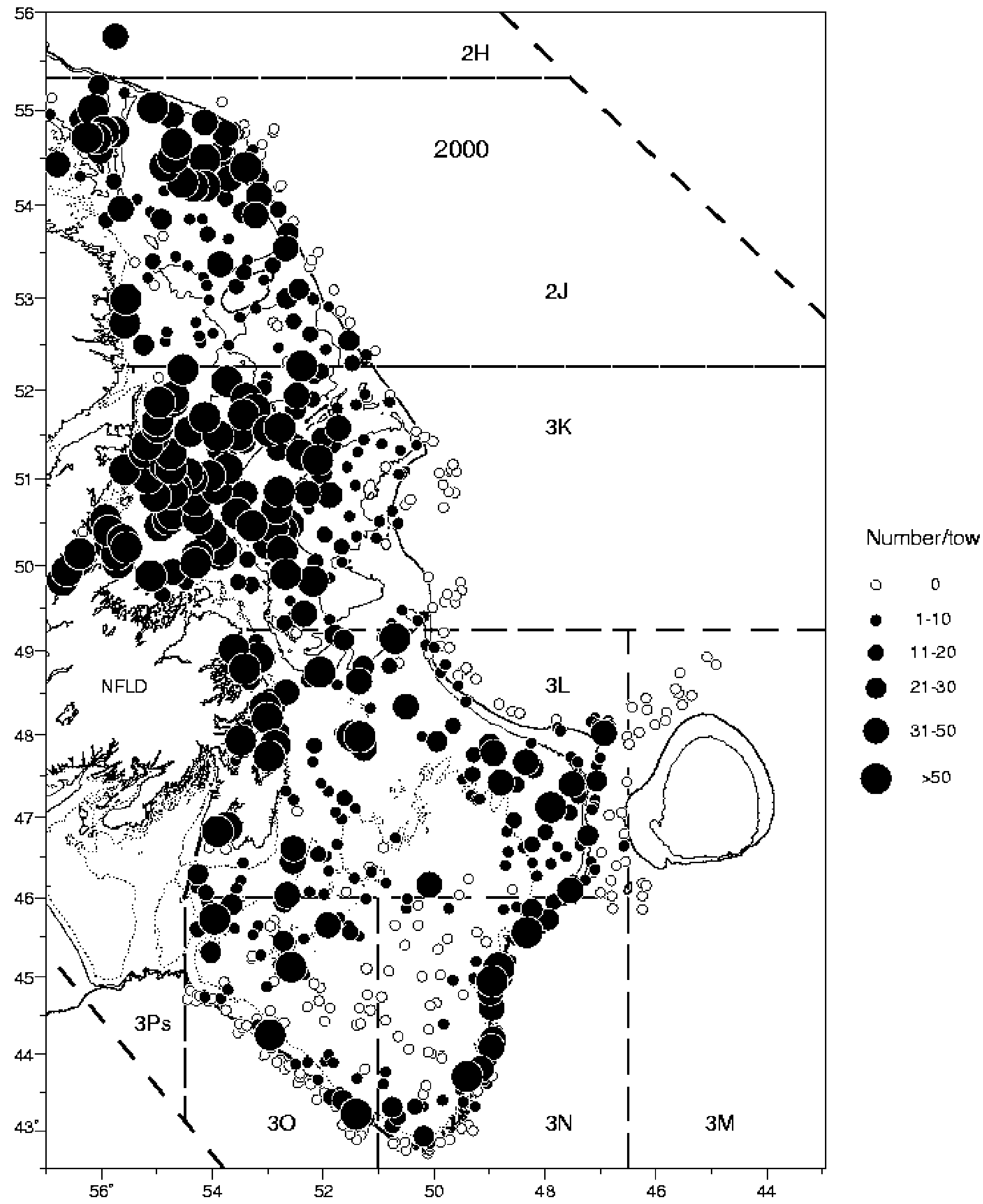

Figure 1. Map of the Newfoundland-Labrador continental shelf showing total snow crab survey catches in 2000 in relation to Northwest Atlantic Fisheries Organization (NAFO) Divisions.

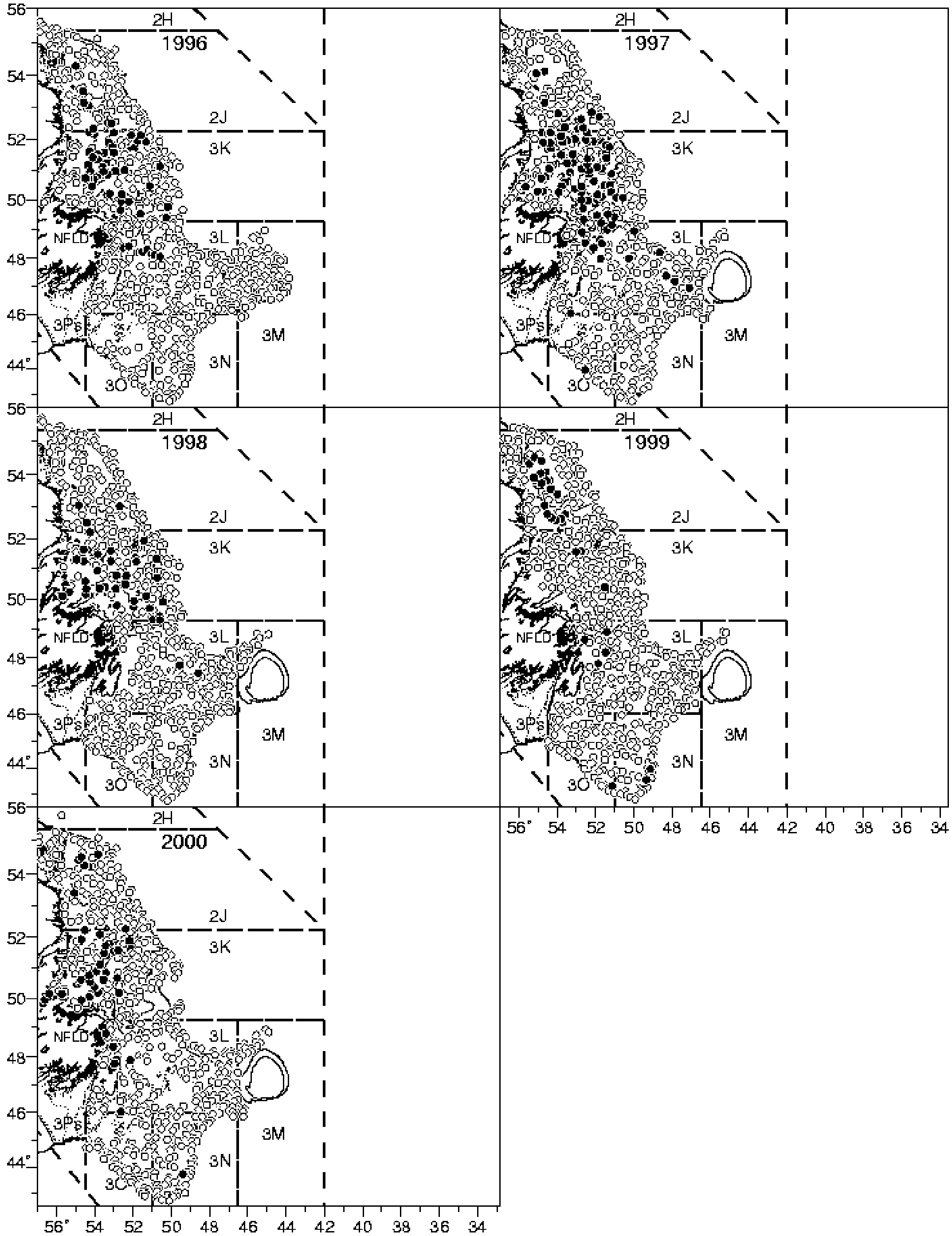

Figure 2. Distribution by year of survey sets where BCD was encountered (closed circles) versus all other sets (open circles).

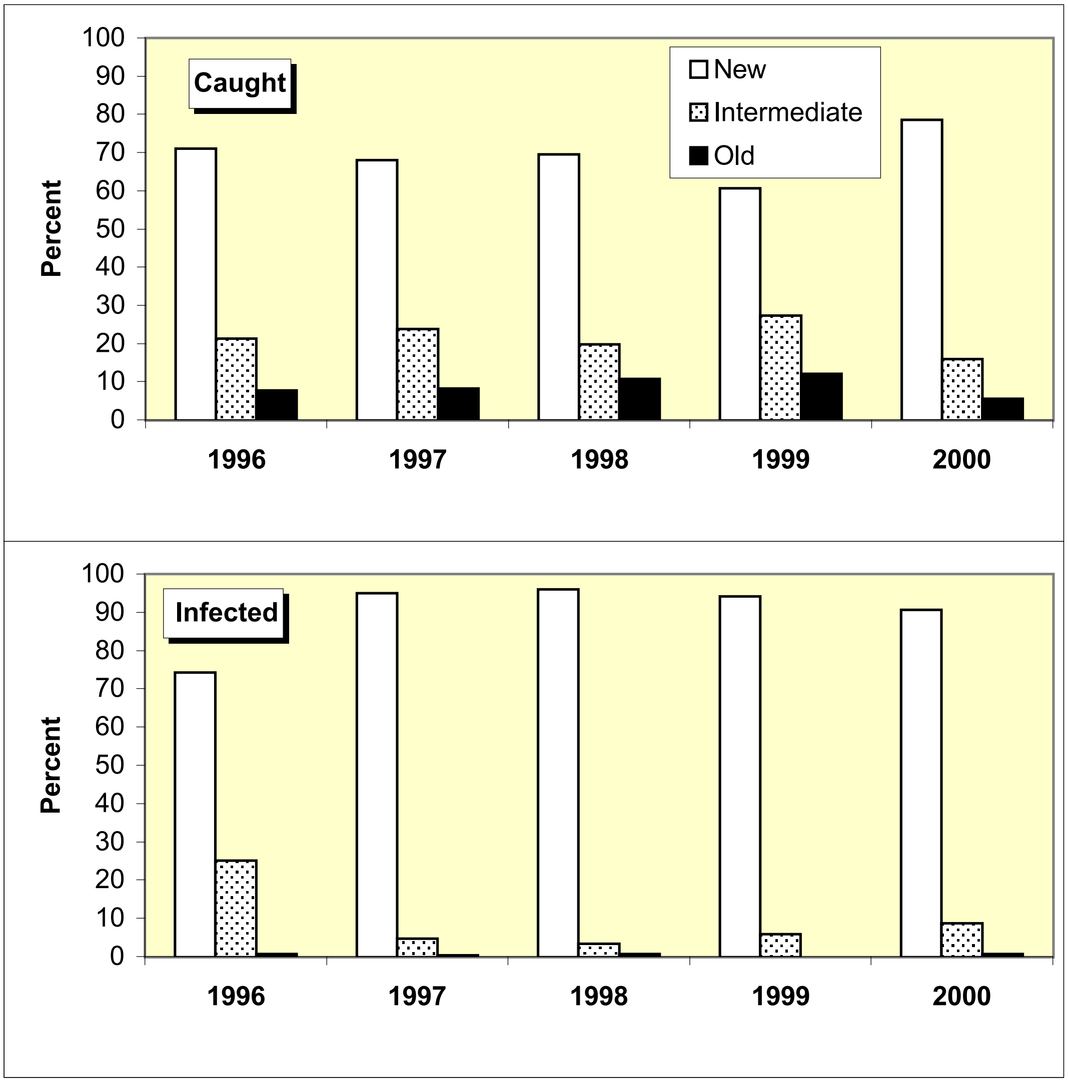

Fig.3. Percentage composition of males caught (above) and of those with BCD (below) by shell condition, for each year, across the entire fall survey area.

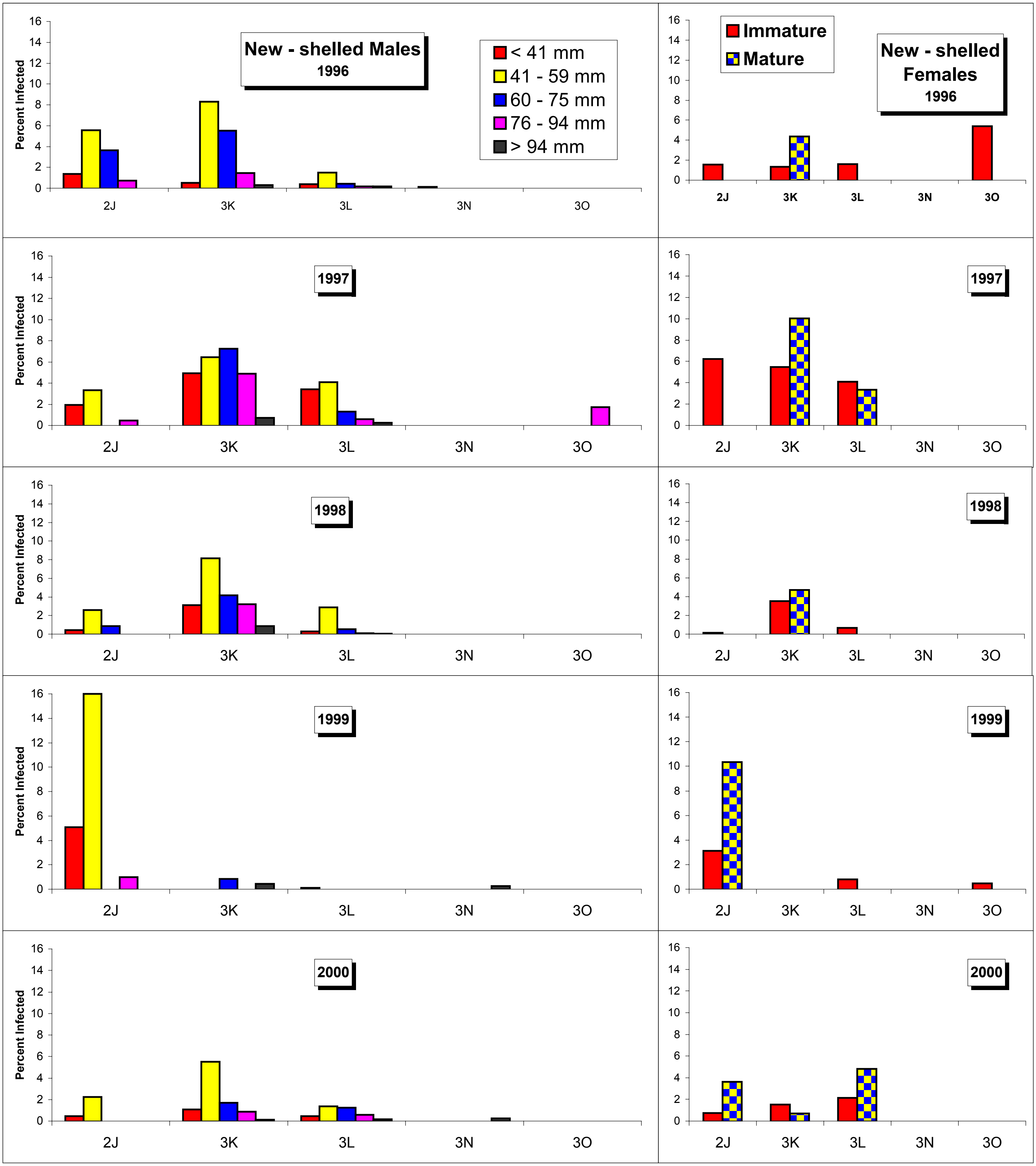

Figure 4. Percentage of crabs with BCD by year, division, sex, size group (for males), and maturity (for females).

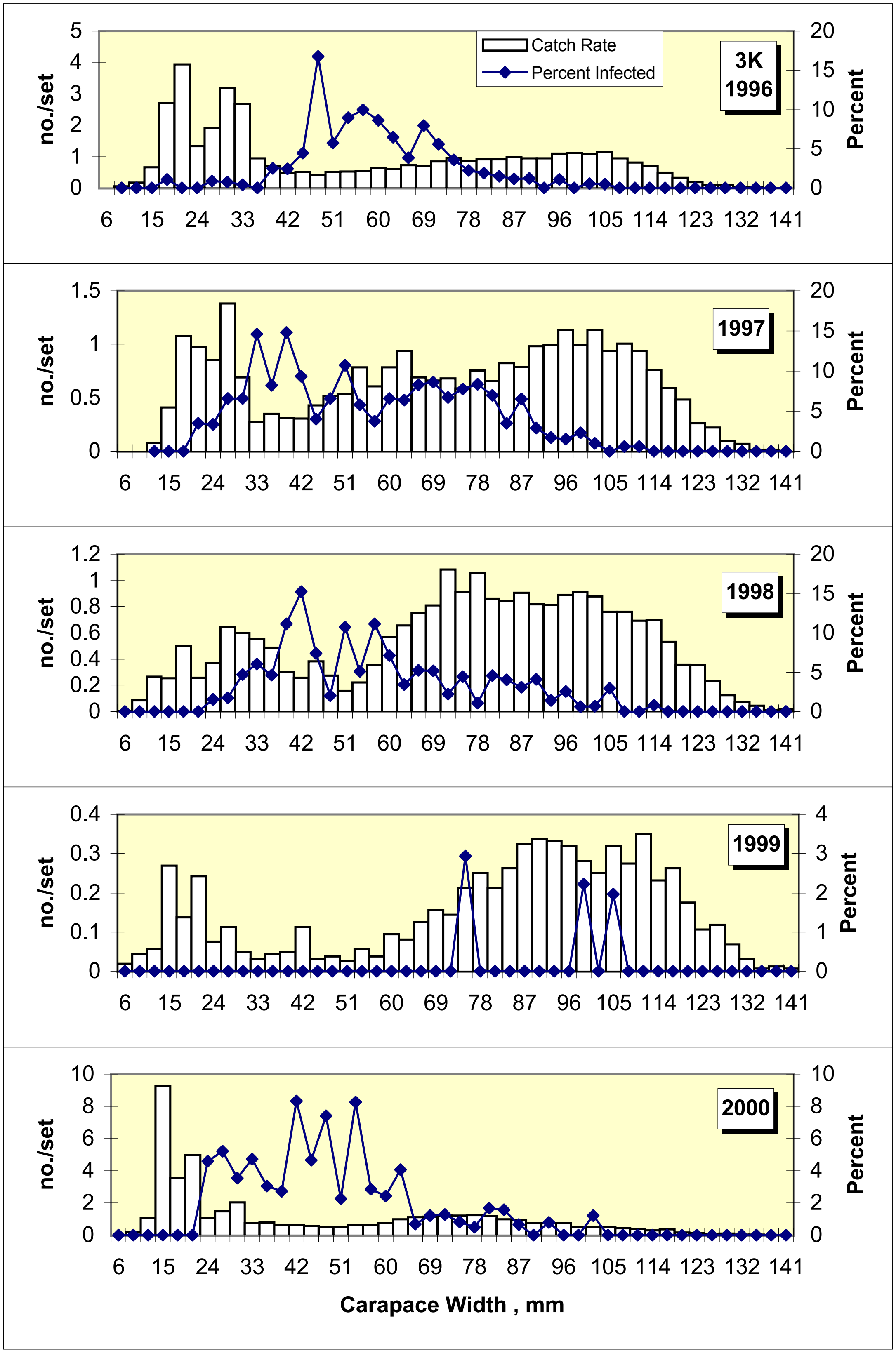

Figure 5. Male carapace width distribution and percent with BCD by year for NAFO Div. 3K.

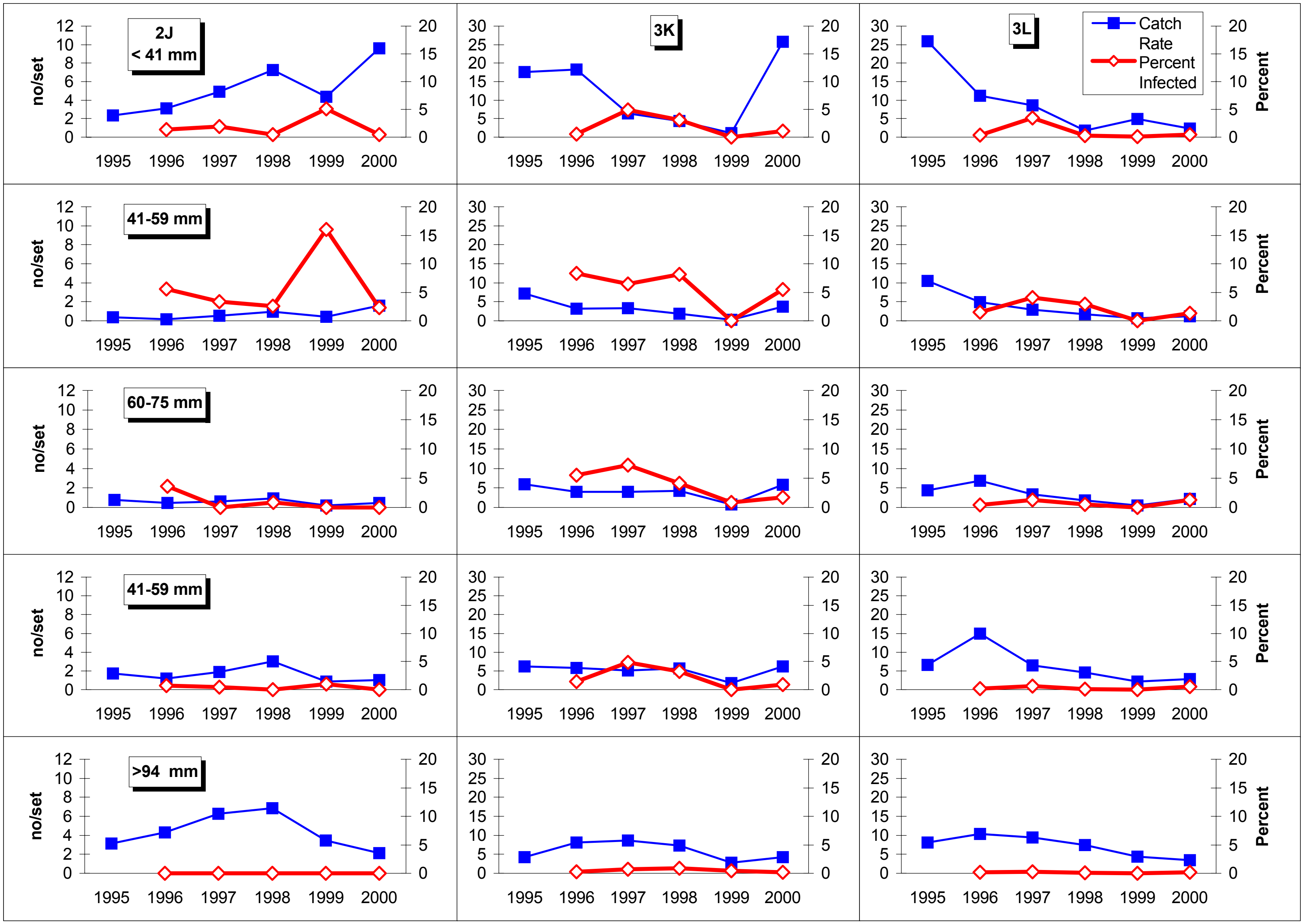

Figure 6. Annual trends in survey catch rate of males and percent with BCD by NAFO Division and size group.