AN ABSTRACT OF THE THESIS OF

Richard K. Childers for the degree of Master of Science

in Fisheries Science presented on May 16, 1994

Title: Prevalence, Geographic Distribution, and Biology of a Dungeness Crab, *Cancer magister*, Microsporidian Parasite

Abstract approved: Redacted for privacy

Robert E. Olson

The microsporidian parasite *Nadelspora canceri* infects Dungeness crabs, *Cancer magister*, along the United States Pacific Northwest coast. The prevalence and seasonal variation of *N. canceri* in Dungeness crabs from Alsea Bay, Oregon, are described based on examination of 2991 crabs collected at monthly intervals from October, 1991 to June, 1993. The average prevalence in monthly samples was 21.0 % and ranged from 8.2 % to 33.0 %. No significant differences in monthly or seasonal parasite prevalence were observed.

A total of 3061 Dungeness crabs was examined from an additional seven Pacific Northwest estuaries and Puget Sound to document the geographic distribution of *N. canceri* and the prevalence of the parasite in these locations. The estuaries sampled and the prevalences observed were: Humboldt Bay, California (14.6%), Coos Bay (10.6%), Yaquina Bay (2.0%), Tillamook Bay (41.2%), and Nehalem Bay, Oregon (14.2%), Willapa Bay (6.9%), and Grays Harbor Washington (0.44%). Dungeness crabs were examined from the Dungeness spit, Kala Point, and Mukilteo areas in Puget Sound and no infected crabs were found.

A total of 9317 male Dungeness crabs > 15.9 cm carapace width (CW) captured in the commercial ocean crab fishery was examined for *N. canceri* and 27 (0.3%) were infected with the parasite.

No infections were found in crabs smaller than 3.0 cm CW and the prevalence of infection generally increased with crab size reaching a peak of 22.2% in 14 cm CW crabs. The overall infection prevalence in male crabs (19.2%) was more than twice that of female crabs (8.0%), and of the 821 infected crabs found, 629 (76.6%) were males.

The mortality of laboratory-held Dungeness crabs naturally infected with *N. canceri* was compared to that of uninfected crabs in two separate experiments and in both cases a significantly higher mortality was observed for infected crabs. *Nadelspora canceri* infections were established in both juvenile and adult Dungeness crabs that were fed parasite spores in laboratory experiments indicating that transmission is direct and intermediate hosts or vectors are not required for transmitting the parasite between hosts.

Prevalence, Geographic Distribution, and Biology of a Dungeness Crab, *Cancer magister*, Microsporidian Parasite

by

Richard K. Childers

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed May 16, 1994

Commencement June 1995

APPROVED:

Redacted for privacy

Associate Professor of Fisheries and Wildlife in charge of major

Redacted for privacy

Head of Department of Fisheries and Wildlife

Redacted for privacy

Dean of Graduate School

Date thesis is presented May 16, 1994

Typed by Richard Childers for Richard Childers

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to Dr. Robert Olson, my major professor, for his advice, guidance, and encouragement throughout this study. I would like to thank Dr. Paul Reno, and Dr. David Sampson for serving on my committee and providing their expertise in the area of data analysis.

I owe special thanks to Karen my wife, whose patients and understanding, saw this project through to completion.

Finally I wish to acknowledge all the graduate students and research assistants who baited crab pots, pulled crab rings, and just smiled when pinched.

TABLE OF CONTENTS

TABLE OF CONTENTS cont.

LIST OF FIGURES

LIST OF TABLES

Prevalence, Geographic Distribution, and Biology of a Dungeness Crab, *Cancer magister*, Microsporidian Parasite

Chapter 1
Introduction

Microsporidians are small, unicellular, spore forming, eukaryotic organisms that are obligate intracellular parasites. An unusual characteristic of these parasites is the absence of mitochondria. This results in an absolute dependence on host cells to meet energy requirements. Microsporidians have been recognized as a distinct protozoan phylum (Microspora) since 1977 (Sprague 1977a, b, Canning 1990). Microsporidians parasitize all five vertebrate classes and several invertebrate phyla (Sprague 1977b, c), but are most common in fishes and arthropods (Weiser 1976, Canning 1977, 1990). The number of known microsporidian species is now about 800 (Canning 1990), with over 140 species described from crustacean hosts (Couch 1983). Microsporidians are among the most common and most pathogenic of crustacean disease agents, and are especially well documented among the decapods (Couch 1983, Sparks 1985, Sindermann 1990). The effects and site of infection vary according to the host and species of parasite. The parasites can be dispersed throughout the host, restricted to a specific tissue or organ, or confined within cysts (Canning 1977). While some microsporidian infections cause no obvious effects on the host (Weiser 1976) many result in extensive tissue damage and ultimately death of the host (Sprague and Vávra 1976, Canning 1982).

The Dungeness crab, *Cancer magister*, occurs along the Pacific coast of North America from central California to Alaska (Pauley et al. 1986, Botsford et al. 1989) and is harvested in commercial and recreational fisheries throughout this range. At present there are very few diseases described in Dungeness crabs (Meyers

et al. 1985) and only two protozoan infections have been reported: a systemic ciliate disease caused by *Paranophrys* sp. (Armstrong et al. 1981, Sparks et al. 1982), and an undescribed microsporidian that infects muscle tissue (Morado and Sparks 1988). *Paranophrys* sp. is considered a opportunistic invader requiring entry into the crab through injury of the exoskeleton. The parasite causes destruction and dysfunction of major organ systems and ultimately death of the host (Sparks et al. 1982). No detailed information exist on the microsporidian that was observed infecting Dungeness crabs by Morado and Sparks (1988) except that spores were oval-shaped and not contained within membranes and this suggests the parasite belongs in the family Nosematidae.

Nadelspora canceri, a microsporidian that differs morphologically from that reported by Morado and Sparks (1988), has recently been found to infect Dungeness crabs caught by recreational crab fishermen in Oregon's Alsea and Yaquina Bays (Olson et al. 1994). *Nadelspora canceri* infects the striated muscle and causes it to become whitish and opaque, rather than the normal translucent appearance. The parasite has unusual needle-shaped spores ($\approx$10 μm x <0.25 μm) that are easily recognized when muscle is viewed under a light microscope. Spores are found in vast numbers in the muscle tissue and in heavy infections the muscle appears to be virtually replaced by spores. Preliminary observations suggested that the prevalence of infected crabs in Alsea Bay was between 15 and 25%, while the prevalence in Yaquina Bay appeared to be less than 1% (R. E. Olson, unpublished observations). Detailed information on the prevalence of infection in the Dungeness crab population and the geographic distribution of the parasite is unknown. The effects of the parasite on individual crabs or the Dungeness crab population are also unknown and no information exists on host specificity or the source, method and time-course of infection.

The purpose of this research was to document the geographic distribution and prevalence of *N. canceri* in Dungeness crabs and determine the mode of infection and effects of the parasite on individuals. The specific research objectives were:

A. To determine the prevalence and seasonal variation of *N. canceri* infection in crabs from Alsea bay.

B. To determine the geographic distribution of *N. canceri* in the Pacific Northwest and its occurrence in the commercial ocean crab fishery.

C. To determine if the prevalence of *N. canceri* varies with the age and/or sex of Dungeness crabs.

D. To determine if the parasite can be transmitted by direct ingestion of *N. canceri* spores in both juvenile and adult Dungeness crabs, or whether transmission requires a vector or intermediate host.

E. To determine if naturally infected crabs have a higher mortality than uninfected crabs when held in the laboratory.

This thesis is arranged in four chapters that follow the manuscript format option. Chapter one is an introduction. Chapter two is a review of the literature pertaining to the research. Chapter three describes *N. canceri* prevalence and seasonal variation in Alsea Bay, its geographic distribution in the Pacific Northwest, and its occurrence in the commercial ocean fishery. Chapter four describes the results of experiments on the laboratory transmission of *N. canceri* and its ability to cause mortality in laboratory-held infected crabs.

Chapter 2
Literature Review

Microspora

The first microsporidian to be observed and described was *Nosema bombycis* by Nägeli in 1857 (Canning 1990). *Nosema bombycis* was determined by Pasteur and other scientists to be the etiological agent of "pebrine" or silkworm disease, an epidemic disease that caused a decline in the European silk industry in the mid-nineteenth century. Other microsporidians were soon discovered and Balbiani (1882, cited in Sprague 1977a) was the first to assign these organisms a position in the Protozoa, creating the order Microsporidia to accommodate them. Labbé (1899, cited in Sprague and Vávra 1976, Sprague 1977a) produced the first complete literature review of the order Microsporidia and listed 33 named and 20 unnamed species. About 800 species of microsporidians have now been described (Canning 1990) and Sprague (1977a, b) proposed a separate phylum (Microspora) for the microsporidians in 1977. Weiser (1976) speculated that as many as 10,000 microsporidian species have yet to be discovered.

All microsporidians produce resistant spores that serve to transmit infections between hosts (Canning 1990). Microsporidian spores are among the smallest of those produced by protozoans (Canning 1977) and range in size from 1-20 μm, but are typically less than 5 μm (Larsson 1986, Canning 1990, Perkins 1991). Microsporidian spores characteristically contain a membrane bound organelle, often coiled within the spore, called a polar filament that can be extruded when the spore is properly stimulated. Extruded polar filaments often exceed 100 μm in length but are less than 0.1μm wide (Weidner 1972, 1976, Weidner et al. 1984, Canning 1990, Perkins 1991). Microsporidian infections are initiated when an extruded polar filament pierces a host

cell and the sporoplasm (the infectious agent within the spore) is transferred through the polar filament and is inoculated into the host cell cytoplasm (Weidner 1972, Canning 1990, Perkins 1991). The sporoplasm consists of a nucleus and cytoplasm bounded by a unit membrane.

The development of microsporidian parasites within hosts has been intensely studied at the light microscope and ultrastructural levels and generally includes the entrance of the infective sporoplasm into the host cell, the asexual multiplication stage (merogony) during which the parasites rapidly increase in numbers by binary or multiple fission, and sexual sporogony leading to the formation of durable spores (Canning 1977, Larsson 1986, Canning 1990, Perkins 1991).

Microsporidian infections can be transmitted by different routes, although the most common method is through the digestive tract when spores are ingested by susceptible hosts (Larsson 1986). *Per os* infections have been established experimentally in fishes (Canning and Lom 1986), crustaceans, and insects (Weiser 1976). Transovarian transmission of microsporidian infections has also been reported in fish (Summerfelt 1972) and insects (Weiser 1976). The cannibalistic behavior exhibited by many decapod crustaceans is thought to favor microsporidian transmission that requires ingesting parasite spores (Iversen 1969, Skurdal et al. 1990, Langdon and Thorne 1992). However, few microsporidian species that infect decapods have been successfully transmitted in the laboratory by feeding susceptible hosts infected tissue. Infections were established in several laboratory experiments when muscle tissue from the blue crab, *Callinectes sapidus*, infected with the microsporidian *Ameson michaelis,* was fed to uninfected *C. sapidus* (Sprague et al. 1968, Weidner 1970, Overstreet and Whatley 1975). Langdon and Thorne (1992) were successful at transmitting the microsporidian *Vavraia parastacida per os* to two species of freshwater crayfish, *Cherax tenuimanus* and *Cherax albidus*. It has also been suggested, that in order to become infective, spores from some microsporidian species may need to be conditioned

by passage through non-host animals (Iversen and Kelly 1976, Graham and France 1986, Parsons and Khan 1986). Iversen and Kelly (1976) reported success in infecting the shrimp *Penaeus duorarum* with the microsporidian *Agmasoma* (formerly *Thelohania*) *penaei* only when spores were "conditioned" or "primed" by passing through the digestive tract of fish, prior to feeding to *P. duorarum*. Experiments by Herbert (1988) were unsuccessful at establishing *Thelohania* sp. infections in the redclaw crayfish, *Cherax quadricarinatus*, after feeding spores directly or via fish.

Other evidence suggests that some microsporidians have more complex life-cycles requiring intermediate hosts (Andreadis 1985). Two experimental attempts by Roth and Iversen (1971) failed to transmit the microsporidian *Thelohania duorara* to pink shrimp, *Penaeus duorarum*, by feeding infected shrimp to healthy shrimp. Several experimental attempts by Breed and Olson (1977) were unsuccessful in transmitting the microsporidian, *Pleistophora crangoni*, in three species of crangonid shrimp. This evidence suggests that transmission of certain microsporidians between decapod hosts may require an intermediate host, or that other unknown conditions may also be required before spores become infective.

Microsporidians have been reported to infect a variety of tissues and organs in decapod hosts including the hepatopancreas (Azevedo 1987), ovaries (Kelly 1979, Sparks and Morado 1985), and tegmental glands (Sparks and Morado 1985), although the most common site of infection is within striated muscle (Sparks 1985). Infections that occur in decapod muscle tissue can often be detected by the opaque, white appearance of the muscle either visible through the thin integument (articular membrane) at the appendage joints, or through the abdominal exoskeleton in smaller hosts such as shrimp and crayfish (Sparks 1985, Sindermann 1990). As the number of parasites increases and the infection spreads, lysis of infected and adjacent muscle tissue occurs and parasite spores can become so numerous that they virtually replace the host muscle tissue (Findley et al. 1981, Vivares and Azevedo 1988). As many as 10^9

parasite spores per gram of muscle tissue have been observed in infected decapod hosts (Findley et al. 1981), and as much as 80% of the host muscle destroyed as a result of muscle proteolysis that accompanies parasite development (Vivares and Azevedo 1988).

Hemolymph changes resulting from microsporidian infections that occur in muscle tissue were reported by Findley et al. (1981). Hemolymph biochemical components from blue crabs infected with the *Ameson michaelis* were compared to that of uninfected crabs and hemolymph K^+ ion concentrations increased by 60% and Cl^- and Na^+ ion levels decreased by 15% in infected crabs (Findley et al. 1981). Infected crabs also exhibited significantly higher hemolymph concentration of NH_3, glycogen and seven amino acids tested. Findley et al. (1981) suggested that the hemolymph changes observed in infected crabs could interfere with host oxygen uptake and osmoregulation.

The amount of information describing decapod microsporidiosis is extensive and examples have been abundantly reported in the literature. Studies that provide some information about microsporidiosis in natural decapod populations and host-parasite relations are given below.

Four species of crangonid shrimp *Crangon franciscorum, C. nigricauda, C. nigromaculata* and *C. stylirostris* that occur along the Pacific coast of Oregon, are parasitized by the microsporidian *Pleistophora crangoni.* The biology and seasonal prevalence of P. *crangoni* in *C. franciscorum , C. nigricauda,* and *C. stylirostris*, in the vicinity of Yaquina Bay, Oregon, was described by Breed and Olson (1977) who examined a total of 7107 shrimp over a one year period (June 1975-June 1976). Seasonal differences in infection prevalence were observed in *C. franciscorum* and *C. stylirostris* and were highest in the winter months with 30.3% and 41.0 % respectively. The infection prevalence in *C. nigricauda* was lower than *C. franciscorum* and *C. stylirostris* and remained below 8.0%. The intensity of *P. crangoni* infections increased

with the size of the shrimp and field observations indicate that infections occur during the summer months and only young shrimp are susceptible to infections. *Pleistophora crangoni* infections occurred only in the muscle tissue in both sexes and parasitic castration was observed in female shrimp.

Microsporidian parasites have been reported to infect several commercially important shrimp species from the Gulf of Mexico and South Atlantic coast of the United States. The disease condition commonly known as "cotton shrimp" or "milk shrimp" results from the white discoloration of the muscle tissue caused by microsporidian infections. The microsporidian *Agmasoma penaei* infects the white shrimp, *Penaeus setiferus*, and pink shrimp, *Penaeus duorarum* , (Kelly 1979, Overstreet 1973). *Agmasoma penaei* infections occur in, and destroy, striated muscle and ovaries (Kelly 1979) and could affect population recruitment under epizootic conditions as a result of the parasitic castration of female shrimp (Sparks 1985). *Ameson* (formerly *Nosema*) *nelsoni* infects the brown shrimp, *Panaeus aztecus*, (Overstreet 1973) and the microsporidian *Pleistophora* sp. has been reported to infect *P. setiferus P. duorarum* and *P. aztecus* (Baxter et al. 1970). Both *Ameson nelsoni* and *Pleistophora* sp. infect the striated muscle, and as much as 50% of the host muscle tissue can be replaced with parasite spores (Kelly 1979).

Pink shrimp, *Pandalus jordani*, a commercially important species in California, Oregon, and Washington, hosts four species of microsporidian parasites: *Thelohania butleri*, *Pleistophora crangoni*, and two undescribed species placed in the collective genus *Microsporidium* . All four species parasitize the striated muscle giving shrimp a white, opaque appearance. The prevalence of microsporidian infection in *P. jordani* was studied over a six year period (1975-1980) and was found to be very low; of 207,942 shrimp examined only 0.19% were infected with these parasites (Olson and Lannan 1984). Heavy commercial exploitation of the host shrimp, resulting in reduced

population density, is suggested as a possible explanation for the low prevalence of infection in the *P. jordani* population (Olson and Lannan 1984).

The microsporidian *Ameson* sp. infects three species of shrimp; *Penaeus semisulcatus, P. merguiensis,* and *P. esculentus* at low prevalence levels in Northern Australia. Two hundred thousand of these shrimp were examined and less than 0.1% were infected with *Ameson* Sp. (Owens and Glazebrook 1988). *Ameson* sp infections occur primarily in the striated muscle tissue of the abdomen (Owens and Glazebrook 1988). The microsporidian *Thelohania* sp. infects *P. semisulcatus, P. latisulcatus,* and *P. longistylus* along Australia's Great Barrier Reef also at a prevalence level below 0.1% (Owens and Glazebrook 1988). *Thelohania* sp. infections in these shrimp are not restricted to striated muscle tissue, but also infect the intestinal wall, gonads, heart, hepatopancreas, and nerve cord (Owens and Glazebrook 1988).

Northern shrimp, *Pandalus borealis*, examined from trawl samples taken off the Northwest Atlantic coast of Labrador were found to be infected at low levels with a species of microsporidian from the collective genus *Microsporidium* (Parsons and Khan 1986). A total of 180,886 *P. borealis* was examined and 0.04% were infected with the parasite. This parasite infects only the striated muscle in *P. borealis*, although poorly developed vasa deferentia and ovaries were found in infected males and females respectively (Parsons and Khan 1986).

Several species of freshwater crayfish are parasitized by microsporidians and a number of studies have reported infection levels in natural populations (Herbert 1988, Skurdal et al. 1990, Langdon 1991). The microsporidian *Thelohania contejeani* infects a wide range of crayfish species (Skurdal et al. 1988), and infects the noble crayfish, *Astacus astacus*, throughout its area of distribution in Europe (Skurdal et al. 1990). Samples of *A. astacus* collected from seven locations (lakes and rivers) in S. E. Norway revealed *T. contejeani* infection prevalences ranged from 0.4% to 1.7% (Skurdal et al. 1988). A six year study (1981-1986) determined that 0.29% of *A. astacus* examined

from Lake Steinsfjorden, S. E. Norway, were infected with T. *contejeani* (Skurdal et al. 1990). This microsporidian parasite causes extensive muscle destruction and possibly death of the crayfish host (Cossins 1973).

Cherax quadricarinatus, a tropical crayfish of Northern Australia, is parasitized by the microsporidian *Thelohania* sp. (Herbert 1988). An infection prevalence of 7.8% was reported from a sample of 129 *C. quadricarinatus* collected from the Mitchell River, North Queensland (Herbert 1988).

An undescribed microsporidian belonging to the family Pleistophoridae was found to parasitize the crayfish *Cherax tenuimanus* in western Australia (Langdon 1991). An infection prevalence of less than 2% was found in crayfish being reared in a aquaculture facility (Langdon 1991). Parasite spores were reported to be so numerous in the abdominal musculature of infected crayfish that muscle liquefaction occurred, leaving only small localized areas of uninfected muscle (Langdon 1991).

Several microsporidian parasites have been reported to infect brachyuran crabs (Sprague 1965, 1970, 1977b, Vivares and Sprague 1979, Sparks and Morado 1985, Azevedo 1987, Overstreet 1988, Morado and Sparks 1988), although little information has been reported on infection prevalence in natural crab populations. The microsporidian *Ameson michaelis* (formerly *Nosema michaelis*) infects the muscle tissue of the blue crab, *Callinectes sapidus*, (Sprague, 1965). *Ameson michaelis* infections are widely distributed along the Gulf and Atlantic coast of the United States, but prevalence appears to be low (Overstreet and Whatley 1975, Sparks 1985). Several thousand *C. sapidus* were examined from Mississippi Sound and only one crab infected with *A. michalis* was found. (Overstreet and Whatley 1975). Vegetative growth occurs in hemocytes, and in the connective tissue surrounding the midgut, followed by sporogony in the skeletal muscle throughout the body (Weidner 1970). The parasite causes lysis of the infected muscle tissue (Sprague 1970) and in the final stages of

infection crabs become weakened and laboratory experiments by Weidner (1970) indicate *A. michaelis* infections are lethal to the blue crab host.

Two microsporidian species, *Ameson pulvis* and *Thelohania maenadis*, parasitize the skeletal muscle of the European shore crabs *Carcinus mediterraneus* and *Carcinus maenas* respectively along the coast of France (Vivares and Sprague 1979, Vivares and Cuq 1981). *Thelohania maenadis* infection causes extensive muscle destruction, decreases in hemolymph glucose and total protein, and increases in muscle lactate levels (Vivares and Cuq 1981).

The microsporidian *Thelohania petrolisthis* is known to infect the crab *Petrolisthes armatus* along the gulf coast of Louisiana (Sprague 1970). Infections cause extensive muscle destruction and ultimately death of the host (Sprague 1970). The prevalence of infection and geographic range of this parasite are not known.

Azevedo (1987) described a new genus and species of microsporidian, *Abelspora portucalensis*, which infects the crab *Carcinus maenus* along the Atlantic coast of Portugal. The parasite infects the hepatopancreas, rather than muscle tissue, where it causes large white cysts or xenomas to form. Each xenoma consist of an aggregate of hypertrophied host cells where the parasites develop and proliferate. Xenoma formation is typical of microsporidian infections that occur in vertebrates, but is unusual in invertebrates.

Two undescribed microsporidian species belonging to the genus *Thelohania* have been reported to infect the blue king crab, *Paralithodes platypus*, and the red king crab, *P. camtschatica* respectively (Sparks and Morado 1985). One hundred and thirty four *P. platypus* collected from Bristol Bay, Alaska, were examined and six (4.4%) were infected with *Thelohania* sp. Seventy three *P. camtschatica* were examined from the Pribilof Islands and St. Matthew Island, Alaska, and five (6.8%) were infected with a thelohanid parasite that was different from that found in *P. platypus* . Infected crabs are grossly recognizable by the abnormal "cottage cheese"

appearance of much of the viscera, which led to the disease being referred to as cottage cheese disease. Virtually all organs are invaded by the parasites including: hepatopancreas, gonads, tegmental glands, and the wall of the digestive tract.

Dungeness crab, *Cancer magister*

The Dungeness crab occurs in estuaries and offshore along the Pacific coast of North America from central California to Alaska and range from the intertidal zone seaward to a depth of at least 100 m (Pauley et al. 1986, Botsford et al. 1989, Hobbs et al. 1992). The maximum size for Dungeness crabs is 21 cm carapace width (CW) and the maximum estimated age for the species is eight years (Pauley et al. 1986). Mating of Dungeness crabs occurs once a year from March through July, when a hard-shelled male fertilizes a recently molted soft-shelled female. Females store sperm internally and fertilization occurs as eggs are extruded, usually in October or November (Botsford et al. 1989). Fecundity ranges from 700,000 eggs in younger females to 2.5 million eggs in older females (Pauley et al. 1986). Eggs are brooded in a mass under the abdomen for several months until hatching. Egg development is temperature dependent and hatching occurs primarily in December through January (Pauley et al. 1986).

Dungeness crabs larvae are planktonic and develop through five zoeal and one megalopal stage before settling. The larvae are most likely kept nearshore during their planktonic stage by the prevailing onshore ocean surface currents that occur in the winter and spring months (McConnaughey et al. 1992).

Dungeness crabs molt approximately six times each year for the first two years beyond the first post larval instar. By the third year of life (about 130mm CW) molting becomes less frequent and occurs approximately once each year in larger

crabs (Pacific Fisheries Management Council 1979, Hankin et al. 1985, Warner 1987).

In an effort to determine the extent of Dungeness crab movement along the Pacific coast more than 28,000 Dungeness crabs have been tagged and released and more than 7,000 have been recovered giving useful information on crab movement and migration (Pacific Fisheries Management Council 1979). In general, tag returns show the following: there are no definite patterns to coastal movements and most crabs recovered with tags have been within a few miles of where they were released. Some onshore-offshore movement (Diamond and Hankin 1985) and movements in and out of bays and from bay to bay have been observed, but the rate at which this interchange takes place is not known.

Dungeness crabs stomach analysis by Gotshal (1977) and Stevens et al. (1982) revealed that crustaceans, clams, and fish are the most common food items consumed. Echinoderms, gastropods, and polychaetes are also consumed by Dungeness crabs, but are less common food items. Cannibalism is known to occur in natural crab populations and the frequency of cannibalism in Dungeness crabs was reported by Stevens et al.(1982) who studied 410 crabs and found that 18.2% had consumed other Dungeness crabs.

Commercial and recreational fisheries for Dungeness crabs occur throughout the range of the species. The commercial Dungeness crab fishery is one of the most valuable fisheries on the United States west coast, exceeded only by salmon and the groundfish complex (flatfish, rockfish, cod, and sablefish). Annual commercial landings in excess of 16 million kg. are not uncommon and average annual landings for the period 1986-1991 were 15.6 million kg. with an annual value of about $50 million (O'Bannon 1993). Only male Dungeness crabs ≥15.9 cm may be landed by commercial fishermen and annual exploitation rates for legal size males for the entire west coast is commonly over 75% and can be as high as 95% (Gotshal 1978,

Methot 1990). The annual recreational catch is estimated to be less than two percent of the commercial catch (Barry 1985, Demory 1985), but despite these relatively low landings, the recreational fishery generates a substantial amount of income for the tourist and recreational industries in coastal communities (Carter and Radke 1991). The recreational fishery is mainly concentrated in the coastal estuaries, whereas commercial efforts are primarily in the ocean with only a minor bay component.

A striking and well documented characteristic of the west coast commercial Dungeness crab fishery is the dramatic fluctuation in commercial landings. Landings in some years can be as low as 20% of the catch of high years. Furthermore, the fluctuations seem to be of a cyclical nature. Landings in California, Oregon, and Washington follow a coastwide pattern where 3-4 years of low catch are followed by 3-4 years of high catch (Armstrong 1983, Botsford 1986, Botsford et al. 1989, Methot 1990, Hobbs et al. 1992). Botsford et al. (1983) and Hankin (1985) have concluded that the fluctuations in landings are due to changes in crab abundance (year class strength) and not changes in fishing effort. Attempts to explain the fluctuations in abundance of Dungeness crab can be categorized as population density independent (environmental factors), or density dependent factors. Density independent explanations such as fluctuations in ocean water temperature and their effects on egg survival (Wild 1980), and larval transport resulting from wind-driven ocean currents (Hobbs et al. 1992) have been proposed. Hobbs et al. (1992) and McConnaughey et al. (1992) suggest that Dungeness crab larval survival, and therefore year class strength, are linked to interannual variations in coastal oceanographic conditions. Cannibalism (Botsford and Wickham 1978), and disease (Meyers et al. 1985) are examples of possible density dependent factors influencing abundance. Considerable research continues to focus on the causes of

the fluctuations, but a definitive scientific explanation, including the possible role of disease, is still unavailable.

Chapter 3
Prevalence of the microsporidian *Nadelspora canceri* in Dungeness crab, *Cancer magister*, from eleven Pacific Northwest locations and its occurrence in the commercial ocean crab fishery

INTRODUCTION

Microsporidians parasitize all five vertebrate classes and several invertebrate phyla, (Sprague 1977b, c) but are most common in fishes and arthropods (Weiser 1976, Canning 1977, 1990). The number of known microsporidian species is now about 800 (Canning, 1990), with over 140 species described from crustacean hosts (Couch 1983). Microsporidians are among the most common and most pathogenic of crustacean disease agents (Couch 1983, Sparks 1985) and are especially well documented among the decapods (Sindermann 1990). Most microsporidian infections of decapods affect muscle tissue causing a white discoloration on gross examination, and muscle destruction. Sparks (1985), and Sindermann (1990) have reviewed decapod microsporidiosis.

Microsporidian parasites have been reported in a variety of shrimp species, although infection levels in natural populations are typically less than 1% (Olson and Lannan 1984, Parsons and Khan 1986, Owens and Glazebrook 1988). Breed and Olson (1977) reported the overall infection prevalence the microsporidian *Pleistophora crangoni* in several species of crangonid sand shrimps to be 8.8%. Several species of freshwater crayfish are parasitized by microsporidians and reported infection levels in wild populations range from 0.29% to 7.8% (Herbert 1988, Skurdal et al. 1990, Langdon 1991). Microsporidian parasites have been reported to infect a variety of brachyuran crabs (Sprague 1965, 1970, 1977a, b, Vivares and Sprague 1979, Sparks and Morado 1985, Azevedo 1987, Morado and

Sparks 1988, Overstreet 1988) although little information has been reported on infection prevalences in natural crab populations.

There are at present very few diseases described in Dungeness crab, *Cancer magister*, and only two protozoan infections have been reported (Meyers et al. 1985). A systemic ciliate disease caused by *Paranophrys* sp. has been reported in laboratory held crabs (Armstrong et al. 1981) and from the natural crab population in Puget Sound (Sparks et al. 1982). An undescribed microsporidian (family Nosematidae) with a typical oval-shaped spore that infects Dungeness crabs was observed by Morado and Sparks (1988), although no detailed information on this parasite has been published.

Nadelspora canceri, a microsporidian that differs morphologically from that observed by Morado and Sparks (1988), has recently been found to infect Dungeness crabs caught in Oregon's Alsea and Yaquina Bays (Olson et al. 1994). The parasite infects the striated muscle and causes it to appear whitish and opaque, rather than its normal translucent appearance. *Nadelspora canceri* has unusual needle-shaped spores ($\approx$10 μm x < 0.25 μm) that are easily recognized when muscle is viewed under a light microscope (Olson et al. 1994). Spores are found in vast numbers in the skeletal muscle and in heavy infections the muscle appears to be virtually replaced by spores.

Preliminary observations suggested that the prevalence of infected crabs in Alsea Bay was between 15 and 25%, while the prevalence in Yaquina Bay appeared to be less than 1% (Olson R. E. personal communication). This study was designed to determine the prevalence and seasonal variation of *N. canceri* infection in Dungeness crabs from Alsea Bay, to document the infection levels in other Pacific Northwest locations, and to determine the prevalence of *N. canceri* in the commercial ocean crab fishery.

MATERIALS AND METHODS

Collection of animals

Crabs were collected from all locations except Yaquina Bay, Oregon, (Fig. 1) with baited crab rings and pots. Some rings were lined with 0.6 cm nylon mesh to allow capture of juvenile crabs (≤1.0 cm) as well as larger crabs. In Yaquina Bay samples were collected with crab rings and by towing a 4.8 m semiballoon otter trawl lined with a 0.6 cm nylon mesh cod end.

The carapace width (CW) of each crab was measured to the nearest millimeter just anterior to the 10^{th} anterolateral spine and the sex recorded for crabs ≥ 3.0 cm CW. Crabs ≤ 3.0 cm CW could not be accurately sexed without dissection. Red rock crabs, *Cancer productus*, captured incidentally while sampling, were also examined for infection although the sex, size, and numbers of these crabs were not recorded.

The presence or absence of microsporidian infections was determined macroscopically by examining crab muscle that was visible through the periarticular membrane at the base of each thoracic appendage. Muscle of infected crabs appeared white and opaque in contrast to the translucent appearance of uninfected crab muscle. A sample of striated muscle was removed from all grossly infected crabs and examined under a phase contrast microscope at 400X to confirm the presence of *N. canceri* spores which are morphologically unique (Olson et al. 1994). Muscle tissue from 500 crabs collected from Alsea Bay, Oregon (Fig. 1) that were categorized as uninfected was also examined microscopically to test the accuracy of preliminary diagnosis based on the absence of gross signs of disease.

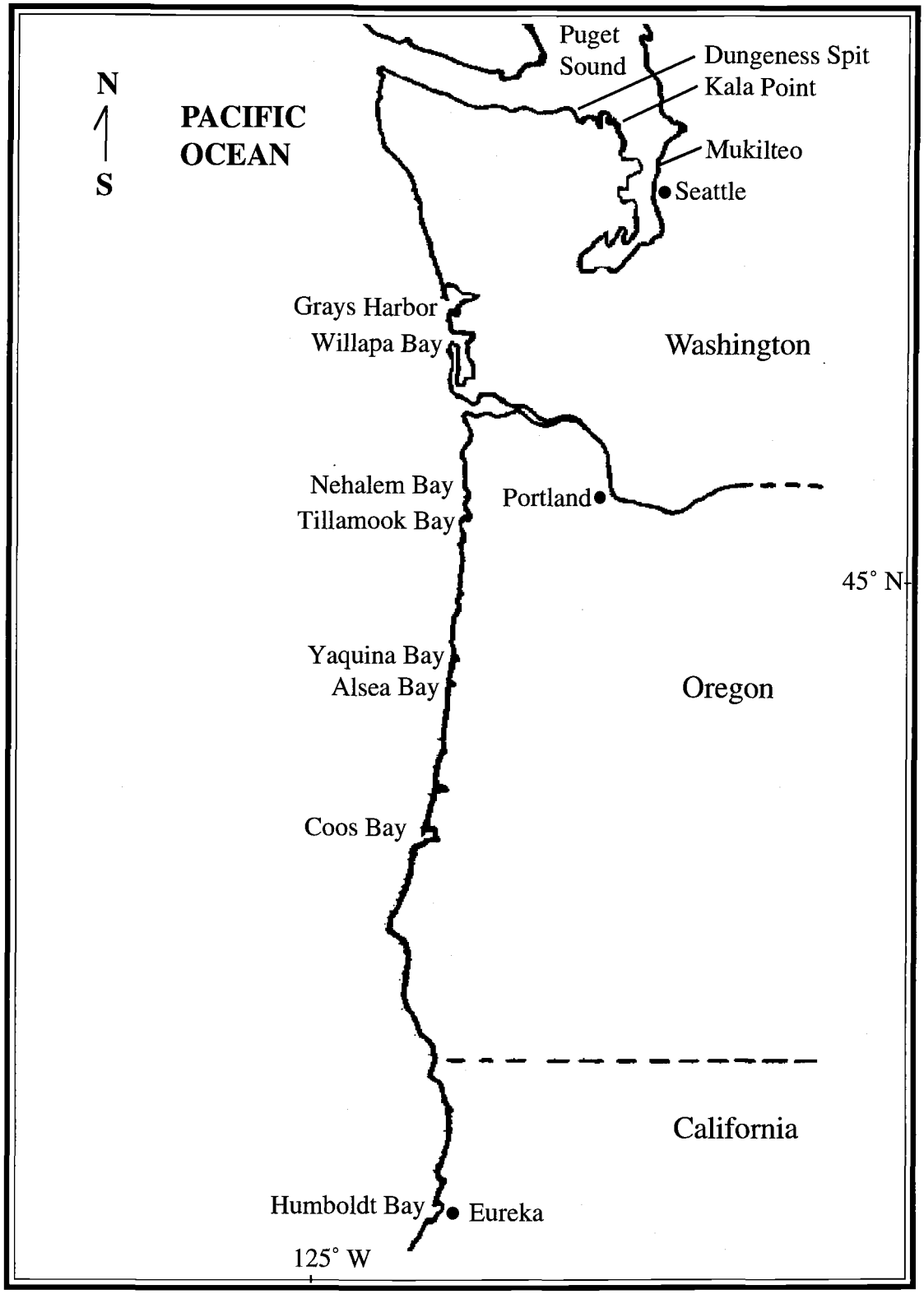

Figure 1. Study area along the North American Pacific coast showing locations of Dungeness crab collection.

Alsea Bay Sampling

Alsea Bay (Fig. 1, 2) was the site of repeated sampling without replacement to examine seasonal variations in infection levels, and differences in parasite prevalence based on crab sex and size. The area sampled was restricted to approximately one kilometer northeast and southwest of the US highway 101 bridge (Fig. 2). Crabs were collected each calendar month beginning in October 1991 and continued through June 1993. The number of days between samples ranged from 19 to 30, although most samples were separated by at least 25 days. Inclement weather prevented sampling in November of 1991. A minimum of 71 crabs was collected in each sample. This sample size provides a 95% confidence level for detecting at least one infected crab in a population of 1,000,000 or more, if the disease prevalence is 5% or greater (Ossiander and Wedemeyer 1973). Samples were collected near high tide during the daylight hours and the crabs were transported alive in insulated coolers to the Hatfield Marine Science Center Fish Disease Laboratory of Oregon State University where they were examined for the microsporidian parasite. In June, 1992, an additional sample of small juvenile crabs was collected by hand at low tide from refuges under algae and eel grass blades.

The data on the occurrence of the microsporidian parasite in Alsea Bay crabs were analyzed using a Generalized Linear Interactive Modeling computer program (Glim 3.77, Royal Statistical Society) to construct a logistic regression model for binomial proportions. Parasite prevalence (proportion of crabs infected with *N. canceri)* was used as a response variable in the model. The independent variables in the model were crab size, crab sex and sample month. The size variable was created by grouping crabs within each sample into one centimeter CW size classes. A logit transformation of the response variable was used to linearize the relationship between the parasite prevalence and the independent variables

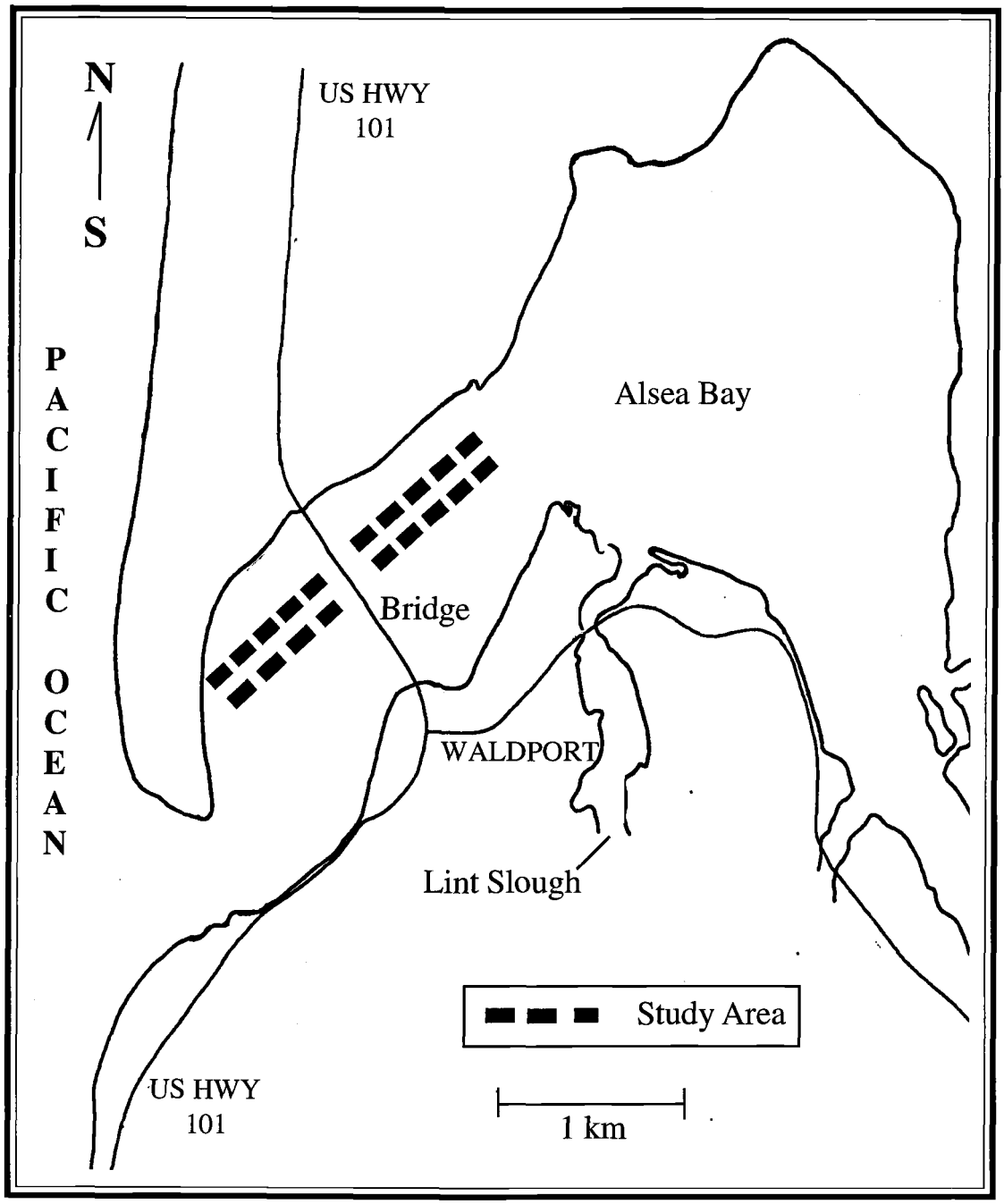

Figure 2. Alsea Bay, Oregon, showing study area

(Healy 1988, Atkin et al 1989). The error distribution in the model was assumed to be binomial. A drop in deviance test (extra sum of squares test) was used to determine if the relationship between the response variable and the independent variables was statistically significant.

Sampling other Pacific Northwest locations

Dungeness crabs were collected near high tide during daylight hours from seven Pacific Northwest estuaries and three locations in Puget Sound between November, 1991, and August, 1993 (Fig. 1). In Oregon, samples were collected from the Yaquina, Tillamook, Nehalem, and Coos estuaries. In Washington samples were collected from the Willapa Bay and Grays Harbor estuaries and from Kala Point, Dungeness Spit, and Mukilteo in Puget Sound. One sample was collected from the South Humboldt Bay estuary in California. The size (CW) and sex of each crab were recorded and the presence or absence of infection determined macroscopically as described above. All crabs were released at the collection site after removing a muscle sample from those that appeared infected by external visual criteria.

Offshore commercial catch sampling

Dungeness crabs landed in the commercial ocean fishery, from 18 separate vessel trips, were sampled at three crab processing plants in Newport, Oregon, during the months of December-February for two consecutive commercial seasons (1991-1992). In Crecent City, California, commercial landings from three vessel trips were examined at a single crab processing plant in April 1992. Only male crabs ≥15.9 cm CW may be landed in the commercial fishery, therefore, the ocean crabs examined consisted only of these large males. Appendage muscle from each

grossly infected crab was examined microscopically to confirm the presence of spores. A Chi square analysis was used to test whether the observed prevalence of *N. canceri* in the offshore crabs was statistically different from the prevalence observed in male crabs ≥ 15.9 cm CW captured in estuaries.

RESULTS

Overall prevalence of *Nadelspora canceri*

A total of 6052 Dungeness crabs was examined from estuaries and Puget Sound and microsporidian infections were detected in 821 (13.5%) crabs. In all cases microscopic examination of muscle tissue from the grossly infected crabs confirmed the presence of *N. canceri* spores. Microscopic examination of 500 crabs that were collected from Alsea bay and categorized as uninfected macroscopically revealed no microsporidian spores.

The sex was determined for 5611 Dungeness crabs over 3.0 cm CW and 57.7% of these crabs were males (Fig. 3). The overall infection prevalence in male crabs (19.2%) was over twice that of female crabs (8.0%), and of the 821 infected crabs found, 629 (76.6%) were males (Fig. 3).

The infection prevalences according to crab size are shown in Figure 4. No infections were found in crabs smaller than 3.0 cm CW and the prevalence generally increased in crabs larger than 3.0 cm CW reaching a peak of 22.2% in 14 cm CW crabs. The infection prevalence then decreased in crabs larger than 14 cm CW and none of the seven crabs larger than 17 cm CW was infected.

Red rock crabs were captured incidentally in the Yaquina, Humboldt, and Coos estuaries and at Kala Point in Puget Sound. The number of red rock crabs examined was estimated to be over 1000, although the exact number was not

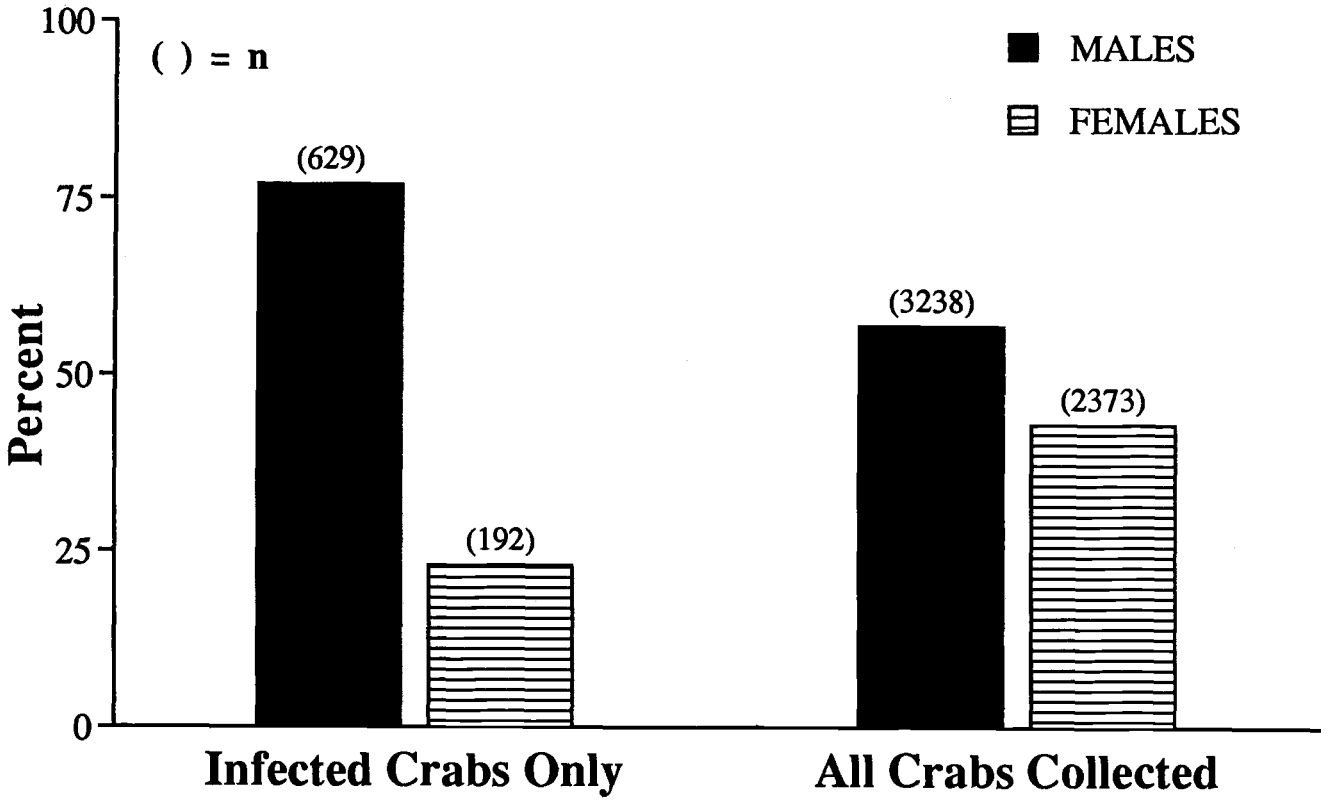

Figure 3. Percentage distribution of male and female Dungeness crabs for all crabs collected and for crabs infected with *Nadelspora canceri*.

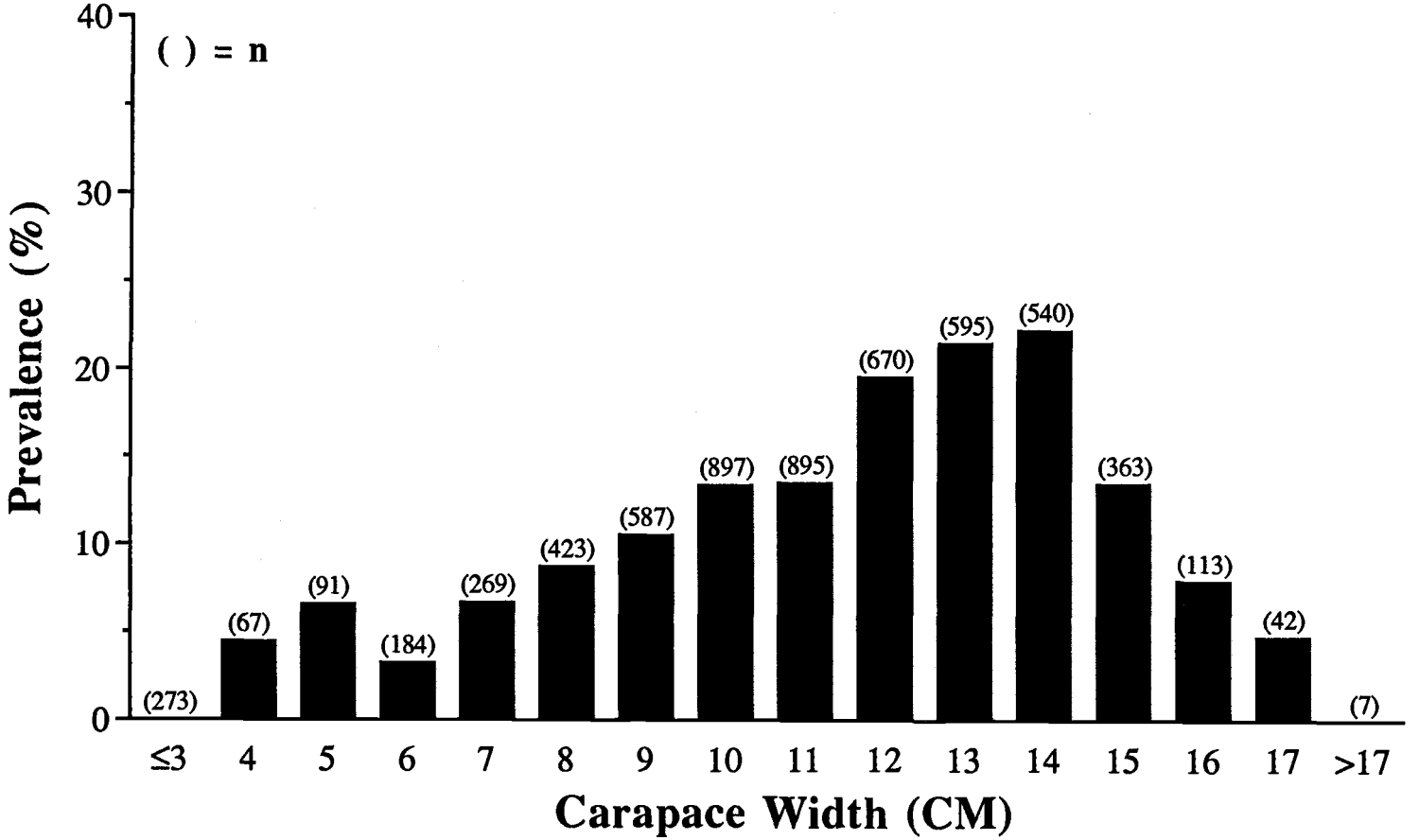

Figure 4. Prevalence (%) of the microsporidian *Nadelspora canceri* in Dungeness crabs from all sampling locations combined.

recorded. Most of the red rock crabs examined were from the Yaquina estuary. A total of three red rock crabs infected with *N. canceri* was found, all from Yaquina Bay.

A total of 8760 male Dungeness crabs (≥ 15.9 cm CW) captured in the commercial ocean fishery was examined at Newport, Oregon, and microsporidian infections were detected in 32 by gross inspection. Twenty six of the 32 infected crabs were infected with *N. canceri* and 6 were infected with an undescribed microsporidian with oval-shaped spores. Five hundred and fifty seven crabs were examined from the commercial ocean catch at Crecent City, California, and one crab infected with *N. canceri* was found. In total, 27 of the Dungeness crabs examined from the commercial ocean catch were infected with *N. canceri* for an overall infection prevalence of 0.3%.

Of the 6052 crabs examined from estuaries and Puget sound only 49 were large males (≥ 15.9 cm CW) that were equal in size to the 8760 crabs examined from the commercial offshore catch. One (2.0 %) of the 49 large males was infected with *N. canceri.* The difference in parasite prevalence between the offshore crabs (0.3%) and the large males from the estuaries (2.0%) is statistically significant (χ^2_1 df = 5.0, P value = 0.02).

Prevalence of *Nadelspora canceri* in Alsea Bay

During the 21 month study period, 2991 Dungeness crabs were examined from Alsea Bay and infected crabs were found in all routine monthly samples. A total of 588 crabs was infected with *N. canceri* (Table 1) giving an overall prevalence of (19.6%). The number of males in monthly samples was often substantially higher than females, although in several samples females

Table 1. Prevalence of *Nadelspora canceri* in monthly samples of Dungeness crabs from Alsea Bay, Oregon, October 1991 to June 1993. Crab sizes reported in cm carapace width (CW)

Month	No. of crabs in sample	Mean size cm CW	Size range cm CW	Males in sample (%)	No. of crabs infected	Total prevalence (%)	Prevalence in males (%)	Prevalence in females (%)
1991 Oct	145	10.4	0.8-18.3	29.1	12	8.28	12.82	7.37
Dec	93	12.1	3.3-18.4	72.5	20	21.51	23.88	15.38
1992 Jan	161	11.4	0.9-16.2	48.1	25	15.53	22.67	9.76
Feb	270	9.2	3.4-16.7	53.3	56	20.70	21.53	19.84
Mar	154	8.9	3.3-14.9	64.3	35	22.73	29.29	10.91
Apr	163	8.6	3.3-13.7	81.6	37	22.70	27.82	0.00
May	138	11.5	4.0-16.4	70.3	37	26.81	24.74	31.71
Jun	106	10.6	5.4-16.1	67.9	32	30.19	40.28	8.82
*Jun	137	0.85	0.7-2.2	--	0	0	--	--
Jul	139	9.6	4.5-15.3	56.1	31	22.30	32.05	9.84
Aug	141	10.2	3.0-16.8	26.4	20	14.18	16.22	13.59
Sep	152	10.0	1.5-16.1	25.1	23	15.13	28.95	10.62
Oct	166	9.8	3.1-16.2	22.8	16	9.64	13.16	8.59
Nov	154	9.1	2.9-15.7	43.8	26	16.88	23.88	11.63
Dec	118	12.6	8.5-16.7	87.3	39	33.05	35.92	13.33
1993 Jan	119	10.7	3.2-15.1.	76.0	26	21.85	26.97	7.14
Feb	71	11.7	24-15.7	78.8	16	22.54	28.85	7.14
Mar	144	10.9	2.5-14.2	80.3	45	31.25	38.18	11.54
Apr	146	10.1	3.1-15.1	86.3	46	31.51	34.92	10.00
May	136	10.1	1.0-17.1	68.1	23	16.91	21.74	6.98
Jun	138	9.6	4.4-16.7	58.7	23	16.67	25.93	3.51
Totals	2991	10.2	0.7-18.4	58.04	588	19.6	26.45	10.86

*Sample of juvenile Dungeness crabs collected by hand from refuges under eel grass blades in June 1992

predominated. In total, males comprised 58% of the crabs collected from Alsea Bay.

The infection prevalence was higher in male crabs in each sample with the exception of May of 1992 (Table 1). The average prevalence of infection for male and female crabs in monthly samples was 26.4% and 10.8% respectively, and of the 588 infected crabs found in Alsea Bay, 77% were males and 23% were females. The difference in infection prevalence between male and female crabs from Alsea Bay over the entire sampling period was highly significant (Table 2) ($F_{stat} = 18.84$, $P < 0.0001$).

The prevalence of infection in Alsea Bay according to crab size is shown in Figure 5. The smallest infected crab found measured 3.2 cm CW, and the prevalence of infection generally increased with crab size up to 13 cm CW where 34.4% of the crabs examined were infected. The prevalence of infection then decreased and none of the 25 crabs over 16 cm CW was infected. The differences in the prevalence of *N. canceri* infection observed between the different size classes was highly significant (Table 2) ($F_{stat} = 23.20$, $P < 0.0001$).

The average prevalence in monthly samples was 21.0 % and varied from 8.2% in October, 1991, to 33.0% in December, 1992. The variable sample month alone was not statistically significant (Table 2) ($F_{stat} = 1.25$, $P= 0.21$) and differences in the prevalence of *N. canceri* in monthly samples can be attributed to differences in the sex and size composition of the samples. The interaction between the variables sex and size was tested and also found to be statistically significant (Table 2) ($F_{stat} = 7.0$, $P= 0.008$). Because of this interactive effect, the influence of the variable sex on the prevalence of *N. canceri* in monthly samples will be different at different size classes. However, when the variables crab sex and size are accounted for in the analysis no significant differences in monthly parasite prevalence or seasonal component of infection occurred.

In June, 1992, 137 small (≤ 2.2 cm CW) juvenile crabs were collected by

Table 2. Analysis of deviance table showing results of drop in deviance test (extra sum of squares test) for association between parasite prevalence (Y) and the independent variables sample month (M), crab size (SZ) and crab sex (SX).

Model	Variables Tested	Deviance	df	Δ Deviance	Δ df	F_{stat}	P_{value}
logit (Y) = $\beta_0 + \beta_1 SX + \beta_2 SZ + \beta_3 M$	--	530.24	386	--	--	--	--
logit (Y) = $\beta_0 + \beta_1 SX + \beta_2 SZ$	M	562.88	405	32.64	19	1.25	0.213
logit (Y) = $\beta_0 + \beta_1 SX + \beta_2 M$	SZ	562.11	387	31.87	1	23.20	<0.0001
logit (Y) = $\beta_0 + \beta_1 SZ + \beta_2 M$	SX	582,02	388	51.78	2	18.84	<0.0001
logit (Y) = $\beta_0 + \beta_1 SX + \beta_2 SZ + \beta_3 M + \beta_4 SX \cdot SZ$	SX•SZ	520.61	385	9.63	1	7.01	0.008
logit (Y) = $\beta_0 + \beta_1 SX + \beta_2 SZ + \beta_3 M + \beta_4 M \cdot SX$	M•SX	490.47	360	39.77	26	1.11	0.32
logit (Y) = $\beta_0 + \beta_1 SX + \beta_2 SZ + \beta_3 M + \beta_4 M \cdot SZ$	M•SZ	494.55	367	35.69	19	1.36	0.143

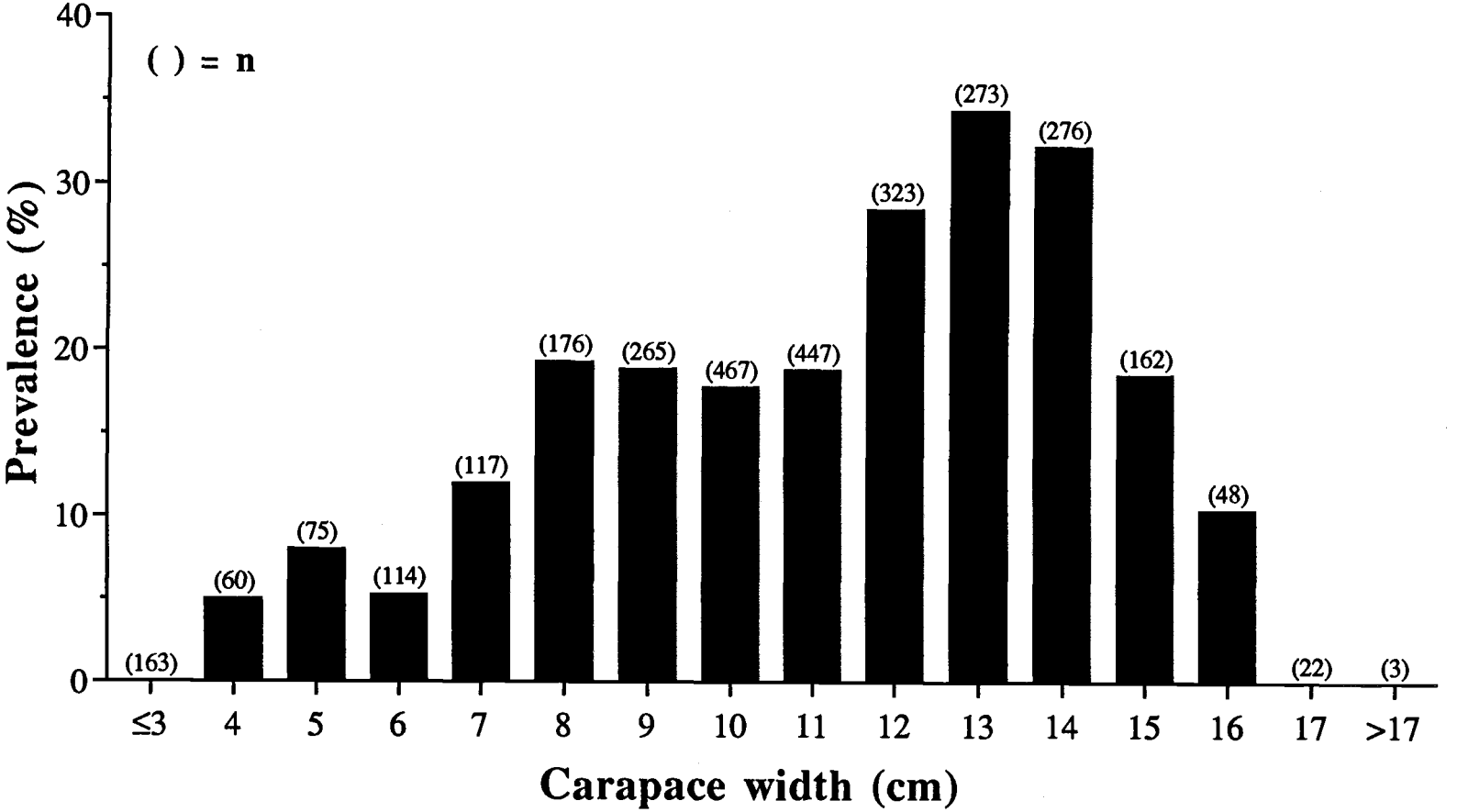

Figure 5. Prevalence (%) of the microsporidian *Nadelspora canceri* in Dungeness crabs from Alsea Bay, Oregon.

hand and no crabs in this sample were infected with *N. canceri*. Data from this sample were not included in the routine monthly collection data because it was the only sample collected that specifically selected juvenile crabs and would have artificially depressed the prevalence level in the June 1992 sample.

Prevalence of *Nadelspora canceri* in other locations sampled

A total of 3061 Dungeness crabs collected from seven Pacific Northwest estuaries and Puget Sound was examined and *N. canceri* infections were detected in 233 (Table 3). The parasite was found in all estuaries sampled and in each estuary the prevalence in male crabs was higher than in females. Of the 233 infected Dungeness crabs collected in these estuaries 74% were male.

The most southerly sample collected was in South Humboldt Bay, California. A total of 171 crabs was examined and 25 (14.6%) were infected with *N. canceri*. Many large (>140 mm) female crabs were captured in Humboldt Bay resulting in a sample with lower proportion of male crabs than in all other estuaries sampled (Table 3). In Oregon the parasite was common in Coos Bay (10.6%), Nehalem Bay (14.2%), and Tillamook Bay (41.2%), and uncommon in eight Yaquina Bay samples (Table 4). The overall prevalence of *N. canceri* in Yaquina Bay was 2.0%. The prevalence of *N. canceri* in five otter trawl samples (661 crabs) from Yaquina bay was 3.3 % and ranged between 0.7% and 9.8%. Two samples (402 crabs) were collected from Yaquina Bay using crab rings and the infection prevalence was 0.7 % and 2.3 %. One sample (235 crabs) of small juvenile crabs (< 2.0 cm CW) was collected by hand in Yaquina Bay from refuges under algae and eel grass blades and none were infected with the parasite. Infection prevalence decreased in the more northerly samples from Washington estuaries where levels were 6.9% in Willapa Bay and 0.4% in Grays Harbor. However, he average crab

Table 3. Prevalence of the microsporidian *Nadelspora canceri* in Dungeness crabs from seven Pacific Northwest estuaries and Puget Sound. Crab sizes reported in cm carapace width (CW).

Location	No. of crabs sampled (No. samples)	Mean size cm CW	Size range cm CW	Males in sample (%)	No. of crabs infected	Total prevalence (%)	Prevalence in males (%)	Prevalence in females (%)
Humboldt Bay, Ca	171 (1)	13.6	7.4-18.9	39.2	25	14.62	16.42	13.46
Coos Bay, Or	264 (1)	11.8	8.6-17.8	66.3	28	10.61	12.00	7.87
Yaquina Bay, Or	1298 (8)	10.4	0.7-18.1	49.6	26	2.00	2.73	1.55
Tillamook Bay, Or	245 (1)	10.5	1.0-15.3	75.9	101	41.22	45.90	29.31
Nehalem Bay, Or	211 (1)	9.3	5.0-14.2	60.6	30	14.22	17.97	8.43
Willapa Bay, Wa	303 (2)	8.5	6.5-15.0	60.3	21	6.93	10.97	3.92
Grays Harbor, Wa	453 (2)	6.4	1.0-15.9	71.3	2	0.44	0.85	0.00
Puget Sound, Wa*	116 (3)	13.3	9.1-17.4	31.0	0	0	0	0
Totals	3061 (19)	9.7	0.7-18.9	51.4	233	7.38	10.9	4.8

* Samples from three locations combined: Kala Point, Dungeness Spit, And Mukilteo.

Table 4. Prevalence of the microsporidian *Nadelspora canceri* in eight samples of Dungeness crabs collected from Yaquina Bay, Oregon, Feburary, 1992 to September, 1993. Crab sizes reported in cm carapace width(CW)

Month	Sampling method	No. of crabs in sample	Mean size cm CW	Size range cm CW	Males in sample (%)	No. of crabs infected	Total prevalence (%)	Prevalence in males (%)	Prevalence in females (%)
1992 Feb	Trawl	113	12.3	4.4-18.1	48.6	2	1.7	1.8	1.7
May	Trawl	273	8.8	4.2-16.6	40.2	2	0.7	1.8	0
Jun	Ring	273	8.6	5.1-16.9	40.4	2	0.7	0.9	0.6
Jul	Hand*	235	1.2	0.7-1.9	--	0	0	0	0
Dec	Trawl	89	12.0	9.3-15.2	67.2	5	2.1	2.5	1.2
1993 Mar	Trawl	51	12.5	5.1-16.1	72.5	5	9.8	10.8	7.1
Jun	Trawl	135	10.9	1.8-15.7	48.1	7	5.1	4.6	5.7
Sep	Ring	129	11.5	7.8-16.1	67.4	3	2.3	3.4	0
Totals		1298	10.4	0.7-18.1	49.6	26	2.0	2.73	1.55

*Sample of juvenile Dungeness crabs collected by hand from refuges under eel grass blades in July 1992

size was smaller in the two Washington estuaries sampled compared to the other estuaries (Table 3) and the prevalence may be greater in larger crabs in these estuaries. No infected crabs were found out of the 116 crabs examined from the three areas in Puget Sound.

The prevalence of infection according to crab size when samples from these locations are combined is shown in Figure 6. No *N. canceri* infections were found in 205 crabs smaller than 7 cm CW that were examined and the prevalence generally increased with crab size up to 13 cm CW where 13.6% of the crabs examined were infected. The prevalence decreased beyond 13 cm CW before increasing to a prevalence of 8.3% in 20 crabs 17 cm CW. Four crabs larger than 17 cm CW were uninfected.

DISCUSSION

Nadelspora canceri was not observed in Dungeness crabs until 1985 (Olson, R. E. pers. comm.) and it is not known if the parasite was recently introduced to the Pacific Northwest or if it had simply not been observed previously. It is also possible that *N. canceri* has always been present in the Pacific Northwest, but that unknown conditions are currently favoring the transmission of the parasite and allowing high infection prevalences to occur.

The overall prevalence of *N. canceri* infection found in this study (13.5%) and the prevalence in the most intensely sampled location, Alsea Bay, (19.6%) in particular, is substantially higher than most microsporidian infection prevalences that have been reported in other decapod populations. Overstreet and Whatley (1975) examined several thousand blue crabs from Mississippi Sound and only one crab infected with the microsporidian *Ameson michalis* was found. Other studies show that the prevalences of microsporidian infections in natural decapod

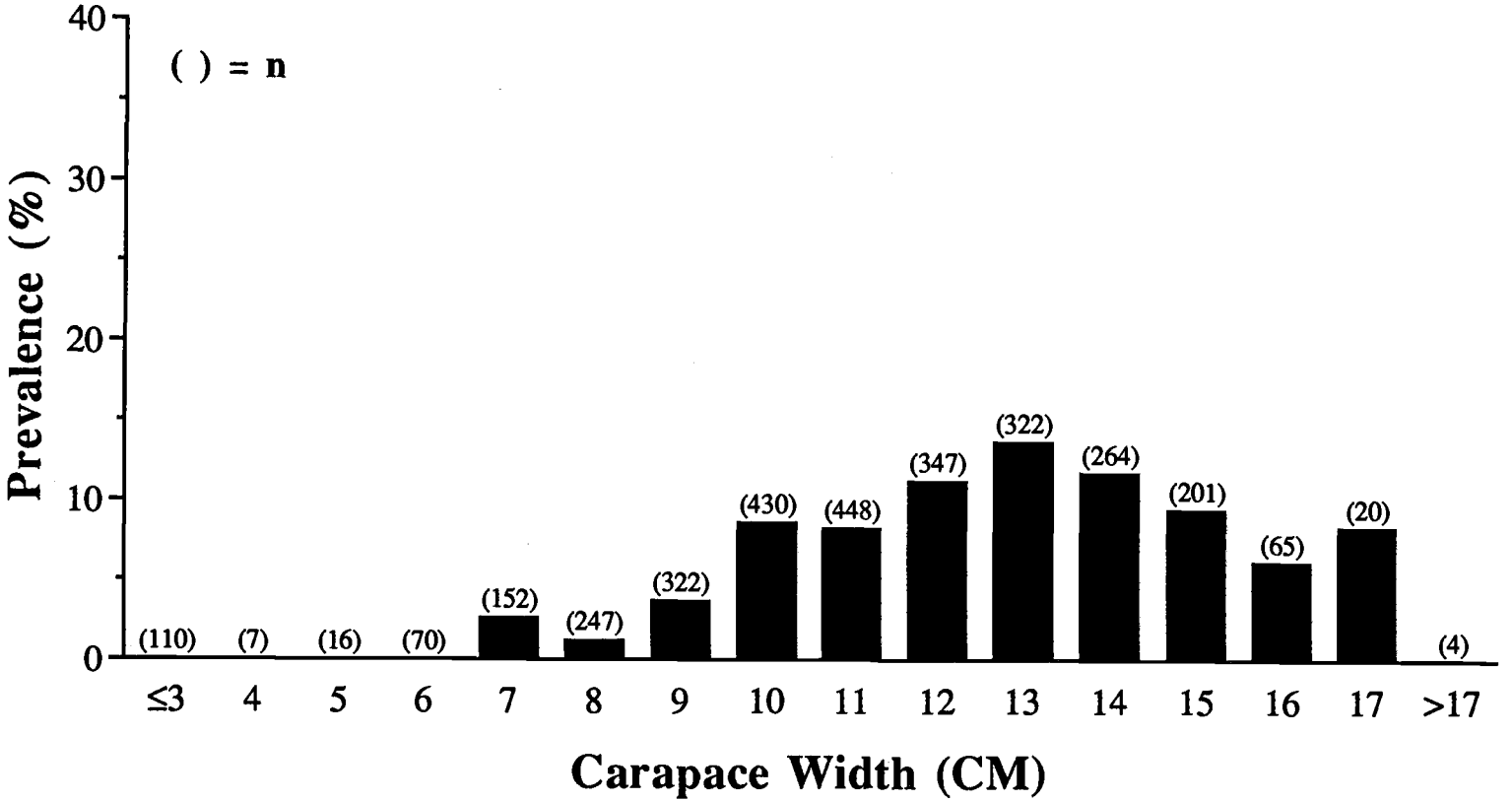

Figure 6. Prevalence (%) of the microsporidian *Nadelspora canceri* in Dungeness crabs from all sampling locations excluding Alsea Bay, Oregon.

populations are typically below 1.0%, especially in commercially exploited species, (Olson and Lannan 1984, Parsons and Kahn 1986, Owens and Glazebrook 1988, Skurdal et al. 1988), although infection prevalences greater than 1.0% have been reported. For example the microsporidian *Pleistophora crangoni* parasitizes several species of crangonid sand shrimps and was studied by Breed and Olson (1977) who examined over 7000 of these shrimp along the Oregon coast and found the overall infection prevalence to be 8.8% and levels in some monthly collections to be as high as 41% in *Crangon stylirostris*. Observations of microsporidian infection levels greater than 1.0% have also been reported in crayfish (Herbert 1988), and king crabs (Sparks and Morado 1985), although the number of animals examined in these studies was less than 200.

In contrast to Alsea Bay, the overall prevalence of *N. canceri* in Yaquina Bay, an estuary located 17.2 km from Alsea Bay (Fig. 1), was only 2.0% (Table 3, 4). Over the course of this study the prevalence of infection in 8 Yaquina bay samples did not vary greatly and was consistently lower than the prevalence found in Alsea Bay. Furthermore, no statistical differences in parasite prevalence occurred in 20 monthly samples from Alsea Bay. These results suggest that the prevalence levels of *N. canceri* in both Alsea and Yaquina Bays are relatively stable. Reasons for the differences in prevalence between Alsea and Yaquina Bays are not known. Physical differences in estuarine characteristics or differences in crab population densities between these estuaries could influence the rate of parasite transmission and therefore prevalence of *N. canceri* , although these factors have not been examined.

Environmental factors such as temperature and salinity were not measured in this study although these conditions are known to change seasonally in estuaries like Alsea Bay that have high fresh water flows during winter months. Because no monthly or seasonal differences in the prevalence of *N. canceri* in Alsea Bay

occurred, that could not be accounted for by crab size and sex ratio differences, no indication of environmental influences on parasite prevalence in Alsea Bay were detected. The interactive effect between the size and sex variables in monthly samples from Alsea Bay makes it difficult to determine to what degree the parasite prevalence in monthly samples depends on sex alone since the prevalence will depend on sex differently at different size classes of crabs .

Initially, the differences in infection prevalences observed between Yaquina Bay (≈1%) and Alsea Bay (≈20%) and the typically low (<1.0%) microsporidian prevalences reported in other decapod populations suggested that the high prevalence of *N. canceri* in Alsea Bay was unusual. However, when Dungeness crabs were examined from other estuaries along the Pacific Coast it became apparent that high prevalences of *N. canceri* were not uncommon (Table 3).

Dungeness crabs infected with *N. canceri* were common in estuaries from Humboldt Bay to Willapa bay, uncommon in Grays Harbor and not found in Puget Sound. Since only 116 Dungeness crabs were examined from Puget Sound and 69 % of these crabs were females it is possible that *N. canceri* occurs at low levels in Puget Sound and was simply not detected. However, since the parasite was least common or absent in the two northern most sampling locations, the northern limit of the geographic range of this parasite may be on the coast of Washington. No indication of the southern limit of the geographic range of the parasite was found because it was common in Humboldt Bay, the southern most area sampled.

The total number of male and female crabs captured and examined was nearly equal, although *N. canceri* infections were substantially more common in male crabs (Fig. 3). In the few studies of microsporidian infection prevalences in decapod species where male and female infection prevalences were reported, the male and female infection levels were approximately equal (Parsons and Kahn 1986, Herbert 1988, Skurdal et al. 1990). Reasons for the higher infection prevalence of *N. canceri* in male

crabs are not known, although behavioral differences between male and female Dungeness crabs could facilitate a higher or more effective parasite transmission rate in male crabs. Examples of these differences include the extent of movements (Diamond and Hankin 1985) and segregation by sex (Pacific Fisheries Management Council 1979). An apparent difference in infection prevalences between male and female crabs could result from our sampling procedures if infected female crabs exhibited feeding or other behavioral changes that reduce their susceptibility to capture in baited traps.

Transmission of *N. canceri* was accomplished in several laboratory experiments (Chapter 4 this thesis) when infected crab tissue was fed to uninfected crabs, indicating that vectors or intermediate hosts are not required to establish infections. Male and female crabs, both juveniles and adults, were experimentally infected and no differences in susceptibility based upon crab sex or crab age were observed. The cannibalistic behavior exhibited by Dungeness crabs (Butler 1954, Tegelberg 1972, Stevens et al. 1982) is therefore a probable mechanism for transmitting *N. canceri* between hosts in feral populations. A higher infection prevalence could result in male crabs if the rates of cannibalism in the natural population were greater for male than for female crabs. No information on differences in rates of cannibalism between male and female Dungeness crabs is available.

Significantly different infection prevalences were observed in different size classes of Dungeness crabs (fig. 3, 4 ,5). The infection prevalence was lowest in small, less than 6 cm CW, crabs which are 0^+ age juveniles (Gunderson et al. 1990). Studies by Stevens et al. (1982) showed that cannibalism was greatest among 0^+ age Dungeness crabs, however, small Dungeness crabs are known to segregate from older crabs in estuaries (Stevens and Armstrong 1984) and this behavior could reduce the chances of these younger crabs being exposed to infected crabs.

The prevalence of infection, in crabs larger than 6 cm CW, generally increased with crab size up to 13-14 cm CW which are 2^+ years of age (Gunderson et al. 1990).

Nadelspora canceri infections are lethal to the crab host (Chapter 4 this thesis) and the higher infection prevalences observed in 2^+ age infected crabs may be the result of lower mortality rates in the older infected crabs compared to 0^+ juvenile infected crabs in the estuaries. It is also conceivable that the probability of being exposed to the parasite may increase with the age of the crab and the amount of time spent in the estuary.

Possible explanations for the decrease in the prevalence of *N. canceri* in crabs larger than 14 cm CW include: High mortality rates in the smaller 10-14 cm CW infected crabs. Infected crabs may migrate out of the estuary, or as infected crabs increase in age and the infection progresses, feeding may be reduced thereby reducing the chances of capturing larger heavily infected crabs in baited traps. A reduction in molting frequency in the smaller (10-14 cm CW) infected crabs could also result in fewer infected large crabs; however, infected crabs held in the laboratory were observed to molt regularly (chapter 4).

The prevalence of *N. canceri* in large (≥15.9 cm CW) male crabs examined from the offshore commercial ocean fishery was significantly lower than that found in male crabs of the same size examined from estuaries and overall prevalence differences between estuaries and offshore crabs was substantial. These substantial differences in infection prevalence could reflect density dependent differences in transmission efficiency. Lower crab densities have been reported for 0^+ and 1^+ age Dungeness crabs from the offshore crab populations off the Washington coast compared to adjacent inshore estuaries (Armstrong and Gunderson 1985, McConnaughey et al. 1992). For example; juvenile crab densities between 1000 and 4000 per hectare were not uncommon in the Grays Harbor estuary, whereas densities offshore were usually less than 300 per hectare (Armstrong and Gunderson 1985). The prevalence of the parasite in offshore female crabs and sub-legal male crabs was not determined.

Red rock crabs are members of the same genus and often share the same habitat as Dungeness crabs in estuaries, therefore, finding three red rock crabs infected with the parasite was not surprising. However, observations made during this study indicate that *N. canceri* infects the red rock crab population at a prevalence level well below that observed in Dungeness crabs. Reasons for this difference are unknown.

Chapter 4
Experimental transmission of the microsporidian *Nadelspora canceri* to Dungeness crabs, *Cancer magister*, and mortality in laboratory held *N. canceri* infected crabs.

INTRODUCTION

Microsporidian diseases are well documented in decapod crustaceans (Sprague 1977b, c), and are reviewed by Sparks (1985), and Sindermann (1990). Microsporidian infections have been reported in a variety of tissues and organs in decapod hosts including the hepatopancreas (Azevedo 1987), ovaries (Kelly 1979, Sparks and Morado 1985), and tegmental glands (Sparks and Morado 1985), although the most common site of infection is within striated muscle (Sparks 1985, Sindermann 1990). Microsporidian infections that occur in decapod muscle tissue can cause extensive muscle necrosis (Sprague 1970, Breed and Olson 1977, Kelly 1979, Parsons and Kahn 1986, Skurdal et al. 1988, Vivarès and Azevedo 1988 , Langdon 1991), and death of the host (Sprague 1970, Weidner 1970, Sprague and Vávra 1976, Canning 1982, Skurdal et al. 1988).

The most common method for microsporidian transmission is through the digestive tract when infectious spores are ingested by susceptible hosts (Larsson 1986). The cannibalistic behavior exhibited by many decapod species is thought to favor microsporidian transmission that requires ingesting parasite spores (Iversen 1969, Skurdal et al. 1990, Langdon and Thorne 1992). The blue crab *Callinectes sapidus* has been experimentally infected with the microsporidian *Ameson michaelis* by feeding infected tissue directly to uninfected crabs (Sprague, et al. 1968; Weidner, 1970; Overstreet and Whatley 1975) and the crayfish *Cherax tenuimanus* and *Cherax albidus* have been infected with *Vavraia parastacida* also by direct feeding (Langdon and Thorne 1992). The only other report of experimental microsporidian transmission to a decapod host was that of Iversen and

Kelly (1976) who could infect *Penaeus duorarum* with *Thelohania penaei* only after spores were "conditioned" or "primed" by passing through the digestive track of fish. Other experimental attempts to transmit microsporidian infections *per os* to decapod hosts have been unsuccessful (Roth and Iversen 1971, Breed and Olson 1977, Graham and France 1986, Parsons and Khan 1986, Herbert 1988) indicating that intermediate hosts or other unknown conditions may be necessary for transmitting these parasites between hosts.

Nadelspora canceri a microsporidian that infects the commercially important Dungeness crab, *Cancer magister*, along the United States Pacific Northwest coast was recently described by Olson et al. (1994). The geographic distribution and prevalence of *N. canceri* in the Dungeness crab population was described by Childers (Chapter 3 this thesis). *Nadelspora canceri* spores are found in vast numbers in the striated muscle causing it to appear whitish and opaque, rather than having a normal translucent appearance. Infections are easily detected by viewing the white opaque muscle tissue through the ventral articular membrane at the base of each thoracic appendage. The parasite has unusual needle shaped spores ($\approx$10 μm x $<$ 0.25 μm) that are easily recognized when muscle is viewed under a phase contrast microscope at 400X (Olson et al. 1994). The effects of the parasite on individual crabs or the Dungeness crab population are unknown and no information exists on host specificity or the mode of infection.

The purpose of this study was to determine if *N. canceri* infections caused mortality in laboratory-held crabs and if *N. canceri* infections could be established *per os* in Dungeness crabs. These questions were addressed in four laboratory experiments. Two experiments were designed to compare the mortality of Dungeness crabs naturally infected with *N. canceri* to that of uninfected crabs held under the same laboratory conditions. These experiments differed from each other in that infected crabs held in the first mortality experiment shared a common

saltwater aquarium that was isolated from the aquarium holding uninfected crabs. In a second experiment individual crabs were isolated to allow monitoring of crabs on a individual basis and to prevent spreading of *N. canceri* in control crabs if one or more were sub-clinically infected with the parasite at the beginning of the observation period. Laboratory transmission of *N. canceri* to Dungeness crabs was attempted in two experiments by feeding muscle tissue from crabs naturally infected with the parasite directly to uninfected 5 to 11 cm Carapace width (CW) crabs in one experiment and to juvenile crabs < 2.0 cm CW in a second experiment.

MATERIALS AND METHODS

Animal collection and maintenance

The mortality and transmission experiments were conducted at the Oregon State University, Hatfield Marine Science Center, Fish Disease Laboratory. The carapace width of each crab was measured to the nearest millimeter just anterior to the 10^{th} anterolateral spine. Crabs in experiments were fed daily on a diet consisting of shrimp or herring and held in 9 to 12°C salt water that was sand filtered and ultraviolet light irradiated. Juvenile Dungeness crabs (< 2.0 cm CW) used in experiments were collected by hand at low tide from refuges under algae blades and transported alive to the laboratory in five gallon buckets. Larger crabs (> 2.0 cm CW) were collected with baited crab rings. To prevent aggressive behavior and injury the larger crabs were individually wrapped in wet burlap after capture and placed in insulated coolers for transporting to the laboratory.

The presence or absence of *N. canceri* infection in Dungeness crabs collected and used in mortality experiments was determined by visual examination

of the muscle tissue through the periarticular membrane at the base of the thoracic appendages.

Mortality in laboratory-held *N. canceri* infected Dungeness crabs: Experiment One

Twenty Dungeness crabs infected with *N. canceri* (14 males, 6 females) and thirteen uninfected crabs (6 males, 7 females) were collected from Alsea Bay, Oregon, (Fig. 1, 2) on January 11, 1992. The average size of infected crabs was 12.1 cm CW and ranged from 7.1 to 14.7 cm CW, whereas the average size of uninfected crabs was 10.4 cm CW and ranged from 7.2 to 14.3 cm CW. The mean size of infected and uninfected crabs collected for this experiment was not statistically different (F_{stat} = 1.3, P value = 0.32).

These crabs were held in the laboratory by enclosing each crab individually within a covered 16 x 18 x 22 cm basket constructed of 4.0 cm mesh rigid plastic. The baskets holding infected and uninfected crabs were held separately in identical 0.2 x 1.2 x 1.4 m fiberglass saltwater aquaria. Uninfected crabs shared a common water supply as did all infected crabs. Flowing salt water maintained at a rate of 8-10 liters/min passed once through the mesh baskets containing crabs before draining to a 2-4 ppm chlorinated effluent. When crabs died wet mount muscle smears were examined under a phase contrast microscope at 400X to confirm the presence or absence of *N. canceri* spores. After 157 days the experiment was terminated and a sample of muscle tissue was taken from each of the surviving crabs to document the presence or absence of the microsporidian. The product limit survival analysis developed by Kaplan and Meier (1958) was used to statistically compare individual survival times of infected and uninfected crabs. A computer program (STATISTICA/Mac™) was used to perform the product limit analysis.

Mortality in laboratory-held *N. canceri* infected Dungeness crabs: Experiment Two

Forty-four Dungeness crabs infected with *N. canceri* (33 males, 11 females) and 45 uninfected crabs (27 males, 18 females) were collected from Alsea bay, Oregon, on July 12, 1992. The average size of infected crabs was 9.9 cm CW and ranged from 4.5 to 14.2 cm CW. The average size of uninfected crabs was 10.3 cm CW and ranged from 5.5 to 14.0 cm CW. The difference in the mean size of infected and uninfected crabs was not statistically significant (F_{stat} = 1.2, P value = 0.22)

Crabs were isolated individually in the laboratory by holding each crab in a numbered, round plastic, 12 liter, container. Removable lids on each container prevented crabs from escaping and allowed easy access for feeding and cleaning. A separate saltwater line entering through the lid of each container supplied water to the containers at a flow rate of 24-48 liters each hour. Salt water drained from the containers through 1.5 cm holes located 26 cm from the bottom of each container. The containers were placed in four rectangular fiberglass troughs measuring 0.4 x 0.5 x 4.8 m. This arrangement resulted in each crab in the experiment being on its own salt water supply throughout the experiment and no crabs encountered salt water that had come in contact with another crab. The containers holding infected crabs were placed in two troughs and containers holding uninfected crabs were held in two separate troughs. Salt water draining from the containers and into the troughs was used to maintain a uniform temperature water bath around the containers. A 20 cm high standing drain pipe in each trough held the water level at a constant 20 cm depth.

The containers were emptied and cleaned every other day to remove un-eaten food and feces, and were sanitized weekly with iodiphor disinfectant to prevent the buildup of bacterial film on the inner surface. When crabs died, wet

mount muscle smears were examined under a phase contrast microscope at 400X to determine the presence or absence of *N. canceri* spores. After 275 days the experiment was terminated and a sample of muscle tissue was taken from each surviving crab to document the presence or absence of the microsporidian. The survival of the infected and uninfected crabs was compared statistically using a product limit survival analysis (Kaplan and Meier 1958).

Transmission of *N. canceri* in juvenile and adult Dungeness crabs

Twenty-four Dungeness crabs measuring 5-11 cm CW, that were uninfected by external visual criteria, were collected on July 2, 1992 in Yaquina Bay, Oregon, (Fig. 1) an estuary with a low prevalence of *N. canceri* (chapter 3 this thesis). After transporting to the laboratory, twelve crabs (7 males, 5 females) that averaged 8.7 cm CW were randomly assigned to a treatment group, and twelve crabs (6 males, 6 females) that averaged 7.9 cm CW were randomly assigned to a control group. Each crab was housed individually in a round plastic 12 liter containers as described above.

The containers were held in two 0.2 x 1.2 x 1.4 m fiberglass water bath aquaria. Containers holding treatment and control crabs were placed in separate aquaria. Water draining from the containers maintained a 19 cm deep water bath around the containers in each aquarium. This arrangement prevented any mixing of salt water or physical contact between individual crabs.

Four adult male Dungeness crabs infected with *N. canceri* were collected in Alsea Bay, Oregon, on July 3, 1992, and served as the source of parasite spores. Muscle tissue from each crab was examined microscopically to confirmed the presence of *N. canceri* spores. Approximately three grams of infected muscle tissue, dissected free of the shell, was placed as a food source in each container holding a

treatment crab on four separate parasite exposure days: the second, fourth, sixth, and tenth day of the experiment. Infected muscle tissue from a different infected crab was used for each of the exposure days. Less than two hours elapsed between the time when infected tissue was dissected free of the shell and when it was introduced into the containers. No other food was present in the containers on exposure days and infected muscle tissue remained in the containers for 24 hours before it was removed. The control crabs were fed shrimp on exposure days and were never exposed to *N. canceri* spores.

Each crab was inspected, at approximately seven day intervals, for gross signs of infection (white discoloration of muscle) by examining muscle tissue through the articular membrane. Muscle tissue from crabs that died during the experiment and crabs that survived to the end of the experiment (372 days) was examined under a phase contrast microscope at 400X to determine if *N. canceri* spores were present.

Transmission of *N. canceri* in 1.0-2.0 cm CW juvenile Dungeness crabs

Sixty-nine apparently healthy juvenile Dungeness crabs (1.0-2.0 cm CW) were collected from Yaquina Bay on July 1, 1992, and transported to the laboratory. *Nadelspora canceri* was not detected in any of the crabs when muscle tissue was examined through the articular membrane under a dissecting microscope.

Each crab was randomly assigned to a treatment or control group and placed into a 6.2 x 15.8 x 24 cm basket constructed of (0.9 cm) mesh rigid plastic. Thirty-two crabs were assigned to the treatment group and thirty-seven crabs were assigned to the control group. Removable lids, of the same mesh construction, on each basket prevented crabs from escaping and allowed easy access for feeding.

The baskets were submerged into two 600 liter flow-through saltwater aquaria. Baskets holding treatment crabs were placed in a separate aquarium from that holding

control crabs and the flow of salt water entering these tanks was maintained at 500-600 liters each hour. This arrangement resulted in a common water supply for all control crabs and a separate, common water supply for all crabs in the treatment group.

Four naturally infected Dungeness crabs were collected in Alsea Bay, Oregon, on July 3, 1992, and served as a source of *N. canceri* spores. Approximately one gram of infected muscle tissue was placed as a food source in each basket holding a treatment crab on the second, fourth, sixth, and tenth day of the experiment. No other food was present in the baskets on these days and the infected tissue was left in the baskets for 24 hours before it was removed. The control crabs were fed shrimp and were never exposed to *N. canceri* spores.

Both treatment and control crabs were examined under a dissecting microscope for external signs of infection (white muscle tissue) at intervals that ranged from six to ten days. On the 40th day of the experiment 26 crabs (15 control and 11 treatment) were removed from the experiment and muscle tissue was dissected from each crab and examined under a phase contrast microscope at 400X to determine if *N. canceri* spores were present. Muscle tissue from all crabs that died during the experiment and from all crabs that survived to the end of the experiment (75 days) was examined microscopically to determine the presence or absence of *N. canceri* spores.

RESULTS

Mortality in laboratory-held *N. canceri* infected Dungeness crabs: Experiment One

The mortality of laboratory-held Dungeness crabs (12.1 cm average CW) naturally infected with *N. canceri*, held in a common water source, was compared to that of uninfected crabs (10.4 cm average CW), held in a separate common water source, and the results are shown in Figure 7. Eighty-five percent of the infected crabs

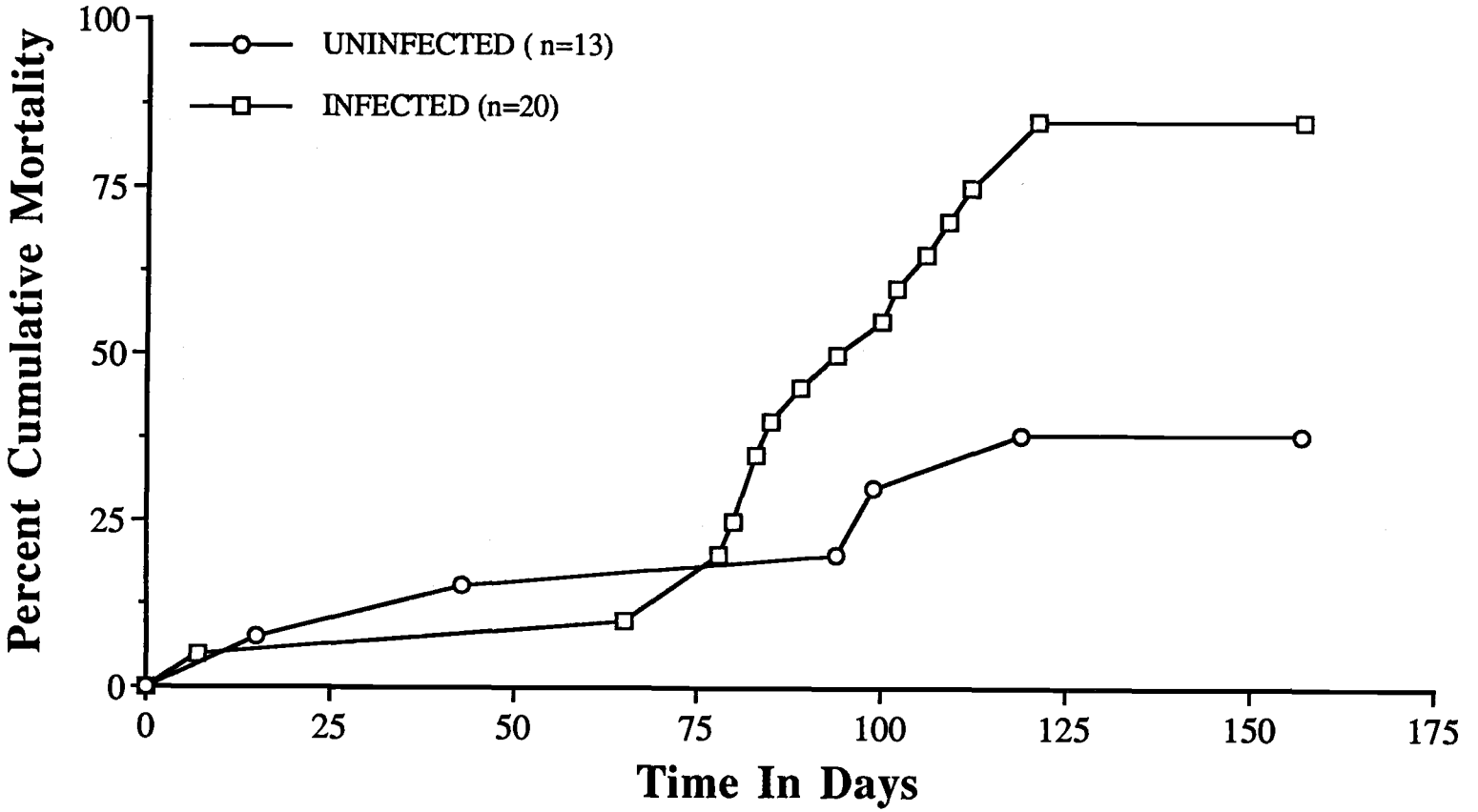

Figure 7. Percent cumulative mortality of *Nadelspora canceri* infected and uninfected Dungeness crabs held in separate common water aquaria.

died during the 157 day observation period while 39% of the uninfected crabs died. The mean day to death for infected and uninfected crabs was 93 and 123 days respectively. The observed difference in mortality between infected and uninfected crabs was statistically significant (F_{stat}=2.8, P value = 0.01). Microscopic examination of muscle tissue from each crab confirmed the presence of *N. canceri* spores in all presumptively infected crabs and no indications of infection remission was detected in any of these crabs. No spores were detected in the presumptively uninfected crabs.

Mortality in laboratory-held *N. canceri* infected Dungeness crabs: Experiment Two

The mortality of 44 naturally infected crabs (9.8 cm average CW) isolated in individual containers was compared to that of 45 identically held presumptively uninfected crabs (9.9 cm average CW) and the results are shown in Figure 8. Thirty-seven (84%) of the 44 infected crabs died before the end of the 275 day observation period and the mean day to death was 147 days. The presence of *N. canceri* was confirmed microscopically in all crabs in the infected group.

One uninfected crab died from unknown causes 89 days after the beginning of the 275 day observation period. All other crabs in this group survived until the end of the experiment. Two crabs that appeared to be uninfected by gross visual examination at the start of the observation period developed visual signs of *N. canceri* (white discoloration of muscle) 35 and 75 days after observations began. Both crabs survived to the end of the observation period when the presence of *N. canceri* spores was confirmed in each crab microscopically. These two crabs were not included in the data analysis. No *N. canceri* spores were detected in any other crabs in the uninfected group. The differences in mortality observed between the infected and uninfected crabs held in this experiment were significant (F_{stat}= 24.0, P value = < 0.0001).

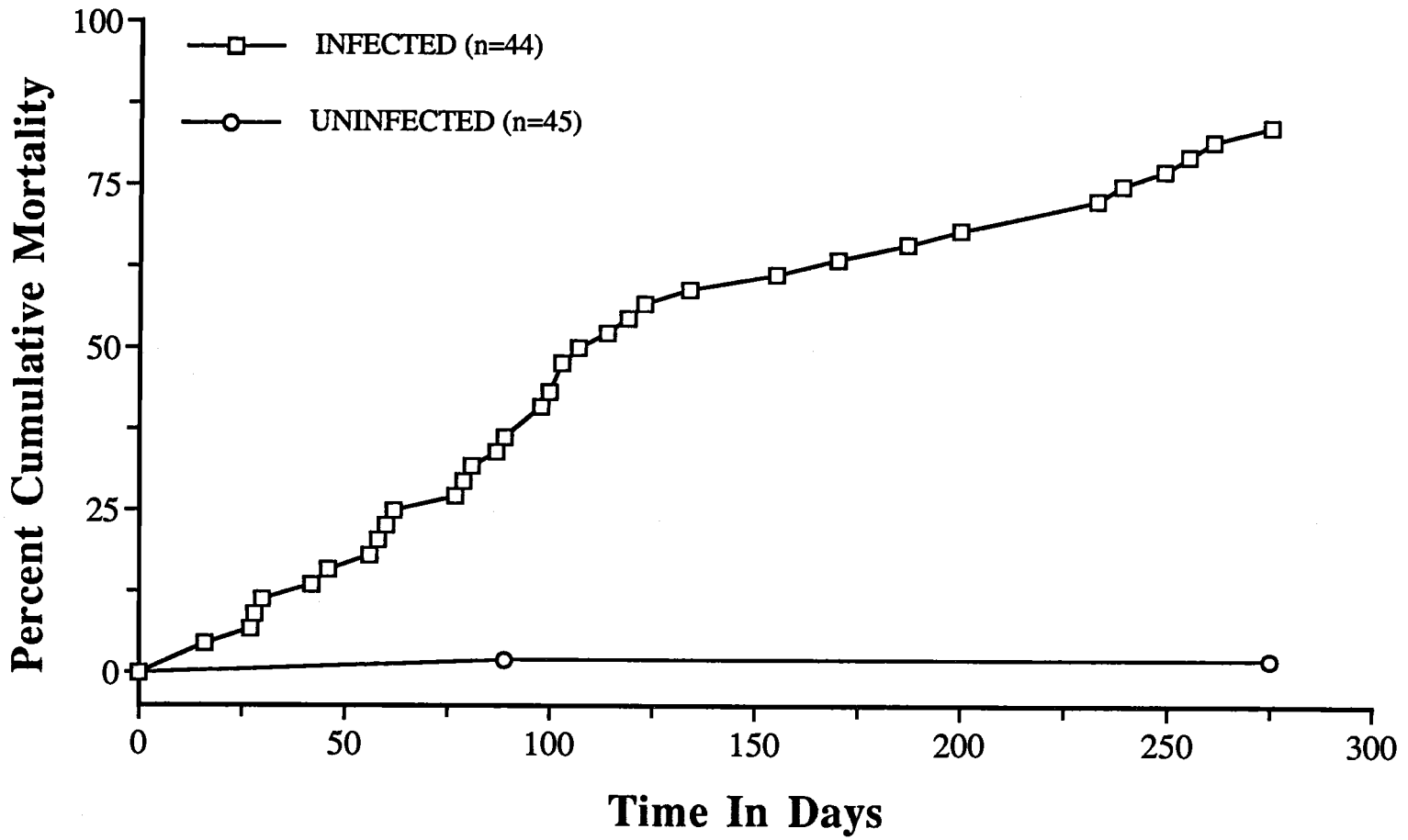

Figure 8. Percent cumulative mortality of *Nadelspora canceri* infected and uninfected Dungeness crabs held individually in 12 liter containers in the laboratory.

Molting and behavior

Both infected and uninfected crabs molted during the observation period. Five of the seven infected crabs that survived until the end of the experiment molted as did 41 of the 44 surviving uninfected crabs. The number of infected and uninfected crabs that survived and did not molt was not statistically different ($\chi^2_{,1\ df} = 3.23$, P value = 0.07). The two surviving infected crabs that did not molt were 12.4 and 13.0 cm CW and the three surviving uninfected crabs that did not molt were also large and measured 13.7, 14.0 and 13.6 cm CW. Twelve of the 37 infected crabs that died molted prior to death.

Infected crabs were lethargic and weak compared to uninfected crabs and were often unable to grasp or pinch with the chelipeds. Feeding was observed in infected and uninfected crabs, although crabs in the premolt stage and several infected crabs stopped feeding prior to molting and death respectively. No indication of infection remission was observed in any of the infected crabs.

Egg extrusion was observed in two infected female crabs on experiment day 94 and 102 respectively. No microsporidian spores were detected in these eggs when samples were removed from the crabs abdomen and examined microscopically.

Transmission of *N. canceri* in juvenile and adult Dungeness crabs

Twelve crabs averaging 8.7 cm CW, that were experimentally exposed to *N. canceri* spores and twelve unexposed crabs averaging 7.9 cm CW were held in individual containers and the results are shown in Table 5. *Nadelspora canceri* infections were established in eleven (6 males, 5 females) out of twelve Dungeness crabs that were exposed to parasite spores and no infections were observed in the twelve control crabs (Table 5, Fig. 9). Seven of the exposed crabs that became infected died before the end of the 372 day observation period. Individual deaths were recorded

Table 5. *Nadelspora canceri* infections observed in Dungeness crabs exposed to *N. canceri* spores by feeding, and crabs not exposed to spores.

	EXPOSED CRABS				UNEXPOSED CRABS			
	n	Mean size and (range) cm CW*	Mean day to death	No. infected	*n*	Mean size and (range) cm CW*	Mean day to death	No. infected
Crabs dying during experiment	7	9.1 (7.2-11.3)	295	7	1	6.5 (---)	372	0
Crabs surviving to end of experiment	5	8.3 (5.5-10.3)	---	4	11	8.0 (6.6-10.6)	---	0
Totals	12	8.7 (5.5-11.3)		11	12	7.9 (6.6-10.6)		0

*Mean crab size and size range recorded at the beginning of the experiment.

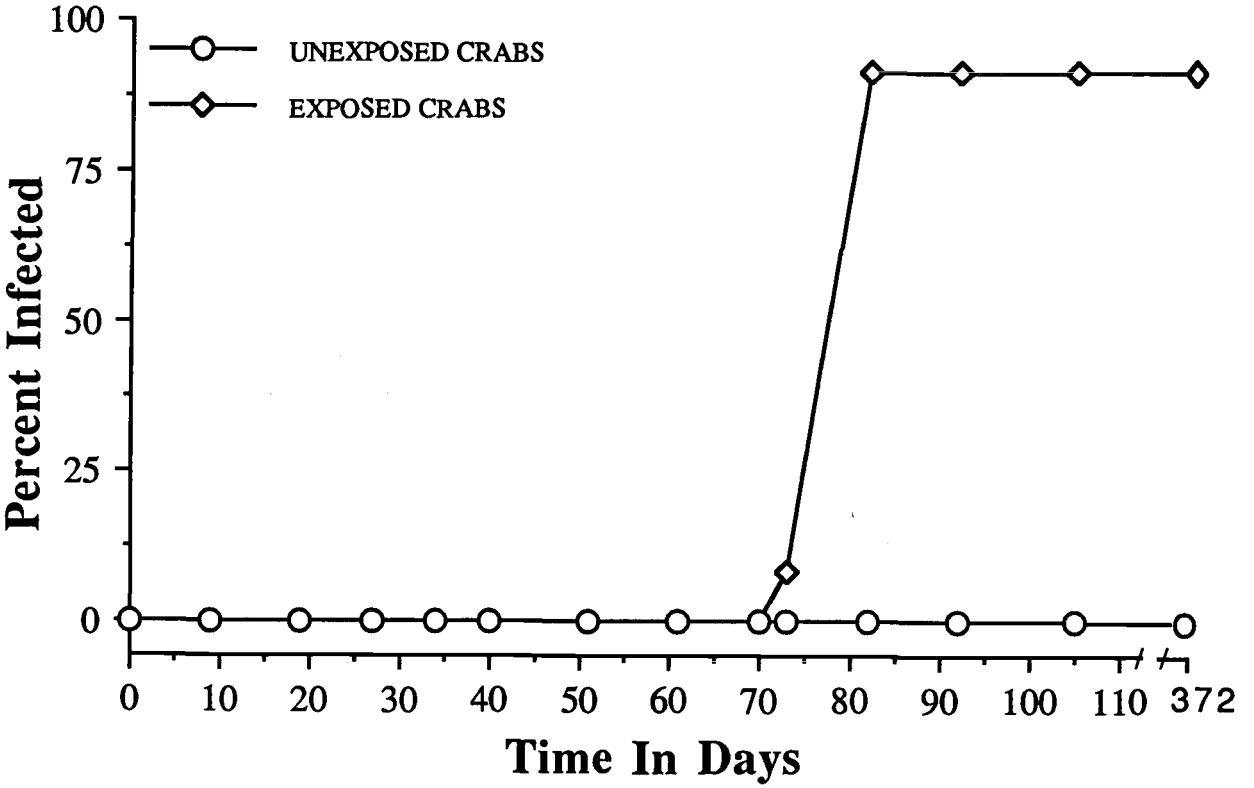

Figure 9. Percentage of *Nadelspora canceri* infections observed in 5-11 cm carapace width Dungeness crabs exposed to parasite spores by feeding, and crabs not exposed to parasite spores.Infections determined by macroscopic examination of muscle tissue through appendage articular membranes.

on days 105, 229, 297, and four crabs died between days 336 and 341 of the test. The mean day to death for these seven crabs was 295 days. Five exposed (4 infected) and all twelve unexposed crabs survived to the end of the 372 day test.

External signs of infection (white muscle) were first visible in a single exposed crab on day 73 post parasite exposure and in all eleven infected crabs when they were next examined on day 82 (Fig. 9). No indication of infection remission was detected in the eleven infected crabs and spores of *N. canceri* were confirmed in each of these crabs microscopically. No parasite spores were detected microscopically in the twelve control crabs or the single exposed crab that remained uninfected after gross examination.

Transmission of *N. canceri* in < 2.0 cm CW juvenile Dungeness crabs

Thirty-two juvenile crabs, < 2.0 cm CW, were fed crab tissue containing *N. canceri* spores and held in a common saltwater aquarium and thirty-seven unexposed control crabs < 2.0 cm CW were held in a separate saltwater aquarium. The crabs were observed regularly for the presence of *N. canceri* infection over a 75 day period and the results are shown in Table 6.

Both exposed and unexposed crabs died at various times throughout the experiment and muscle tissue from these crabs was examined microscopically for infection. Prior to day 40, two exposed and seven unexposed crabs died; all were negative for the presence of the parasite. On day 40, 11 exposed and 15 control crabs were sacrificed for microscopic examination and no evidence of infection was detected. The first macroscopic sign of infection in exposed crabs was on day 51 post parasite exposure when six of 19 crabs showed the white opaque muscle indicative of infection (Fig. 10). By day 61 post parasite exposure all exposed crabs remaining alive appeared infected by gross examination. The first spores to be detected microscopically were

Table 6. *Nadelspora canceri* infections observed in juvenile Dungeness crabs exposed to *N. canceri* spores by feeding, and crabs not exposed to *N. canceri* spores. Infections determined by microscopic examination of muscle tissue. Crabs were < 2.0 cm CW on experiment day one.

	EXPOSED		UNEXPOSED	
	n	No.Infected	*n*	No. Infected
Crabs dying before experiment day 40	2	0	7	0
Crabs sacrificed for microscopic examination on day 40	11	0	15	0
Crabs dying between day 41 and 72	8	7	6	0
Crabs surviving until end of experiment (day 75)	11	11	9	0
Totals	32	18	37	0

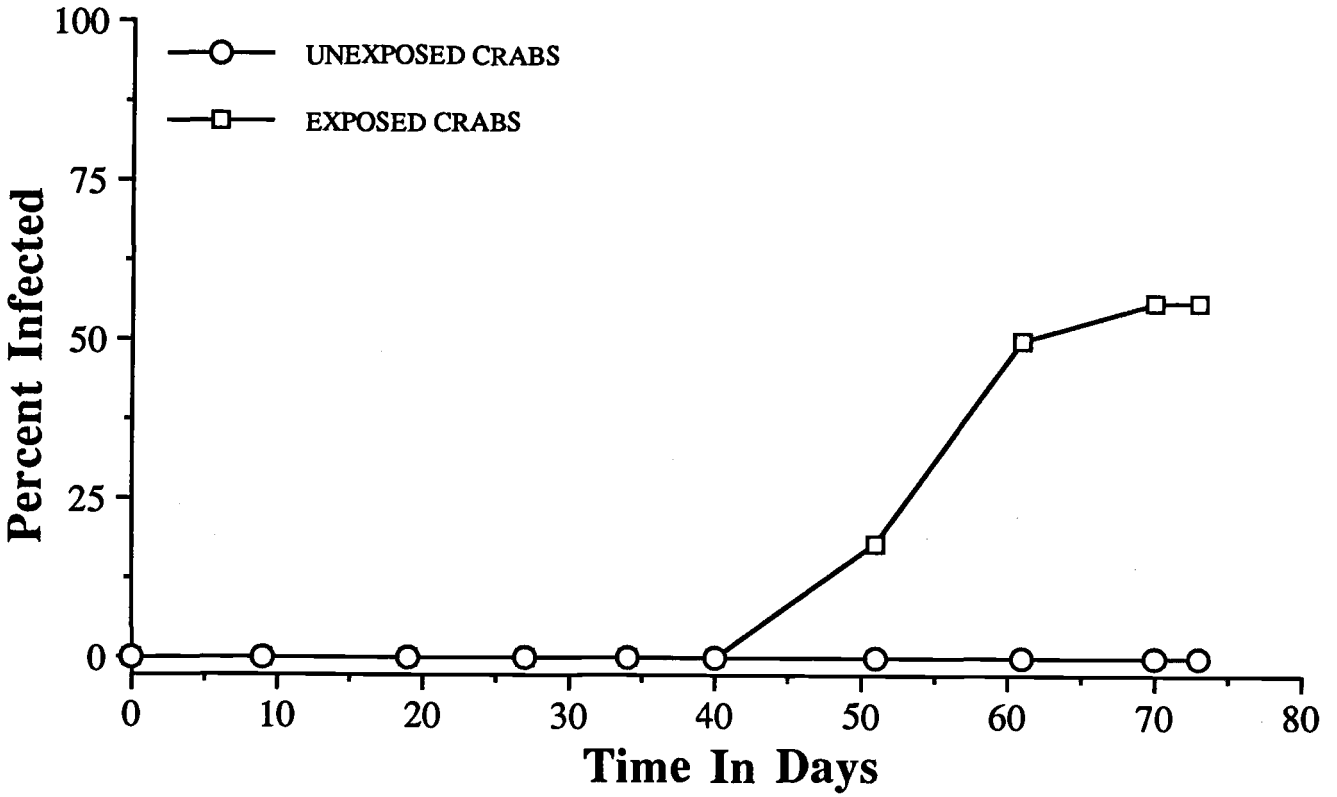

Figure 10. Percentage of *Nadelspora canceri* infections observed in < 2.0 cm carapace width Dungeness crabs exposed to parasite spores by feeding, and crabs not exposed to parasite spores. Infections determined by macroscopic examination of muscle tissue through appendage articular membranes.

from an exposed crab that died on day 54 post parasite exposure. In total, *N. canceri* infections were detected in 18 of 32 exposed crabs and in 18 of 19 exposed crabs that lived for at least 51 days (Table 6). No infections were observed in the 37 unexposed crabs (Table 6, Fig. 10).

DISCUSSION

Nadelspora canceri infections were established experimentally in 29 out of 44 Dungeness crabs (18 juveniles and 11 adults) that were fed infected muscle tissue and of the 31 crabs that were held long enough to detect *N. canceri* spores, only two (one juvenile and one adult) did not become infected. This shows that transmission is direct and that intermediate hosts or vectors are not required for establishing infection in crab hosts. Since only two crabs that were exposed and observed long enough to detect infection did not become infected, it is possible that these individuals did not ingest infected muscle tissue. The single large crab that was exposed and did not become infected molted several weeks after exposure and was therefore in a premolt condition during exposure. Crabs that are in a premolt condition often do not eat for several weeks prior to molting.

The ingestion of infectious spores by a susceptible host is believed to be a common mechanism for transmitting microsporidians between hosts in natural populations (Olson 1976, Weiser 1976, Larsson 1986), although researchers have had varying success when attempting to establish *per os* microsporidian infections in decapod hosts. *Ameson michaelis* infections have been established in blue crabs, *Callinectes sapidus,* by feeding infected tissue directly to uninfected crabs (Sprague et al. 1968, Weidner 1970, Overstreet and Whatley 1975) and *Vavraia parastacida* infections have been established experimentally in the crayfish *Cherax tenuimanus* and *Cherax albidus* also by direct feeding (Langdon and Thorne 1992).

Thelohania penaei infections were established in *P. duorarum* only after spores were "conditioned" or "primed" by passing through the digestive track of fish prior to feeding to shrimp (Iversen and Kelly 1976). Herbert (1988) failed to establish *Thelohania sp.* infections in the freshwater crayfish *C. quadricarinatus* by feeding spores directly, injecting spores, or by passing spores through fish. *Thelohania contejeani* spores were fed to the crayfish, *Orconectes virilis,* and no infections were detected after holding 20 exposed crayfish for nine months (Graham and France 1986). Muscle tissue infected with *T. duorara* was fed to 24 pink shrimp, *P. duorarum*, over a three day period and no recognizable stages of infection were detected after holding the exposed animals 30 days (Roth and Iversen 1971). A variety of experiments were conducted by Breed and Olson (1977) to determine the conditions necessary to infect crangonid sand shrimp with the microsporidian *P. crangoni* and none were successful.

Ingesting parasite spores through cannibalism has been documented as a mechanism for transmitting microsporidian infections in insects (Kellen and Lindegren 1968) and is believed to be a mechanism for transmitting microsporidians in natural decapod populations (Iversen 1969, Skurdal et al. 1990, Langdon and Thorne 1992). Cannibalism is known to occur in natural crab populations. The frequency of cannibalism in Dungeness crabs was reported by Stevens et al.(1982) who examined the stomach contents of 410 crabs and found that 18.2% had consumed other Dungeness crabs. It was shown in the present study that ingesting *N. canceri* spores by Dungeness crabs results in infection and it seems likely that cannibalism among feral Dungeness crabs is at least one mechanism for transmitting *N. canceri.*

In this study external signs of infection (white muscle) were first visible in small juvenile crabs 51 days after being exposed to *N. canceri* spores and in larger crabs 73 days after exposure. It is highly likely that only high concentrations of parasite spores, and not prespore developmental stages, cause the white discoloration of the muscle

tissue. Recognizing prespore stages of this parasite under a light microscope is difficult and methods for detecting these stages have not been refined.

Similar observations were made by Langdon and Thorne (1992) who reported clinical signs of *V. parastacida* infections in the crayfish, *C albidus*, 60 days after being exposed to parasite spores. In contrast, blue crabs experimentally infected with the microsporidian *A. michalis* showed clinical signs of "heavy infection" 15-30 days after being exposed to parasite spores (Sprague et al. 1968, Weidner 1970).

One notable difference between the experiments conducted by Weidner (1970), and Langdon and Thorne (1992), and the experiments reported here was water temperature. Weidner (1970) held infected blue crabs in 22°C water, Langdon and Thorne (1992) held infected crayfish in 18°C water, and the Dungeness crabs infected with *N. canceri* held in this study were in 9-12°C water. Temperature may influence the rate that microsporidian infections progress in decapod hosts. Olson (1976, 1981) showed that temperature strongly influenced the ability of the microsporidian *Glugea stephani* to infect fish hosts, and that parasite development rates in fish was directly related to temperature. Similar experiments on microsporidian infections in decapod hosts have not been done.

Both the mortality experiments that involved naturally infected crabs, and the transmission experiments indicated that *N. canceri* causes chronic, long term infections that are eventually lethal. Although some infected crabs lived for extended periods, mortality rates in infected crabs were always high compared to control crabs except in the transmission study involving juvenile crabs where infected and uninfected crabs died at about the same rate. Juvenile crabs may be more susceptible to handling stress compared to adult crabs and this could account for the mortality observed in both infected and uninfected juvenile crabs.

In contrast to the chronic infections caused by *N. canceri*, *C. albidus* infected with *V. parastacida* were moribund 120 after being exposed to spores (Langdon and

Thorne 1992) and *C. sapidus* infected with *A. michaelis* died within 30 days of being exposed to parasite spores (Weidner, 1970). However, as mentioned previously, these experimental animals were held at warmer water temperatures which may have influenced the rate that these infections progressed.

Although it was not intended in this study to rigorously compare the ability of infected crabs to molt with that of uninfected crabs, some infected crabs were observed to molt throughout the observation periods in all experiments.

Both infected and uninfected crabs were observed to feed actively throughout the experiments. Although as individual infected crabs became moribund, anorexia, weakness, and inability to grasp or pinch with the chilepeds was common.

In conclusion, *N. canceri* infections can be established in Dungeness crabs by direct consumption of infected crab muscle. Furthermore, infections are chronic and slowly debilitating, and ultimately result in death of the host.

Bibliography

Andreadis, T. (1985). An experimental transmission of a microsporidian pathogen from mosquitoes to an alternate copepod host. Proc. Natl. Acad. Sci. USA 82: 5574-5577

Armstrong, D. A., Burreson, E. M., Sparks, A. K. (1981). A ciliate infection *Paranophrys* sp. in laboratory held Dungeness crabs, *Cancer magister*. J. Invert. Path. 37: 201-209

Armstrong, D. A. (1983). Cyclic crab abundance and relationships to environmental causes. In: Wooster, W. (ed.) From Year to Year: Interannual variability of the environment and fisheries of the Gulf of Alaska and eastern Bering Sea. Washington Sea Grant Publ. WSG-WO 83-3, p.102-110

Armstrong, D. A., Gunderson, D. R. (1985). The role of estuaries in Dungeness crab early life history: A case study in Grays Harbor, Washington. In: B. R. Melteff (symposium coordinator). Proceedings of the symposium on Dungeness crab biology and management, 1984 Alaska Sea Grant Report 85-3, Fairbanks, p 145- 170

Atkin, M., Anderson, D., Francis, B., Hinde, J. (1989). Statistical modeling in GLIM. In: Copas, J. B., Dawid, A. P., Eagleson, G. K., Silverman, B. W. (ed.) Oxford statistical science series. Clarendon Press, Oxford

Azevedo, C. (1987). Fine structure of the microsporidian *Abelspora portucalensis* gen. n. sp. n. (microsporidia) parasite of the hepatopancreas of *Carcinus maenas* (Crustacea, Decapoda). J. Invert. Path. 49: 83-92

Barry, S. (1985). Overview of the Washington coastal Dungeness crab fishery. In: Melteff, B. R. (symposium coordinator) Proceedings of the symposium on Dungeness crab biology and management. 1984 Alaska Sea Grant Report 85-3, Fairbanks, Alaska, USA, p. 33-36

Baxter, K. N., Rigdon, R. H., Hanna, C. (1970). *Pleistophora* sp. (Microsporidia : Nosematidae) a new parasite of shrimp. J. Invert. Path. 16: 289-291

Botsford, L. W., Wickham, D. E. (1978). Behavior of age specific density dependent models and the northern California Dungeness crab, *Cancer magister*, fishery. J. Fish. Res. Board Can. 35: 833-843.

Botsford, L. W., Methot, R. D.., Johnston, W. E. (1983). Effort dynamics of the Northern California Dungeness crab, *Cancer magister*, fishery. Can. J. Fish. Aquat. Sci. 40: 337-346

Botsford, L. W. (1986). Population dynamics of the Dungeness crab, *Cancer magister*. In: Jamieson, G. S., Bourne, N. (ed.) North Pacific Workshop on Stock Assessment and Management of Invertebrates. Can. Spec. Publ. Fish. and Aquat. Sci. 92: 140-153

Botsford, L. W., Armstrong, D. A., Shenker J. M. (1989). Oceanographic influences on the dynamics of commercially fished populations. In: Landry, M. R., Hickey, B. W. (ed.) Coastal Oceanography of Washington and Oregon. Elsevier, Amsterdam, and New York, p. 511-566

Breed, G. M., Olson, R. E. (1977). Biology of the microsporidian parasite *Pleistophora crangoni* n. sp. in three species of crangonid sand shrimps. J. Invert. Path. 27: 407-408

Butler, T. (1954). Food of the commercial crab in the Queen Charlotte Islands region. Prog. Rep. Pacif. Cst. Stns. 99: 3-5

Canning, E. U. (1977). Microsporidia. In: Kreier, J. P. (ed.) Parasitic Protozoa, Vol. IV. Academic Press, New York, San Francisco, London, p. 155-196

Canning, E. U. (1982). An evaluation of protozoal characteristics in relation to biological control of pests. Parasitology 84: 119-149

Canning, E. U., Lom, J. (1986). The microsporidia of vertebrates. Academic Press. London

Canning, E. U. (1990). Phylum Microspora. In: Margulis, L., Corliss, J. O., Melkonian, M., Chapman. D. J. (ed.) Handbook of Protoctista. Jones and Bartlett, Boston, p. 53-72

Carter, C. N., Radke, H. (1991). Selected economic aspects of the Alsea Bay crab fishery. Oregon. Dept. Fish. Wildlife Report, July 1991

Cossins, A. R. (1973). *Thelohania contajeani* Henneguy. Microsporidian parasite of *Austropotamobius pallipes* Lereboullet-an histological and ultrastructural study. Freshwater Crayfish 1: 151-164

Couch, J. A. (1983). Diseases caused by Protozoa. In: Provenzano, A. J. Jr. (ed.) The Biology of Crustacea, Vol. 6, Pathobiology. Academic Press, New York, San Francisco, London. p. 155-196

Demory, D. (1985). An overview of Oregon's Dungeness crab fishery with management concept for the future. In: Melteff, B. R. (symposium coordinator) Proceedings of the symposium on Dungeness crab biology and management. 1984 Alaska Sea Grant Report 85-3, Fairbanks, Alaska, USA, p. 27-32

Diamond, N., Hankin, D. G. (1985). Movements of female Dungeness crabs, *Cancer magister*, in Northern California based on tag recoveries. Can. J. Fish. Aquat. Sci. 42: 919-926

Findley, A. M., Blakeney, E. W. Jr., Weidner, E. H. (1981). *Ameson michaelis* (Microsporidia) in the blue crab, *Callinectes sapidus* : Parasite induced alterations in the biochemical composition of host tissues. Biol. Bul. 161: 115-125.

Gotshall, D. W., (1977). Stomach contents of Northern California Dungeness crabs, *Cancer magister*. Calif. Fish and Game 63 (1): 43-51

Gotshall, D. W., (1978) Catch-per-unit-of-effort studies of Northern California Dungeness crab, *Cancer magister*. Calif. Fish and Game 64: 189-199

Graham, L., France, R. (1986). Attempts to transmit experimentally the microsporidian *Thelohania contejeani* in freshwater crayfish *Orconectes virilis*. Crustaceana 51: 208-211

Gunderson, D. R., Armstrong, D. A., Shi, Y., McConnaughey, R. A. (1990). Patterns of estuarine use by juvenile English sole, *Parophrys vetulus*, and Dungeness crab, *Cancer magister*. Estuaries 13: 59-71

Hankin, D. G. (1985). Proposed explanations for fluctuations in abundance of Dungeness crab: a review and critique. In: Melteff, B. R. (symposium coordinator) Proceedings of the symposium on Dungeness crab biology and management. 1984 Alaska Sea Grant Report 85-3, Fairbanks, Alaska, USA, p. 305-326

Hankin, D. G., Diamond, N., Mohr, M. (1985). Molt increments, annual molting probabilities and survival rates of adult female Dungeness crabs in Northern California. In: Melteff, B. R. (symposium coordinator) Proceedings of the symposium on Dungeness crab biology and management. 1984 Alaska Sea Grant Report 85-3, Fairbanks, Alaska, USA, p. 189-206

Healy, M. J. R. (1988). GLIM an introduction. Clarendon Press, Oxford

Herbert, B. W. (1988). Infection of *Cherax quadricarinatus* (Decapoda: Parastacidae) by the microsporidian *Thelohania* sp. (Microsporidia: Nosematidae). J. Fish Dis. 11: 301-308

Hobbs, R. C., Botsford, L. W., Thomas. A.(1992). Influence of hydrographic conditions and wind forcing on the distribution of Dungeness crab, *Cancer magister*, larvae. Can. J. Fish. Aquat. Sci. 49: 1379-1388

Iversen, E. S., (1969). Microsporidiosis in commercial penaeid shrimp. In: Mistakidis, M. N. (ed.) Proceedings of the world scientific conference on the biology and culture of shrimps and prawns, Vol. 3, Experience Papers. FAO Fish. Rep. No. 57 p. 1135-1140

Iversen, E. S., Kelly, J. F. (1976). Microsporidiosis successfully transmitted in Pink Shrimp. J. Invert. Pathol., 27: 407-408

Kaplan, E. L., Meier, P. (1958). Nonparametric estimation from incomplete observations. J. Amer. Statist. Assoc. 53: 475-481

Kellen, W. R., Lindegren, J. E. (1968). Biology of *Nosema plodiae* sp. n., a microsporidian pathogen of the Indian meal moth, *Plodia interpunctella* (Hübner), (Lepidoptera: Phycitidae). J. Invert. Path. 11:104-111

Kelly, J. F. (1979). Tissue specificities of *Thelohania duorara*, *Agmasoma penaei*, and *Pleistophora sp.*, microsporidian parasites of pink shrimp, *Penaeus duorarum*. J. Invert. Pathol. 33: 331-339

Langdon, J. S. (1991). Microsporidiosis due to a pleistophorid in marron, *Cherax t enuimanus* (Smith), (Decapoda: Parastacidae) J. Fish Dis. 14: 33-44

Langdon, J. S., Thorne, T. (1992). Experimental transmission *per os* of microsporidiosis due to *Vavraia parastacida* in the marron, *Cherax tenuimanus* (Smith), and yabby, *Cherax albidus* (Clark). J. Fish Dis. 15: 315-322

Larsson, R. (1986). Ultrastructure, function, and classification of microsporidia. In: Corliss, J. O., Patterson, D. J. (ed.) Progress in Protistology, Vol. 1. Biopress Ltd., Bristol U. K. p.325-390

McConnaughey, R. A., Armstrong, D. A., Hickey, B. M., Gunderson, D. R (1992). Juvenile Dungeness Crab (*Cancer magister*) recruitment variability and oceanic transport during the pelagic larval phase. Can. J. Fish. Aquat. Sci. 49: 2028-2044

Methot, R. D. Jr. (1990). Management of a cyclic resource: the Dungeness crab fishery of the pacific coast of North America. In: Caddy, J. F. (ed.) Marine invertebrate fisheries: their assessment and management. Wiley Publ., New York. p. 205-223

Meyers, T. R., Schirley, T. C., Schirley, S. M., Sparks, A. K., Morado, J. F.(1985). A review of Dungeness crab diseases in the Pacific Northwest and Alaska. In: Melteff, B. R. (symposium coordinator) Proceedings of the symposium on Dungeness crab biology and management. University of Alaska Sea Grant Report 85-3, Fairbanks, Alaska, USA, p. 209-222

Morado, J. F., Sparks, A. K. (1988). A review of infectious diseases of the Dungeness Crab, *Cancer magister*. In: Shumway S. E. (ed.) Proceedings of the annual meeting of the National Shellfish Association. J. Shellfish. Res. Vol. 7, No. 1. p.127

O'Bannon, B. K. (1993). Fisheries of the United States, 1991. National Marine Fisheries Service, Fisheries Statistics Division, NOAA, Silver Spring, MD.

Olson, R. E. (1976). Laboratory and field studies on *Glugea stephani* (Hagenmuller), a microsporidian parasite of pleuronectid flatfishes. J. Protozoology 23(1): 158-164

Olson, R. E. (1981).Effects of low temperature on the development of the microsporidian *Glugea stephani* in English sole, *Parophrys vetulus*. J. Wild. Dis. Vol. 17, No. 4. p 559-582

Olson, R. E. Lannan, C. A. (1984). Prevalence of microsporidian infection in commercially caught pink shrimp *Pandalus jordani*. J. Invert. Path. 43: 407-413

Olson, R. E, Tiekotter, K. L., Reno, P. W. (1994) *Nadelspora canceri* N. G., N. Sp., an Unusual Microsporidian Parasite of the Dungeness crab, *Cancer magister* J. Euk. Microbiol. 4 (14): 337-347

Ossiander, F. J., Wedemeyer, G. (1973). Computer program for sample sizes required to determine disease incidence in fish populations. J. Fish Res. Board Can. 30: 1383-1384

Overstreet, R. M. (1973). Parasites of some penaeid shrimps with emphasis on reared hosts. Aquaculture 2: 105-140

Overstreet, R. M., Whatley, E. C., (1975). Prevention of microsporidiosis in the blue crab, with notes on natural infections. In: Avault, J. W., Miller, R. (ed.) Proceedings of the sixth annual meeting world mariculture society. Louisiana State University, Baton Rouge, Louisiana, p. 335-355

Overstreet, R. M. (1988). Microsporidiosis of the blue crab. In: Sindermann, C. J., Lightner, D. V. (ed.) Developments in aquaculture and fisheries science. Vol. 17, Disease diagnosis and control in North American marine aquaculture. Elsevier, Amsterdam, p. 200-202

Owens, L., Glazebrook, J. S. (1988). Microsporidiosis in prawns from Northern Australia. Aust. J. Mar. Freshwater Res. 39: 301-305

Pacific fisheries Management council (1979). Fishery management plan for the Dungeness crab Fishery of Washington, Oregon and California. Portland, Oregon

Parsons, D. G., Khan, R. A. (1986). Microsporidiosis in northern shrimp, *Pandalus borealis*. J. Invert. Path. 47: 74-81

Pauley, G. B., Armstrong, D. A., Heun, T. W. (1986). Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest)--Dungeness Crab. U.S. Fish Wildl. Serv. Biol. Rep. 82(11.63). U.S. Army Corps of Engineers, TR EL-82-4

Perkins, F. O. (1991). Sporozoa: Apicomplexa, Microsporidia, Haplosporidia, Paramyxea, Myxosporidia, and Actinosporidia. In: Harrison, F. W., Corliss, J. O. (ed.) Microscopic Anatomy of Invertebrates, Vol. 1. John Wiley and Sons, Inc. New York, p. 261-331

Roth, J. N., Iversen, E. S. (1971). Attempts to transmit experimentally the microsporidian *Thelohania duorara*, parasitizing the pink shrimp, *Penaeus duorarum*. Trans. Amer. Fish. Soc. 100: 369-370

Sindermann. C. J. (1990). Principle Diseases of Marine Fish and Shellfish. Second Edition, Vol. 2, Diseases of Marine Shellfish. Academic Press, Inc. New York, Boston, London. p. 93-144

Skurdal, J., Taugbøl, T., Fjeld, E., Hessen, D. O., Hasten, T. (1988). *Thelohania contajeani* Henneguy parasitizing the noble crayfish, *Astacus astacus* L., in Norway. J. Fish Dis. 11: 433-435

Skurdal, J., Qvenild, T., Taugbøl, T., Fjeld, E. (1990). A 6-year study of *Thelohania contejeani* parasitism of the noble crayfish, *Astacus astacus* L., in Lake Steinsfjorden, S. E. Norway. J. Fish Dis. 13: 411-415

Sparks, A. K., Hibbits, J., Fegley, J. C. (1982). Observations on the histopathology of a systemic ciliate *Paranophrys* sp. disease in the Dungeness crabs, *Cancer magister*. J. Invert. Path. 39: 219-228

Sparks, A. K. (1985). Synopsis of invertebrate pathology. Elsevier Science Publishers, Amsterdam, Oxford, New York

Sparks, A. K., Morado, J. F. (1985). A preliminary report on the diseases of Alaskan king crabs. In: Melteff, B. R. (symposium coordinator) Proceedings of the international king crab symposium. 1985 Alaska Sea Grant Report 85-12, Anchorage, Alaska, USA, p333-338

Sprague, V. (1965). *Nosema* sp. (Microsporidia Nosematidae) in the musculature of the crab *Callinectes sapidus* . J. of Protozoology. 12(1): 66-70

Sprague, v., Vernick, S. H., Lloyd, B. J. (1968). The fine structure of *Nosema sp.* Sprague, 1965 (Microsporidia, Nosematidae) with particular reference to stages in sporogony. J. Invert. Path. 12: 105-117

Sprague, V. (1970). Some protozoan parasites and hyperparasites in marine decapod crustacea. In: Snieszko, S. F. (ed.) A symposium on diseases of fish and shellfish. Spec. Pub. 5, Amer. Fish. soc. Washington D. C., p. 416-430

Sprague, V., Vávra, J. (1976). Preface. In: Bulla, L. A. Jr. Cheng, T. C (ed.) Comparative Pathobiology, Vol. 1, Biology of the Microsporidia. Plenum Press, New York and London, p. ix-xiii

Sprague, V. (1977). Classification and phylogeny of the microsporidia. In: Bulla, L. A. Jr., Cheng, T. C. (ed.) Comparative Pathobiology, Vol. 2, Systematics of the Microsporidia. Plenum Press, New York and London, p. 1-30

Sprague, V. (1977). Annotated list of species of Microsporidia. In: Bulla, L. A. Jr., Cheng, T. C. (ed.) Comparative Pathobiology, Vol. 2, Systematics of the microsporidia. Plenum Press, New York and London, p. 31-334

Sprague, V. (1977). The zoological distribution of Microsporidia In: Bulla, L. A. Jr., Cheng, T. C. (ed.) Comparative Pathobiology, Vol. 2, Systematics of the Microsporidia. Plenum Press, New York and London, p. 335-386

Stevens, B. G., Armstrong, D. A., Cusimano, R. (1982). Feeding habits of the Dungeness crab, *Cancer magister*, as determined by the index of relative importance. Mar. Biol. 72: 135-145

Stevens, B. G., Armstrong, D. A. (1984). Distribution, abundance, and growth of juvenile Dungeness crabs, *Cancer magister*, in Grays Harbor Estuary, Washington. Fish. Bull. U. S. 82: 469-483

Summerfelt, R. C. (1972). Studies on the transmission of *Pleistophora ovariae*, the ovary parasite of the golden Shinner, *Notemigonus crysoleucas* . Final Report, Project No. 4-66-R. Nat. Marine Fish. Service p. 1-19

Tegelberg, H. (1972). State of Washington Dungeness crab Study, August 1972. A. Prog. Rep. U. S. Fish and Wildl. Serv. Project No. 1-76-R: 15-18

Vivares, C. P., Sprague, V. (1979). The fine structure of *Ameson pulvis* (Microspora, Microsporidia) and its implications regarding classification and chromosome cycle. J. Invert. Path. 33: 40-52

Vivares, C. P., Cuq, J. L. (1981). Physiological and metabolic variations in *Carcinus mediterraneas* (Crustacea: Decapoda) parasitized by *Thelohania maenadis* (Microspora: Microsporidia): an ecophysiopathological approach. J. Invert. Path. 37: 38-46

Vivares, C. P., Azevedo C. (1988). Ultrastrucural observations of the life cycle stages of *Ameson atlantica* Sp. nov., a microsporidian parasitizing *Cancer pagurus* L. J. Fish Dis. 11: 397-387

Warner, R. W. (1987) Age and growth of male Dungeness crabs, *Cancer magister*, in Northern California. Calif. Fish and Game 73: 4-20

Weidner, E. (1970). Ultrastructure study of microsporidian development I. *Nosema* sp. Sprague, 1965 in *Callinectes sapidus*. Zeitschrift Zellforsch. 105: 34-54

Weidner, E. (1972). Ultrastructure study of microsporidian invasion into cells. Zeitschrift Parasitenk. 40: 227-242

Weidner, E. (1976). The microsporidian spore invasion tube. The ultrastructure, isolation, and characterization of the protein comprising the tube. J. Cell Biol. 71: 23-34

Weidner, E., Byrd, W., Scarborough, A., Pleshinger, J., Sibley, D. (1984). Microsporidian spore discharge and the transfer of polarplast organelle membrane into plasma membrane. J. Protozool. 31(2): 195-198

Weiser, J. (1976). Microsporidia in invertebrates: host-parasite relations at the organism level. In: Bulla, L. A. Jr. Cheng, T. C. (ed.) Comparative Pathobiology, Vol. 1, Biology of the Microsporidia. Plenum Press, New York and London, p. 163-202

Wild, P. W. (1980). Effects of sea water temperature on spawning, egg development, hatching success, and population fluctuations of the Dungeness crab, *Cancer magister*. CalCOFI Rep. 21:115-120