2004

Genetic analysis of larval dispersal of dungeness crab, Cancer Magister

Valliammal Chockalingam
San Jose State University

GENETIC ANALYSIS OF LARVAL DISPERSAL OF DUNGENESS CRAB, *CANCER MAGISTER*

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San Jose State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

Valliammal Chockalingam

May 2004

UMI Number: 1420434

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

APPROVED FOR THE DEPARTMENT OF BIOLOGICAL SCIENCES

Dr. Leslee Parr

Dr. Vida Kenk

Dr. Shannon Bros

Dr. Curtis Roegner, National Marine Fisheries, National Oceanographic and Atmospheric Administration.

APPROVED FOR THE UNIVERSITY

ABSTRACT

GENETIC ANALYSIS OF LARVAL DISPERSAL OF DUNGENESS CRAB, *CANCER MAGISTER*

By Valliammal Chockalingam

Genetic variability of *Cancer magister* megalopae was compared over a three-year period (1997 – 1999). The genetic structure of megalopae within and among larval groups collected at sea or during recruitment to the Coos Bay estuary was evaluated by sequencing 350 base pairs of the Cytochrome Oxidase I subunit of mitochondrial DNA. Fifty-two different haplotypes were obtained from 137 megalopae. Phylogenetic analysis showed six maternal lineages. Nucleotide diversity both within and among larval groups was <1.6%. Haplotype diversity was found to be high within patches/cohorts but no heterogeneity in haplotype frequency was found among patches/cohorts. This suggests that the larvae are mixing at sea from different sources. Oceanic current systems differed during the collection years, which provided an opportunity to determine current system effects on larval dispersal. Genetic differentiation was also found among megalopae collected from different years ($P<0.05$).

ACKNOWLEDGEMENTS AND DEDICATIONS

I wish to thank those people who in some way helped me in the completion of this work.

First and foremost, I am indebted to Dr. Leslee Parr, my graduate advisor, for so much of her invaluable time and energy spent offering constant support, patience, and guidance. Her generosity of spirit and temperate manner made the process of learning satisfying and enjoyable. I was given a chance to develop ideas on my own and make mistakes I could learn from, but I always knew that support would be there when I asked for it. At times the support came before I knew I needed to ask, which was greatly appreciated as well.

I would like to thank my committee members Dr. Vida Kenk, Dr. Shannon Bros, and Dr. Curtis Roegner (NOAA), for their enthusiastic support and interest in the research that led to this thesis. I thank them for their time and patience reading an early draft and helping to shape it into something that holds together and covers the appropriate grounds.

This project would not have been possible without Dr. Curtis Roegner (NOAA) and Dr. Alan Shanks (University of Oregon) who graciously provided samples of *Cancer magister*. I also like to thank them for their helpful discussion about oceanography and biology of Dungeness crab.

I sincerely thank these individuals for their support: Darcy Levee and Christine Nelson (Biology department) for sequencing my samples on time, Natasha Khuri for helping me choose the right software to analyze the results, Danielle Palmer and Amy Fontarensky (lab members) especially for their friendship and support.

Financial support for this research came from Dr. Leslee Parr, Professional development funds from the College of Science, Arthur and Karin Nelson Scholarship, and Albert and Dorothy Ellis Research Fellowship from the Biology Department Scholarship Committee.

Most importantly, thanks to my husband for his countless hours of patience and understanding in allowing me to complete this work and pursue an advanced degree. Without his support and encouragement, this effort would not have been possible. I wish to thank my parents for their motivation and support during this entire journey. This work is dedicated to my husband Ramasamy Subramanian, my daughter Srinidhi Meenakshi Ramasamy, my parents Chockalingam Ramanathan and Suseela Chockalingam.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

In general, species with the lowest potential for dispersal have the highest genetic variability among different locales but are relatively homogeneous within populations, whereas species characterized by highest dispersal potential, may show little or no spatial genetic variation among populations but may have high or low variability within populations (Avise 1994). Many marine species have a long duration planktotrophic larval stage and thus, a high dispersal phase. In these species, the absence of genetic variation over vast areas in the majority of marine species is not surprising. Many marine species do not show any apparent spatial genetic segregation across vast areas for example, populations of the bivalves *Mya arenaria* in New England (Caporale et al. 1997) and *Donex deltoid* in SE Australia (Murray-Jones & Ayre 1997). Investigations of the gene flow of mid-ocean ridge vent organisms has revealed extremely high levels of homogeneity among populations over vast areas, indicating that larval transport is great enough to prevent isolation of uncommon alleles (Karl et al. 1996). Similarly, Silberman et al. (1994) observed low genetic variation among populations in post larval spiny lobster (*Panulirus argus*) recruiting to the Florida Keys at different seasons and they concluded that larvae were mixing at sea.

However, there have been several cases in which high genetic variation among populations has been observed in species with long dispersal phases. Stevens (1991) detected genetic differentiation in pea crabs despite the possibility for extensive gene flow through dispersal of zoeal and megalopal stages. Similarly, Bunch et al. (1998) measured high diversity in Tanner crab populations within the Gulf of Alaska and related

the pattern of heterogeneity to larval transport. In association with this, Rocha-Olivares and Vetter (1998) compared mtDNA sequences of rockfish populations from the coasts of California, Oregon, British Columbia and the Gulf of Alaska. It was found that significant population structure existed between these populations of rockfish and that a biogeographic division between the Oregonian and Aleutian Provinces might be restricting transport of larvae. Moran et al. (1997) found very large differences between salmon populations in both a single watershed and along the coast of northern California. Tam et al. (1996) and Van Syoc (1994) measured variation in the genetic structure of populations of mole crabs and goose barnacles respectively, across the equatorial boundary between North and South America. Lavery et al. (1995) found that allozymes in the coconut crab *Birgus latro* were significantly different between the populations in the Indian Ocean and those in the Pacific. It is now clear that, while organisms lacking a long dispersal phase are more likely to exhibit heterogenetic populations, a long planktonic life does not guarantee widespread gene flow.

High genetic variation among population of species with high dispersal may be due to several reasons. Most species of marine benthic invertebrates reproduce with planktonic larvae that spend days to months in the water column before settlement to the substrate. Spatio-temporal variation in this planktonic dispersal phase directly affects population dynamics, gene flow, speciation and genetic structure of adult populations (Hedgecock 1986; Avise 1994; Roughgarden et al. 1988; Grosberg and Levitan 1992). One of the reasons may be, even though there is unrestricted larval transport, differential mortality of the larvae selects for different genotypes among populations. Another reason

might be restricted larval transport. Either oceanographic barriers (Rocha-Olivares and Vetter 1998) or behavioral mechanisms can restrict larval transport between populations. Larval swimming behavior coupled to physical oceanographic processes determines dispersal pattern (Young & Chia 1987; Young 1990), which in turn affects mixing of larvae. Brasher et al., 1992 distinguished populations from southern Australia from New Zealand populations of *Jasus verreauxi* in spite of their long pelagic phase. In the same geographical area Ovenden et al. 1992 found genetically homogenous populations of *Jasus edwardsii.* The only difference between these two lobster species was that *Jasus verreauxi* seem to regulate their distributions by vertical migratory behavior in combination with current of different directions to remain close to their initial habitat (Brasher et al. 1992). If there is restricted larval transport, there won't be any mixing of populations, which lead to high genetic variation.

Genetic studies of larvae are necessary to fully understand the larval mixing process that ultimately controls the gene flow among the populations. Until recently, studies of marine population ecology have concentrated on adults. Studying adult population structure allows inferences to be drawn about the net effects of gene flow (i.e. larval dispersal) and local selection, but studies of larvae are necessary to fully characterize the sources of genetic variation in adult populations (Hedgecock 1986; Grosberg and Levitan 1992). The genetic characteristics of adults are reflected in their progeny, and so the genetic variability within and among larval settlement cohorts (groups of megalopae returning together) reflects present dispersal trajectories and

oceanic mixing processes. To our knowledge, the genetics of marine larvae have been measured in only one previous investigation (Silberman et al. 1994).

Cancer magister is a large, mobile crustacean that inhabits sandy and sand-silt habitats in estuaries and near shore zones from southern California to the Aleutian Islands. Mating occurs from May to June in Washington in offshore locations. Female crabs migrate to high salinity waters to release zoeae, thus ensuring rapid transport of larvae to the coastal ocean. Eggs are extruded between October to March in Oregon, October to December in Washington. An egg mass may contain from 1 to 2 million eggs (Wild 1983) and a female may produce up to five million eggs in 3 or 4 broods during her lifetime (MacKay 1942). Eggs mature in about 2 to 3 months. The hatching season commonly shortens from north to south along the Pacific coast. Eggs hatch in coastal waters from January to April in Oregon and Washington coast.

The pelagic larvae of *Cancer magister* (Dungeness crab) (Dana 1852) are good candidates for examining genetic variation in a species with a long distance dispersal phase. The Dungeness crab, like many benthic decapod crustaceans, has a complex life cycle characterized by a relatively long (3 to 4 month) pelagic phase but its genetics have not been examined. However, the length of the pelagic period is temperature dependent (Wild 1983); estimates of development times range from 70 to 180 days, with faster development times in southern regions (Mohoney et al. 1994). Depending on latitude, larvae undergo 5 zoeal stages before molting into the large (10mm carapace) and strong swimming megalopae. From January to June, larvae are found in the coastal ocean and Puget Sound (Reilly 1983, Hobbs & Botsford 1992), although few zoeae are found in

estuaries. Once larvae have molted into the megalopae, their ecological role is to procure a settlement site. Megalopae settle and metamorphose into first crab instars exclusively in the near shore zone and estuaries, usually in less than 20mdepth (McConnaughey et al. 1992). Very high settlement densities occur in estuaries compared to coastal sites and both growth and survival are enhanced in estuaries (Dumbauld et al. 1993). The crabs mature after two to three years with a lifespan of 8 to 10 years. Adult crabs are large, mobile crustacean that inhabits sandy and sand-silt habitats in estuaries and near shore zones from southern California to the Aleutian Islands.

Although Dungeness crab's relatively long pelagic phase maximizes the potential for dispersal and mixing of populations (Avise 1994), there are four compelling reasons to expect detectable genetic variation among populations of this species. They are larval swimming behavior, patchy aggregations at sea, and recruitment pulses in estuarine systems and variation in oceanic currents.

Larval swimming behavior can significantly affect dispersal (Jacoby 1982; Booth et al. 1985; Jameison and Phillips 1988; Shenker 1988; Hobbs et al. 1992). *C. magister* larvae migrate vertically, and because horizontal currents vary with depth, this behavior has been shown to retard larval transport in both oceanic (Peterson et al. 1979; Bucklin et al. 1989) and estuarine systems (Bousfield 1955; Cronin & Forward 1982). This might also influence the direction and speed of oceanic transport. Their swimming process allows for extensive excursions that provide the potential to exploit currents in deep water masses. Jamieson and Phillips (1993) found very high accumulations of megalopae below 140 m in Puget Sound. They related it to behaviorally mediated retention within

system. Jacoby (1982) and Shenker (1988) speculated that vertical migrations in response to light and pressure could form subsurface aggregations of larvae in the ocean. These vertical migration patterns might strongly affect dispersal; reduce the mixing of larvae in ocean, which in turn increase genetic variation among this species.

Investigations of crab dispersal (Shenker 1988) have revealed that larvae are aggregated into discrete patches at sea on horizontal scale of 5 km. Some of these aggregations may be due to accumulation of megalopae in the convergent zones of frontal features. Another reason for this aggregation might be that the female crabs release the eggs as an egg mass. An egg mass may contain from 1 to 2 million eggs (Wild 1983) and a female may produce up to five million eggs in 3 or 4 broods during her lifetime (MacKay 1942). When the eggs mature to form zoeae, these zoeae migrate to the ocean. The patchy cross-shelf patterns indicates larvae are subjected to varying horizontal flow regimes, which in turn suggests differing dispersal trajectories for the different patches. It is unknown if these aggregations persist from the time of larval release or if the megalopae from various sources mix at sea. If these aggregations are from different sources and not the result of mixing of various sources at sea, this might cause genetic variation among Dungeness crab population.

Roegner et al. 1999 found that the megalopae recruit to estuarine systems (Coos Bay) in discrete pulses (a recruitment cohort). There are commonly large imports of megalopae to the estuary, lasting several days, separated by low or zero counts. The megalopae recruiting at one particular day are considered as a separate recruitment event (a recruitment cohort). This episodic nature of larval delivery may be related to the

patchy distributions of megalopae at sea. If larvae are segregated at sea and delivery is non-uniform among the coastal sites, then population genetic structure can result. Thus determining the genetic relatedness of the larval patches at sea and the recruitment cohorts at bay would greatly improve our understanding of Dungeness crab larval dispersal processes.

Finally, variation in ocean currents can affect larval dispersal. Transport of the larvae will depend in part on the predominant currents in this region. The California current system off Washington, Oregon and northern California consists of southward flowing California Current and the northward flowing California Undercurrent which surfaces as the Davidson Current in the fall and winter (Hickey 1979, 1989). Flows during the winter are typically southward in the upper layer offshore and northward inshore and beneath the upper layers offshore. After the spring transition, the California current moves inshore and flows is typically southward at the surface and northward below (Hickey 1979, 1989; Strub et al. 1987b). Thus during the first 3 months of the year, larvae will be transported north by the Davidson current (Johnson et al. 1986). After the spring transition, larvae will be transported south by the California current.

Previous investigations of Dungeness crab populations based on morphology suggest population differentiation. DeBrosse et al. (1990) found significant differences in size of megalopae between Puget Sound and those captured in the Strait of Juan de Fuca, offshore Washington and Southwest Vancouver Island. In addition to the megalopal size differences, DeBrosse et al. also found *Cancer magister* megalopae differed in number and morphology of spines and setae between Puget Sound and oceanic stocks. McMillan

(1995), Dinnel et al. (1993), Oresanz and Gallucci (1988) and DeBrosse et al. (1990) reported two distinct pulses of *C. magister* megalopae settlement in the Juan de Fuca Strait and Puget Sound. They concluded that the differences were probably due to a combination of genotypic and phenotypic factors unique to distinct spawning stocks, but that there must be some degree of import/export of larvae between Puget Sound and the Pacific Ocean. If such traits are heritable, it may be possible to detect genetic differences among seasonal cohorts of Dungeness crab megalopae and so may provide direct evidence of differential dispersal of megalopae along the Pacific coast.

The aim of this study is to determine if larval crab populations are genetically homogenous or if there are genetically distinct populations because of physical barriers to larval dispersal or larval behavior that create differential larval transport. DNA sequence analysis can be used to detect genetic variability of Dungeness crab megalopae collected along the coastline of Washington and Oregon. Genetic variability was measured both within and among populations of megalopae collected at sea as patches and also those recruiting to the Coos Bay estuary. If the larvae are mixing at sea it is likely that the genetic variation will be low between patches/cohorts but can be high, low or moderate within patches/cohorts. If the larvae don't mix at sea then genetic variation will be low within population but high genetic variation between patches/cohorts.

Oceanographic current systems prevalent during the larval dispersal period vary between years, especially during El Niño and La Niña events. This gives us an opportunity to determine if oceanic currents could be responsible for larval population segregation because samples were taken during these events. The El Niño-Southern

Oscillation (ENSO) phenomenon is an interannual disturbance of the climate system characterized by weak upwelling and warmer surface temperatures in the equatorial Pacific Ocean every 4-7 years. El Niño is usually followed by La Niña, which is dominated by low temperatures and strong upwelling. During El Niño, there is enhanced pole ward transport while during La Niña transport is minimized. Samples were collected for the current study during the period from 1997 to 1999. 1997 was considered a normal year for current patterns. El Niño began during August 1997 and was measurable off the Pacific Northwest through early summer 1998. La Niña occurred during 1999. Therefore, Megalopae collected during 1997 would have likely come from local crab populations. However, during El Niño in 1998, the source of megalopae would be primarily from the south and during La Niña in 1999, the source of megalopae would be more from the North.

I chose to use mitochondrial DNA analysis (MtDNA) rather than nuclear DNA for my analysis in this study. MtDNA analysis has been used extensively to examine genetic population structure in natural populations, and it often shows greater resolution of genetic differences than nuclear genes (Moritz. 1994). MtDNA is especially useful to detect variation between populations because it is usually more prone to genetic drift than are nuclear loci (Birky et al. 1989). The transmission of mtDNA is usually maternal and non-recombinational and has a rapid rate of evolution. MtDNA genealogies have been applied extensively to the study of population structure and in many cases have revealed genetic patterns that are readily interpreted in terms of habitat distribution, geography and life histories of the particular organisms, such as mating systems, and larval distribution

(reviewed by Avise. 1994). High genetic variation of mitochondrial Cytochrome Oxidase subunit (COI) gene has been observed in several taxa related to Dungeness crab including tanner crabs, mole crabs, penaeid shrimps and barnacles.

MATERIALS AND METHODS

Megalopae were collected both at sea and at coastal sites. During 1997, 1998 and 1999 Dr. Curtis Roegner (NOAA Fisheries) and Dr. Alan Shanks (University of Oregon) collected megalopal or larval samples of *Cancer magister* from ocean surveys. The 1997 and 1998 cruises were made off the coast of Coos Bay, Oregon between 43.3 and 44.0° N latitude during cruises of the NOAA ship R/V *McArthur* (Figure 1). The cruise dates were 24 to 28 June 1997, and 26 May to 1 June 1998. The 1999 cruise was conducted on the trawler *Lady Kaye* in an area offshore of Willapa Bay and Grays Harbor, Washington (46.5 to 47.2° N and 125.0 to 124.3° W) (Figure 1). All collections were made using a grid of stations extending from the near shore zone to well off the continental shelf. During the Lady Kaye cruise, the grid was sampled twice in late May 1999: Survey 1 from 23-25 May and Survey 2 from 29-30 May. Each survey took ~1.75 days to complete.

The surface concentration of megalopae was sampled with a Manta style neuston net (1.5 x 0.5 m mouth dimensions, 300 μm mesh net), which is designed to sample organisms near the ocean surface. The net was towed for 9 to 11 minutes at 2 knots, and the volume of water filtered through the net (averaging 400 m^3) was measured with a General Oceanics™ flowmeter. The larvae used for the genetics analysis were sub sampled from the net and either placed in 70% ethanol (1997) or frozen (1998, 1999). For this study, we used 20 samples from 1997, 18 from 1998 and 22 from 1999.The remaining larvae were sorted in the laboratory and concentration was standardized by tow

volume to yield individuals m^{-3} (ind. m^{-3}). Oceanographic conditions were measured during the cruise with a Seabird CTD model SBE 19.

Within the estuaries, light traps were used to collect time series of larval crab. Light traps function as behavioral samplers and depend upon the positive phototropism of organisms towards artificial illumination. The traps, which floated at the surface, were constructed from semi-opaque 5gallon plastic jugs, and plankton gained entry through 1 x 3 cm openings cut into the apex of translucent plastic funnels. The light source was a 6 Watt AC fluorescent bulb sealed in an acrylic tube. Captured specimens were concentrated in a cod end (250 μm mesh) attached to the base of the trap and sorted in the laboratory. The light traps were located near the Charleston Coast Guard station in Coos Bay. Samples were collected for a month-long period in 1997, and then daily from April 1998 to December 2000. Larvae tended to occur in the light traps in distinct pulses of abundance. We sampled larvae from a single date in spring 1997, from several dates spanning the recruitment period in 1998, and from two dates in autumn 1999.For this study, we used 12 samples from 1997, 37 samples from 1998 and 28 samples from 1999.

DNA was extracted and amplified for sequencing. DNA was extracted with the Wizard Genomic Purification Kit (Promega) ™, labeled (Table 1) and then stored at – 20°C until needed. The extracted DNA was used for amplifying 350bp of Cytochrome Oxidase I subunit of Dungeness crab. Amplification conditions for this locus were optimized. A 25μl PCR reaction mixture was used. The final concentration of the reaction mixture was 1U *Taq* polymerase, 2μl template DNA, and 0.5 mM each of the forward and reverse primers, 2mM magnesium ion, 0.2mM dNTPs and 1X PCR buffer.

PCR reactions were performed in an Eppendorf Gradient Mastercycle™ programmed for 30 cycles; denaturing at 94°C for 30 Sec, annealing at 50°C for 30 Sec, extension at 72°C for 60 Sec and final extension at 72°C for 10min. PCR product was also stored at -20°C until needed. Negative controls were set up along with the DNA samples. In this reaction mixture 2µl of sterile water was used instead of template DNA. Primers were designed based on the sequence published by Harrison and Crespi (1999). These primers (Forward primer: GGAGGATTTGGAAATTGATT;
Reverse primer: GTACAGGAAGGGATAGTAGT) are specific to Dungeness crab. The amplified DNA was resolved on 2% Agarose gels with Ethidium bromide to confirm the presence of PCR product except in negative control.

PCR product was sequenced using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit™. Plasmid (pGEM3Zf) and water was used as positive and negative controls respectively. Purification of sequencing reaction was done as suggested in the sequencing kit. Sequences obtained from 137 samples were aligned using the computer program ClustalW™ (Pearson and Lipman, 1988).

To determine if larvae are mixing at sea, genetic variability was measured within and between patches/cohorts of megalopae collected at sea/coastal site during all the three years. Genetic variability was measured at nucleotide level and at individual level by using DnaSP program version 3.0™ (Rozas and Rozas 1999). Nucleotide diversity (at nucleotide level) is the average number of nucleotide differences per site between all possible pairs of sequences in the sample (Nei 1987, equation 10.5). Nucleotide divergence is the average number of nucleotide substitutions per site between populations

(Nei 1987, equation 10.20). Haplotype diversity (Individual level) is a DNA sequence analogue to heterozygosity used in the study of protein polymorphism. It is defined as the probability that two haplotypes randomly chosen from a population are different (Nei 1987, equation 8.4). The distribution of haplotypes among patches/cohorts collected during all the years was evaluated for homogeneity using the Chi-square test with DnaSP program™

Pairwise sequence divergences were calculated with the Tamura-Nei model, then the resulting distances were clustered using neighbor-joining and a bootstrap with 500 replications was run to test the confidence of the topology of the phylogenetic tree by using MEGA software package™ (Molecular Evolutionary Genetic Analysis, Version 2.1; Kumar et al. 2001). Phylogenetic trees give an indication of the degree of genetic dissimilarity among the populations; the longer the branches separating a pair of populations, the more genetically dissimilar are individuals sampled from those patches or year. Genetic distance is measured by number of nucleotide substitutions per site between a pair of sequences. Sequences are compared nucleotide by nucleotide.

Phylogenetic relationships among the nucleotide sequences were also inferred from a minimum spanning network of the unique haplotypes by using TCS version 1.13™, a computer program to estimate gene geneologies (Clement et. al., 2000). Haplotypes were connected by a series of mutational events to all other haplotypes through a set of equally parsimonious pathways. The haplotype with the highest probability is displayed as a square, while other haplotypes are displayed as ovals. The size of the square or oval corresponds to the haplotype frequency.

To determine whether different oceanic current patterns during the study period had any effect on genetic composition of the Dungeness crab population, genetic variability was compared among years. This is done Genetic compositions for different years were obtained by using DnaSP™ program as haplotype distribution. Chi-Square test was done, using haplotype diversity measures, to evaluate the homogeneity of haplotype distribution among the years. Phylogenetic tree was obtained by using all the samples as explained above.

Table 1: Sample Identification

Serial No:	Actual sample information/labels		Sample ID
	Date	Labels	
1997			
1	9705	OB#1 II	7OB01M
2	9705	OB#1 II	7OB02M
3	9705	OB#1 II	7OB03M
4	9705	OB#1 II	7OB04M
5	9705	OB#1 II	7OB05M
6	9705	OB#1 II	7OB06M
7	9705	OB#1 II	7OB07M
8	9705	OB#1 II	7OB08M
9	9705	OB#1 II	7OB09M
10	9705	OB#1 II	7OB10M
11	9705	OB#1 II	7OB11M
12	9705	OB#1 II	7OB12M
13	6.22.97	CRPT2 13 MILE	7MCRP2J1
14	6.22.97	CRPT2 13 MILE	7MCRP2J2
15	6.22.97	CRPT2 13 MILE	7MCRP2J3
16	6.22.97	CRPT2 13 MILE	7MCRP2J4
17	6.26.97	MA97 2603	7M2603J1
18	6.26.97	MA97 2603	7M2603J2
19	6.26.97	MA97 2603	7M2603J3
20	6.26.97	MA97 2602	7M2602J1
21	6.26.97	MA97 2602	7M2602J2
22	6.26.97	MA97 2602	7M2602J3
23	6.26.97	MA97 2602	7M2602J4
24	6.26.97	MA97 2602	7M2602J5
25	6.26.97	MA97 2602	7M2602J6
26	6.25.97	MA97 2501	7M2501J1
27	6.25.97	MA97 2501	7M2501J2
28	6.25.97	MA97 2501	7M2501J3
29	6.25.97	MA97 2501	7M2501J4
30	6.25.97	MA97 2501	7M2501J5
31	6.25.97	MA97 2501	7M2501J6
32	6.25.97	MA97 2501	7M2501J7
1998			
1	27MAY1998	CBG3.4N MA98	8M27MC1
2	27MAY1998	CBG3.4N MA98	8M27MC2

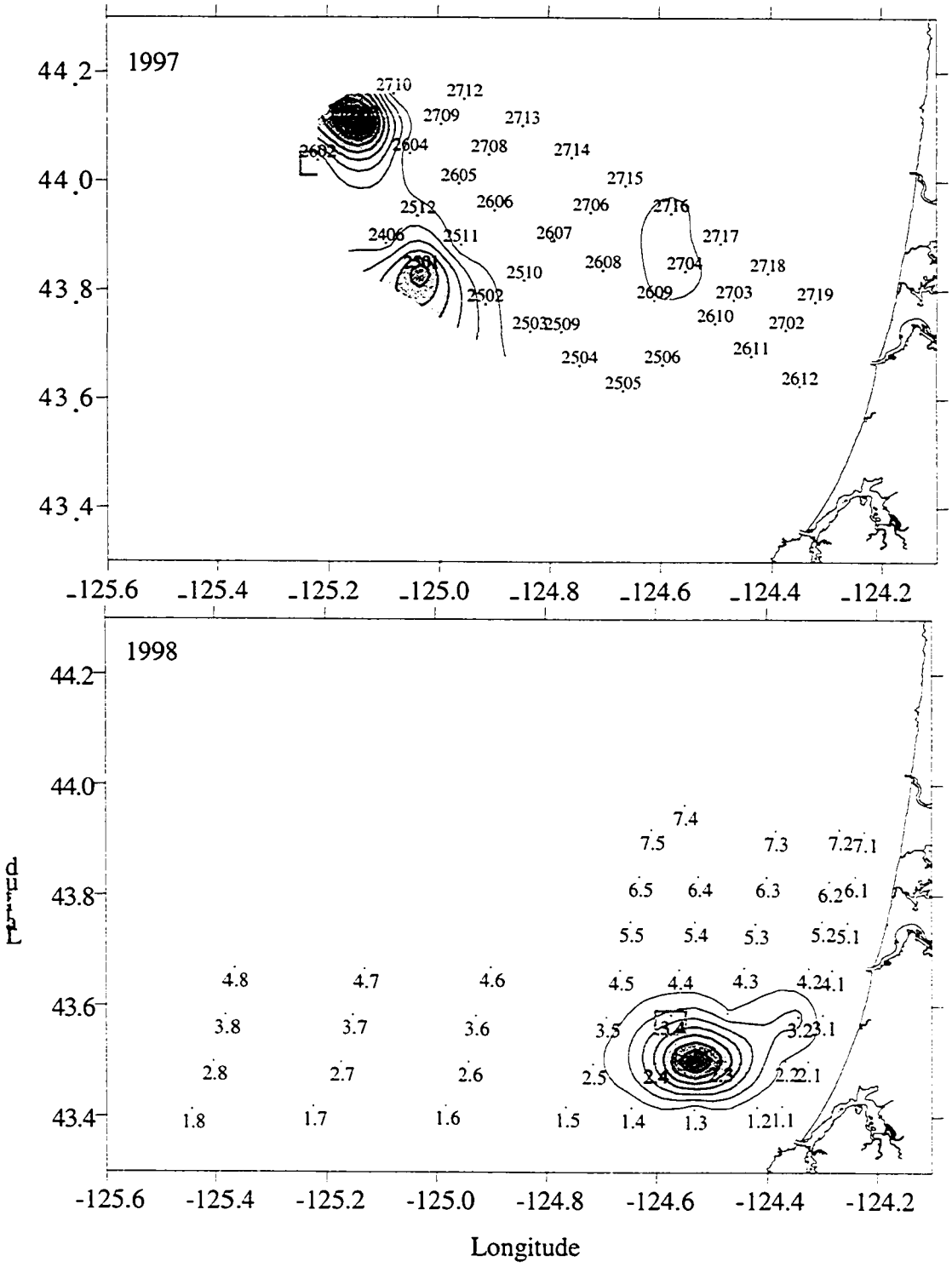
3	27MAY1998	CBG3.4N MA98	8M27MC3
4	27MAY1998	CBG3.4N MA98	8M27MC4
5	27MAY1998	CBG3.4N MA98	8M27MC5
6	27MAY1998	CBG3.4N MA98	8M27MC6
7	27MAY1998	CBG3.4N MA98	8M27MC7
8	27MAY1998	CBG3.4N MA98	8M27MC8
9	27MAY1998	CBG3.4N MA98	8M27MC9
10	27MAY1998	CBG3.4N MA98	8M27MC10
11	27MAY1998	CBG3.4N MA98	8M27MC11
12	27MAY1998	CBG3.4N MA98	8M27MC12
13	27MAY1998	CBG2.4	8M27MA1
14	27MAY1998	CBG2.4	8M27MA2
15	27MAY1998	CBG2.4	8M27MA3
16	27MAY1998	CBG2.5 MA98	8M27MB1
17	27MAY1998	CBG2.5 MA98	8M27MB2
18	27MAY1998	CBG2.5 MA98	8M27MB3
19	27APRIL1998	OB#2,JAR – 4 9804	8OB27A1
20	27APRIL1998	OB#2,JAR – 4 9804	8OB27A2
21	27APRIL1998	OB#2,JAR – 4 9804	8OB27A3
22	27APRIL1998	OB#2, JAR - 4	8OB27A4
23	27APRIL1998	OB#2, JAR - 4	8OB27A5
24	27APRIL1998	OB#2, JAR - 4	8OB27A6
25	27APRIL1998	OB#2, JAR - 4	8OB27A7
26	27APRIL1998	OB#2, JAR - 4	8OB27A8
27	27APRIL1998	OB#2, JAR - 4	8OB27A9
28	980506	OB#3 - 7	8OB11J1
29	980506	OB#3 – 6	8OB10M1
30	980506	OB#3 – 6	8OB10M2
31	980506	OB#3 – 6	8OB10M3
32	980506	OB#3 – 5	8OB04M4
33	980506	OB#3 – 5	8OB04M5
34	980506	OB#3 – 5	8OB04M6
35	980506	OB#3 – 5	8OB04M7
36	980506	OB#3 – 5	8OB04M8
37	980506	OB#3 – 5	8OB04M9
38	980506	OB#3 – 5	8OB04M10
39	980506	OB#3 - 5	8OB04M11
40	980612	OBB	8OB12J1
41	980612	OBB	8OB12J2
42	980612	OBB ROEGNER	8OB12J3
43	980612	OBB ROEGNER	8OB12J4
44	980612	OBB ROEGNER	8OB12J5

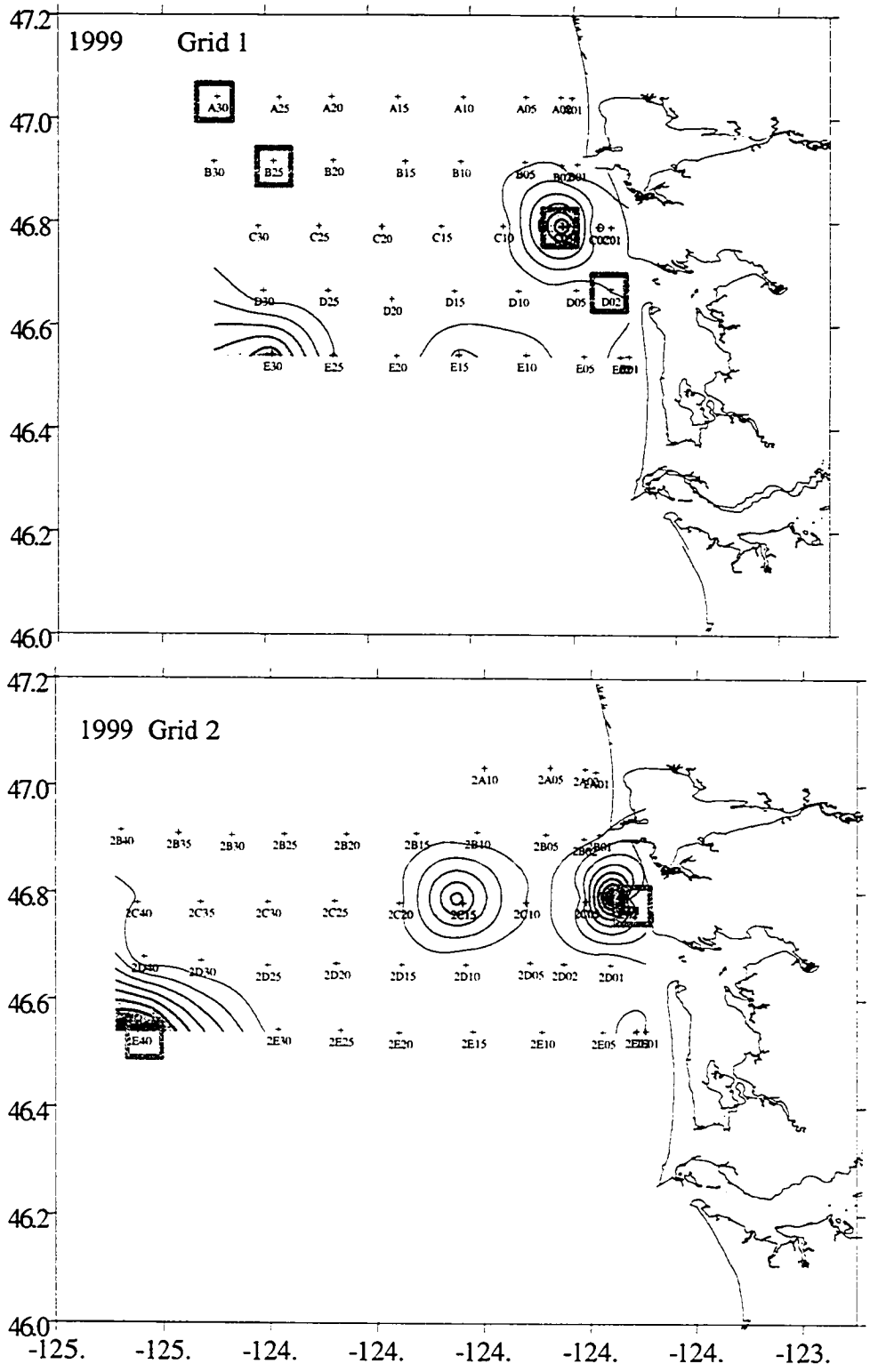
45	980612	OBB	8OB12J6
46	980612	OBB	8OB12J7
47	980612	OBB	8OB12J8
48	980612	OBB ROEGNER	8OB12J9
49	980612	OBB ROEGNER	8OB12J10
50	980612	OBB ROEGNER	8OB12J11
51	980612	OBB ROEGNER	8OB12J12
52	980612	OBBOB 20CM	8OB12J13
53	980612	OBBOB 20CM	8OB12J14
54	981025	OB#4	8OB25O1
55	981029	OB#4 9811	8OB29O1
1999			
1	990526	GBF102 – 5A	9LGBF1
2	990524	LK L30 – 1A	9LKL30
3	990529	2E40 - 1B	9L2E401
4	990529	2E40 - 4B	9L2E402
5	990529	2E40 - 2B	9L2E403
6	990528	WCEO1-02 – 5B	9LWCE1
7	990528	WCEO1-02 – 2A	9LWCE2
8	990526	GAFO4 – 3B	9LGAF1
9	990525	LK DO2 – 1B	9L2D021
10	990526	LK DO2 – 1B	9L2D022
11	990525	LK F30 – 3B	9L2F301
12	990525	LK F30 – 2A	9L2F302
13	990525	LK F30 – 3A	9L2F303
14	990525	LK F30 – 4A	9L2F304
15	990524	LK CO5 – 8B	9L2C051
16	990524	LK CO5 – 4B	9L2C052
17	990524	LK CO5 – 1B	9L2C053
18	990524	LK CO5 – 1A	9L2C054
19	990524	LK CO5 – 11A	9L2C055
20	990523	LK B25 – 2A	9L2B251
21	990523	LK B25 – 3A	9L2B252
22	990523	LK B25 – 4A	9L2B253
23	990901	OBB 2 OF 5 – 1B	90B01S1
24	990901	OBB 3 OF 10	90B01S2
25	990901	OBB 2 OF 10	90B01S3
26	990907	OBB 8 OF 10 – 1B	90B07S4
27	990907	OBB 7 OF 10	90B07S5
28	990907	OBB 5 OF 10	90B07S6
29	990907	OBB 10 OF 10	90B07S7

30	990908	OBB17 OF 26 – 2B	90B08S8
31	990908	OBB 21 OF 26 – 4B	90B08S9
32	990908	OBB 19 OF 26	90B08S10
33	990908	OBB 26 OF 26	90B08S11
34	990908	OBB 9 OF 26	90B08S12
35	990908	OBB 13 OF 26	90B08S13
36	990908	OBB 2 OF 26	90B08S14
37	990908	OBB 16 OF 26	90B08S15
38	990908	OBB 1 OF 26	90B08S16
39	990908	OBB 6 OF 26	90B08S17
40	990908	OBB 11 OF 26	90B08S18
41	990908	OBB 9 OF 10	90B08S19
42	990911	OBB 8 OF 10	90B11S20
43	990911	OBB 3 OF 10	90B11S21
44	990911	OBB 4 OF 10	90B11S22
45	990911	OBB 5 OF 10	90B11S23
46	990911	OBB 10 OF 10	90B11S24
47	990911	OBB 1 OF 5	90B11S25
48	990827	OBB 3 OF 4-1B	9OB27A26
49	990827	OBB 4 OF 4	9OB27A27
50	990827	OBB 1 OF 4	9OB27A28

7 – 1997, 8 – 1998, 9 – 1999; In Bay – OB followed by the date and then month of collection, At sea: M – McArthur98 cruise, L – Lady Kaye cruise followed by transect site (place where the samples are collected at sea). 1997: M – May, J – June; 1998: A – April, M – May, J- June, O – October; 1999: S – September. A – August; Replicate number is given at the end of the label.

Figure 1: *Cancer magister* distributions during 1997, 1998, and 1999




1999 Grid 1
1999 Grid 2
47.2
47.0
46.8
46.6
46.4
46.2
46.0
-125.
-125.
-124.
-124.
-124.
-124.
-124.
-123.

RESULTS

A total of 137 sequences were obtained from individual megalopae collected during 1997, 1998 and 1999. The portion of Mitochondrial Cytochrome Oxidase I gene region that was aligned comprised of 320 positions (excluded the sites with gaps/missing data), of which 47 were variable. (Table 2, Table 3). 52 different haplotypes were obtained from 137 individuals (Table 3).

Nucleotide diversity and genetic distance measures were found to be low both within and between patches/cohorts of Dungeness crab megalopae collected at sea/coastal sites. Nucleotide diversity and genetic distance values range from 0.20 to 1.7%and 0.20 to 1.85% (Table 7 and Table 8).

However, haplotype diversity was found to be high within patches/cohorts but low between patches/cohorts. Haplotype diversity within patches/cohorts ranges from 81 to 100% (Table 7). Chi-Square analysis based on haplotype diversity showed no significant differences between patches/cohorts (Table 11).

Phylogentic analyses showed that there were no distinct patches/cohorts. The molecular phylogenetic trees were constructed with different neighbor-joining model by MEGA and they were presented in the figures 12 - 24. The phylogenetic trees obtained were similar regardless of whether neighbor-joining dendogram was constructed using Jukes-Cantor, Kimura-2-parameter, Tamura-Nei, Tajima-Nei or Tamura-3-parameter model. Thus, the trees constructed using Tamura-Nei were shown in all cases. The phylogenetic trees obtained didn't show any significant clustering related to particular patch at sea or to particular cohort at coastal site (Figures 12 - 23).

Haplotype composition and haplotype diversity vary annually. Twenty-four different haplotypes were seen in megalopae collected during 1997, 23 in 1998 and 15 in 1999. The most common haplotype (H1) occurred in 24 individuals (17.5%) followed by H5, which was seen in 18 individuals (13.13%). These two haplotypes (H1 and H5) occurred in all 3-year samples representing the entire spatial and temporal span of the sample collection (Table 4 and Figure 2- 5). 3 haplotypes (H7, H16, H42) were shared by samples collected during 1997 and 1999. H38 was shared between 1997 and 1998; H33 was shared between 1998 and 1999. Forty-five of the fifty-two haplotypes were unique to different years. Among these 45 unique haplotypes, the samples collected during 1997 had 19 unique haplotypes, 1998 had 18 and 1999 had only 8 (Table 4, Figure 2- 4). Most of the haplotypes were shared between the samples collected at sea and at bay during 1998 and 1999 except for 1997 where all the haplotypes seen at bay were unique compared to the ones found at sea (Table 5 and Figures 6 – 11). Chi-square analysis based on haplotype diversity showed a significant genetic variation across years (Table 11).

Phylogenetic analyses showed that the megalopae collected during 1997 were genetically dissimilar. The branch lengths were comparatively long for most of the megalopae from 1997 (Figure 23). There was one cluster, which was predominantly formed by samples from 1997 (Figure 23). Similarly, the phylogenetic tree obtained by pooling the samples collected during all three years at sea showed distinct cluster of 1997 (Figure 21)

Haplotype tree formed 4 different clusters (Figure 24). One cluster contained approximately 80% of the total individual, which constitute 72% of 1997, 73% of 1998 and 94% of 1999 samples. The next major cluster was formed by 20% of 1998 samples, 2% and 6% of 1999 and 1997 samples respectively. 12.5% of 1997 samples group together to form the 3rd cluster. The 4th cluster consists of 2% of 1999 samples and 9% of 1997 samples.

Minimum spanning network tree (Figure 25) showed 6 different maternal lineages. Among these, 4 were independent ancestral haplotype from 1997 samples. Individuals from all the years formed the major lineage. In this major lineage, 7 individuals from 1999, 5 and 1 from 1998 and 1997, formed the ancestral haplotype respectively. Most of the haplotypes in this lineage consists of individuals from 1998 and 1999 rather than 1997. Another lineage had 8 individuals of which 7 were from 1997 and 1 from 1998.

Table 2: Number of variable sites and sample size for different group of populations

Grouping	Sample size	Number of positions included for alignment	Number of variable sites
All sample	137	320	47
Sea	60	321	28
Bay	77	323	36
1997	32	323	31
1998	55	325	23
1999	50	328	19

Table 3:

52 haplotypes and corresponding sequence changes, variable sites within subunit I of the cytochrome oxidase I gene of *Cancer magister* mitochondrial DNA.

Relative nucleotide position in compared sequences

```
     12578111112223334581111111111122222222233333333
          356783890248160112556677800445688800112344
                        5481892917015077013557013756

H1   GATAGTCCTCTTAATTTTAAAACCCAGCAATGAAGTACAGTTATCTC
H2   ...............................A...............
H3   .......................................TG......
H4   .........................C.....................
H5   ..................................A............
H6   ...................G..............A............
H7   A.................................A............
H8   T.................................A.T..........
H9   T.................................A............
H10  T.G...............................A............
H11  T.G........C......................A............
H12  A.G.........................G.....A............
H13  T...........................G.....A............
H14  T....G..A...................G.....A............
H15  T.......A.........................A............
H16  A...........................G.....A............
H17  ............................G.....A............
H18  T.............C...................A............
H19  A.............C...................A............
H20  ..............C...................A............
H21  ...........................T......A............
H22  T..........................T......A............
H23  A..GC.............................A............
H24  A..GC..........................................
H25  A..GC.....................................C....
H26  AT.GC..............G...........................
H27  ...GCC.TCA.........................C...........
H28  T..GC....................................C.....
H29  ...GC...........................G........C.....
H30  ...GC.............................A............
H31  ..................................A.......C....
H32  ........A.................................C....
H33  .............................................CG
H34  ..................................A..........CG
H35  ....................................C..........
H36  ........................T......................
```

```
H37  ................G.......G................G.....
H38  ................................G..............
H39  ...........................................C...
H40  A.....................................G........
H41  ......................................G........
H42  A..............................................
H43  A...................G................A.........
H44  C.........................T...C.............A..
H45  T..............................................
H46  T....G.......C.......GA........................
H47  T.G............................................
H48  T.G..........................G.................
H49  T.G..G.......C.C.CC....T.........G.............
H50  T.GGCG............................A............
H51  T.GC.GT...C.CC....................A............
H52  T....G.......C....G............................
```

A dot indicates identity with the consensus sequence. H indicates haplotype

Table 4: Distribution of the 52 haplotypes among 3 different years

ID	N	Haplotype frequency %	Sample ID	1997 N	Haplotype frequency %	1998 N	Haplotype frequency %	1999 N	Haplotype frequency %
H1	24	17.52	7M2602J3, 7MCRP2J1, 7MCRP2J3 8M27MB2, 8OBO4M7, 8OB27A8, 8OB04M4, 8OB12J8,8OB12J5, 8OB27A3,8OB12J7 9LWCE1, 9LWCE2, 9L2C055, 9L2CO52, 9L2B253, 9L2B251, 9OB07S07, 9OB27A28, 9OB08S17, 9OB07S6, 9OB11S24, 9OB08S19, 9OB11S22	3	9.38	8	14.55	13	26
H2	1	0.73	8OB10M3	0	0	1	1.82	0	0
H3	1	0.73	8OB04M9	0	0	1	1.82	0	0
H4	1	0.73	9OB01S2	0	0	0	0	1	2
H5	18	13.14	7M2602J1, 7M2501J7, 7M2602J4 8M27MC10, 8M27MC11, 8M27MB3 8OB04M6, 8OB27A4, 8OB27A9, 8OB04M11 9L2F3O2, 9LGBF1, 9L2F3O3, 9L2F3O4, 9OB11S20, 9OB08S18, 9OB11S25, 9OB07S5	3	9.38	7	12.72	8	16
H6	1	0.73	7M2603J2	1	3.13	0	0	0	0
H7	9	6.57	7M2602J5, 7MCRPT2J4, 7M2603J3, 9LGAF1, 9L2E403, 9L2L1, 9L2C051, 9OBO8S11, 9OB08S9	3	9.38	0	0	6	12
H8	1	0.73	7M2602J6	1	3.13	0	0	0	0
H9	4	2.92	8M27MC4, 8M27MA1, 8OB12J13, 8OB12J3,	0	0	4	7.27	0	0
H10	1	0.73	8M27MC1	0	0	1	1.82	0	0
H11	1	0.73	8OB10M1	0	0	1	1.82	0	0
H12	1	0.73	7M2501J4	1	3.13	0	0	0	0
H13	5	3.65	8M27MA3, 8OB12J12, 8OB11J1, 8OB25O, 8OB12J14	0	0	5	9.09	0	0
H14	1	0.73	7OBM02	1	3.13	0	0	0	0
H15	1	0.73	9OB01S3	0	0	0	0	1	2

H16	2	1.46	7OBM11, 9L2CO53,	1	3.13	0	0	1	2
H17	7	5.11	8OB04M10, 8OB27A1, 8OB12J1, 90BO8S10, 9OB27A27, 9OB08S16,	0	0	3	5.45	4	8
			9OB11S23						
H18	1	0.73	8OB27A7	0	0	1	1.82	0	0
H19	1	0.73	9OB01S1	0	0	0	0	1	
H20	1	0.73	8OB27A5	0	0	1	1.82	0	0
H21	1	0.73	8M27MC7	0	0	1	1.82	0	0
H22	1	0.73	8OB12J9	0	0	1	1.82	0	0
H23	1	0.73	7OBM04	1	3.13	0	0	0	0
H24	1	0.73	7OBM07	1	3.13	0	0	0	0
H25	1	0.73	7OBM06	1	3.13	0	0	0	0
H26	1	0.73	7OBM08	1	3.13	0	0	0	0
H27	1	0.73	8OB12J6	0	0	1	1.82	0	0
H28	1	0.73	7OBM05	1	3.13	0	0	0	0
H29	1	0.73	7OBM09	1	3.13	0	0	0	0
H30	1	0.73	7OBM10	1	3.13	0	0	0	0
H31	1	0.73	7OBM01	1	3.13	0	0	0	0
H32	1	0.73	9OB08S12	0	0	0	0	1	2
H33	5	3.65	8M27MC9, 8M27MC2, 8M27MC5, 8M27MC8, 9OB08S14,	0	0	4	7.27	1	2
H34	1	0.73	8M27MC3	0	0	1	1.82	0	0
H35	1	0.73	7OBM12	1	3.13	0	0	0	0
H36	1	0.73	8OB10M2	0	0	1	1.82	0	0
H37	1	0.73	8OB04M5	0	0	1	1.82	0	0
H38	3	2.19	7M2603J1, 8OB27A6, 8OB12J2	1	3.13	2	3.64	0	0
H39	1	0.73	8OB29O	0	0	1		0	0
H40	2	1.46	9OB08S13, 9OB27A26	0	0	0	0	2	4
H41	1	0.73	8M27MC12	0	0	1	1.82	0	0
H42	9	6.57	7M2501J6, 9L2E402, 9L2CO54, 9L2B252, 9L2E401 9L2D021, 9L2F301, 9OB08S15, 9OB07S4	1	3.13	0	0	8	16
H43	1	0.73	9OB11S21	0	0	0	0	1	2
H44	1	0.73	8OB04M8	0	0	1	1.82	0	0
H45	7	5.11	8M27MB1, 8M27MA2, 8M27MC6, 8OB27A2, 8OB12J4 8OB12J11, 8OB12J10,	0	0	7	12.73	0	0
H46	1	0.73	9L2DO22	0	0	0	0	1	
H47	3	2.19	7M2501J1, 7M2602J2, 7M2501J2	3	9.38	0	0	0	0
H48	1	0.73	7M2501J3	1	3.13	0	0	0	0
H49	1	0.73	7M2501J5	1	3.13	0	0	0	0
H50	1	0.73	9OB08S3	0	0	0	0	1	
H51	1	0.73	7MCRPT2J2	1	3.13	0	0	0	0

H52	1	0.73	7OBM03	1	3.13	0	0	0	0

N – Number of haplotypes; ID – Haplotype ID;

Table 5: Haplotype frequency distribution at sea and in bay for all 3 years

Year	At sea			In Bay		
	Haplotype ID	Number of Haplotype	Haplotype frequency (%)	Haplotype ID	Number of Haplotype	Haplotype frequency (%)
1997	H1	3	15	H14	1	8.33
	H5	3	15	H16	1	8.33
	H6	1	5	H23	1	8.33
	H7	3	15	H24	1	8.33
	H8	1	5	H25	1	8.33
	H12	1	5	H26	1	8.33
	H38	1	5	H28	1	8.33
	H42	1	5	H29	1	8.33
	H47	3	15	H30	1	8.33
	H48	1	5	H31	1	8.33
	H49	1	5	H35	1	8.33
	H51	1	5	H52	1	8.33
1998	H1	1	5.55	H1	7	18.91
	H5	3	16.66	H2	1	2.7
	H9	4	22.22	H3	1	2.7
	H10	1	5.55	H5	4	10.81
	H13	1	5.55	H11	1	2.7
	H21	1	5.55	H13	4	10.81
	H33	4	22.22	H17	3	8.11
	H34	1	5.55	H18	1	2.7
	H41	1	5.55	H20	1	2.7
	H45	3	16.66	H22	1	2.7
				H27	1	2.7
				H36	1	2.7
				H37	1	2.7
				H38	2	5.41
				H39	1	2.7
				H44	1	2.7
				H45	4	10.81
1999	H1	6	27.27	H1	7	25
	H5	4	18.18	H4	1	3.57
	H7	4	18.18	H5	4	14.28
	H16	1	4.55	H7	2	7.14
	H42	6	27.27	H15	1	3.57

	H46	1	4.55	H17	4	14.28
				H19	1	3.57
				H32	1	3.57
				H33	1	3.57
				H40	2	7.14
				H42	2	7.14
				H43	1	3.57
				H50	1	3.57

Figure 2: Haplotype frequencies for the samples collected during 1997

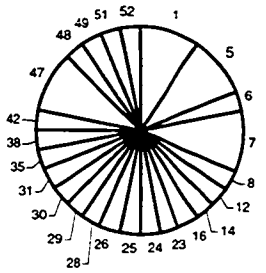


Figure 3: Haplotype frequencies for the samples collected during 1998

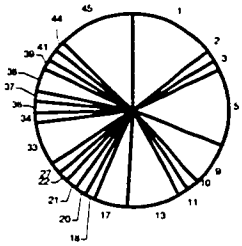


Figure 4: Haplotype frequencies for the samples collected during 1999

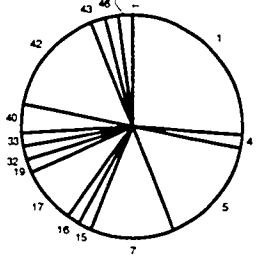


Figure 5: Haplotype frequencies for the samples collected during '97, '98 and '99

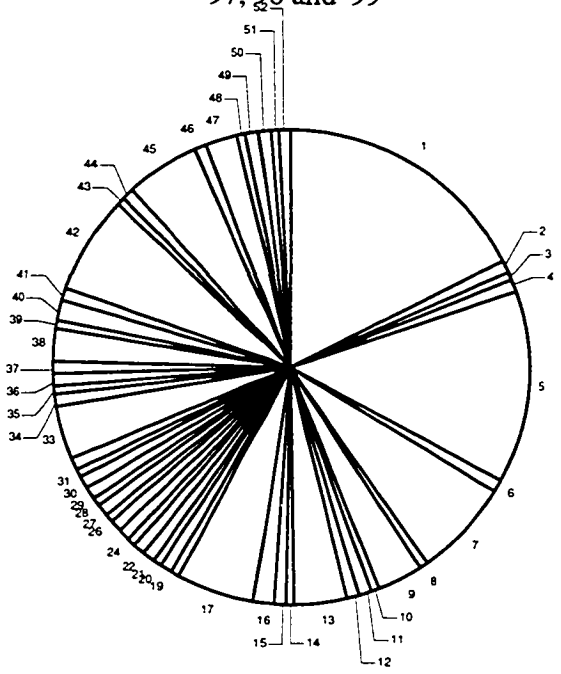


Figure 6: Haplotype frequency distribution for the samples collected at bay during 1997

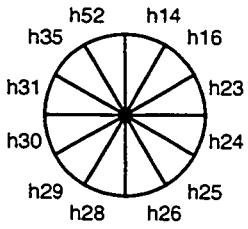


Figure 7: Haplotype frequency distribution for the samples collected at sea during 1997

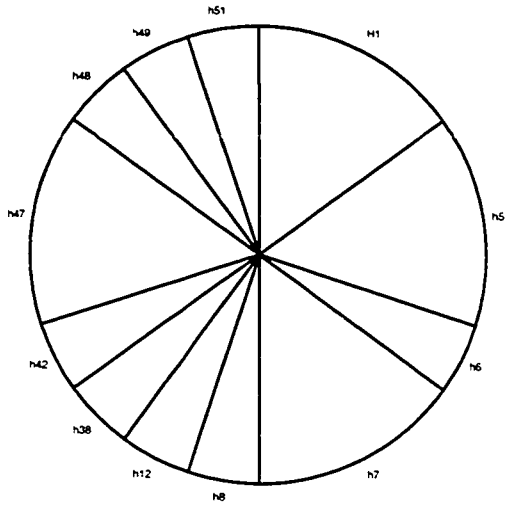


Figure 8: Haplotype frequency distribution for the samples collected at sea during 1998

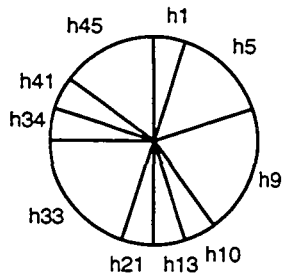


Figure 9: Haplotype frequency distribution for the samples collected at bay during 1998

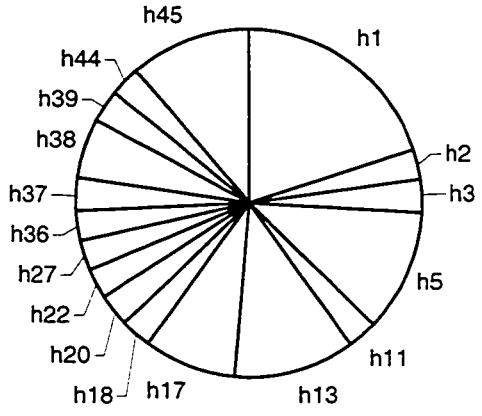


Figure 10: Haplotype frequency distribution for the samples collected at bay during 1999

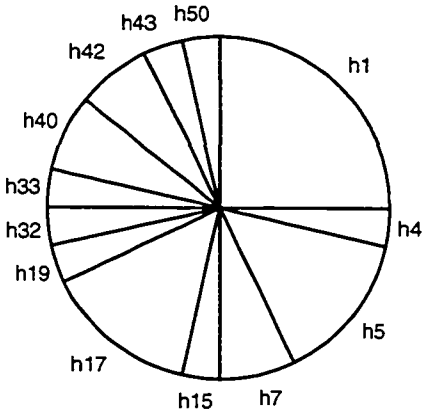


Figure 11: Haplotype frequency distribution for the samples collected at sea during 1999

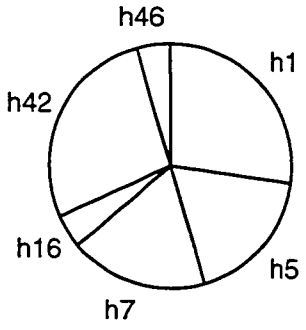


Table 6: Measures of genetic diversity in Dungeness crab megalopae collected at sea and at bay during 1997, 1998 and 1999.

Groups	Sample size	Nucleotide diversity %	Haplotype diversity %	Mean genetic distance within subpopulation
Group 1: All Samples	137	0.891	93.8	0.9
1997	*32*	*1.324*	*97.581*	*1.347*
1998	55	0.814	93.535	0.823
1999	50	0.622	87.265	0.631
At Sea	60	0.797	91.9	0.80
At Bay	*77*	*0.952*	*94.634*	*0.97*
Group 2: At Sea	60	1.020	91.9	1.0
1997	**20**	**1.569**	**94.737**	**1.602**
1998	18	0.725	91.503	0.716
1999	22	0.561	81.818	0.587
Group 3: At Bay	77	0.837	94.2	0.8
1997	**12**	**1.215**	**100**	**1.233**
1998	37	0.794	93.393	0.804
1999	28	0.705	90.476	0.715
Group 4: 1997	32	2.157	97.6	2.2
At Sea	20	1.947	95.789	1.988
At Bay	*12*	*2.528*	*100*	*2.595*
Group 5: 1998	**55**	**0.745**	**93.5**	0.753
At Sea	18	0.716	91.503	0.722
At Bay	*37*	*0.739*	*93.393*	*0.747*
Group 6: 1999	**50**	**0.595**	**87.3**	0.603
At Sea	22	0.494	81.818	0.500
At Bay	*28*	*0.673*	*90.476*	0.681

Table 7: Genetic variation within each patch of megalopae collected at sea and at bay

Groups	Sample size	Nucleotide diversity %	Haplotype diversity %	Mean genetic distance within subpopulation
All Samples				
7M2602J	6	0.60	93.33	0.60
7MCRP2J	**4**	**1.56**	**83.33**	**1.59**
7M2603J	3	0.83	100.00	0.85
7M2501J	**7**	**1.31**	**95.23**	**1.33**
9L2F30	4	0.31	50.00	0.31
9L2CO5	5	0.50	90.00	0.50
9L2B25	3	0.20	66.66	0.21
9L2E40	3	0.20	66.66	0.21
8M27MC	12	0.78	89.39	0.79
8M27MB	3	0.41	100.00	0.41
8M27MA	3	0.41	100.00	0.42
70BM	**12**	**1.46**	**100.00**	**1.49**
8OB04M	8	0.94	92.85	0.95
8OB27A	9	0.55	94.44	0.56
8OB12J	14	0.84	91.20	0.85
8OB10M	**3**	**1.25**	**100.00**	**1.26**
9OB08S	12	0.91	95.45	0.93
9OB27A	3	0.83	100.00	0.85
9OB11S	6	0.60	86.66	0.61
9OB07S	4	0.31	83.33	0.31
9OB01S	**3**	**1.14**	**100.00**	**1.15**

Table 8: Estimates of genetic distance (below diagonal) and nucleotide divergence (above diagonal) between 3 years.

Overall	1997	1998	1999
1997		*1.128*	*1.010*
1998	1.145		*0.767*
1999	1.032	0.760	
At sea	1997	1998	1999
1997		*1.294*	*1.160*
1998	1.312		*0.734*
1999	1.196	0.740	
At bay	1997	1998	1999
1997		*1.035*	*1.007*
1998	1.049		*0.766*
1999	0.975	0.772	

Table 9: Genetic distance (below diagonal) and nucleotide divergence (below diagonal) between the samples collected at sea and at bay during 1997, 1998, 1999.

1997	At Sea	At Bay
At Sea		*2.20*
At Bay	2.274	
1998	At Sea	At Bay
At Sea		*0.758*
At Bay	0.765	
1999	At Sea	At Bay
At Sea		*0.586*
At Bay	0.599	

Table 10: Genetic distance (below diagonal) and Nucleotide divergence (below diagonal) between patc

	7M2602M	8OB10M	7MCRPT2	8OB27MA	9OB07S	8OB04M	9OB01S	9L2CO5	9L2B25	9OB27A	9OB08S
7M2602M		0.83	0.97	0.56	0.46	0.80	0.76	0.55	0.50	0.67	0.73
8OB10M	0.84		1.25	0.84	0.70	1.02	1.11	0.85	0.69	0.93	1.24
7MCRPT2	0.99	1.27		1.01	0.87	1.23	0.01	0.96	0.88	1.09	1.14
8OB27MA	0.57	0.85	1.03		0.43	0.74	0.76	0.56	0.46	0.63	0.73
9OB07S	0.47	0.71	0.89	0.44		0.59	0.70	0.37	0.20	0.46	0.59
8OB04M	0.81	1.04	1.25	0.75	0.60		1.05	0.75	0.59	0.80	0.93
9OB01S	0.77	1.12	1.24	0.77	0.71	1.07		0.75	0.72	0.90	0.94
9L2CO5	0.56	0.87	0.99	0.57	0.38	0.76	0.76		0.35	0.54	0.66
9L2B25	0.51	0.70	0.90	0.47	0.21	0.61	0.74	0.36		0.45	0.59
9OB27A	0.69	0.95	1.11	0.65	0.48	0.82	0.91	0.55	0.46		0.75
9OB08S	0.74	1.04	1.17	0.74	0.60	0.95	0.96	0.67	0.61	0.77	
7M2501J	1.06	1.27	1.42	1.17	1.02	1.40	1.34	1.07	0.98	1.21	1.27
9L2F30	0.42	0.76	0.89	0.42	0.32	0.64	0.66	0.41	0.37	0.53	0.60
8M27MC	0.77	1.04	1.22	0.75	0.64	0.96	1.03	0.78	0.66	0.85	0.91
9OB11S	0.60	0.88	1.05	0.56	0.42	0.75	0.84	0.54	0.44	0.62	0.74
8M27MB	0.45	0.70	0.90	0.43	0.29	0.63	0.70	0.44	0.28	0.53	0.63
7M2603	0.65	0.99	1.11	0.63	0.53	0.87	0.88	0.62	0.56	0.74	0.82
8OB12J	0.73	1.01	1.18	0.72	0.63	0.96	0.96	0.72	0.63	082	0.91
8M27MA	0.06	0.91	1.06	0.62	0.60	0.92	0.77	0.61	0.63	0.74	0.81
7OBM	1.25	1.56	1.61	1.27	1.12	1.48	1.44	1.16	1.10	1.30	1.32
9L2E40	0.54	0.08	0.95	0.62	0.37	0.82	0.70	0.34	0.32	0.56	0.66

w diagonal) between patches/cohorts of megalopae collected at Sea and at Bay during all 3 years.

	9L2B25	9OB27A	9OB08S	7M2501J	9L2F30	8M27MC	9OB11S	8M27MB	7M2603	8OB12J	8M27MA	7OBM	9L2E40
	0.50	0.67	0.73	1.04	0.41	0.76	0.59	0.45	0.64	0.72	0.55	1.23	0.538
	0.69	0.93	1.24	1.25	0.75	1.02	0.86	0.69	0.80	0.99	0.90	1.53	0.868
	0.88	1.09	1.14	1.39	0.87	1.20	1.02	0.88	1.09	1.16	1.04	1.58	0.93
	0.46	0.63	0.73	1.15	0.41	0.74	0.55	0.42	0.62	0.71	0.61	1.25	0.61
	0.20	0.46	0.59	1.00	0.31	0.63	0.41	0.28	0.52	0.62	0.76	1.10	0.36
	0.59	0.80	0.93	1.37	0.63	0.95	0.73	0.62	0.85	0.94	0.91	1.45	0.80
	0.72	0.90	0.94	1.32	0.65	1.01	0.83	0.69	0.86	0.94	1.22	1.42	0.69
	0.35	0.54	0.66	1.05	0.40	0.77	0.53	0.43	0.60	0.71	0.60	1.14	0.33
		0.45	0.59	0.96	0.36	0.65	0.43	0.27	0.55	0.62	0.62	1.08	0.31
	0.46		0.75	1.19	0.52	0.84	0.60	0.52	0.72	0.80	0.69	1.27	0.55
	0.61	0.77		1.25	0.59	0.89	0.72	0.62	0.80	0.89	0.79	1.30	0.65
	0.98	1.21	1.27		1.07	1.31	1.18	0.98	1.26	1.20	1.01	1.70	0.98
	0.37	0.53	0.60	1.09		0.63	0.41	0.33	0.46	0.64	0.54	1.12	0.41
	0.66	0.85	0.91	1.34	0.64		0.76	0.62	0.85	0.92	0.83	1.46	0.80
	0.44	0.62	0.74	1.20	0.42	0.78		0.45	0.64	0.75	0.59	1.25	0.57
	0.28	0.53	0.63	1.00	0.34	0.63	0.46		0.55	0.58	0.48	1.12	0.45
	0.56	0.74	0.82	1.29	0.48	0.87	0.65	0.56		0.84	0.76	1.29	0.59
	0.63	082	0.91	1.23	0.66	0.94	0.77	0.59	0.86		0.62	1.31	0.72
	0.63	0.74	0.81	1.03	0.55	0.84	0.70	0.49	0.77	0..63		1.22	0.59
	1.10	1.30	1.32	1.73	1.14	1.49	1.28	1.14	1.32	1.34	1.24		1.85
	0.32	0.56	0.66	1.00	0.42	0.82	0.58	0.46	0.60	0.74	0.60	1.10	

Table 11: Haplotype frequency comparisons based on chi-square test

Samples compared	Df	Chi-square value	P value	Result
Group 1: Among different years	102	158.154	0.0003***	Reject H̃o
1997 vs 1998	43	65.719	0.0144*	Reject H̃o
1997 vs 1999	33	48.346	0.0413*	Reject H̃o
1998 vs 1999	33	60.098	0.0027**	Reject H̃o
Group 2: In Bay				
1997 vs 1998	28	45	0.0238*	Reject H̃o
1997 vs 1999	23	36	0.0394*	Reject H̃o
1998 vs 1999	27	36	0.1248	**Accept H̃o**
Group 3: At sea				
1997 vs 1998	20	29	0.0883	**Accept H̃o**
1997 vs 1999	14	16.8	0.2670	**Accept H̃o**
1998 vs 1999	13	30	0.0054**	Reject H̃
Group 4: Among the patches of megalopae	980	958.224	0.6846	**Accept H̃o**
Group 5: 1997				
At Sea vs In Bay	22	25	0.3024	**Accept H̃o**
Group 6: 1998				
At Sea vs In Bay	22	28	0.2009	**Accept H̃o**
Group 7: 1999				
At Sea vs In Bay	14	17	0.2420	**Accept H̃o**

H̃o There is no genetic variation between the populations.
There is significant difference in haplotype frequency among the sample collections grouped by year (Group 1). Significant variation is also observed between megalopal patches at sea during 1999 and 1998. Regardless of the year of collection, low genetic differences were found within each patch of sample collection.

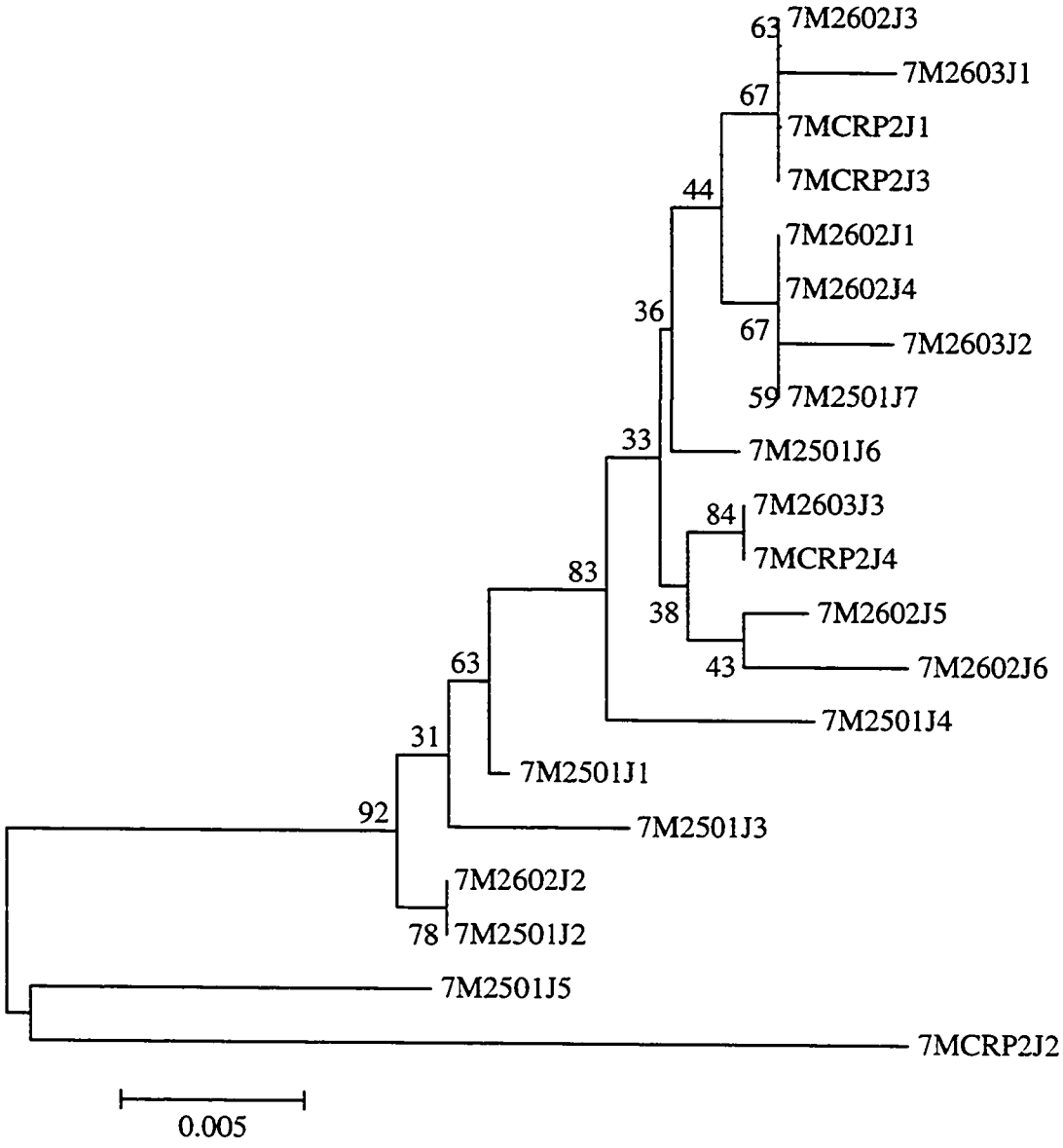


Figure 12: Phylogenetic tree of COI sequences of the Dungeness crab megalopae collected at sea during 1997. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using the Tamuara-Nei correction in MEGA.

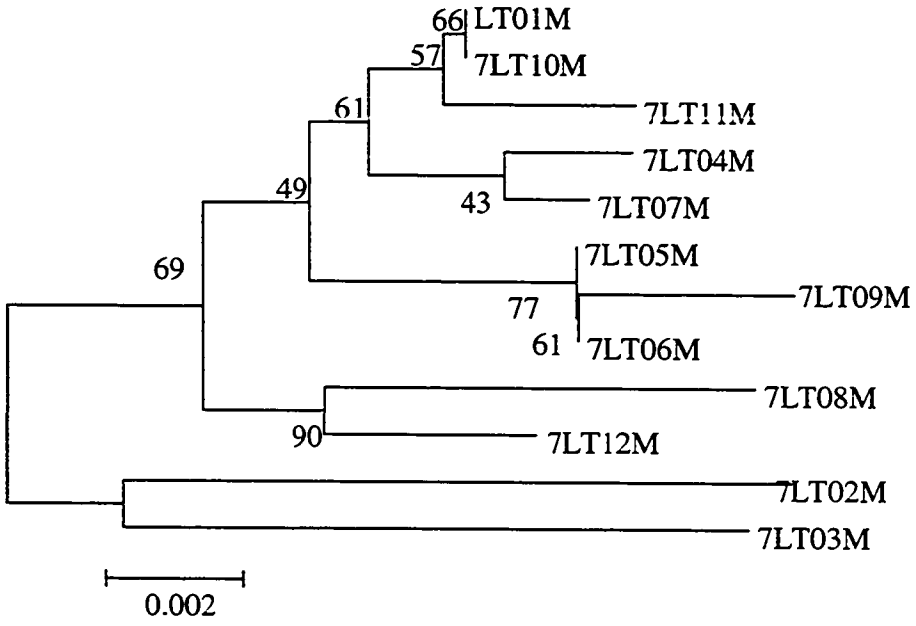


Figure 13: Phylogenetic tree of COI sequences of the Dungeness crab megalopae collected at bay during 1997. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using the Tamuara-Nei correction in MEGA

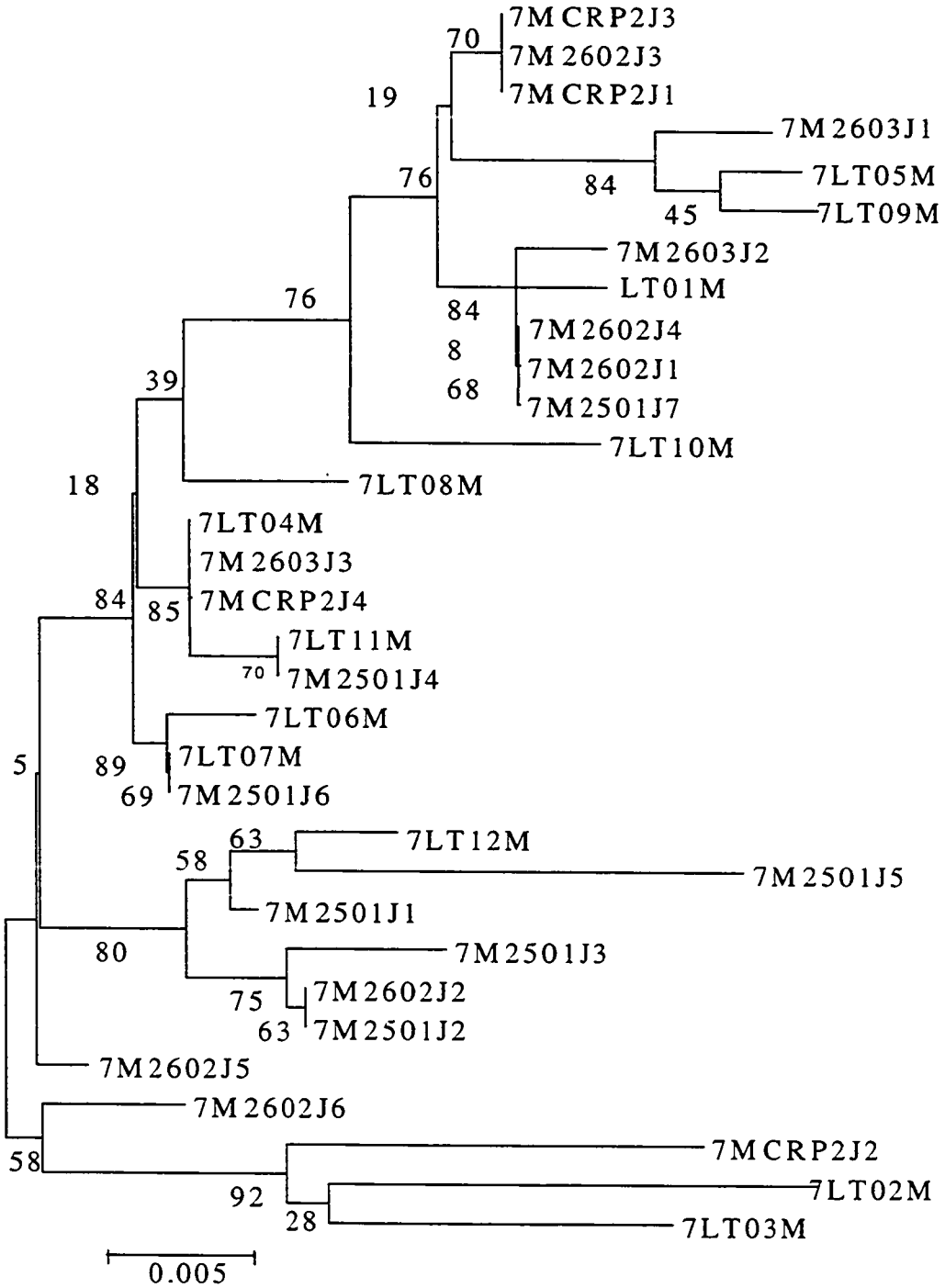


Figure 14: Phylogenetic tree of COI sequences of the Dungeness crab megalopae collected during 1997 at sea and at bay. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using the Tamuara-Nei correction in MEGA

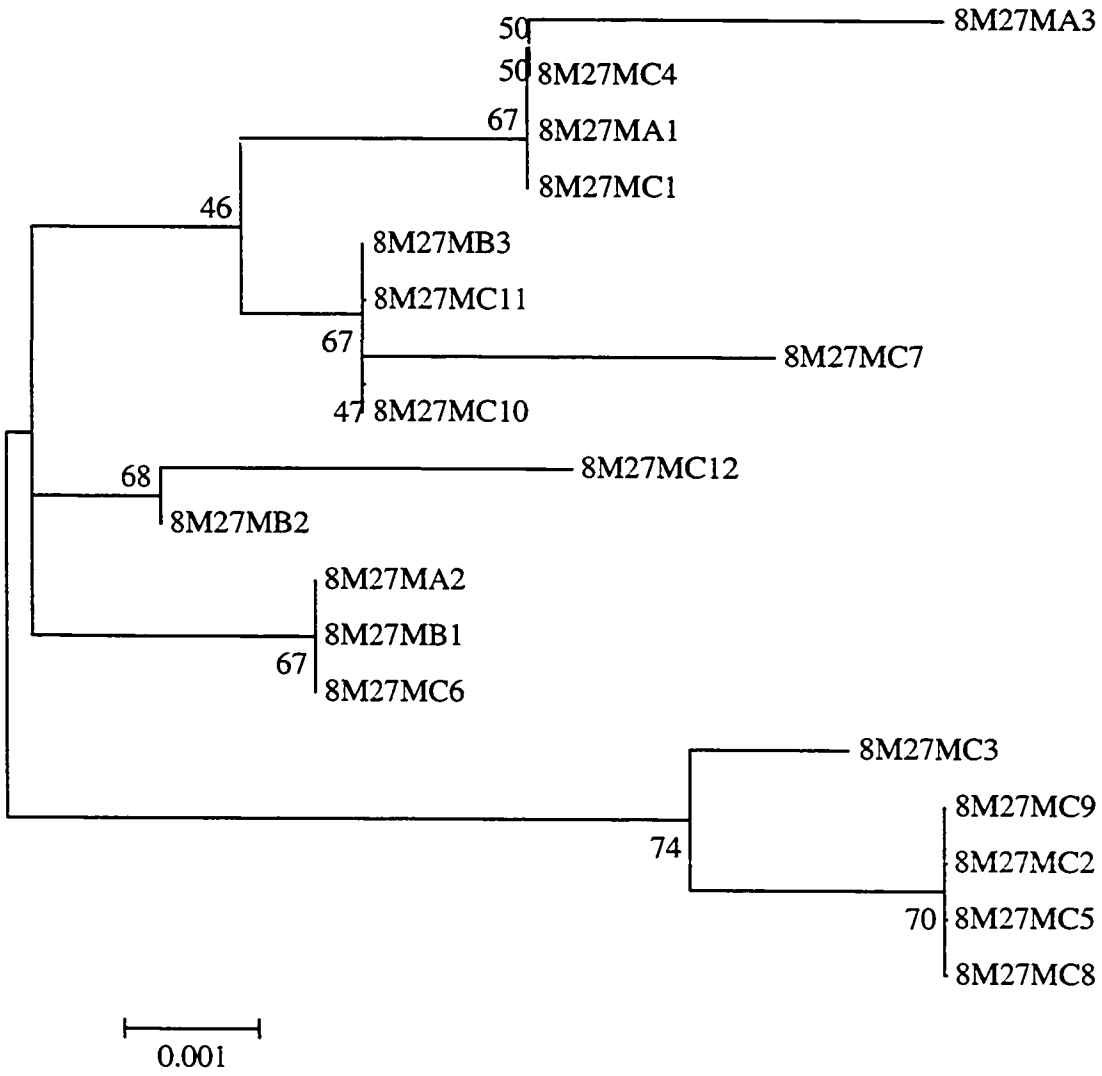


Figure 15: Phylogenetic tree of COI sequences of the Dungeness crab megalopae collected at sea during 1998. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using the Tamuara-Nei correction in MEGA

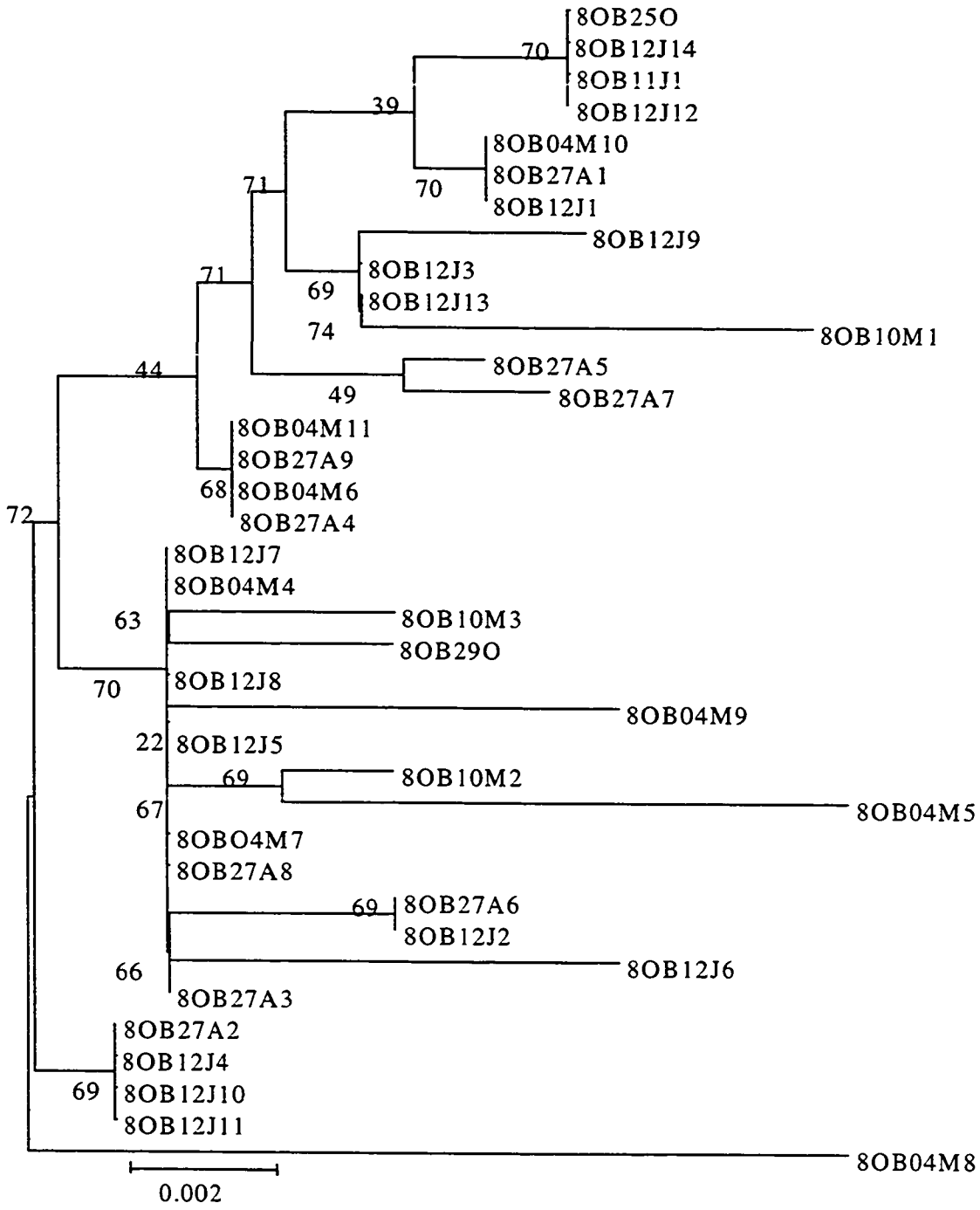


Figure 16: Phylogenetic tree of COI sequences of the Dungeness crab megalopae collected at bay during 1998. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using the Tamuara-Nei correction in MEGA

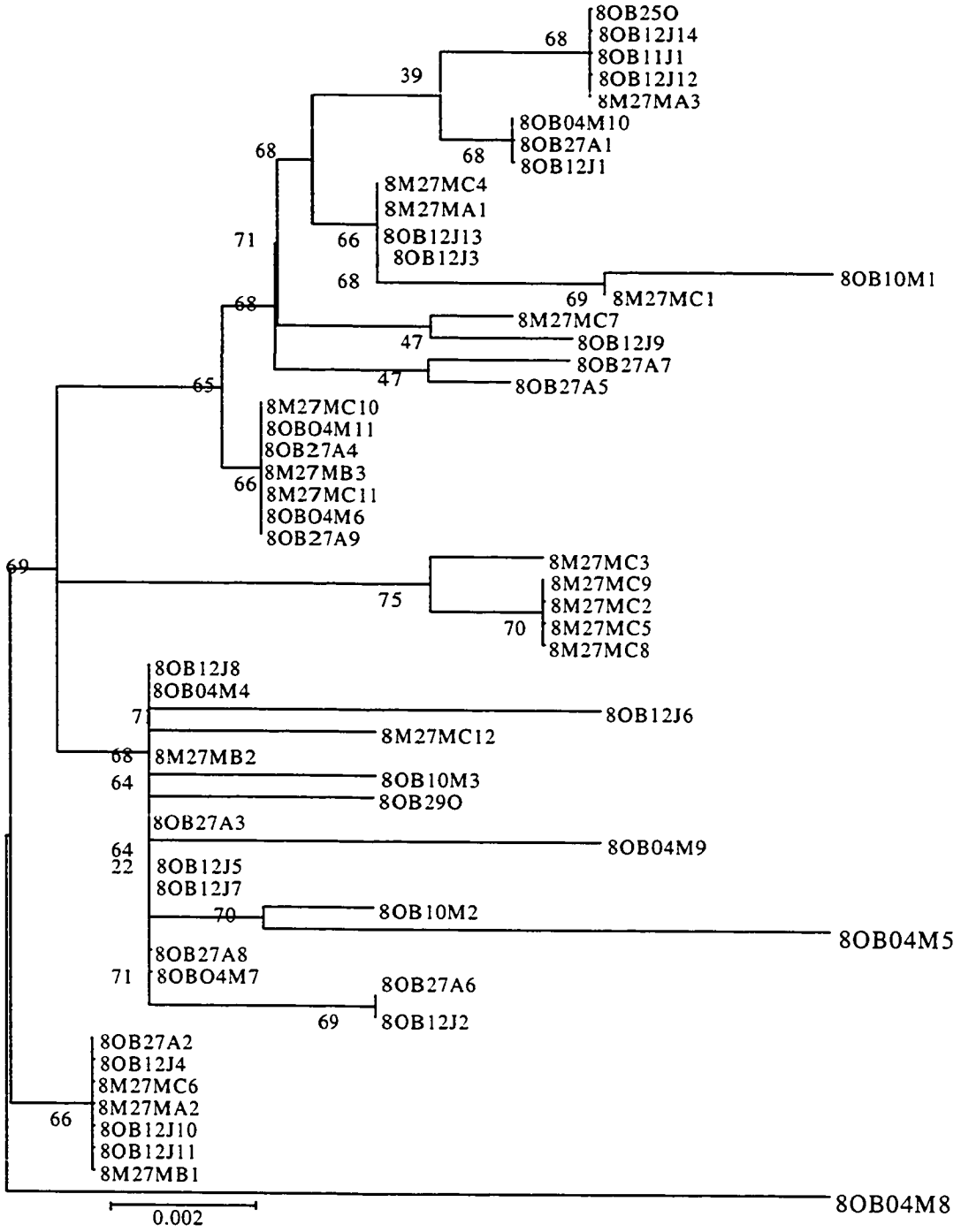


Figure 17: Phylogenetic tree of COI sequences of the Dungeness crab megalopae collected during 1998 both at sea and at bay. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using the Tamuara-Nei correction in MEGA

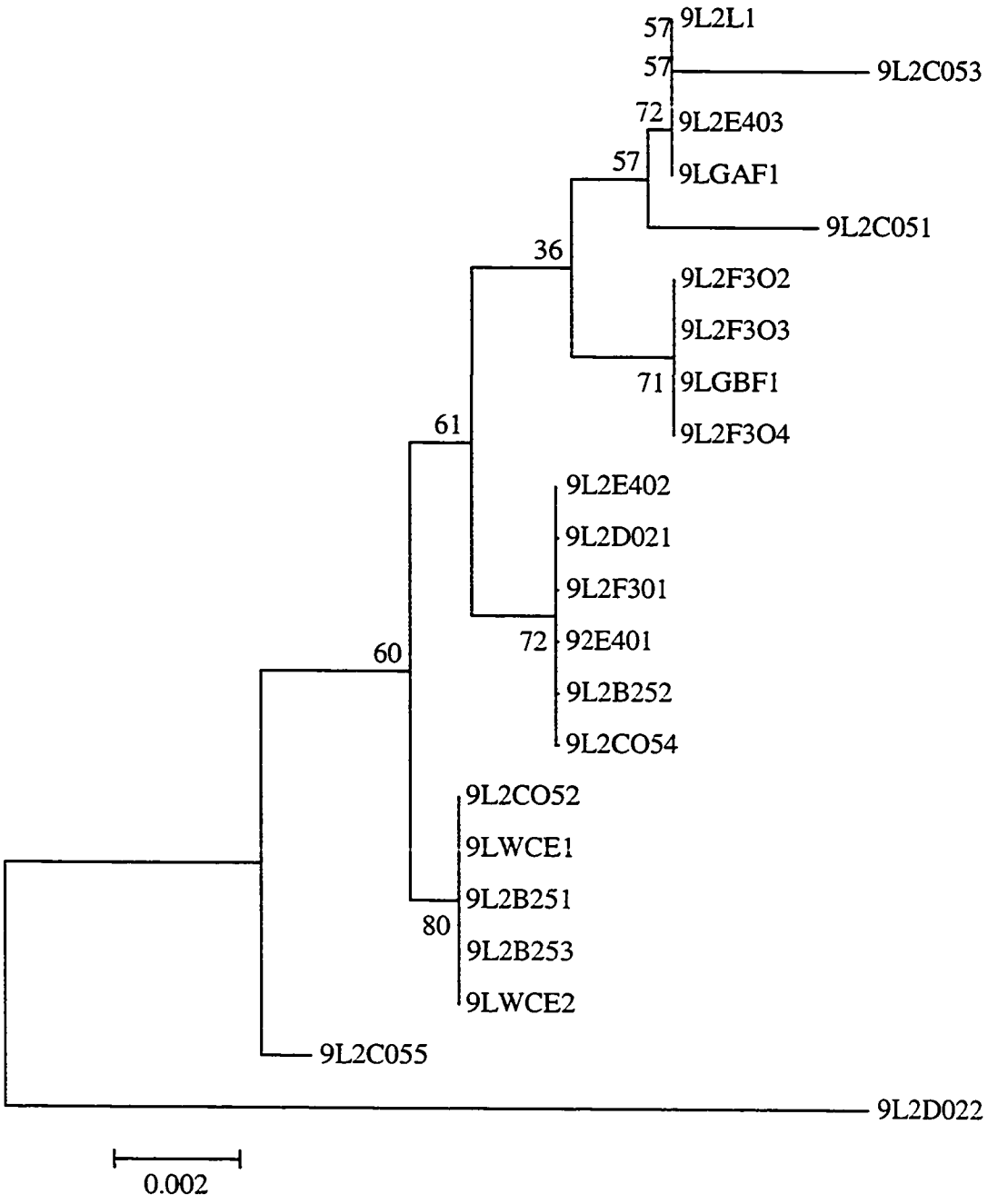


Figure 18: Phylogenetic tree of COI sequences of the Dungeness crab megalopae collected at sea during 1999. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using the Tamuara-Nei correction in MEGA

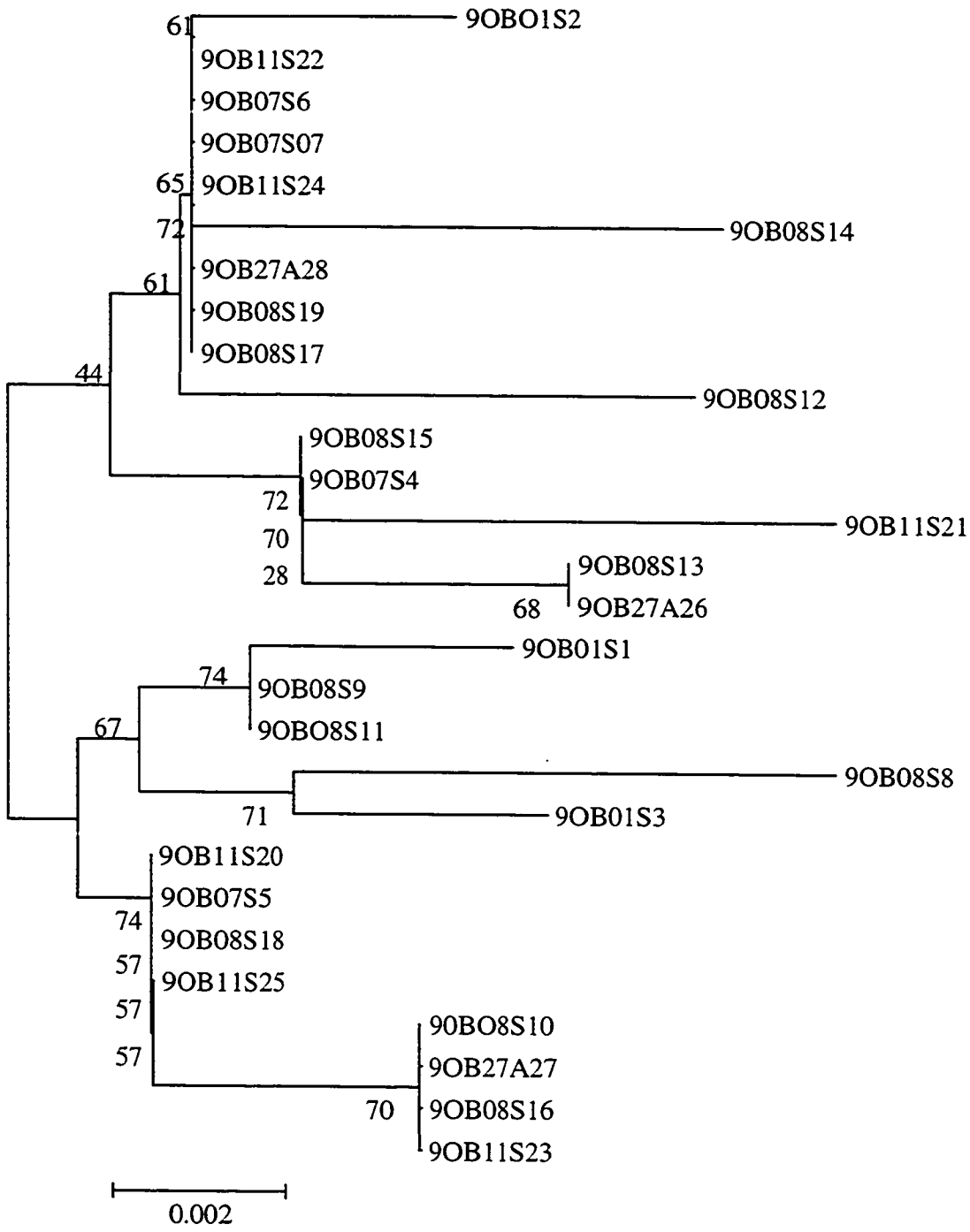


Figure 19: Phylogenetic tree of COI sequences of the Dungeness crab megalopae collected at bay during 1999. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using the Tamuara-Nei correction in MEGA

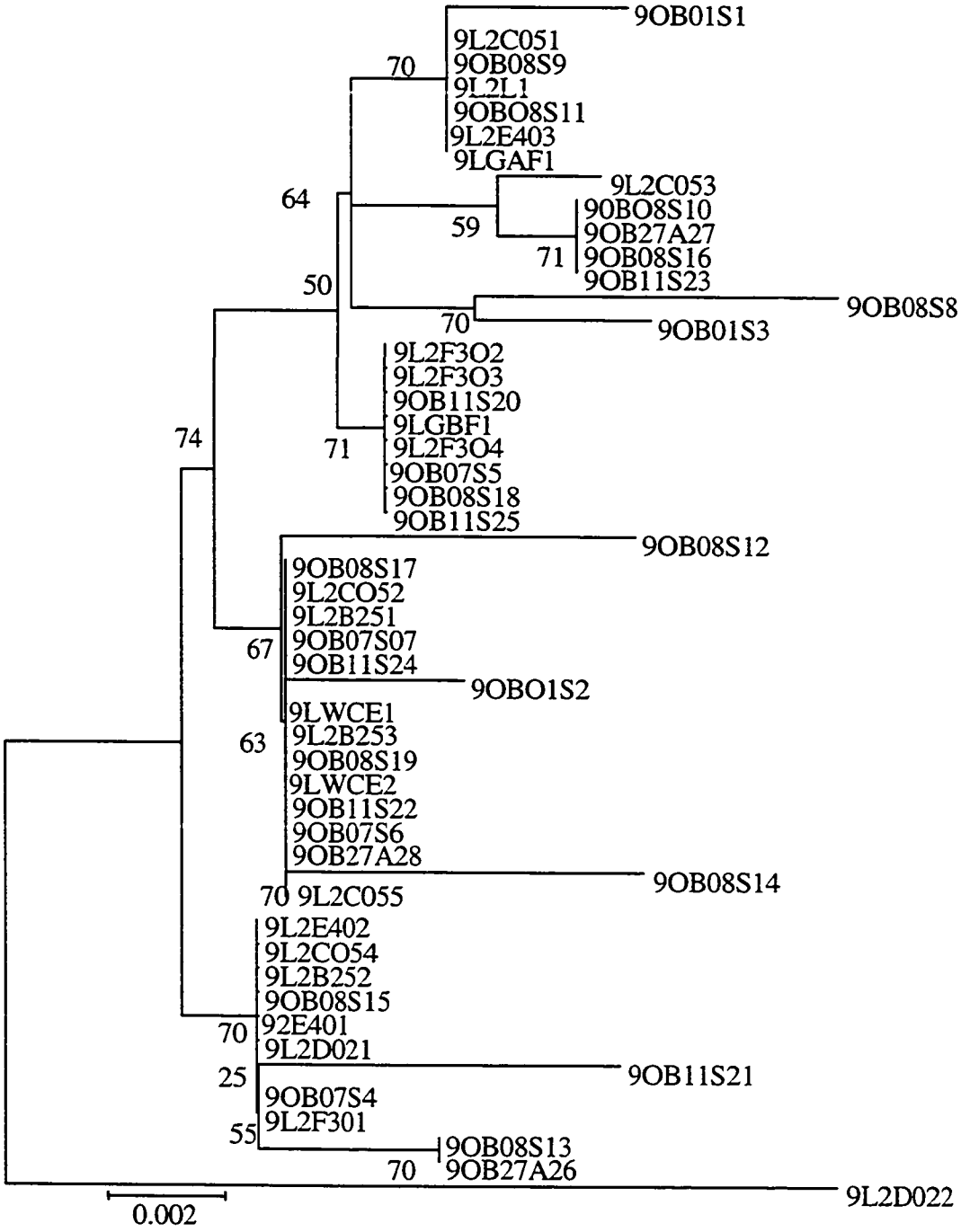


Figure 20: Phylogenetic tree of COI sequences of the Dungeness crab megalopae collected at sea and at bay during 1999. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using the Tamuara-Nei correction in MEGA

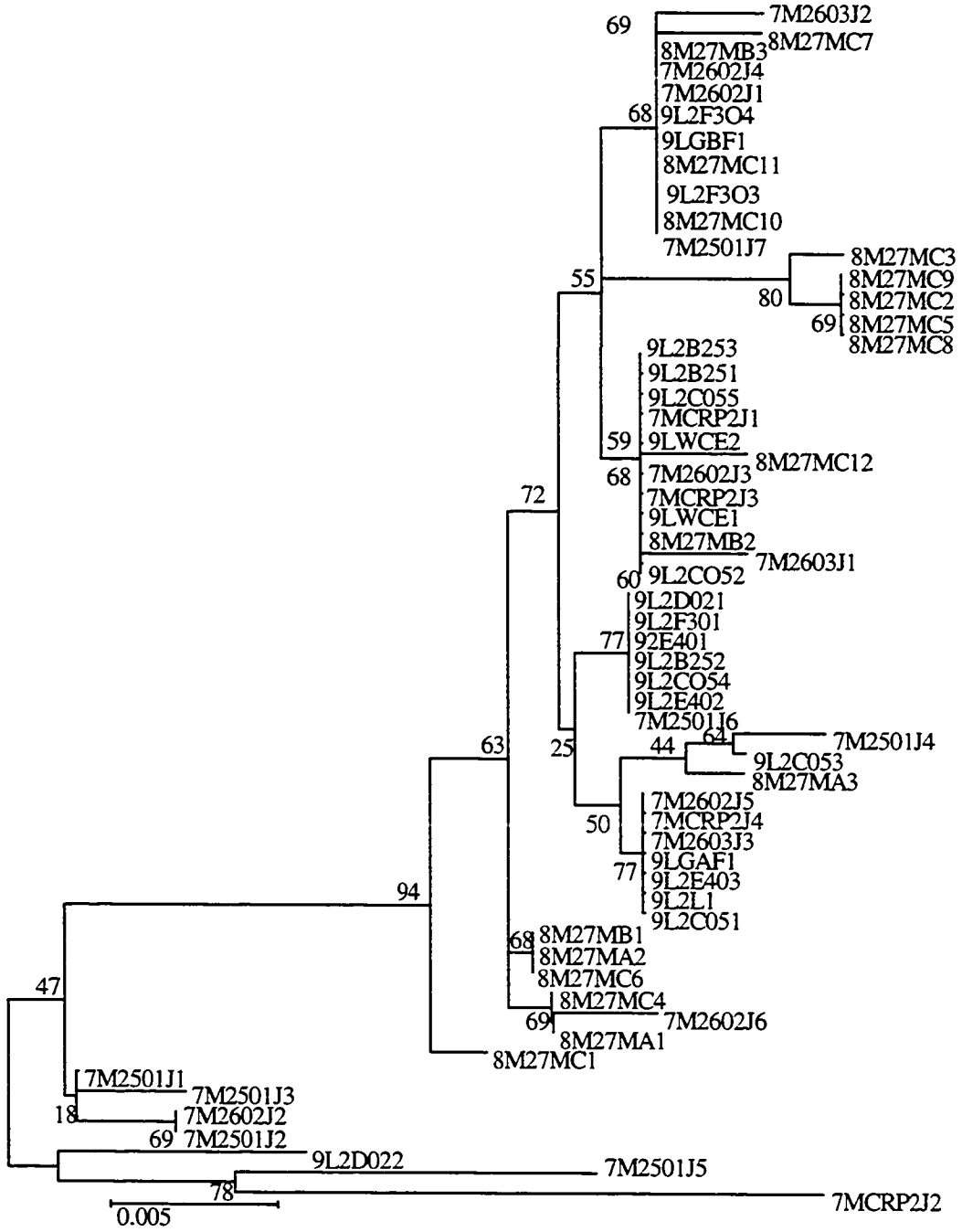


Figure 21: Phylogenetic tree of COI sequences of the Dungeness crab megalopae collected at sea during all three years. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using the Tamuara-Nei correction in MEGA

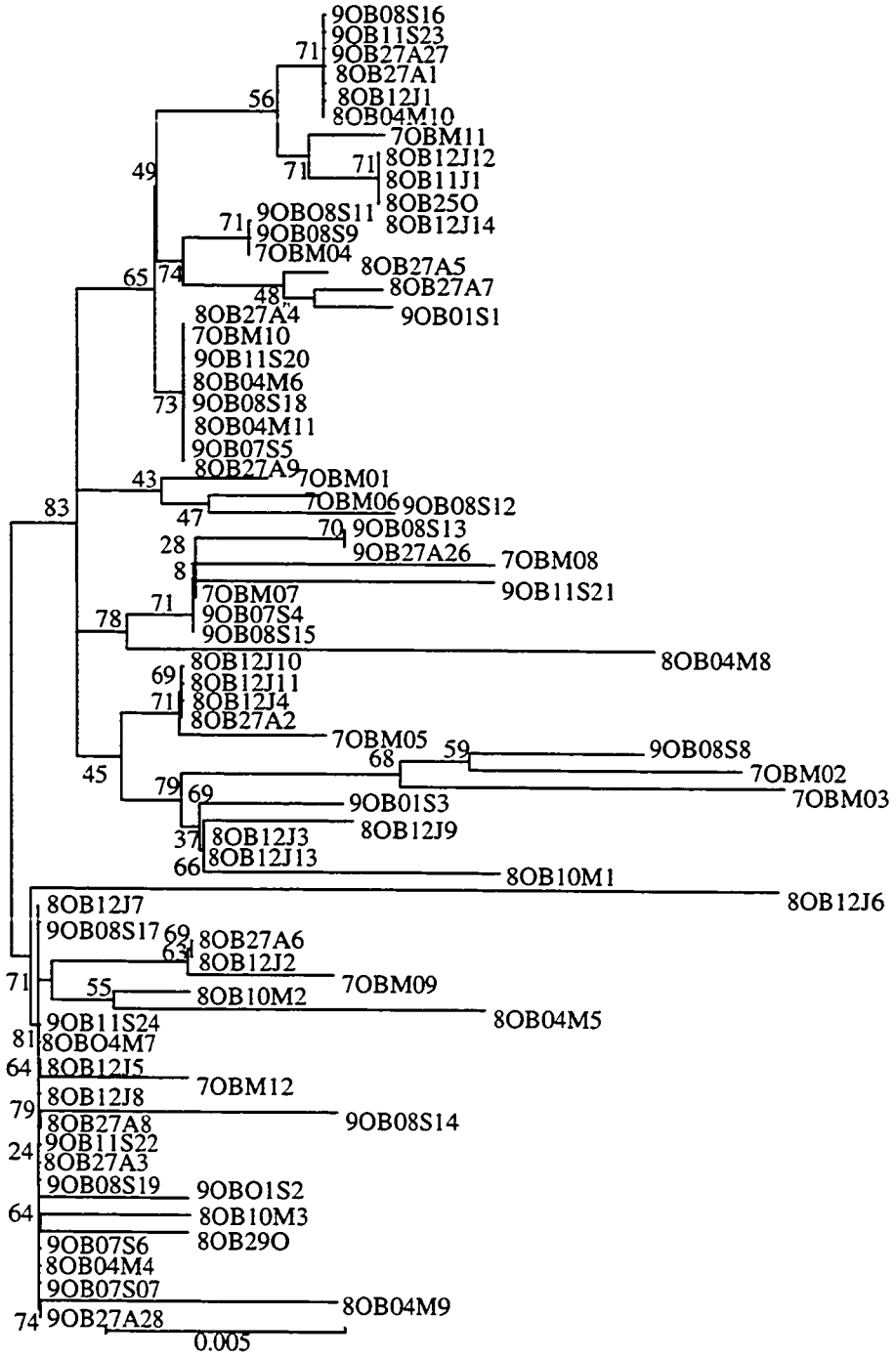
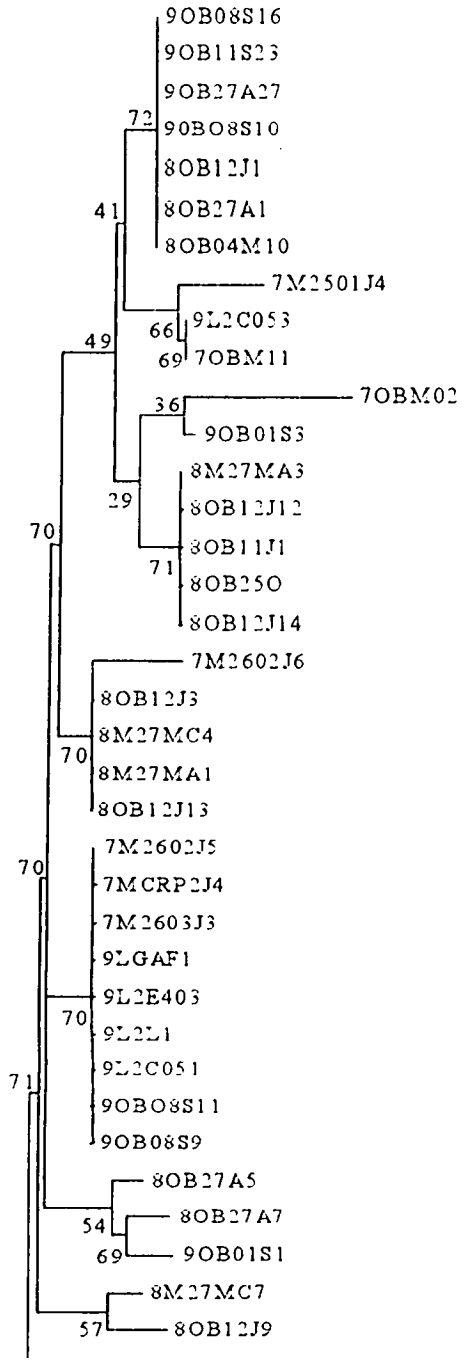
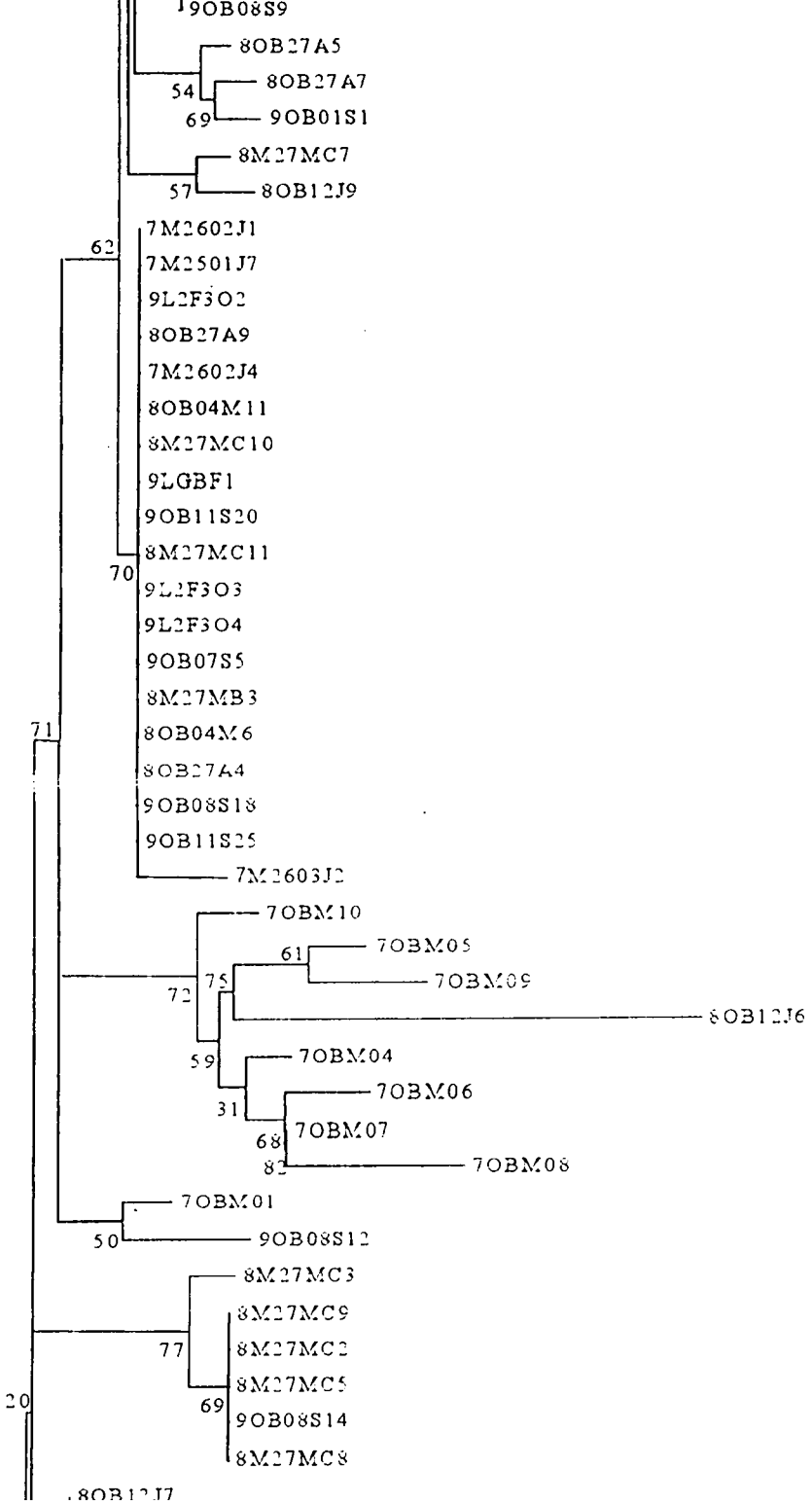
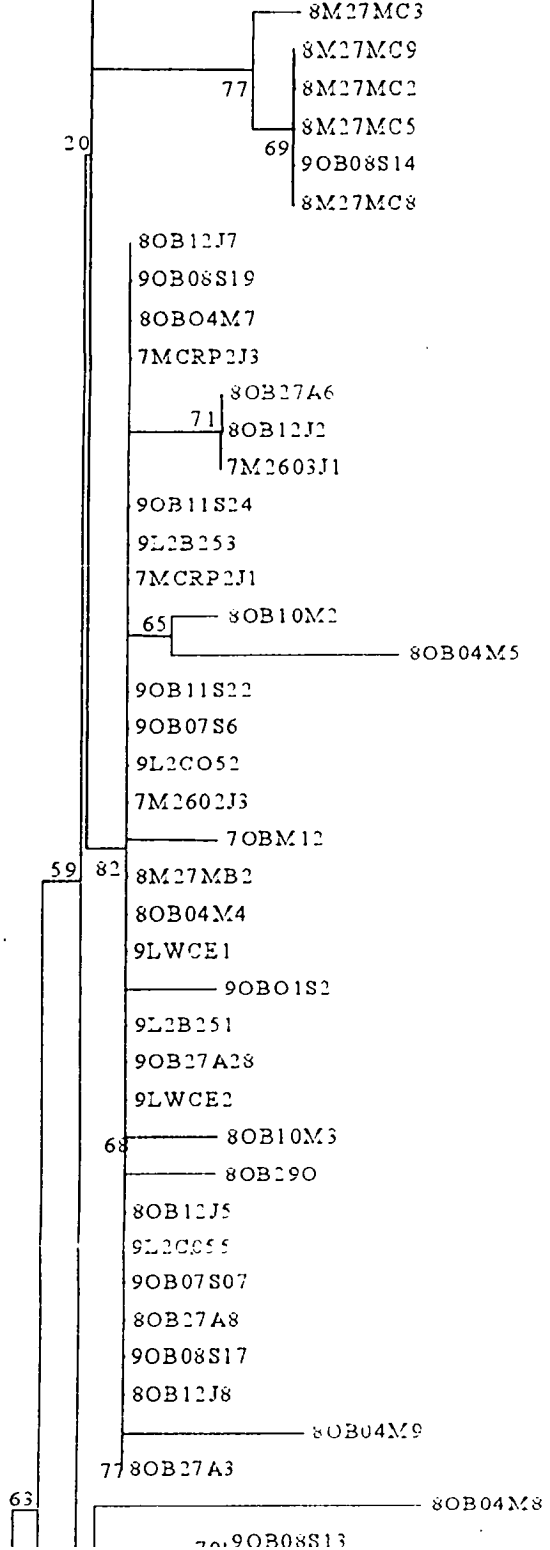


Figure 22: Phylogenetic tree of COI sequences of the Dungeness crab megalopae collected at bay during all three years. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using the Tamuara-Nei correction in MEGA


9OB08S16
9OB11S23
9OB27A27
72
90BO8S10
8OB12J1
41
8OB27A1
8OB04M10
7M2501J4
66
9L2C053
49
69
7OBM11
7OBM02
36
9OB01S3
8M27MA3
29
8OB12J12
70
8OB11J1
71
8OB25O
8OB12J14
7M2602J6
8OB12J3
8M27MC4
70
8M27MA1
8OB12J13
7M2602J5
70
7MCRP2J4
7M2603J3
9LGAF1
9L2E403
70
9L2L1
9L2C051
71
9OBO8S11
9OB08S9
8OB27A5
54
8OB27A7
69
9OB01S1
8M27MC7
57
8OB12J9


9OB08S9
8OB27A5
8OB27A7
54
9OB01S1
69
8M27MC7
8OB12J9
57
7M2602J1
62
7M2501J7
9L2F3O2
8OB27A9
7M2602J4
8OB04M11
8M27MC10
9LGBF1
9OB11S20
8M27MC11
70
9L2F3O3
9L2F3O4
9OB07S5
8M27MB3
71
8OB04M6
8OB27A4
9OB08S18
9OB11S25
7M2603J2
7OBM10
61
7OBM05
75
7OBM09
72
8OB12J6
59
7OBM04
31
7OBM06
68
7OBM07
82
7OBM08
7OBM01
50
9OB08S12
8M27MC3
8M27MC9
77
8M27MC2
8M27MC5
20
69
9OB08S14
8M27MC8
8OB12J7


8M27MC3
8M27MC9
77
8M27MC2
8M27MC5
20
69
9OB08S14
8M27MC8
8OB12J7
9OB08S19
8OBO4M7
7MCRP2J3
8OB27A6
71
8OB12J2
7M2603J1
9OB11S24
9L2B253
7MCRP2J1
65
8OB10M2
8OB04M5
9OB11S22
9OB07S6
9L2CO52
7M2602J3
7OBM12
59
82
8M27MB2
8OB04M4
9LWCE1
9OBO1S2
9L2B251
9OB27A28
9LWCE2
8OB10M3
68
8OB29O
8OB12J5
9L2C855
9OB07S07
8OB27A8
9OB08S17
8OB12J8
8OB04M9
77
8OB27A3
63
8OB04M8

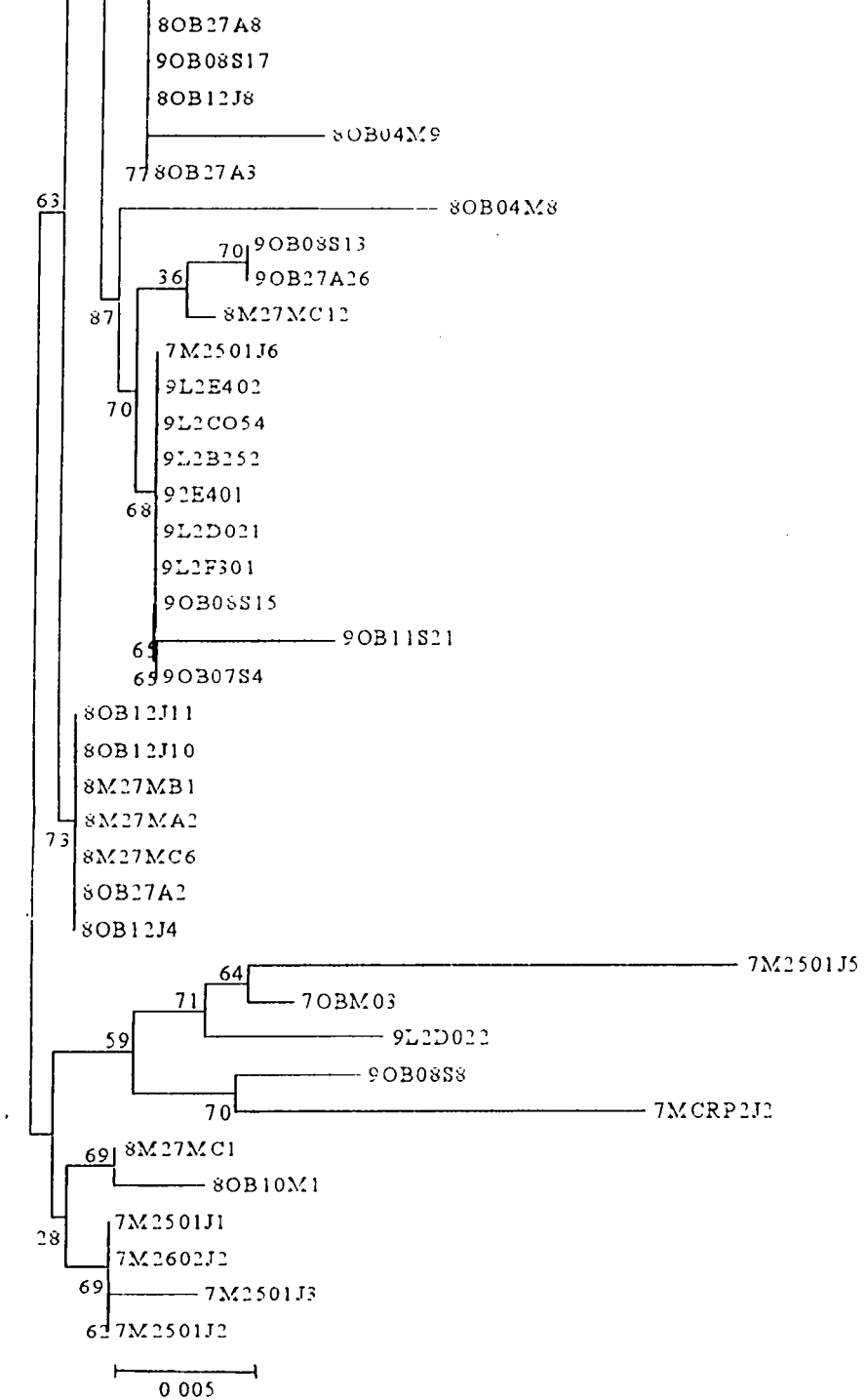


Figure 23: Phylogenetic tree of COI sequences of the Dungeness crab megalopae collected at sea and at bay during all three years. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using Tamura-Nei correction in MEGA

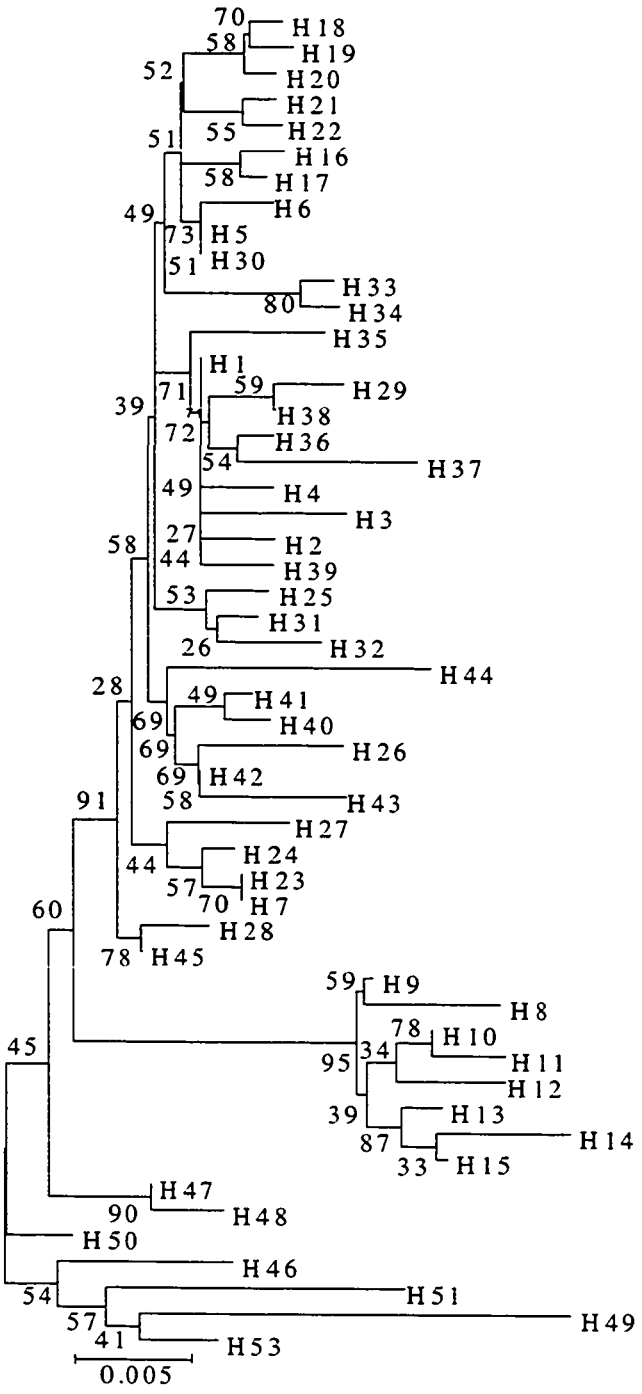


Figure 24: Phylogenetic tree relating 52 haplotypes observed among the 137 Dungeness crab mtDNA sequences. The tree represents 500 bootstrap replicates constructed by MEGA. The scale bar represents divergence using the Tamuara-Nei correction in MEGA

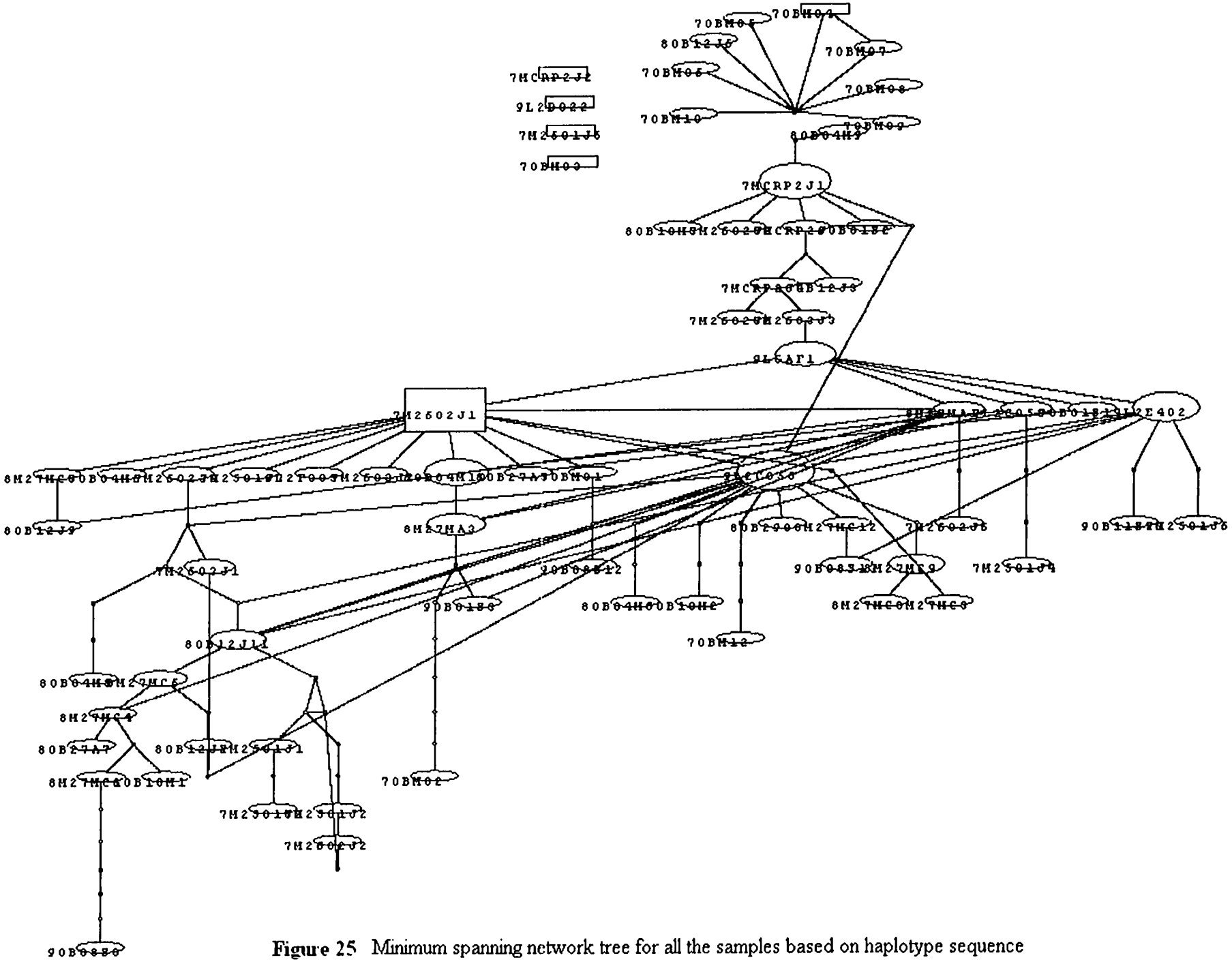


Figure 25 Minimum spanning network tree for all the samples based on haplotype sequence

DISCUSSION

Mitochondrial DNA has been widely used as a marker in population studies; however, few works have been devoted to crustacean mtDNA. Thus, in order to assess the appropriateness of the COI gene for this study, Tajima's neutral mutation hypothesis (i.e., all mutations are selectively neutral) was tested for COI gene of the mtDNA of Dungeness crab. This was done by a statistical method developed by Tajima (1983), which uses the relationship between the number of segregation sites (Kimura 1983) and the average number of nucleotide differences (Tajima 1983). Neutral mutation is a mutation that has no effect on the fitness of the individual. The Tajima statistic value D (-2.45) found by applying the Tajima statistical method to the part of COI gene of the mtDNA sequence of the 137 samples was not significantly different from 0. So, it was concluded that the DNA variation of the Dungeness crab mtDNA sequences might be influenced by neutral mutation. Therefore, it is reasonable to choose the COI gene segment for studying the sequence variation and population genetic studies of Dungeness crab. The present study is the first attempt at determining the degree of genetic differentiation due to larval dispersal in Dungeness crab, *Cancer magister*. This study focused on the megalopal population that encompasses the Oregon and Washington coast.

Genetic variation within and between patches/cohorts of megalopae collected at sea and at bay

The present study based on nucleotide variation suggests that megalopae from various sources are mixing at sea, which would homogenize the genetic composition of settling megalopae, regardless of genetic composition of adult stocks. Nucleotide diversity (Tables 7 and 10) was less than 1.6% except for only one case in both within and between patches/cohorts, which represent both temporal and spatial distribution of megalopae. Lack of variation at nucleotide level might be due to low rate of mtDNA differentiation in that particular part of CO1 gene. Similarly, genetic distance data (Tables 7 and 10) (another way to interpret nucleotide diversity) also shows that the megalopae are mixing at sea. The results from our study agree with other study based on protein electrophoresis (Soule and Tasto, 1983) of adult Dungeness crab population along the Pacific coast. They found no significant differences in the allele frequencies at the esterase locus and concluded that Dungeness crab is dispersing widely which would homogenize this population. The low nucleotide diversity reported from this study is similar to that observed in spiny lobster post larval (Silberman et al., 1994). They also found low amounts of genetic diversity (<1.6% of nucleotide diversity) among seasonal cohorts of spiny lobster post larvae and concluded that they are mixing at sea. In addition to this, several studies report lower genetic differentiation in species such as mole crabs (Tam et al., 1996), gooseneck barnacles (Van Syoc, 1994) and red rock lobsters (Ovenden et al., 1992). Since nucleotide diversity was less than 1.6% in most of the cases, it is likely that Dungeness crab larvae are mixing at sea.

The findings from our study are consistent with other studies done on related crab species. Gomez-Uchida et al. (2003) investigated genetic diversity within and between adult populations of the Hairy Edible crab, *Cancer setosus*, using allozyme and AFLP markers, covering a range of approximately 2500km along the Chilean coast. Genetic diversity estimates within populations were low for allozyme loci but much higher for AFLP loci. They also found that the differentiation among samples was statistically significant for allozymes but not for AFLPs. They concluded that Hairy Edible crab stocks off of Chile are composed of genetically homogeneous populations even though allozyme data suggest small, but significant, population differentiation. In addition, McMillen-Jackson et al. (1994) found an overall genetic homogeneity of allelic frequencies in the Blue crab populations, *Callinectes sapidus*, inhabiting the Atlantic and Gulf of Mexico coasts.

Haplotype diversity data and phylogenetic analysis also suggest that the Dungeness crab larvae are mixing at sea. This is because the haplotype diversity was high due to different source population or one source population with high genetic variation. In a study assessing the intraspecific genetic structure of *Balanus glandula* from southern California to Alaska, Hedgecock (1994) found that 96% of the total genetic diversity was found among individuals within samples and only 4% was accounted for by differences among populations. This lack of geographic structure in vertebrate and invertebrate populations from Alaska to California has been attributed to high levels of gene flow via larval dispersal (Hedgecock 1994; Stepien 1995). I recommend that genetic analyses of both the adult and the megalopae populations at each geographic location should be done

in future. This can be used to determine the sources of the megalopae. This is the only way to eliminate the within the patch/cohort but no significant differences were found among the patches/cohorts (Table 7). High haplotype diversity within patches might be possibility that post larval mortality, rather than larval transport, is structuring the population. The phylogenetic tree (Figures 12-21) also shows that the larvae are mixing at sea since there is no cluster representing a specific patch collected at sea or a specific cohort collected at coastal site.

In contrast to the homogeneity (low diversity measures) found in patches at sea, genetic diversity measures are comparatively high for cohorts of megalopae recruited in bay (Table 6). This might be due to either differential survival of genotypes after recruitment or by temporal variation in the genetic composition of recruits.

Effect of Oceanic currents on dispersal and recruitment of Dungeness crab megalopae

This study also reveals the fact that the varying oceanic current during different years has an effect on Dungeness crab larval dispersal. Genetic composition in terms of haplotype frequency distribution varies annually. Unique haplotypes found during different years may be representative of the source population depending on current pattern. This suggests that the larval dispersal depends on the annual current pattern. For instance H13, H9 and H45 are some of the unique haplotypes to 1998, since El Niño was predominant during 1998, they might be representative of southern population. Likewise H40, H46, H4 from 1999 may be from northern population and H47, H6, H51 from 1997 may be from the local population. Haplotype diversity data also suggests that the annual variation in oceanic current pattern has effect on Dungeness crab dispersion. This is because significant differences were found across years.

The samples collected during 1997 are more variable than other two years in several ways (Tables 6 – 10). First, most genetic diversity is seen within patches of megalopae collected at sea and in bay during 1997 (Tables 6 and 7). Also, nucleotide divergence and genetic distance between the megalopae found at sea and in bay for 1997 are high compared to other two years (Tables 8 -10). Secondly, most of the haplotypes were shared and no significant genetic differences are observed between the megalopae caught at sea and in bay during 1998 and 1999 (Figures 8 - 11 and Tables 5 and 11). Even though, all the haplotypes found in bay are unique compared to those at sea during 1997 (Figures 6 and 7) no significant genetic variation is observed between them (Tables 5 and

11). This suggests that the megalopae recruited in bay during all three years might be genetically similar to the ones found at sea at that particular year. However, significant variation is seen when comparing haplotype frequency among different years in bay (Table 11). This shows that the megalopae recruited in bay during 1997 are genetically distinct from those in 1998 and 1999 (Figures 6, 9 and 10). The fact that little genetic variation is found between megalopae captured in bay during the El Niño/La Niña years suggests other factors than average current velocities such as swimming behavior are influencing crab larval dispersal and recruitment. Thirdly, the phylogenetic tree drawn by considering all the samples collected both at sea and in bay from different years show a distinct clustering of 1997 samples with higher genetic distance (Figure 23). Similarly, the phylogenetic tree obtained by pooling the samples collected during all three years at sea showed a distinct clustering of 1997 individuals (Figure 21). There are two possible reasons. In 1999, megalopae were collected near Willapa Bay, in 1997 and 1998 megalopae were collected near Coos Bay but at different locations. This might be one of the reasons. Another reason might be the megalopae present during 1997 are coming from different source population than those present during other two years. This suggests the varied dispersal ability of Dungeness crab megalopae.

However, some of the samples from 1997 do cluster with other years (Figures 21 – 24) suggesting that these shared haplotypes could be from the same source population. In addition to this, the two most common haplotypes H1 and H5 (Table 4 and Figures 2 - 11) are seen during the entire spatio-temporal scale of this study. In general, it is likely that these two haplotypes are abundant in the Dungeness crab population.

Furthermore, chi-square analysis (Table 11) shows a significant genetic variation among the sample collections grouped by year and between megalopae collected at sea during 1998 and 1999. This might be again due to following reasons 1. Sampling season in Bay is different during different years. In 1997, megalopae were collected in early June whereas in 1998 they were collected during April through June and in 1999 during august through September. 2. Variation in sampling areas at sea during different years i.e., samples collected during 1999 are from Willapa Bay (little bit north), 1997, and 1998 are from Coos Bay (south) 3. Sampling locations at Coos Bay during 1997 and 1998 are different 4. Annual current patterns are different (1997 normal, 1998 el Niño and 1999 La Niña), thus source population might be different during the study period 5. Larval swimming behavior. Also, the haplotype frequency distribution at sea and in bay is unique during different years (Figures 6, 9, 10 and Figures 7, 8, 11).

The minimum spanning network tree and haplotype tree suggests the presence of different source population. The minimum spanning network (Figure 25) tree demonstrates the existence of six different maternal lineages. Five of these lineages were associated with the megalopae collected in 1997. In addition, the haplotype tree (Figure 24) showed four different clusters or lineages. Thus, the results obtained from this study suggest that the source of recruits does vary annually.

Two factors suggested that Dungeness crab population might have high genetic variation among patches. First, distinct patches observed by Roegner et al. (1999) indicating apparent isolation at sea. Secondly, De Brosse et al.(1990) have observed morphological differences in size and number of spines. However, our study suggests that

the degree of mixing is higher than one would expect under these circumstances. Daniel S. R. et al., (1998) found similar results in potamonautid river crab, *Potamonautes parvispina*. They found that the river crab populations in 2 Western Cape Rivers, South Africa, were genetically homogenous even though the river crabs in these regions vary morphometrically. They suggested that environmental factors might be responsible for the large degree of phenotypic variation.

The larval morphometric variability might have been induced by environmental factors (e.g. temperature, salinity, food). Dinnel et al. (1993) found recruitment of Dungeness crab in Puget Sound originates from at least 3 identifiable cohorts. Each of the cohorts settles at a different time, attains a different size. In some years one or more of these cohorts may fail to settle and, when settlement does occur, it can be quiet variable from year to year. Thus, effective recruitment to future fishery stocks can vary depending on the strength of each cohort in a given year and on survival and growth rate. They suggested that the differences in size might be due to different diets and energetic regimes. Variation in the timing of recruitment may be due to differences in temperature regimes associated with mating, spawning, egg incubation and larval development. Shirley et al. (1987) investigated differences in Dungeness crab larva between California and Alaska stocks, including laboratory experimentation, and concluded that larval morphology could be greatly affected by water temperature alone. In this study they found that *C. magister* zoea raised in the laboratory at varying temperature had longer spines and body lengths at colder temperatures. It is still left to be determined why

megalopae appear at sea in patches and recruit as cohorts in bay and yet we find no distinct genetic differences.

The Dungeness crab is economically important as a commercially harvested species and also supports a valuable sport fishery along the coasts of Northern California and the Pacific Northwest. Because of the economic importance of the Dungeness crab fishery to the west coast of United States, there is a great deal of interest in improving Dungeness crab fishery management. The establishment of marine reserves has been proposed (Roegner, Curtis and Shanks, Alan Personal communication) as one way to accomplish this. The spacing between reserves depends on the dispersal distance of the propagules. More number of samples should be collected during all seasons and along the entire range of the pacific coast. This will help us to determine the probable dispersal distance of Dungeness crab. These data can be compiled with the available information on the dispersal distance of the porpagules of other Pacific coast benthic marine organisms to design both size and spacing of the marine reserves. Marine reserves should be spaced far enough apart that dispersing propagules released from one reserve could settle in adjacent reserve.

REFERENCES

Armstrong, D. A., Stevens, B. G., and Hoeman, J. C. 1982. Distribution and abundance of Dungeness crab and *Crangon* shrimp, and dredging-related mortality of invertebrates and fish in Grays Harbor, Washington, U.S. Army Corps of Engineers, Seattle. Tech. Rep. 349

Avise, J.C. 1994. Molecular Markers, Natural History and Evolution. Chapman & Hall, New York.

Birky, C.W., Fuerst P. & Maruyama T. 1989. Organelle gene diversity under migration, mutation and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells and comparison to nuclear genes. Genetics 121: 613-627

Booth, J., Phillips, A. & Jamieson, G.S. 1985. Fine scale spatial distribution of *Cancer magister* megalopae and its relevance to sampling methodology. In: Proceedings of the Symposium on Dungeness crab Biology and Management. Meltcaff, B.R., ed. Alaska Sea grant Report. 85 (3): 273-286.

Botsford, L. W., Moloney, C. L., Hastings, A., Largier, J. L., Powell, T. M., Higgens, K. & Quinn, J. F. 1994. The influence of spatially and temporally varying oceanographic conditions on meroplanktonic metapopulations. Deep-Sea Research II. 41: 107-145.

Bousfield, E. L. 1955. Ecological control of the occurrence of barnacles in the Miramichi estuary. Nat. Mus. Canada, Bull. 137. Biol. Ser. 46: 1-69.

Brasher, D.J., Ovenden, J. R., Booth, J. D., & White, R. W. G. 1992. Genetic subdivision of Australian and New Zealand populations of *Jasus verreauxi* (Decapoda: Palinuridae)-preliminary evidence from the mitochondrial genome. New Zealand Journal of Marine and Frewhwater Research. 26: 53-58.

Bucklin, A., Riennecker, M.M. & Mooers, C. N. K. 1989. Genetic tracers of zooplankton transport in coastal filaments off northern California. J. Geophys. Res. 94: 8277-8288.

Bunch, T., Highsmith, R.C., Shields, S.F. 1998. Genetic evidence of dispersal of larvae of Tanner Crabs (*Chionecties bairdi*) by the Alaskan coastal Current. Mol. Mar. Sci. Biotech. 7: 153-159.

Burton, R. S., and Feldman, M. W., 1982. Population genetics of coastal and estuarine invertebrates: does larval behavior influence population structure?. In V. Kennedy [ed.], Estuarine comparisons. 537 – 551.

Caporale, D.A., Beal, B.F., Roxby, R., Van Beneden, R.J. 1997. Population structure of *Mya arenaria* along the New England coastline. Mol. Mar. Sci. Biotech. 6: 33-39

Cleaver, F. C. 1949. Preliminary results of the coastal crab (*Cancer magister*) investigation. Wash. State Dep. Fish, Olympia. Biol. Rep. 49A: 74-82.

Clement, M. Posada, D., and Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. Molecular ecology (9) 1657-1659.

Cronin, T.W. & Forward, R.B.Jr. 1982. Tidally timed behavior: Effects on larval distributions. In Estuarine Comparisons. Kennedy, V.S., ed.. Academic press, Ny. 505-520.

Daniel, S.R., Stewart, B.A., and Gibbons, M.J. 1998. Genetic and morphometric variation in the potamonautid river crab *Potamonautes parvispina* (Decapoda: Potamonautidae) from two Western Cape rivers, South Africa. 32: 1245-1258.

DeBrosse, G., Sulkin, S. & Jamieson, G. 1990. Intraspecific morphological variability in megalopae of three sympatric species of the genus *Cancer* (Brachyura: Cancridae). J. Crustacean Biol. 10: 315-329.

Dinnel, P. A., Armstrong, D. A., McMillan, R. O. 1993. Evidence for multiple recruitment-cohorts of Puget Sound Dungeness crab, *Cancer magister.* Marine Biology 115: 53 – 63.

Dinnel, P.A., Armstrong, D.A., and McMillan, R.O. 1993. Evidence for multiple recruitment cohorts of Puget Sound Dungeeness Crab, *Cancer magister.* Mar. Biol. 115: 53 –63.

Dumbauld, B. R., Armstrong, D. A. & McDonald, T.L. 1993. Use of oyster shell to enhance intertidal habitat to mitigate loss of Dungeness crab (*Cancer magister*) cased by dredging. Can. J. Fish. Aquat. Sci. 50: 381-390.

Gomez-Uchida, Daniel, David Weetman, Lorenz Hauser, Ricardo Galleguillos and Marco Retamal., 2003. Allozyme and AFLP analysis of genetic population structure in the hairy edible crab *Cancer setosus* from the Chilean Coast.

Grosberg, R. K. 1991. Sperm-mediated gene flow and the genetic structure of a population of the colonial ascidian *Botryllus schlosseri.* Evolution 45: 130 – 142.

Grosberg, R.K., and Levitan, D.R. 1992. For adults only? Supplyside ecology and the history of larval biology. Trend Ecol. Evol. 7: 130-133.

Harrison, K., and Crespi, J. 1999. Phylogenetics of *Cancer* crabs (Crustacea: Decapoda : Brachyura). Molecular Phylogenetics and Evolution 12 (2) 186 - 199.

Hedgecock, D. 1986. Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? Bull. Mar. Sci. 39: 550-564.

Hedgecock, D. 1994. Temporal and spatial genetic structure of marine animal populations in the California current. 35: 73-81.

Hickey, B.M. 1989. Patterns and processes of circulation over the Washington continental shelf and slope p. 41- 116. In M.R. Landry and Hickey, B. M., [ed.] Coastal oceanography of Washington and Oregon. Elsevier, Amsterdam and New York p.607.

Hickey, B.M. 1979. The California Current system – hypotheses and facts. Prog. Oceanogr. 8: 191 – 279.

Hobbs, R. C. & Botsford, L. W. 1992. Diel vertical migration and timing of metamorphosis of larvae of the Dungeness crab, *Cancer magister*. Mar. Biol. 112: 417-428.

Jacoby, C.A. 1982. Behavioral responses of the larvae of *Cancer magister* Dana (1852) to light, pressure and gravity. Mar. Behav. Physiol. 8: 267-283.

Jamieson, G.S. & Phillips, A.C. 1988. Occurrence of *Cancer* crab (*C. magister* and *C. oregenesis*) megolopae off the west coast of Vancouver Island, British Columbia. Fish. Bull. 86: 525-542.

Jamieson, G.S. & Phillips, A.C. 1993. Megalopal spatial distribution and stock separation in Dungeness crab (*Cancer magister*). Can. J. Fish. Aquat. Sci. 50: 416-429.

Johnson, D. F.L., Botsword, W., Methot, R. D and Wainwright, T. C., 1986. Wind stress and cycles in Dungeness crab catch off California, Oregon and Washington. Can. J. Fish. Aquat. Sci. 43: 838 – 845.

Karl, S. A., Schultz, S., Desbruyeres. D., Lutz, R., Vrijenhoek, R. C. 1996. Molecular analysis of gene flow in the hydrothermal vent clam (*Calyptogena magnifica*). Mar. Sci. Biotech. 5: 193-202.

Kimura, M. 1983. The neutral theory of molecular evolution. London: Cambridge Univ. Press.

Kumar, S., Tamura, I. Jakonsen, B., and Nei, M. 2001. MEGA2: Molecular Evolutionary Genetics analysis Software. Bioinformatics. 17 (12): 1244-1245.

Larvey, S., Moritz, C., and Fielder, D.R. 1995. Changing patterns of population structure and gene flow at different spatial scales in the coconut crab (Birgus latro). Heredity. 74: 531-541.

MacKay, D.C.G. 1942. The Pacific edible crab, *Cancer magister*. Fish.Res. Board Can. Bull. No.62.32pp.

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.

McMillan, R.O., Armstrong, D.A. & Dinnel, P.A. 1995. Comparison of intertidal habitat use and growth rates of two northern Puget Sound cohorts of 0+ age Dungeness crab, (*Cancer magester*). Estuaries 18: 390-398.

McMillen-Jackson, A.L., Bert, T.M., and Steele, P. 1994. Population genetics of the blue crab *Callinectes sapidus*: modest population structuring in a background of high gene flow. Mar. Biol. 118: 53-65.

Mohoney, C.L., Botsford, L. W. & Largier, J. L. 1994. Development, survival and timing of metamorphosis of planktonic larvae in a variable environment: the Dungeness crab as an example. Mar. Ecol. Prog. Ser. 113: 61-79.

Moran, P., Dightman, D.A., Waples, R.S., Park, L.K. 1997. PCR_RFLP analysis reveals substantial population-level variation in the introns of Pacific salmon (*Oncorhycchus* spp). Mol. Mar. Sci. Biotech. 6: 315-32.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Mol. Ecol. 3: 401-411.

Murray-Jones, S.E., Ayre, D.J. 1997. High levels of gene flow in the surf bivalve *Donax deltoids* (Bivalvia: Donacidae) on the east coast of Australia. Mar. Biol. 128: 83-89.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Orensanz, J.M & Gallucii, V.F. 1988. Comparative study of post larval life-history schedules in four sympatric species of *Cancer* (Decapoda: Brachyura: Cancridae). J. Crust. Biol 8: 187-220.

Ovenden, J. R., Brasher, D.J., and White, R.W.G., 1992. Mitochondrial DNA analysis of the red rock lobster, *Jasus edwardsii*, supports an apparent absence of population subdivision throughout Australia. Mar. Biol 112: 319 – 326.

Palumbi, S. R. & Benzie, J. 1991. Large mitochondrial DNA differences between morphologically similar penaeid shrimp. Mol. Mar. Biol. tech. 1: 27-34.

Palumbi, S. R., and Kessing, B. D., 1991. Population biology of the trans-arctic exchange: MtDNA sequence similarity between Pacific and Atlantic sea urchins. Evolution 45: 1790 – 1805.

Pearson, W.R. (1990) Rapid and Sensitive Sequence comparison with FASTP and FASTA. Methods in Enzymology 183:63 – 98.

Pearson, W.R. and Lipman D.J. (1988) Improved tools for Biological Sequence comparison. PNAS 85: 2444-2448.

Peterson, W. T., Miller, C.B. & Hutchindson, A. 1979. Zonation and maintenance of copepod populations in the Oregon upwelling zone. Deep Sea Res. 26A: 467-494.

Rielly, P.N. 1983. Dynamics of Dungeness crab, *Cancer magister*, larvae off central and northern California. In: Wild, P.W. & Tasto, R.N. Life History, Environment, and the Mariculture Studies of the Dungeness Crab, Cancer magister, With Emphasis on the Central California fishery Resource. Fish. Bull, Calif Dept. Fish Game. 172: 57-84.

Rocha-Olivares, A., Vetter, R.D. 1998. Effects of oceanographic circulation on the gene flow, genetic structure, and phylogeography of the rosethorn rockfish (*Sebastes helvomaculatus*). Can. J. Fish. Aquat. Sci 56: 803-813.

Roegner, G.C., Shanks, A., Armstrong, D.A. 1999. Larval transport and recruitment. In PNCERS 1998 Annual Report, J.K. Parrish and S. Breslow, eds. NOAA. Coastal Oceans Program.

Roughgarden, J., Gaines, s. and Possingham, H 1988. Recruitment dynamics in complex life cycles. Science 241: 1460 – 1466.

Rozas, J. and Rozas, R. (1999). DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15: 174-175.

Shenker, J. M. 1988. Oceanographic associations of neustonic larval and juvenile fishes and Dungeness crab megalopae off Oregon. Fish. Bull. 86: 299-317.

Shirley, S. M., Shirley, T. C., and Rice, S. D. (1987). Latitudinal variation in the Dungeness crab, *Cancer magister*: zoel morphology explained by incubation temperature. Marine Biology. 95: 371-376.

Silberman. J.D., Sarver, S.K., Walsh, P.J. 1994. Mitochondrial DNA variation in seasonal cohorts of spiny lobster (*Panulirus argus)* post larvae. Mol. Mar. Sci. Biotech. 3: 165-170.

Soule, M., Tasto, R. N. (1983). Stock identification studies on the Dungeness crab, *Cancer magister*. Fish Bull. Calif. 172: 39-42.

Stepien, C.A. 1995. Population genetic divergence and geographic patterns from DNA sequences: examples from marine and freshwater fishes. In Evolution and the aquatic ecosystem: defining unique units in population conservation. Edited by J.L. Nielsen and D.A. Powers. American Fisheries Society, Bethesda, Md. 263-287.

Stevens, B. G., and Armstrong, D. A. 1984. Distribution, abundance and growth of juvenile Dungeness crabs, *Cancer magister*, in Grays Harbor Estuary, Washington. U.S.Natl. Mar. Fish. Serv. Fish. Bull. 82 (3): 469-483.

Stevens, P.M. 1991. A genetic analysis of the pea crabs (Decapoda: Pinnortheridae) of New Zealand. Mar. Biol. 108: 403-410.

Strub, P. T, Allen, J. S., Huyer, A., and Smith, R. L., 1987b. Large-scale structure of spring transition in the coastal ocean off western North America. J. Geophys. Res. 92 (C2): 1527 – 1544.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite population. Genetics. 105: 437-460.

Tam, Y.K., Kornfield, I., Ojeda, F.P. 1996. Divergence and zoogeography of mole crabs, *Emerita* spp. (Decapoda: Hippidae), in America. Mar. Biol. 125: 489-497.

Van Syoc, R.J. 1994. Genetic divergence between subpopulations of the eastern Pacific goose barnacle *Pollicipes elegans*: mitochondrial cytochrome c subunit I nucleotide sequences. Mol. Mar. Sci. Biotech. 3: 338-346.

Wild, P.W. 1983. Effects of seawater temperature on spawning egg development, hatching success and population fluctuations of the Dungeness crab, *Cancer magister*. In: Wild, P.W. & Tasto, R.N. Life History, Environment, and the Mariculture Studies of the Dungeness Crab, *Cancer magister*, with emphasis on the Central California Fishery Resource. Fish. Bull. Calif. Dept. Fish Game. 172: 197-213.

Williams J. G. 1979. Estimation of intertidal harvest of Dungeness crab, *Cancer magister* on Puget Sound, Washington beaches. U.S. Natl. Mar. Fish. Serv. Fish Bull. 77 (1): 287 – 292.

Young, C. & Chia, F.S. 1987. Abundance and distribution of pelagic larvae as influenced by predation, behavior and hydrographic factors. In: Reproduction of Marine Invertebrates, IX, General Aspects: Seeking Unity in Diversity. Giese, A.C., Pearse, J.S., & Pearse, V.B. eds. Blackwell Scientific. 385-463.

Young, C. Behavior and locomotion during the dispersal phase of larval life. In: Ecology of Marine Invertebrate Larvae. L.R. McEdward, ed. CRC Press Inc. Boca Raton, Florida. 249-277.