GENETIC ANALYSES ON THE DEEP-SEA SHRIMP *ACANTHEPHYRA PELAGICA* IN THE NORTH-WEST ATLANTIC.

by

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To my biggest support and love of my life,

Iván Vera

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ABSTRACT

Connectivity and gene flow in the marine environment is difficult to predict due to the apparent absence of physical barriers and discrete limits to dispersal. Here I examine the population structure, gene flow and mating system in *Acanthephyra pelagica*, a micronekton species abundant in the deep waters of the Northwest Atlantic. Samples were obtained in different areas of the Northwest Atlantic, from northern Newfoundland to the Scotian Shelf, with emphasis in the Sable Gully Marine Protected Area where sampling was conducted on four consecutive years. Twenty six novel microsatellite loci were developed for this species to aid in this research. The use of the polymorphic loci allowed the assessment of different aspects of population structure. I evaluated generational, temporal and spatial differences. For the generational assessment, cohorts were determined using carapace length measurements. The cohort information was also used to infer the reproductive cycle of *A. pelagica*. The reproductive strategy of the species was also investigated through the use of molecular techniques in order to determine the existence of multiple paternity.

Out of the twenty six microsatellite markers only 12 were polymorphic and out of those, 10 were free of null alleles. Four cohorts were found in each year of sampling, and a total of six cohorts were identified in from 2007 to 2010. It was possible to infer an average four years life span and seasonal spawning. The population was found to be panmictic among locations spatial at scales of less than 1500 km. Significant genetic structure and isolation by distance was found at a higher spatial scale. There were also no genetic differences among years or among generations. Multiple paternity was found to be prevalent in the population with 2-4 fathers in each brood with skewed contribution of each father to the brood. The lack of genetic structure in the population suggests high dispersal and connectivity for this species in this environment. Multiple paternity may be playing a role in the high gene flow detected in this species.

List of Abbreviations and Symbols Used

AMOVA - Analysis of Molecular Variance

dNTP - Deoxynucleotide triphosphates

DVM - Diel Vertical Migration

EDTA - Ethylenediaminetetraacetic acid

FCA - Factorial Correspondence Analysis

H_O - Observed Heterozygosity

H_E - Expected Heterozygosity

HWE - Hardy-Weinberg Equilibrium

IBD – Isolation by distance

mM - Millimolar

MPA - Marine Protected Area

NAFO - North Atlantic Fisheries Organization

N_e – Effective Population Size

PCoA - Principal Coordinate Analysis

PCR - Polymerase Chain Reaction

PLD - Planktonic Larval Duration

PD - Pelagic Larval Duration

PrDM - Probability of detecting multiple mating

µL - Microliter

µM – Micromolar

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Chapter 1: Introduction

Predicting spatial structure in oceanic environments may be challenging due to the difficulties in distinguishing physical limits that are driving genetic structure, also the large population size of organisms and the lack of discrete physical barriers lead to high levels of gene flow and consequently to resisting genetic divergence (Palumbi, 1992). Genetic differentiation is subject to a balance between the forces of genetic drift, gene flow, selection and mutation. Additionally, connectivity in the sea is hard to estimate due to the life cycles of most of marine organisms having highly mobile larvae that are susceptible to being carried by oceanic currents (Hohenlohe, 2004) and different pressures (physiology, predation, food supply) determining the survivorship of individuals carried by currents. In pelagic species, the high degree of habitat continuity, the typically large effective population sizes, and the passive dispersal of larvae can lead to a low level of genetic differentiation (Carvalho and Hauser, 1998). Nevertheless, exceptions to this trend can be found, mainly due to factors such as behavioural mechanisms, complex oceanographic patterns, and barriers to gene flow that could be creating and maintaining population differentiation (Palumbi, 1992).

For micronekton species, that is, small but actively swimming organisms ranging from ~1-2 cm to ~12.5 cm (Pearcy, 1983) and occurring mainly in the mesopelagic zone (200-1000 m depths), the most important physical factors influencing gene flow are mesoscale oceanographic processes (e.g., currents, eddies, coastal upwelling, etc.), since these processes influence dispersion and connectivity among different zones. Physiology and behaviour also influence connectivity and can have a huge impact on gene flow. Understanding these factors together with genetic information can help us to

comprehend how the population is structured in the sea. Micronekton exhibit the ability to swim and are consequently able to avoid or minimize passive drifting with currents, however, the larvae commonly swim slowly, more slowly than a typical horizontal ocean current. The larval stages of my focus species, *Acanthephyra pelagica,* are planktonic and therefore may be advected to other areas. Ocean currents may transport larvae hundreds of kilometers or more (Kinlan and Gaines, 2003; Cowen, 2006; Leis, 2014). Drifting as larvae may be important for the migration in and out of the Sable Gully, one of the main areas of study in this thesis. As adults, micronekton can avoid transportation, nevertheless, these organisms are relatively weak swimmers, and so oceanographic features may be a large barrier for them. In this case, the main oceanographic feature of Atlantic Canada is the Labrador Current which flows southeastward over the continental shelves and slopes of Labrador and Newfoundland (Talley and McCartney, 1982; Lazier and Wright, 1993). Further south on the Scotian Shelf, water circulation is dominated by a southward flow of waters of northern origin, primarily the Nova Scotian Current on the inner shelf and an extension of the Labrador Current along the shelf edge (Hannah *et al*. 2001). On the Sable Gully, the mean circulation is complex with presence of gyres and with seasonal and stratified variations and tidal influences (Shan *et al*, 2013; Greenan *et al*, 2014). These environmental characteristics may be of great importance in maintaining distinct populations among a series of samples distributed temporally, generationally and spatially in this canyon.

The focus species of this investigation is the deep-sea decapod shrimp *A. pelagica.* As many other pelagic shrimps, *A. pelagica*, is considered micronekton. As noted above, the micronekton includes a large group of organisms defined by their size and swimming capacity. The concept includes all mobile organisms capable of withstanding significant current speeds (four body lengths per second over prolonged periods) (Pearcy, 1983).

The micronekton group comprises, among others, larval and juvenile fish, small pelagic fish, krill, pelagic small squids, and shrimps. The most abundant micronekton species in the main study area, Sable Gully, include the fish *Benthosema glaciale* and several shrimp species including *A. pelagica*. The high biomass and abundance levels of *A. pelagica*, suggest this species plays an important ecological role (Company *et al*., 2001). The daily vertical migration of this shrimp species plays a major role in the transfer of energy between pelagic and benthic environments (Foxton, 1970; Morris, 1973).

Acanthephyra pelagica is found in the North and South Atlantic as well as in the South Pacific Oceans. Its bathymetric distribution is between 200-2000m depth, but most individuals are usually found between 700 and 1800m depth off the Azores (Burukovsky and Andreeva, 2010). Within the Sable Gully, MacIsaac et al. (2014), found that the distribution of *A. pelagica* was restricted to depths below 250 m, it concentrates in the 250–750 m stratum, although a considerable portion of the individuals are found deeper, below 1250 m, showing also variations in vertical distribution between day and night samples. Burukovsky and Andreeva (2010) show that off the Azores, spawning occurs in August and maximum recruitment occurs in September. Females grow faster than males and are often found at more than 1000m depth. Pairing with males apparently occurs at a depth of 800-900 m, spawning and brooding eggs at depths exceeding 1000 m. Absolute realized fecundity (ARF – quantity of eggs present on a female's pleopods) varies from 560 to 3700 eggs with an average longest egg diameter of 1.15 mm (Burukovsky and Andreeva, 2010). Until now the biology of this species in the northwest Atlantic has not been studied extensively.

An important objective of my thesis is to improve knowledge on the biology of *A. pelagica* and understand how these biological aspects may be influencing the genetic structure of the species. Several phenotypic and genotypic measurements were made in

order to understand the reproductive strategy of *A. pelagica*. This thesis examines the evidence for multiple mating in a species that inhabits the deep-sea environment where direct observation is difficult. Therefore the application of polymorphic genetic markers is of great importance. The existence of multiple mating is relevant to the ecology and evolutionary biology of the species since it affects the strength of natural and sexual selection (Fleming and Gross, 1994; Evans and Magurran, 2000), the effective population sizes (Sugg and Chesser, 1994; Martinez *et al*., 2000), and the genetic variability. My goal is to estimate the degree of multiple mating (number of individuals genetically contributing to each brood (Neff and Pitcher, 2002) as well as the frequency of multiple mating (proportion of broods in a population that exhibits multiple mating) (Kelly *et al*., 1999; Zane *et al*., 1999). Both aspects will contribute to improving our understanding of the mating system and reproductive strategies in *A. pelagica*.

As mentioned previously, the main sampling area for this study is the Sable Gully, which is the largest submarine canyon off the coast of eastern North America (Greenan *et al.,* 2014). Submarine canyons are common on the continental margins of North America (Kunze *et al*., 2002; Harris and Whiteway, 2011). Canyons are usually areas that present higher species diversity and great biological productivity (Hickey, 1995; Kenchington *et al.*, 2013; Greenan *et al.,* 2014), canyons also help in transport of material from offshore to the continental slope (Shephard *et al.,* 1974; Nittrouer and Wright, 1994; Kunze *et al*., 2002). Moreover, canyons present an increased flow of nutrient because of the presence of upwellings (Freeland and Denman, 1982; Kunze *et al*., 2002; Greenan *et al.,* 2014). The Sable Gully (65 km long and 15 km wide) is unique among canyons of the eastern Canadian margin (Figure 3.3) because of its great depth (>2000 m), steep slopes, and extension onto the continental shelf. It is situated about 200 km off the Nova Scotia peninsula, to the east of Sable Island on the edge of the Scotian Shelf (Greenan *et al*.,

2014). It was an important fishing area for many years (Gordon and Fenton, 2001), until 2004 when it was designated a Marine Protected Area (MPA) under Canada's Oceans Act. The Sable Gully was recognized for being an area of high biodiversity and productivity (Fisheries and Oceans Canada, 1998). It is the core area of distribution of the northern bottlenose whale (*Hyperoodon ampullatus*) (Whitehead *et al.*, 1997) and supports some of the richest coral growths known in Canadian waters (Kenchington, 2010). Patterns of circulation in The Gully suggest the canyon may play an important role as a retention area, and in the larger scale transport of materials onto and off of the shelf (Gordon and Fenton, 2001). Because of those features, the Sable Gully is a zone of high biological productivity with several marine organisms that are present in high abundances in this canyon.

This thesis encompasses diverse objectives organized in three chapters. Chapter 2 is dedicated to the development and design of microsatellite markers. These novel markers will then be used in two subsequent chapters and are also expected to contribute to future research on *A. pelagica*. Chapter 3 focuses on understanding the population genetic structure of *A. pelagica* through the use of the neutral polymorphic DNA markers developed in Chapter 2. I attempt to distinguish genetic structure at a spatial, temporal and generational scale, taking into consideration oceanographic features and biological characteristics of the species. This goal was accomplished testing the null hypothesis: "*There is no significant genetic differentiation among samples from different locations, different generations, and different years".* In Chapter 4, I used some of the polymorphic markers developed in Chapter 2 to examine the evidence for the presence of multiple paternity in *A. pelagica*, testing the null hypothesis: "*There is only one father contributing to the each brood on A. pelagica".*

Chapter 2: Isolation and Characterization of Twenty Six Novel Microsatellite Loci in the Deep-Sea Shrimp *Acanthephyra pelagica.*

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2.1 Abstract

Acanthephyra pelagica is a widespread deep-sea decapod crustacean that is highly abundant and thus, of high ecological significance, within the Sable Gully Marine Protected Area (MPA). Despite its importance no genetic analyses have previously been conducted on this species. Here I present a total of 26 novel species-specific microsatellites isolated and characterized in 132 individuals collected from one wild population within the Sable Gully. The number of alleles observed in polymorphic loci ranged from 6 to 18 with an average of 8.1. The observed and expected heterozygosities varied from 0.156 to 0.723 and from 0.156 to 0.913 respectively. These novel microsatellites are a contribution to future studies on *A. pelagica* and will have a positive impact on the future studies on biology and ecology of this deep-sea species.

2.2 Introduction

Acanthephyra pelagica (Risso, 1816) (Fig. 2.1) is a crustacean that belongs to the order Decapoda, family Acanthephyridae. The species is found in the North and South Atlantic, and South Pacific Oceans (Fig. 2.2). *Acanthephyra pelagica* is a deep living species with a bathymetric distribution between 200 - 2000m depth, but most individuals

are usually found at depths between 700 and 1800m. The species has no economic value, although its high biomass in areas of great biological productivity suggest it is of great ecological importance.

A total of 26 novel species-specific microsatellites were developed to examine population structure and analyze patterns of paternity. No other microsatellites had been developed before for this species. The microsatellites were developed with individuals sampled at the Sable Gully Marine Protected Area (MPA) on the Atlantic coast of Canada. These novel microsatellites will be used in population genetic analyses and on paternity analyses of *A. pelagica* in this thesis research (Chapters 3 and 4).

2.3 Methods

Acanthephyra pelagica individuals were collected from the Sable Gully MPA on the Scotian Shelf (44°N 59°W), off the Atlantic coast of Canada. Upon collection, samples were stored at -20 °C. Tissue samples for DNA analysis were stored in 95% ethanol and ethanol replaced after one week.

Muscular tissues were digested with 400 µg/mL proteinase K (Bio Basic Inc., Markham, ON, Canada) and 300 µL of digestion buffer (10 mM Tris–HCl, pH 8.3, 1 mM EDTA, 400 mM NaCl containing 0.8% sodium dodecyl sulfate (SDS)), for five hours at 55°C on a shaker working at 200 rpm. DNA was extracted using a glass-milk protocol (Elphinstone *et al*., 2003).

DNA extracts of two individuals were sent to The McGill University and Génome Québec Innovation Centre (Montreal, QC, Canada) for sequencing (454 Life Sciences Corp.,

Bradford, CT). The software Msatcommander (Rozen and Skaletsky, 1999; Faircloth, 2008) and QDD (Meglécz *et al*., 2010) were used for primer design. A total of 59 primers were tested. Microsatellite loci were amplified in 7.5 µL total volume containing 1.65 µL of dd H2O, 1 µL of 10x reaction buffer $(NH_4)2SO_4$ (Bio Basic Inc., Markham, Ontario), 1 µL of 25mM MgSO4 (Bio Basic Inc., Markham, Ontario), 0.1 µL of 1 µM of fluorescently labeled M13 tag, 0.1 µL of 1 µM of un-tailed primer (either forward or reverse), 0.1 µL of 0.1 µM of M13 tailed primer (either forward or reverse), 1 µL of 2.5 µM dNTPs (Bio Basic Inc., Markham Ontario), 0.05 µL of TSG Polymerase (Biobasic Inc.,Markham, Ontario), and 1.3 µL approximately 50 ng/µL of genomic DNA. PCR conditions were as follows: 5 min at 94°C, 35 cycles of denaturation (94°C for 30s), annealing (57 – 65°C for 30s), and extension (72°C for 30s).

To visualize the PCR products, 1 µL of PCR product was mixed with 9 µL of formamide. The mix was loaded on a polyacrylamide gel that was run and imaged on a Li-COR machine (Li-COR Bioscience, Lincoln, Nebraska). The genotypes of each individual were obtained using SAGA Automated Microsatellite Software 3.3. The genotype data were analyzed with the MICRO-CHECKER 2.2.3 software (van Oosterhout *et al*., 2004) to test for the presence of null alleles or possible scoring inconsistencies. Tests for Hardy–Weinberg Equilibrium were conducted with Arlequin 3.5.1.2 Software (Excoffier and Lischer, 2010) with a posterior Bonferroni correction.

2.4 Results and Conclusions

Of the 59 primers tested, 26 microsatellites were screened successfully for *A. pelagica*. Twelve out of the 26 microsatellites designed were polymorphic whereas the other 14 were monomorphic. The number of alleles of the polymorphic markers ranged from 6 to 18. The observed and expected heterozygosities varied from 0.156 to 0.723 and from 0.156 to 0.913 respectively. Two loci showed evidence of null alleles when analysed with MICRO-CHECKER, namely Acpe26 and Acpe43. The same two loci deviated significantly from Hardy-Weinberg equilibrium.

These 26 novel microsatellites are a contribution to future studies on *A. pelagica* and will have a positive impact on the research of deep-sea species.

Table 2.1: Characteristics of 26 microsatellite loci. The table shows locus name, primer sequence (5'-3'), repeat motif, size range (bp), annealing temperature (Ta), number of observed alleles (Na), expected heterozygosity (He), observed heterozygosity (Ho), number of successfully amplified individuals (N) and p-value of Hardy-Weinberg equilibrium test (HWE p-value).

Locus	Primer sequence (5'-3')	Repeat	Size Range	Ta(°C)	Na	He	Ho	N	HWE p-value	GenBank Accesssion Number
Acpe03	F: TTGAGAACATGGAATCAGCG	(ACAG)5	170-214	64	10	0.572	0.412	131	0.4410	KJ541077
	R: GCTGAACAAATTCGGATGGA									
Acpe06	F: TACGAGCCGGACAAATTCAA	(ACGG)6	109-145	65	9	0.302	0.273	132	0.0885	KJ541078
	R: CGCATGAATGTATTACAAGACATAAG									
Acpe14	F: TAATCGGACAAATATGGGTGG	(ACTC)6	87-107	65	6	0.418	0.287	129	0.0075	KJ541079
	R: TGCATCTGGAGTTATTGGTCG									
Acpe17	F: CCTGCGGCGACATTATGA	(AAAT)6	189-217	62	6	0.156	0.156	128	1.0000	KJ541080
	R: AGGGTTGCTTCTTCTCATGC									
Acpe20	F: CAATGCTCTTCTTTGCGTAACA	(ACAG)7	176-220	62	9	0.524	0.454	130	0.0240	KJ541081
	R: TGGATGATGCAGTTTGCAGT									
Acpe22	F: TCATAGACCCAAAGATGGCAA	(ACAT)10	150-170	62	6	0.576	0.583	132	0.9409	KJ541082
	R: GATCACAGCGCTTTATCCCA									
Acpe32	F: ATGCACAAACCACTCCACAA	(AG)7	135-147	56	6	0.529	0.420	131	0.0070	KJ541083
	R: CCAATTGCTACACATTCAACCA									
Acpe51	F: GCGGTTCTCGAGTTATAAGGTG	(ACAG)^8	207-235	59	7	0.582	0.723	130	0.0772	KJ541084
	R: GGCGTTTGGGAACGACTG									
Acpe52	F: GCACGCACCCGGTAAAG	(ACAT)^10	261-293	63	7	0.589	0.535	129	0.2914	KJ541085
	R: CGTCAGAATACGATCACAGCG									
Acpe57	F: TCCATACTCTGCAGTTCGC	(ACTG)^8	187-243	57	15	0.763	0.664	131	0.0239	KJ541086
	R: CTGTCAGCCAGTTCTTCCG									
Acpe26	F: TTCTGCAGCAGGAAGTATTGC	(AG)7	214-254	57	18	0.913	0.522	103	0.0000	KJ541087
	R: AAAGCACTCAACCGAAGAAGTT									
Acpe43	F: GGTTTCCACCCTTTCAGACC	(AC)9	135-159	65	12	0.823	0.644	112	0.0001	KJ541088
	R: AATAGGCTGGCTATGGCTCC									
Acpe02	F: AGTAGAACCACGCAAGCACC	(AGAT)5	112	60	1	NA	NA	10	NA	KJ541089
	R: GAACGAGGCGTCAGAGCTA									
Acpe08	F: TTGTTCGACAACGAAACCTG	(AAAC)5	92	60	1	NA	NA	10	NA	KJ541090
	R: TCAAAGACTAACTACCAAATGGTCTG									
Acpe09	F: CAAGCATATTTGCCTCAGGG	(ACAG)5	102	60	1	NA	NA	10	NA	KJ541091
	R: GACTTCACGGGTGCTCTCAT									
Acpe13	F: CATACAGAACCACAGACAGACC	(AAAC)5	90	65	1	NA	NA	10	NA	KJ541092
	R: CGAACGTTTAGTACGTCCGGT									
Acpe19	F: TCCCATTCTAGCTTGAAATATTGG	(AGAT)6	94	63	1	NA	NA	10	NA	KJ541093
	R: TGCATCTATCTGTCTGCTTATCTACC									

Table 2.1 continued

Locus	Primer sequence (5'-3')	Repeat	Size Range	Ta(°C)	Na	He	Ho	N	HWE p-value	GenBank Accesssion Number
Acpe21	F: TATTGGCCAAACAGACTCGG	(ACTG)7	182	62	1	NA	NA	10	NA	KJ541094
	R: GTTCGTCCTACCTGCTGTCG									
Acpe24	F: TGCAGAGGTTTAGACTTCTAGATCAAC	(AATCC)5	108	60	1	NA	NA	10	NA	KJ541095
	R: CTGTGCGTTTCCTTAGGGC									
Acpe25	F: CAGCTGGTATAGGTAGTTCCCG	(AT)7	90	63	1	NA	NA	10	NA	KJ541096
	R: GCGAGATGCCTTAGTGCTTG									
Acpe30	F: AGAGGAAATGTTCTGCTGCG	(AC)7	121	64	1	NA	NA	10	NA	KJ541097
	R: CCGTGGAAGAGTCCAGGTAG									
Acpe31	F: TCCCATAGGGAGAATCAGGTC	(AT)7	95	65	1	NA	NA	10	NA	KJ541098
	R: GTCGTACACTGCACACTCGC									
Acpe34	F: TTATGCATTGCCCTTCTTCC	(AG)7	106	65	1	NA	NA	10	NA	KJ541099
	G: AAAGGAAGGAGACACGTGGG									
Acpe40	F: CAACAACTGCTCCCATAA	(AG)8	229	60	1	NA	NA	10	NA	KJ541100
	R: TCGCGTCCATGTACACAACT									
Acpe42	F: GCTTGTCCAGCTTCCTCTC	(AG)9	102	62	1	NA	NA	10	NA	KJ541101
	R: TCTGGTCCCTTTCCCTCTTT									
Acpe56	F: ATGTGCTGAGTTGCCAAGG	(CTGT)6	133	63	1	NA	NA	10	NA	KJ541102
	R: CTTGCTTTGCTCATCCC									

Figure 2.1. Photo of *Acanthephyra pelagica* (Risso, 1816) with size scale. (From MacIsaac *et al*., 2014)

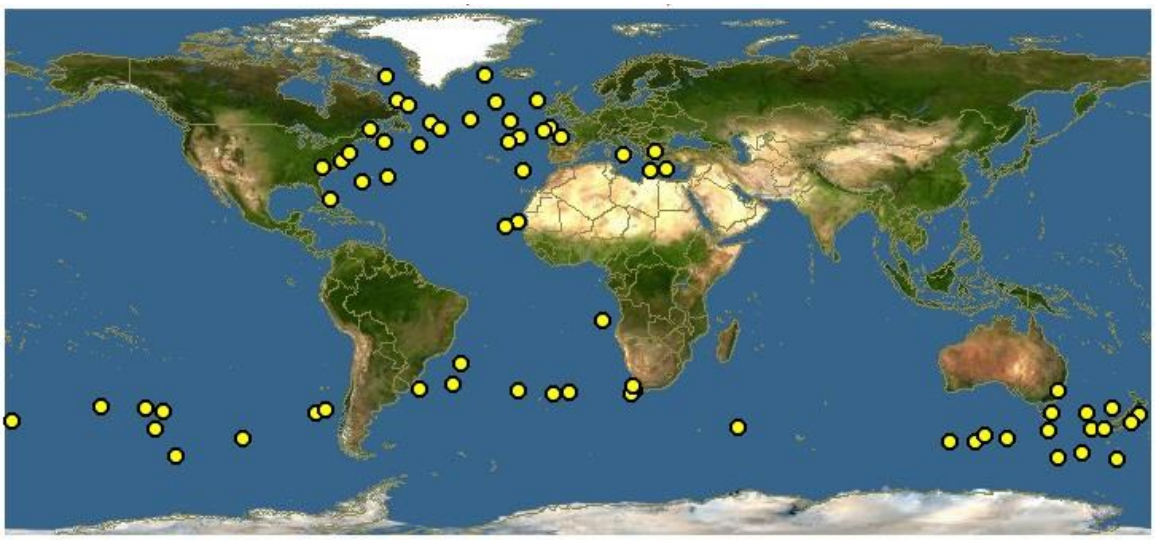


Figure 2.2. World distribution of *Acanthephyra pelagica*. http://www.boldsystems.org.

Chapter 3: Population Structure

3.1 Introduction

In oceanic environments it is difficult to distinguish physical limits that are driving genetic structure: the large population size of organisms and the lack of discrete physical barriers leads to high levels of gene flow and thus acts to resist genetic divergence (Palumbi, 1992). Furthermore, the estimation of connectivity among populations of most of marine organisms is challenging because of their characteristic high fecundities and the frequent existence of a larval phase both of which can be passively dispersed (Cowen and Sponaugle, 2009; Hohenlohe, 2004; Bradbury *et al*., 2008). Larval phase is the dominant dispersal stage, and thus the focus stage to understand population connectivity in marine systems (Cowen and Sponaugle, 2009). Larval dispersal was defined by Cowen and Sponaugle, 2009 as "the intergenerational spread of larvae away from a source to the destination site at the end of the larval stage". Larval dispersal is driven by biological processes such as offspring production, growth, development, survival and behavior. It is also driven by physical processes such as advection and diffusion as well as by the interaction between larval traits and physical properties of the environment (Cowen and Sponaugle, 2009). Dispersal distances are difficult to measure and recent studies have shown extreme heterogeneity in the range of distances across a variety of taxa (meters to thousands of kilometers) (Kinlan and Gaines, 2003; Bradbury *et al*., 2008; Cowen and Sponaugle, 2009). Some investigations have demonstrated, for benthic species, that the dispersal distances are directly related with the planktonic larvae duration (PLD) (Shanks *et al*., 2005; Bradbury *et al*., 2008; Shank, 2009). At the

same time longer PLD is associated with larger latitudinal and greater depth distribution (Bradbury *et al*., 2008) Nevertheless, many other factors affect dispersal distance, especially behavioural traits of larvae and adults (Hedgecock *et al*., 2007). Understanding dispersal and connectivity is important as they are influencing the evolutionary stability and persistence of species and communities (Bradbury *et al*., 2008) having a direct impact on the genetic structure of distant populations. Genetic approaches are used as an indirect method to estimate dispersal distances. Genetic markers can provide a view of genetic connectivity taking place over many generations (Hedgecock *et al*., 2007; Bradbury *et al*., 2008), although there are limitations, the use of Bayesian analytical techniques have proven useful to discern small spatial patterns in population structure and connectivity (Cowen and Sponaugle, 2009).

To understand what processes affect genetic structure in marine communities it is necessary to investigate several factors that may limit connectivity, and therefore gene flow, in marine animals. These factors may be roughly divided into physical and biological factors (Hohenlohe, 2004). Probably the most important physical factor influencing gene flow are the mesoscale oceanographic processes (e.g., currents, eddies, coastal upwelling, *etc*.), since they are influencing dispersion and therefore connectivity among different zones. The more relevant biological factors are the life cycle, physiology and behaviour of the species under study. Knowledge on these factors together with the genetic data can help us to understand how the population is structured in the sea. However genetic differentiation is also subject to a balance between the forces of genetic drift, gene flow, selection and mutation.

Most of the research for marine species dispersal has been focused on benthic sessile species. The present study is instead, focused on *Acanthephyra pelagica*, which is a

pelagic species classified as micronekton by its movement capacities. Micronekton exhibits the ability to swim and is thus able to avoid or minimize drifting with currents. On the other hand, the larval stages of *A. pelagica* are planktonic, and therefore are subject to advection to other areas, increasing population dispersal. As adults, micronekton can avoid transportation, nevertheless, these organisms are relatively weak swimmers, and so oceanographic features may be a large barrier for them. The presence of diel vertical migration (DVM) could also have a large impact on the transport of organisms. North *et al*. (2008) found that when planktonic organisms had this behaviour, there were significant consequences for particle transport, influencing dispersal distances, transport success, and the degree of connectivity between subpopulations.

The main circulation feature of Atlantic Canada is the Labrador Current. The Labrador Current flows southeastward over the continental shelves and slopes of Labrador and Newfoundland. It transports cold water with relatively low salinity and high oxygen content (Talley and McCartney, 1982; Lazier and Wright, 1993). At Hamilton Bank, the current has two branches, one small inshore stream and another main stream over the upper continental slope (Fig. 3.1) (Lazier and Wright, 1993). Further south on the Scotian shelf, water circulation is dominated by an equatorward flow of waters of northern origin, primarily the Nova Scotian Current on the inner shelf and an extension of the Labrador Current along the shelf edge (Hannah *et al*., 2001). In the Sable Gully proper there are two predominant circulations. In the shallower areas above 200 m the water flows to the southwest and is not affected by canyon topography. However in the deeper water below 500 m, the flow over the shelf slope is affected by the larger shelf-scale circulation which is dominated by flow of water from the canyon mouth to the canyon head on the order 0.02 ms-1 (Greenan *et al.*, 2014; Shan *et al.*, 2013). This flow is affected by the strong tides observed in the Gully and make it unique among canyons

along the shelf break. Additionally there is evidence of greater mixing in the Gully, approximately 20 times that observed on the Scotian Shelf (Greenan *et al.*, 2014). There is a persistent seasonally variable offshore flow on the Sable Island Bank which is part of a counter clockwise circulation system over the Gully. In spring, summer, and fall there is an onshore flow on its eastern side passing through. The Gully across its northern side and out along its southern side creating a partial cyclonic gyre. During spring this partial gyre breaks down and there is an on-shelf transport that extends across most of the Sable Gully and provides a cross-shelf supply to the inner shelf (Gordon and Fenton, 2001). The presence of this gyre in the Sable Gully and the slow velocity of the water may make this canyon act as a retention area. This feature is very important because it explains, in part, the huge biomass found in the Sable Gully and is also important for population analysis because it could have a large effect on the number of migrants moving in and out of the Gully.

Little research has been conducted in the field of population genetics in the marine environment as compared to freshwater or land populations. Genetic studies on marine organisms have been mainly focused on species of commercial relevance and particularly on fish. In the northwest Atlantic Ocean some of the fishes investigated with respect to their population genetic structure are: the Greenland Halibut (*Reinhardtius hippoglossoides*) (Roy *et al*., 2014); White hake (*Urophycis tenuis*) (Roy *et al*., 2012); redfish (*Sebastes fasciatus*) (Valentin *et al*., 2014); Deepwater redfish (*Sebastes mentella*) (Stefánsson *et al*., 2009; Roques *et al*., 2002); Bigeye tuna (*Thunnus obesus*) (Gonzalez *et al*., 2008); Blue hake (*Antimora Rostrata*) (White *et al*., 2011); and Cod (*Gadus morhua*) (Bentzen *et al*., 1996; Ruzzante *et al*., 1996; Ruzzante *et al*.,2000; Beacham et al., 2002). There has also been some investigation in population genetics of other organisms in the area, such as the green sea urchin *Strongylocentrotus*

droebachiensis (Addison and Hart, 2004) and a few on crustaceans: the barnacle *Semibalanus balanoides* (Holm and Bourget, 1995); the North atlantic copepod *Calanus finmarchicus* (Provan *et al*., 2009); the snow crab (*Chionoecetes opilio*) (Puebla *et al*., 2008); the northern krill (*Meganyctiphanes norvegica*) (Papetti *et al*., 2005); and the American lobster (*Homarus americanus*) (Kenchington *et al*., 2009) to name a few. So although the marine system has been investigated in this area for a few organisms, the information with respect to genetic population structure available in the region is still scarce, and particularly so for pelagic crustaceans or micronektonic organisms in general. As discussed previously, biological traits of the species play a key role in the dispersal of organisms and thus the connectivity of populations. Consequently, it is not possible to make an accurate prediction on the genetic structure of a species based on previous studies or physical traits of the environment only.

Here I present the first genetic study on the species *A. pelagica* and on the genus *Acanthephyra*. I examine the genetic structure of this species at two differing geographic scales, first at the level of the Northwest Atlantic and second, at the more localized scale of the Sable Gully and surrounding areas. Furthermore this study will give key insights for micronekton invertebrate connectivity in Atlantic Canada. I estimate contemporaneous gene flow and resolve temporal, generational and spatial population structure in *A. pelagica* using a subset of the 26 microsatellite DNA markers developed in Chapter 2. This study aims to infer patterns of connectivity correlating oceanographic information of the area with the genetic structure of within the Sable Gully and other areas of Atlantic Canada.

3.2 Methods

3.2.1 Sampling and Tissue Collection.

Acanthephyra pelagica individuals were collected from several areas of the Atlantic coast of Canada (Fig. 3.2), with special emphasis on the Sable Gully Marine Protected Area (MPA) on the Scotian Shelf (44°N 59°W), where surveys were conducted annually at three fixed-stations (Head, Main, and Deep) between 2008 and 2010 (Kenchington *et al*., 2014). Shrimp were also collected in 2007 at a location east (Offshore station) of the MPA and in 2009 at a location close to one of the canyon walls (Wall station) (Fig. 3.3) (Kenchington et al., 2009).

Shrimp within the Sable Gully were collected with an International Young Gadoid Pelagic Trawl (IYGPT) with a net opening of approximately 60 m^2 and a mesh size decreasing from 100 mm at the headline to 12.7 mm at the cod end. Sampling depth ranged from the surface to 1750 m (Kenchington *et al*., 2009, 2014).

Samples were also collected off Newfoundland (NAFO DIvisions 2H, 2J, 3K, Fig. 3.2), in November/December 2011, as well as within NAFO Division 4W in July 2012. Upon collection, samples were stored at -20°C. Tissue samples for DNA analysis were stored in 95% ethanol (ethanol replaced after 1 week). Individuals chosen for DNA analysis were measured (Carapace length) and sexed. Ovigerous females were identified and egg samples were taken and preserved in 95% ethanol for subsequent paternity analysis.

3.2.2 Phenotypic Analyses

Individual carapace length (CL) was measured to assess the size structure on the Sable Gully population in each year (2007, 2008, 2009 and 2010). This was done for females and males separately. To establish boundaries between these groups, a cluster analysis was performed using SigmaPlot version 12.2. These size-age groups were used to assign individuals to cohorts to assess genetic differences among generations.

3.2.3 DNA Extraction and Amplification

Muscular tissues were digested with 400 µg/mL proteinase K (Bio Basic Inc., Markham, ON, Canada) and 300 µL of digestion buffer (10 mM Tris–HCl, pH 8.3, 1 mM EDTA, 400 mM NaCl containing 0.8% sodium dodecyl sulfate (SDS)), for 5 hours at 55°C on a shaker working at 200 rpm. DNA was extracted using a glass-milk protocol (Elphinstone *et al*., 2003).

Amplification of fragments for each locus and individual were performed. Microsatellite loci were amplified in 7.5 µL total volume containing 1.65 µL of dd H2O, 1 µL of 10x reaction buffer $(NH_4)2SO_4$ (Bio Basic Inc., Markham, Ontario), 1 µL of 25mM MgSO4 (Bio Basic Inc., Markham, Ontario), 0.1 µL of 1 µM of fluorescently labeled M13 tag, 0.1 µL of 1 µM of un-tailed primer (either forward or reverse), 0.1 µL of 0.1 µM of M13 tailed primer (either forward or reverse), 1 µL of 2.5 µM dNTPs (Bio Basic Inc., Markham Ontario), 0.05 µL of TSG Polymerase (Biobasic Inc.,Markham, Ontario), and 1.3 µL approximately 50 ng/µL of genomic DNA. PCR conditions were as follows: 5 min at 94°C, 35 cycles of denaturation (94°C for 30s), annealing (57 - 65°C for 30s), and extension (72°C for 30s).

To visualize the PCR products, 1 µL of PCR product was mixed with 9 µL of formamide. The mix was loaded on a polyacrylamide gel that was run and imaged on a Li-COR machine (Li-COR Bioscience, Lincoln, Nebraska). The genotypes of each individual were obtained using SAGA Automated Microsatellite Software 3.3.

3.2.4 Population Structure

The presence of null alleles was evaluated with MICRO-CHECKER 2.2.3 software (Van Oosterhout et al. 2004). Expected (He) and observed (Ho) heterozygosities, allelic diversity, allele frequencies, and inbreeding coefficient (F_{IS}) were estimated with GenAlEx 6.5 software (Peakall and Smouse, 2006). Allelic richness (A_R) was estimated and private alleles (A_P) were identified with HP-Rare version 1.0 software (Kalinowski, 2005). Hardy- Weinberg equilibrium was assessed using the Arlequin 3.5 software (Excoffier and Lischer, 2010) with Bonferroni correction. LOSITAN software (Antao *et al*., 2008) was used to detect loci that might be under balancing or positive selection; detecting loci under selection is important since their inclusion on population genetic analyses could bias results and interpretation (White *et al*., 2010). A regression analysis was performed to assess if allelic richness is standardized for sample size.

Overall and pairwise F_{ST} (Weir and Cockerham, 1984) among 23 sampling sites were estimated with Arlequin 3.5 software (Excoffier and Lischer, 2010). One sample (Head 2010) was eliminated because of its small size. Samples taken on the same location and year exhibiting F_{ST} values not significantly different from zero were pooled. The final sample number is 13 (Table 3.3). Ten of the samples are within the Sable Gully or in the adjacent offshore slope, while the remaining 3 samples originate from other areas of the NW Atlantic Ocean (NAFO Divisions 2H, 2J/3K and 4W). Jackknifing was subsequently

performed across loci to assess the influence of individual loci on F_{ST} estimates. Pairwise F_{ST}'s between samples from different locations within the Sable Gully were estimated again after pooling. This was also done with Arlequin 3.5 software (Excoffier and Lischer, 2010). F_{ST} Values were also plotted using a PCoA function on GenAlEx 6.5 software (Peakall and Smouse, 2006). As the use of F_{ST} has been questioned as a measure of genetic differentiation (Hedrick, 2005; Jost, 2008), I also estimated overall Jost's D using SMOGD software (Crawford, 2010). Genetic relationships across all samples and individuals were shown with a factorial correspondence analysis (FCA) that "detects the best linear combinations of variables and describes the variation between observations" (Teixeira *et al.,* 2012). This was done using the software GENETIX 4.05 software (Belkhir *et al.,* 1996).

Isolation by distance (IBD) was tested using Rousset (1997) data analysis for populations along linear habitats. Previously calculated F_{ST} values were used and geographic distances were calculated using ArcGis desktop 10.1 (ESRI, 2011). The correlation between genetic ad geographic distances was done using GenAlex 6.5 software (Peakall and Smouse, 2006).

Several analyses of molecular variance (AMOVA) were conducted to assess the magnitude of various sources of variation including spatial, generational and temporal groupings (Arlequin 3.5; Excoffier and Lischer, 2010)). Individuals were assigned to cohorts as a function of carapace length (see Phenotypic Analysis above).

The STRUCTURE software (version 2.3.4) was then used to define the number of genetic groups present in our samples. Runs had an initial burn-in of 50000 cycles with 200000 additional cycles. Three iterations were performed for each K (from 1 to 17). Samples from outside Sable Gully were then removed and a similar analysis was then conducted with STRUCTURE 2.3.4 (Pritchard *et al*., 2000) considering only the Sable

Gully samples. Parameters used were the same and K ranged from 1 to 10. Results were assessed using Pritchard's (Pritchard *et al*., 2000) L (K) and Evanno's method (Evanno *et al*., 2005) Δ (K). Structure Harvester software was used to visualize results of the different runs.

The spatial Bayesian clustering program Geneland (Guillot *et al*., 2005) was used to complement STRUCTURE because Geneland is more efficient at detecting populations when there are lower levels of genetic differentiation and may be more efficient even when the loss of connectivity is recent (Chen *et al*., 2007). The software was run with 200x100 iterations. It was run using the 10 samples within the Sable Gully and with all the samples considering the Sable Gully as one sample. To compensate the different sample sizes, Research Randomizer 4.0 software (Urbaniak and Plous, 2013) was used to take a random sample within the Sable Gully sample, this subsample of 150 individuals was the one actually used for the analysis together with samples from NAFO Divisions 4W, 2H and 2J/3K.

The POWSIM software (Ryman and Palm, 2006) was used to estimate if there is sufficient statistical power to reject the null hypothesis (H_O) of genetic homogeneity, for different combinations of sample sizes and effective population sizes to detect differences between samples. It was also used to determine if α error (to reject H_O) is within an acceptable range. Effective population size was estimated with LDNe software (Waples and Do, 2008) for the samples collected within the Sable Gully.

3.3 Results

3.3.1 Distribution by Sex and Carapace Length.

A total of 1512 shrimp individuals were sampled. Individuals were obtained from 26 collections, taken from nine localities, in six different years. All individuals were measured and their sex was determined except when individuals were immature or their first pair of pleopods was missing. The total sample size within the Sable Gully was N=1299 individuals of which 547 were females, 396 were males and 178 were of unknown sex. The sex ratio was thus slightly skewed with 58% females. One hundred of the 547 females were carrying eggs, and the smallest female carrying eggs was 12.6 mm (CL). Size structure across samples in each year showed four peaks indicating a lifespan longer than four years and a likely seasonal pattern of reproduction (Fig. 3.4 and 3.5). Females were found to reproduce at ages 3+ and 4+, although 90% of the females carrying eggs were four years old. The size structure analysis indicated the presence of 6 cohorts over the four years of sampling. Boundaries found with the cluster analysis helped determine individual age and consequently, cohort.

3.3.2 Genetic Population Structure

Multilocus (10 loci) genotypes were obtained for 1026 shrimp individuals collected from nine localities sampled during six consecutive years. The total number of individuals and samples was reduced due to poor DNA quality for most of the 2007 samples and the small size of one of the 2010 samples (Head 2010, N=5). All samples from within the Sable Gully collected in 2007 were eliminated and only the offshore samples collected that year (2007) from east of the MPA were considered in all subsequent analyses.

The 10 loci were chosen based on polymorphism presence of null alleles and neutrality. MICRO-CHECKER detected the likely presence of null alleles in loci Acpe26 and Acpe43. These loci were therefore eliminated from subsequent analysis of population structure. All other loci were free of null alleles and there was no evidence for either divergent or balancing selection for any of them suggesting they are all neutral (Fig 3.6).

The number of alleles per locus (N_A) ranged from 9 (Acpe03) to 17 (Acpe57) over all samples (Appendix A), while the mean number of alleles ranged from 4.6 (Acpe32) to 10 (Acpe57) (Appendix A). The mean number of alleles per locus ranged from 4 (Wall 2009) to 9 (Main 2010 and 3K/2J) (Table 3.1) Average allelic richness varied from A_R: 3.52 (Wall 2009) to A_R: 4.72 (Head 2009 and 4W). Private allelic richness (PA_R) averaged over loci ranged from 0.05 (Head 2008) to 0.25 (4W). The effective number of alleles varied from A_E: 1.93 (Wall 2009) to A_E: 2.32 (Deep 2009). The unbiased expected heterozygosity varied from 0.47 (Wall 2009, 2H) to 0.55 (4W) while observed heterozygosity ranged from 0.37 (Wall 2009) to 0.53 (4W) (Table 3.1). The number of alleles did increase with sample size as shown in Figure 3.9, however, the asymptotic shape of the tendency line in Figure 3.9 is indicating that larger samples would likely not have introduced more alleles.

Only 8 out of the 220 tests (22 samples, 10 loci) exhibited departures from Hardy-Weinberg equilibrium (Table 3.1), a result that can occur by chance alone. No locus/sample combination was therefore considered to be out of HWE.

Pairwise F_{ST} values between samples were not significantly different from zero ($p>0.05$) in most of the cases (Tables 3.2 and 3.3), after Bonferroni correction. Significant pairwise F_{ST} values included the estimates between the sample from 4W and those from Head 2008, Main 2009, 2J/3K and 2H. The highest F_{ST} estimate (0.029) involves the sample from 4W and that from NAFO Division 2H off Newfoundland. The subsequent

PCoA analysis (Fig. 3.7) based on F_{ST} values show graphically how sample 4W is different from the Newfoundland samples particularly from that obtained in NAFO division 2H. These two samples are the most geographically distant and were taken in consecutive years. Jackknifing across loci, indicated that none of the loci exert undue influence on the results. Removing distant locations and focusing on samples within Sable Gully F_{ST} values were not significantly different from zero in most of the cases ($p>0.05$). The IBD analysis showed a significant strong correlation between geographic and genetic distance with a correlation coefficient of 0.59 and a R^2 of 0.34 (Fig. 3.14).The power analysis performed indicates that with the sample size available on this research (>16), effective population size (136), and allele frequencies found in our loci, there is sufficient statistical power to detect differences (>95%) between samples when F_{ST} is ≥0.0037 (Fig. 3.10). Also, Figure 3.11 shows that for a Ne of 136 the probability of rejecting H_O when it is true is under 0.05.

The FCA analysis showed a similar pattern to the PCoA where the 4W sample is different from the rest of the samples, in particular to 2J/3K which in this case is the other sample that is differentiated from the rest. Samples within the Gully tend to be aggregated (Fig. 3.8).

Values of Jost's D show an overall a value of 0.002 and values from 0 to 0.019 for the different loci. These results are in accordance to F_{ST} and G_{ST} results.

Hierarchical AMOVAs failed to show a significant ($P<0.05$) influence for any of the groupings (i.e., year of sampling and locations within years, cohort and years within cohort ($p>0.05$) (Table 3.4a). In other words, there were no differences among sample years or cohorts ($p>0.05$). The only significant effect ($P=0.027$) was observed when comparing samples across broad regions (Newfoundland vs. Scotian Shelf) but the percentage of variation explained was low (0.17 %) (Table 3.4b) and the samples were

collected in different years making it difficult to ascertain whether the effect is due to location or year of sampling. Cohorts were not identified out of the Gully and other trends were not found.

In line with these results, STRUCTURE analysis failed to identify any evidence of population differentiation among samples within the Sable Gully (Fig. 3.13). Also, Geneland analysis failed to identify population differentiation, finding the higher likelihood for a single cluster (Fig. 3.12). The same results were found when test was run with and without samples outside the Sable Gully.

3.3.3 Effective Population Size

The effective population size (N_e) within the Sable Gully was found to be 136 (95% confidence limits, 115.2 – 161.0) using 0.02 as the lowest allele frequency. These results were also used for the power analysis where I demonstrate that there is enough power to detect differences among populations at our smallest sample size.

3.4 Discussion

This study revealed the panmictic nature of the pelagic shrimp *Acanthephyra pelagica* collections within the Sable Gully. Neither the spatial, temporal, nor cohort analyses revealed evidence of population genetic structure within this shrimp species. The study, however, revealed evidence of genetic structure when considering samples from locations outside the Sable Gully in particular those collected off the coast of Nova Scotia in NAFO Division 4W. Despite the little genetic differences among samples within

the Sable Gully in both time, generation and space, a significant correlation between the genetic and geographic distance was found over larger spatial scales. This effect was evidenced by a number of data points and appears to be operative over scales of 1500 km or more with this data set. The information gathered with the 10 microsatellites, allows the interpretation as the result of neutral processes. Lack of power due to low genetic variation can be rejected as an alternative hypothesis as demonstrated with the power analysis. POWSIM results are in direct relation with the N_e calculated, 136, Also α error is increased if N_e is larger. This study suggests there is enough evidence to support the conclusion that *A. pelagica* exhibits panmixia at all but the largest geographic scale in the North West Atlantic

A N_e of 136 is considered low for a marine species. The ratio of effective to census population size (N_e:N_c) in natural populations is considered to be of importance for the conservation of populations. Is hard to estimate a census population size for this species. Considering the abundance values obtained by MacIsaac *et al*. (2014), where they calculate the abundance per set using samples from the same survey. I estimate a density of 0.0014 ind./m^3 (considering a net opening of 60m^2 and 1250m depth). If we are conservative to estimate a distribution of 50 km offshore the platform along a coastal line of 2000 km, within 1250m depth, we have a total of 1.25$e^{+14}$$m^3$, which leads to an estimate of 12.75e^{+12} individuals. This is indicating a N_e: N_c ratio of around 7.8e^{-11}, which is very small even for a marine organism but comparable to that recently estimated for a marine dinoflagellate (Watts *et al*., 2013). The reproductive biology and some life-history aspects are important determinants of Ne, although other factors may also be relevant (Turner *et al*., 2006; Palstra and Ruzzante, 2008)

Although most of the sampling was conducted within the Sable Gully, sampling was geographically widespread, the most distant sample points are separated by

approximately 2400 km. This is a long distance for many organisms. The current system in the area, the Labrador Current, has an average of ~0.3 m s^{-1}. This suggests that a particle can travel from area 2H to 4W in 92.6 days. Furthermore, Bailey *et al.* (2005) found that the species *Acanthephyra eximia* has an average routine swimming speed of 0.18 m s^{-1}. Assuming that *A. pelagica* would present a similar speed, it would take 57.75 days swimming southward to travel from 2H to 4W in. Consequently, in the four year lifespan of this species it can travel a long distance in less than 3 months. This explanation implies one directional gene flow where migrants go from north to south. This migration probably occurs more often among juvenile individuals, since they are not able to withstand the current speed. Shanks and Eckert (2005) discuss how species may have adapted biological traits in order to use small eddies or seasonal oceanographic features to move against the main currents avoiding unidirectional larval drift. Some of the adaptations that may play a role in avoiding unidirectional migration are time and place of reproduction, pelagic larval duration (PD) and diel vertical migration (DVM) (Shanks and Eckert, 2005; North *et al*., 2008).

Deep-sea larvae are exposed to fluctuations in food supply and high predation risk during their planktonic phase in the water column (Ramirez-Llodra, 2002; Baeur, 2004). The high mortality on early life stages could explain, partially, the small effective population size found in this study, even though there are no genetic differences among years or cohorts, this can be explained by the fact that individuals of one cohort may reproduce with individuals of other cohorts.

The pairwise F_{ST} estimates show few genetic differences among samples. Sample 4W located on the Scotian Shelf to the south of the Sable Gully is the most different from all the rest of the samples. Although only five out of 68 comparisons are significant, and this could indicate that these differences are due to chance, four of the five significant results

involve the sample located in NAFO area 4W. This suggests that the shrimp collected within NAFO division 4W are likely genetically distinguishable from the rest of the samples. This sample had a significant F_{ST} value of 0.029 when compared to sample 2H located the furthest north off the coast of Newfoundland. This result, together with the IBD results are indicating that although there is high dispersal, high gene flow and high connectivity between areas, there is some degree of isolation related with geographic distance among locations.

The number of alleles is low for most of the loci, with a few common alleles and many uncommon alleles. This is also reflected in the fact that the effective number of alleles is much smaller than the number of alleles. It would be interesting to develop and include more polymorphic microsatellite markers to detect more accurately differences among populations.

The size distribution analysis together with the count of ovigerous females, suggests that spawning occurs once a year and always at the same time of the year. Burukovsky and Andreeva (2010) found a similar pattern where spawning season peaks in August off the Azores. For the Northwest Atlantic I cannot be sure what month is the most intense for spawning, but ovigerous females were present in August, September and March, although with a different degree of development of eggs on different months. Our age and cohort analysis is in agreement with some aspects of the results found by Burukovsky and Andreeva (2010). Although, I did not find 2 year old individuals reproducing, and sizes are not comparable because they used the total length of the individuals, while in this study I used the carapace length, four clearly distinct size peaks were found with a clear signal of annual reproduction.

Despite the huge advances in the field of population genetics, little research has been conducted on population genetic structure of pelagic invertebrates (Bucklin, 1995;

Benzie, 2000; Zane, 2000). Investigation on marine organisms mostly focus on fish because of their economic importance and the results of these studies have shown in some cases significant levels of genetic differentiation even within small geographic areas (Bowen and Grant, 1997). In the Northwest Atlantic, Roy *et al*. (2014) found evidence of panmixia in the Greenland halibut (*Reinhardtius hippoglossoides*) population. Similar results were found in world-wide scale research on the highly migratory Bigeye tuna (*Thunnus obesus*), where no genetic differences were found across the Atlantic Ocean. For the Blue hake (*Antimora rostrata*), panmixia was also found across the North Atlantic (White *et al*., 2011). On the other hand, in the case of the White hake (*Urophycis tenuis*) Roy *et al*. (2012) found three genetically distinguishable populations. The same result was found when the Deepwater redfish (*Sebastes mentella)* was investigated in the area; three distinguishable clusters were found, suggesting genetic structure (Steffánsson *et al*., 2009). Another study of red fishes *(Sebastes mentella and Sebastes fasciatus*) detected a weak structure at a large geographical scale (Valentin *et al*., 2014). For the cod (*Gadus morhua*) distinct populations were also found under different sampling conditions (Bentzen *et al*., 1996; Ruzzante *et al*., 1996; Ruzzante *et al*., 2000; Beacham et al., 2002). There is not a clear pattern on what biological or physical traits may be causing structure in these latter populations. It could be argued that each case is different, since species differ in their life histories and in the physical characteristics of the environment that affect them, because of differences in their habitat preferences. A few studies have been conducted in this geographical area for crustacean species. The snow crab (*Chionoecetes opilio*) was investigated through the use of microsatellites throughout the Northwest Atlantic. Absence of genetic structure was found across samples in Atlantic Canada, while a genetic break was identified between Greenland and Atlantic Canada (Puebla *et al*., 2008). These results support the panmixia findings of this research for *A. pelagica*.

Similar results were find for the North Atlantic copepod *Calanus finmarchicus* were high levels of dispersal were found across the North Atlantic (Provan *et al*., 2009) On the other hand, in the case of the American Lobster (*Homarus americanus*), significant but weak genetic structure was identified among different areas of Atlantic Coast of Canada (Kenchington *et al*., 2009). Neither of these species is pelagic as adults, so again, it is very hard to compare to *A. pelagica*. Many of the studies performed on marine shrimps relied on the use of allozymes or mitochondrial DNA, which makes comparisons with the results of the present study difficult. Studies based on microsatellite markers are scarce and most of them have used relatively few loci (Ball *et al*., 2003; Maggioni *et al*., 2003; Borrell *et al*., 2004). Some research has been conducted to examine the genetic structure of a penaeid shrimp (Ball *et al*., 2003; Maggioni *et al*., 2003; Borrell *et al*., 2004; Tsoi *et al*., 2007), since some species of this group have economic value and there are some attempts to artificially cultivate some species of this group (Benzie, 2000). Penaeid shrimps are also Decapods but are part of a different Suborder, they feature a different reproductive strategy and their biology is thus not highly similar to that of *A. pelagica.* They do however, exhibit a pelagic stage too. Studies conducted on different species of penaeid shrimps showed little genetic variation over long distances (>1000 km) (Borrell *et al*., 2004; Maggioni *et al*., 2003; Benzie, 2000). Much of the genetic structure in wild populations of these organisms appears to reflect historical events on large biogeographical scales, rather than resulting from patterns of present-day dispersal (Benzie, 2000). Studies on another group of micronektonic pelagic crustaceans, euphausids, commonly known as krill, have come to a similar conclusion. This group has been studied mainly using mitochondrial DNA, showing very little population genetic structure over long distances. *Euphausia superba* in the Antarctic area shows very weak genetic structure while *Meganyctiphanes norvegica* in the North Atlantic shows discrete genetic pools along its distribution range (Papetti *et al*., 2005; Zane and Patarnello,

2000). The difference in the genetic structure exhibited by these two species likely results, at least in part, from differences in the oceanographic conditions in the areas inhabited by the two species (Zane and Patarnello, 2000). Bilodeau *et al*. (2005) investigated the population structure of *Callichirus islagrande*, a taxon closely related to *A. pelagica* but with a different life style (*C. islagrande* is a coastal burrowing crustacean). The research compared results of three different markers, allozymes, mitochondrial DNA and microsatellites. The mitochondrial DNA indicated two lineages, reproductively isolated a million years ago, while the microsatellite information indicated 4 - 5 closed populations. As was expected microsatellites were better indicators of population structure, since they are highly polymorphic markers (Bilodeau *et al*., 2005). These results are indicative of the importance of habitat where organisms live in and their behaviour, for dispersal and gene flow, showing that demersal and/or coastal organisms possess better mechanisms to avoid dispersal and increase recruitment, compared to pelagic organisms. It seems that the pelagic life style plays a key role in preventing genetic differentiation: species with high larval dispersal are genetically more homogeneous than species with lower dispersal capacity (Palumbi, 1996). The low level of genetic differentiation observed in pelagic species could also be explained by the high habitat continuity, the usually large population size, and the passive dispersal of larvae (Carvalho and Hauser, 1998). Nevertheless, exceptions to this general trend emphasize on other factors like behavioural mechanisms, selective processes, complex oceanographic patterns, and barriers to gene flow that may have a role in creating and maintaining population differentiation (Palumbi, 1994).

In the case of *A. pelagica* the little genetic differentiation found among spatially distant samples can be explained by the continuity of the environment and the lack of barriers to gene flow. The planktonic larvae of *A. pelagica* is probably increasing dispersal and the

maternal care of females is probably increasing their chances of survival during their development. Even though the Labrador Current would supposedly induce a unidirectional gene flow, Shank and Eckert (2005) suggest that many organisms with a planktonic stage may have evolved to exploit eddies and countercurrents that may be present in the system. Predominantly unidirectional currents are found along many coastlines. If there were no mechanisms to prevent unidirectional flow, the population would progressively go extinct, this is known as the “Drift paradox” (Muller, 1954; Humphries and Ruxton, 2002; Shank and Eckert, 2005). The active swimming of adults allows the dispersion against current flow and also increases survival by allowing them to escape unsuitable environments. This research has shown that *A.pelagica* exhibits high levels of dispersal and high connectivity within the Northwest Atlantic.

Table 3.1. Summary statistics for *A. pelagica* used in genetic analyses of population structure with microsatellite DNA showing: Sample Size (N), Latitude, Longitude, Depth Range of Capture (m), Carapace Length (mm) and Age (yr) Ranges, Proportion of Gravid Females, Average Number of Alleles per Locus, Observed (H_O) and Expected (H_E) Heterozygosity and Loci out of Hardy–Weinberg equilibrium (HWE).

Group	Geographic location and collection date	N	Latitude	Longitude	Depth Range (m)	Length range (CL, mm)	Age range (Years)	Proportion of gravid females	Mean Alleles per locus	Ho	He	HWE
SABLE GULLY N=815	Head, Sep 2008	74	44°1'10.2''	-59° 0' 41.4"	0 – 750	5.47 – 24.52	1 – 4	0.02	7	0.42	0.48	None
	Head, Aug 2009	15	44°1'10.6''	-59° 0' 41.4"	0 – 750	5.25 – 23.40	1 – 4	0.00	5	0.46	0.50	None
	Main, Sep 2008	132	43°50'49.2''	-58° 54' 56.5"	0 – 1250	6.12 – 24.07	1 – 4	0.07	8	0.45	0.50	Acpe51
	Main, Aug 2009	97	43°50'49.2''	-58° 54' 56.5"	0 – 1250	6.79 – 26.41	1 – 4	0.07	7	0.44	0.50	None
	Main, Mar-2010	130	43°50'48.8''	-58° 54' 56.2"	0 – 1250	4.99 – 25.33	1 – 4	0.08	9	0.44	0.50	None
	Deep, Sep 2008	77	43°43'54.8''	-58° 45' 52.9"	0 – 1250	6.91 – 24.55	1 – 4	0.32	7	0.46	0.51	Acpe14
	Deep, Aug 2009	98	43°43'55.2''	-58° 45' 52.9"	0 – 1250	6.21 – 24.79	1 – 4	0.10	8	0.41	0.49	Acpe52
	Deep, Mar-2010	96	43°43'54.8''	-58° 45' 52.6"	0 -1750	5.77 – 25.01	1 – 4	0.16	8	0.42	0.49	Acpe03, Acpe51
	Wall-44, Aug 2009	17	43°52'59.5''	-58° 54' 14.4"	0 – 750	6.67 – 22.16	1 – 4	0.00	4	0.37	0.47	None
	Offshore, Sep 2007	79	43°17'59.9''	-59° 0' 0"	0 – 1600	7.30 – 25.62	1 – 4	0.25	8	0.45	0.51	Acpe52
NEWFOUNDLAND N=165	3K/2J, Dec 2011	148	50°35'24''/53°10'12''	-49° 49' 47.9"/ -51° 57' 0"	0 – 1325	11.63 – 25.76	2 – 4	0.24	9	0.42	0.49	Acpe22
	2H, Oct 2011	17	56°42'47.88''	-58° 33' 24.2"	0 – 1109	18.30 – 24.55	3 – 4	0.31	5	0.41	0.47	None
SCOTIAN SHELF N=48	4W Scotia, Aug 2012	48	42°46'56.3''	-61° 59' 36.6"	0 – 1200	7.44 – 23.09	1 – 4	0.00	8	0.53	0.55	Acpe14

Table 3.2. Pairwise F_{ST} among 22 samples taken in different locations and years in Atlantic Canada. Name of the sample indicating sampling area and year of sampling. Sample size is shown on top row (N). ** = Significant values after Bonferroni correction (95%).

N	39	40	29	45	33	99	44	33	16	48	49	17	58	40	59	71	44	52	61	17	87	48
	1 - Offshore2007-13	2 - Offshore2007-12	3 - Head2008-29	4 - Head2008-31	5 - Main2008-15	6 - Main2008-53	7 - Deep2008-52	8 - Deep2008-51	9 - Head2009-57	10 - Main2009-39	11 - Main2009-19	12 - Wall2009-44	13 - Deep2009-20	14 - Deep2009-5	15 - Main2010-33	16 - Main2010-21	17 - Deep2010-35	18 - Deep2010-51	19 - Nwfld -2J	20 - Nwfld - 2H	21 - Nwfld -3K	22 - Scotia - 237
1- Offsh2007-13	0																					
2 - Offsh2007-12	0.004	0																				
3 - Head2008-29	0.010	-0.004	0																			
4 - Head2008-31	0.001	0.000	0.006	0																		
5 - Main2008-15	0.009	0.002	0.002	0.010	0																	
6 - Main2008-53	0.004	0.000	0.007	0.000	0.011	0																
7 - Deep2008-52	0.009	-0.002	0.002	0.002	0.001	0.000	0															
8 - Deep2008-51	0.002	0.000	0.014	-0.001	0.008	-0.002	0.001	0														
9 - Head2009-57	-0.006	-0.001	-0.004	-0.005	0.000	-0.004	-0.001	0.002	0													
10 - Main2009-39	0.013	0.000	0.000	0.004	0.007	0.009	0.002	0.014	0.002	0												
11- Main2009-19	0.002	-0.003	-0.002	0.003	0.008	-0.001	0.001	0.003	-0.007	0.004	0											
12 - Wall2009-44	0.013	0.008	0.000	0.003	0.015	0.001	0.001	0.011	-0.002	0.009	-0.002	0										
13 - Deep2009-20	0.008	0.002	0.000	0.004	0.012	0.006	0.005	0.011	-0.003	0.005	0.000	-0.001	0									
14 - Deep2009-5	0.005	0.004	0.009	0.002	0.004	0.000	0.000	0.002	-0.002	0.012	0.005	0.001	0.010	0								
15 - Main2010-33	0.007	0.002	0.012	0.001	0.012	-0.002	0.000	0.001	-0.004	0.008	0.001	0.001	0.007	-0.003	0							
16 - Main2010-21	0.007	0.001	-0.002	0.008	0.004	0.006	0.002	0.009	-0.005	0.010	0.003	0.003	-0.001	0.008	0.010	0						
17 - Deep2010-35	0.012	0.010	0.018	0.010	0.008	0.004	0.003	0.003	0.002	0.025**	0.010	0.012	0.016**	0.006	0.003	0.008	0					
18 - Deep2010-51	0.003	0.003	0.005	0.006	-0.001	0.004	-0.001	0.005	-0.003	0.005	0.003	0.008	0.006	0.002	0.007	0.000	0.007	0				
19 - Nwfld-2J	0.006	-0.002	0.001	-0.004	0.005	0.003	0.001	0.005	-0.002	-0.002	0.001	0.002	0.002	0.004	0.005	0.005	0.015	0.003	0			
20 - Nwfld -2H	0.020	0.023	0.030	0.002	0.031	0.009	0.015	0.012	0.007	0.014	0.019	0.014	0.020	0.009	0.002	0.032	0.020	0.025	0.010	0		
21- Nwfld-3K	0.009	-0.001	-0.001	0.001	0.005	0.001	-0.002	0.007	-0.006	0.001	-0.001	0.000	0.000	0.003	0.003	0.001	0.010	0.001	0.000	0.012	0	
22 - Scotia 237	0.009	0.009	0.015	0.016**	0.003	0.010	0.004	0.008	-0.002	0.019**	0.007	0.014	0.014	0.008	0.008	0.006	0.001	0.005	0.016**	0.029**	0.010**	0

** = Significant values after Bonferroni correction (95%)

Table 3.3. Pairwise F_{ST} values (Below the diagonal) and corresponding P values (above the diagonal) among pooled samples taken in different locations and years in Atlantic Canada. Name of the sample indicating sampling area and year of sampling. Sample size is shown on top row (N). * = Significant values after Bonferroni correction (95%).

N	79	74	132	77	16	97	17	98	130	96	148	17	48
	1 - Offshore 2007	2 - Head 2008	3 - Main 2008	4 - Deep 2008	5 - Head 2009	6 - Main 2009	7 - Wall 2009	8 - Deep 2009	9 - Main 2010	10 - Deep 2010	11 - Nwfld-2J/3K	12 - Nwfld -2H	13 - Scotian shelf
1 - Offshore 2007	-	0.748	0.505	0.396	0.829	0.297	0.153	0.351	0.351	0.081	0.243	0.018	0.018
2 - Head 2008	-0.001	-	0.541	0.297	0.874	0.622	0.459	0.685	0.441	0.045	0.982	0.117	0.000
3 - Main 2008	0.000	0.000	-	0.901	0.910	0.135	0.369	0.541	0.721	0.532	0.351	0.018	0.036
4 - Deep 2008	0.001	0.002	-0.002	-	0.532	0.117	0.315	0.279	0.450	0.505	0.144	0.018	0.045
5 - Head 2009	-0.005	-0.006	-0.005	0.000	-	0.775	0.676	0.883	0.928	0.703	0.757	0.297	0.523
6 - Main 2009	0.001	-0.001	0.002	0.003	-0.003	-	0.387	0.270	0.162	0.000	0.838	0.018	0.000
7 - Wall 2009	0.009	0.001	0.003	0.004	-0.002	0.003	-	0.829	0.595	0.252	0.550	0.243	0.045
8 - Deep 2009	0.002	0.000	0.000	0.002	-0.005	0.002	-0.003	-	0.982	0.126	0.703	0.081	0.009
9 - Main 2010	0.001	0.001	-0.001	0.000	-0.007	0.002	0.000	-0.002	-	0.541	0.450	0.036	0.108
10 - Deep 2010	0.004	0.006	0.000	0.000	-0.003	**0.008***	0.008	0.003	0.000	-	0.036	0.018	0.324
11 - Nwfld-2J/3K	0.002	-0.002	0.001	0.002	-0.004	-0.001	0.001	0.000	0.000	0.005	-	0.090	0.000
12 - Nwfld -2H	0.020	0.012	0.013	0.013	0.007	0.015	0.014	0.013	0.015	0.021	0.011	-	0.000
13 - Scotian shelf	0.009	**0.015***	0.006	0.005	-0.002	**0.013***	0.014	0.010	0.005	0.002	**0.013***	**0.029***	-

Table 3.4. Results of the analysis of molecular variance (AMOVA) a) For samples within the Gully; b) For all samples. Showing the source of variation, degrees of freedom (d.f.), sum of squares, percentage of variation and p-values.

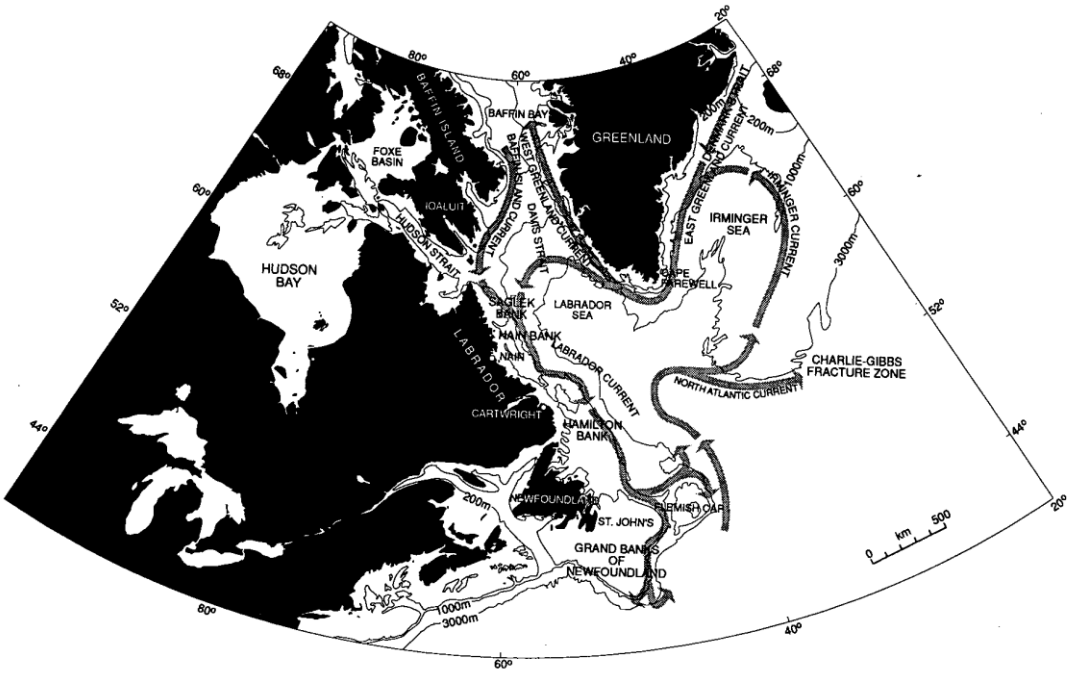
a) Source of variation	d.f.	Sum of squares	Percentage of variation	p-value
Among years	3	10.6	0.12	0.072+-0.004
Among locations within years	6	14.3	-0.01	0.785+-0.007
Among individuals within locations	1616	3915.4	99.89	0.375+-0.007
Among years	3	10.4	0.07	0.125+-0.004
Among cohorts within years	12	33.8	0.16	0.307+-0.007
Among individuals within cohorts	1620	3920.9	99.77	0.149+-0.005
Among Cohorts	5	14.1	-0.04	0.547+-0.007
Among years within cohorts	10	30.1	0.25	0.145+-0.005
Among individuals within years	1620	3920.9	99.79	0.148+-0.005
Among cohorts	5	13.889	-0.01	0.360+-0.008
Among location/year within Cohorts	31	84.918	0.32	0.381+-0.007
Among individuals within location/year	1587	3835.111	99.69	0.369+-0.006
Between Sexes	1	2.373	-0.04	0.620+-0.007
Among cohorts within sex	10	29.128	0.18	0.245+-0.007
Among individuals within cohorts	1390	3388.595	99.86	0.285+-0.006
Among location/year	9	24.9	-0.02	0.183+-0.005
Among Cohort within location/year	29	80.9	0.39	0.286+-0.006
Among individuals within cohort	1587	3834.5	99.63	0.276+-0.006

b) Source of variation	d.f.	Sum of squares	Percentage of variation	p-value
Among years	5	20.359	0.17	0.0274+-0.002
Among locations within years	7	18.296	0.06	0.558+-0.007
Among individuals within locations	2039	4950.038	99.77	0.034+-0.003

Table 3.5. Genetic Diversity for 10 microsatellite loci in 13 populations of *A. pelagica*. Table showing: Inbreeding coefficient (F_{IS}); overall fixation index (F_{IT}); Fixation index (F_{ST}); Number of migrants (Nm); Estimator of actual differentiation (Jost 2008) (D_{EST}); Standardized measure of genetic differentiation (Hedrick 2005) (G'ST_est); Nearly unbiased estimator of relative differentiation (Nei 1983) (GST_est).

Locus	F_{IS}	F_{IT}	F_{ST}	Nm	D est	G_{ST}_est	G'_{ST}_est
Acpe03	0.206	0.215	0.012	20.760	0.002	0.001	0.003
Acpe06	0.213	0.223	0.014	17.981	0.002	0.003	0.005
Acpe14	0.297	0.305	0.011	22.497	0.000	0.001	0.001
Acpe17	0.025	0.037	0.012	20.010	0.001	0.002	0.002
Acpe20	0.149	0.157	0.010	23.586	0.000	0.000	0.000
Acpe22	0.009	0.020	0.012	21.032	0.002	0.001	0.003
Acpe32	0.093	0.119	0.029	8.478	0.021	0.018	0.039
Acpe51	-0.196	-0.178	0.014	17.035	0.005	0.004	0.009
Acpe52	0.231	0.244	0.017	14.293	0.010	0.007	0.016
Acpe57	0.118	0.128	0.011	22.514	0.001	0.001	0.002

a)



b)

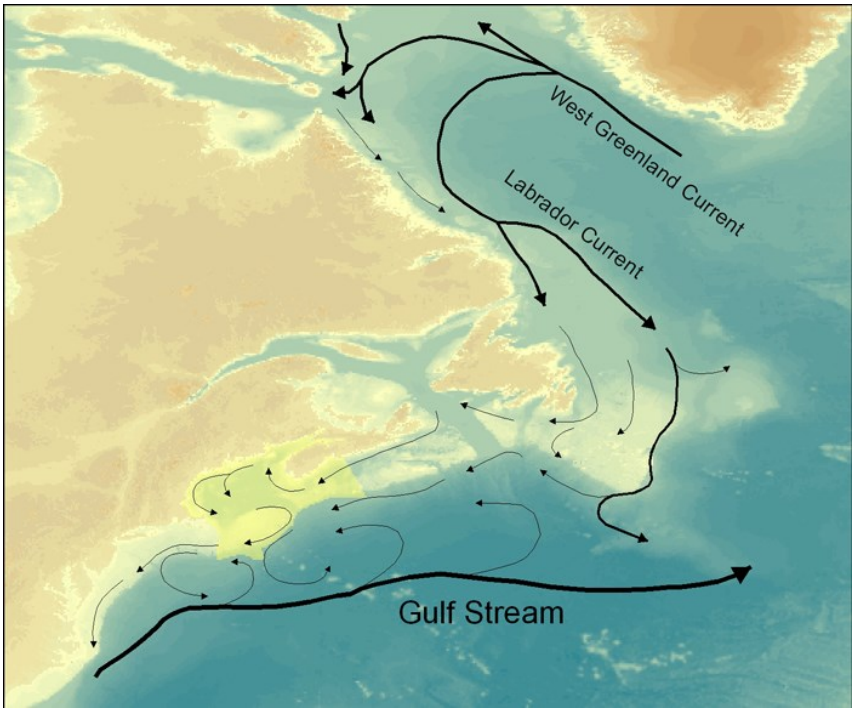


Figure 3.1. Maps showing the major currents in the Atlantic coast of Canada a) Lazier and Wright, 1993; b) Census of marine life web page http://www.gulfofmaine-census.org

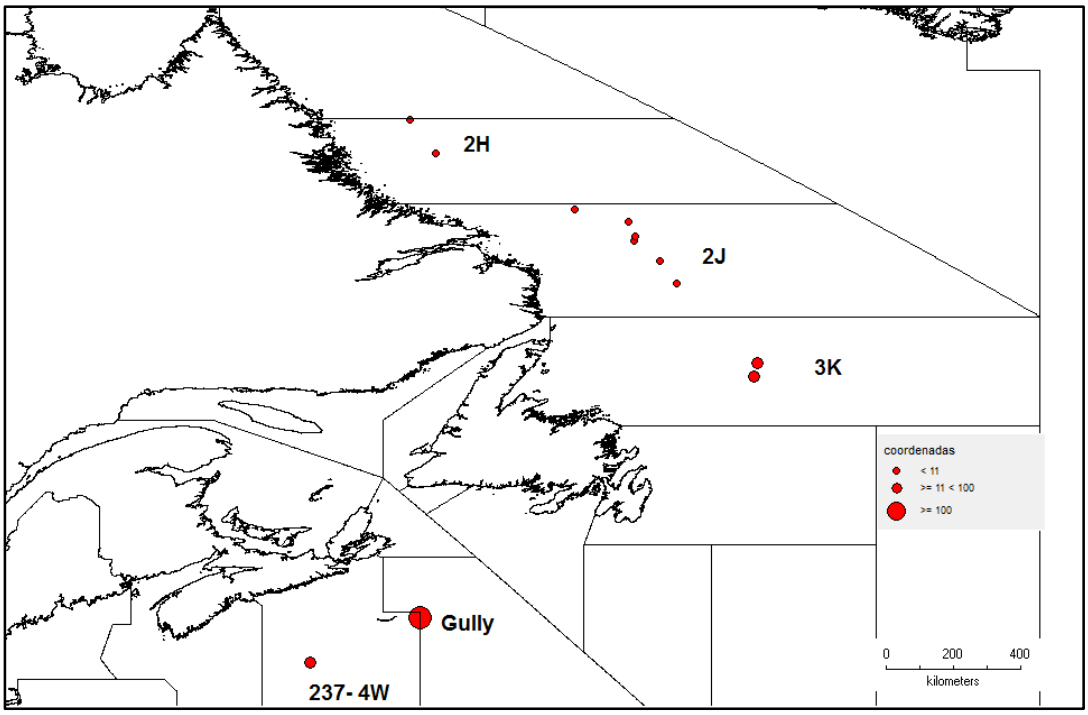


Figure 3.2. Map showing in red the location of the samples of *Acanthephyra pelagica* along the Atlantic coast of Canada. The size of the dots is associated to de sample size (see legend)

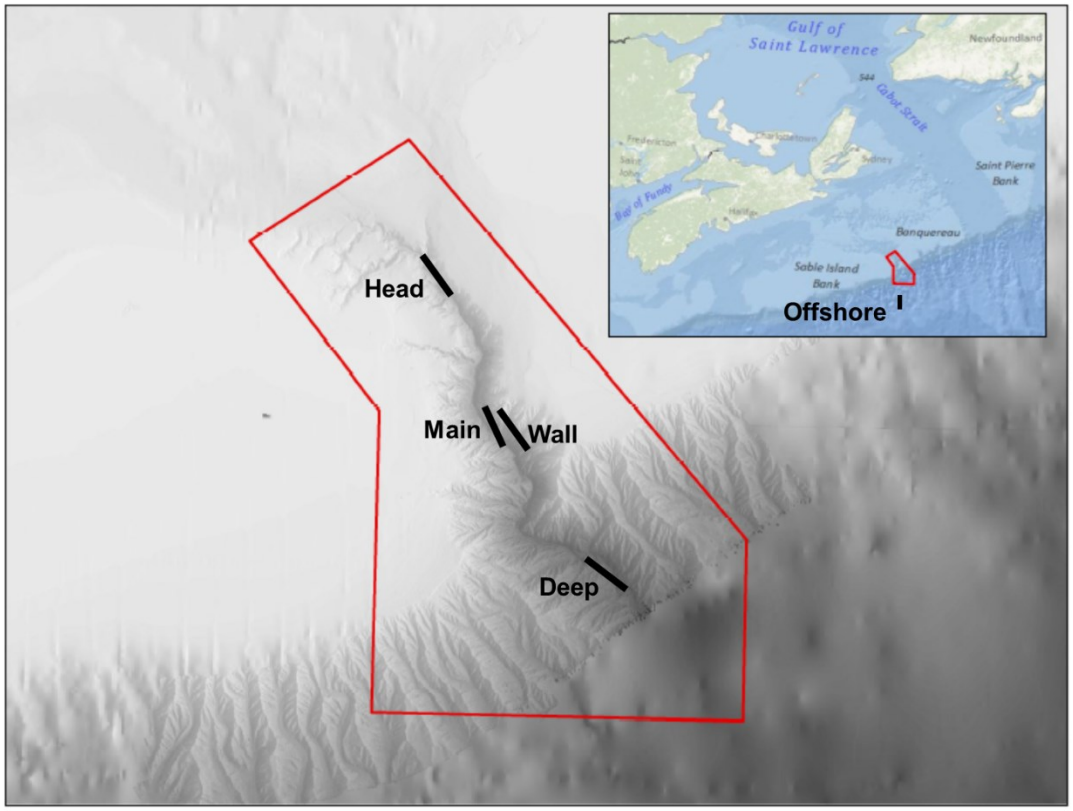


Figure 3.3. Map showing the location of the sampling sites of *Acanthephyra pelagica* within the Sable Gully (Head, Main, Wall, and Deep) and the offshore station.

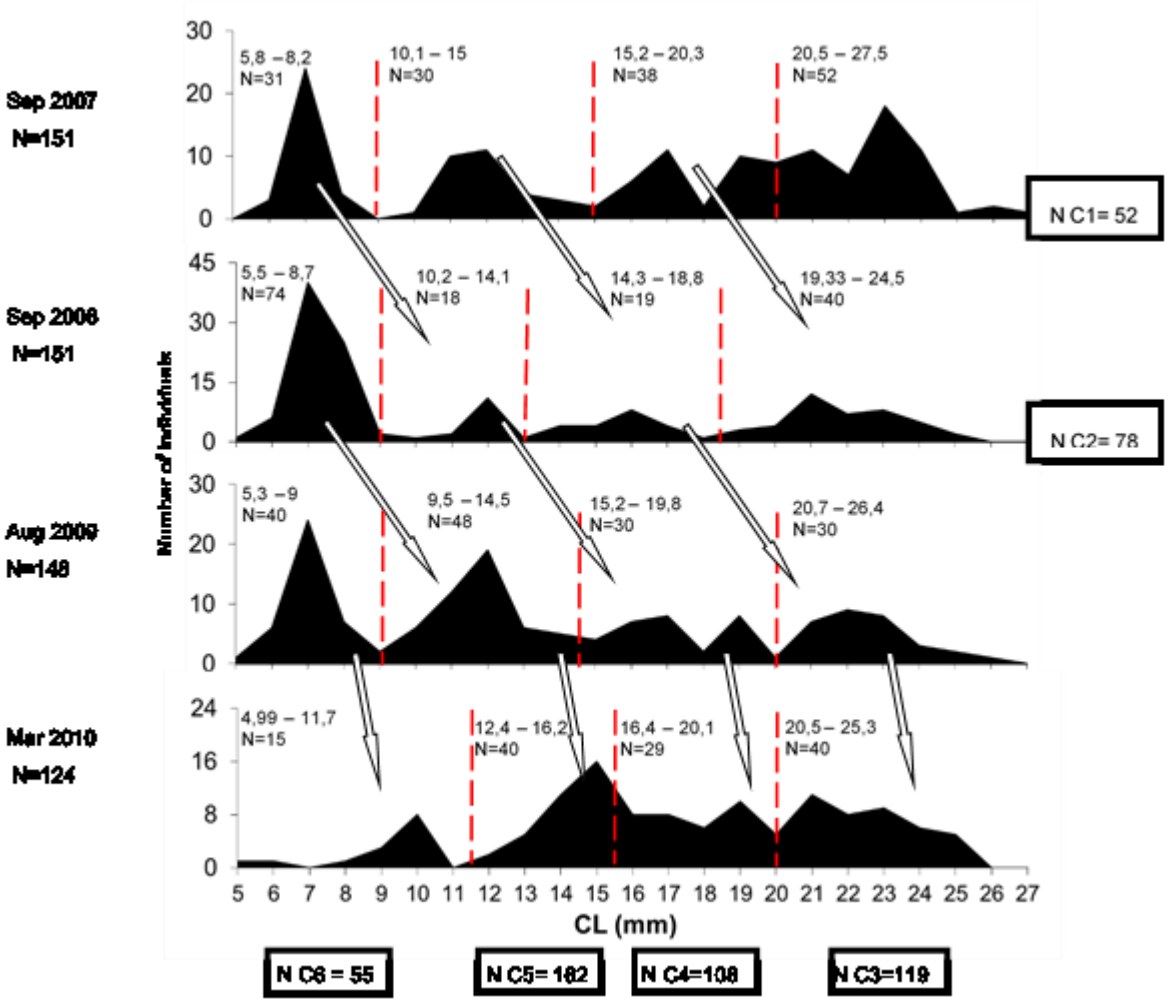


Figure 3.4. Demographic structure in male *Acanthephyra pelagica* in the Sable Gully. Four size-age groups identified for each year for which samples are available. Six cohorts identified among the four years (C1, C2, C3, C4, C5 and C6). Number of individuals per year are indicated on the left side of the graph. Number of individuals per cohort indicated at the bottom and right side of the figure (e.g. N C1= 52). Number of individuals per cohort per year are indicated, together with the size range for each age on each year, above each peak.

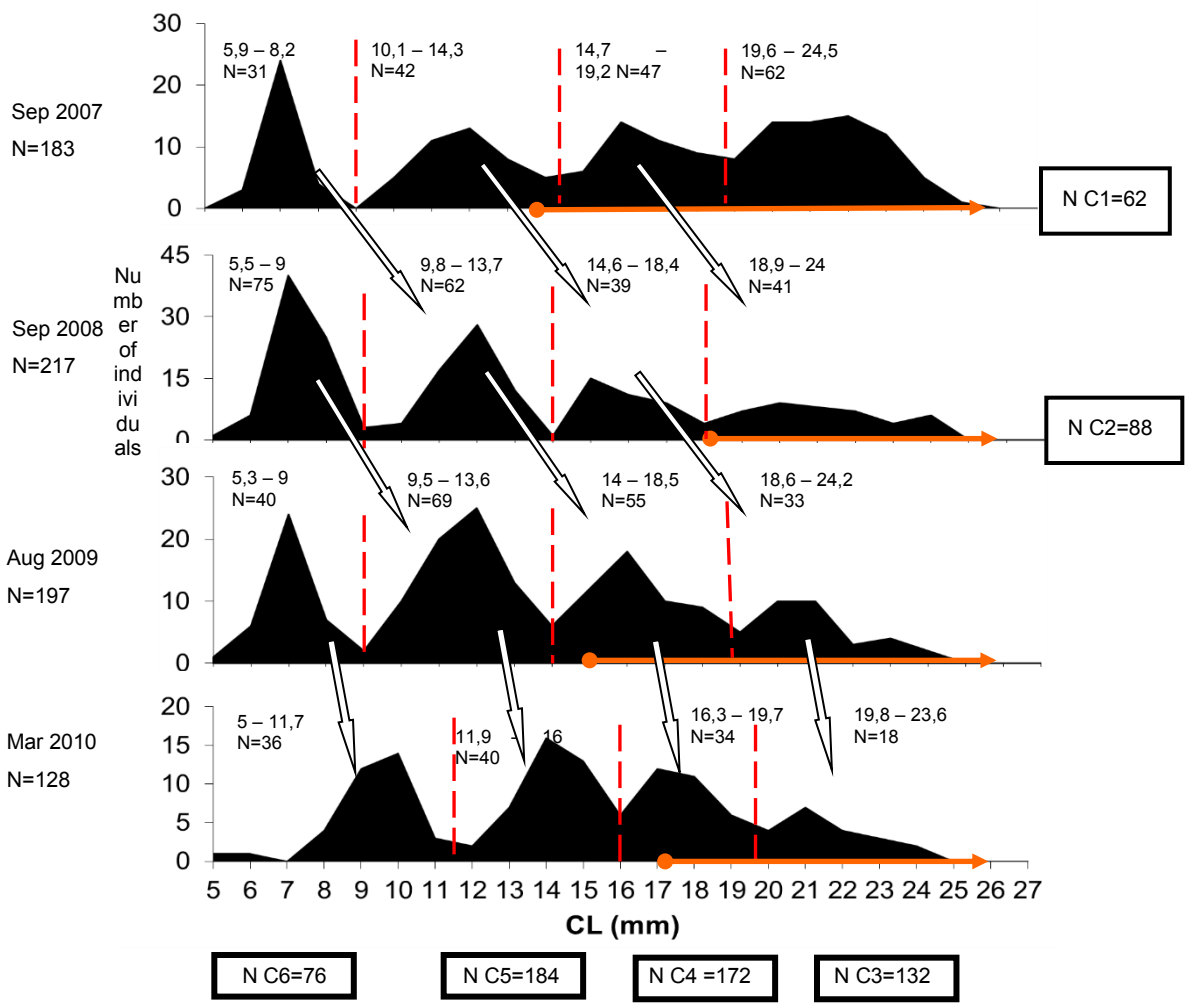


Figure 3.5. Demographic structure in female *Acanthephyra pelagica* in the Sable Gully. Four size-age groups identified for each year for which samples are available. Six cohorts identified among the four years (C1, C2, C3, C4, C5 and C6). Number of individuals per year are indicated on the left side of the graph. Number of individuals per cohort indicated at the bottom and right side of the figure (e.g. N C1= 52). Number of individuals per cohort per year are indicated, together with the size range for each age on each year, above each peak. Orange arrow show size of first appearance of gravid females.

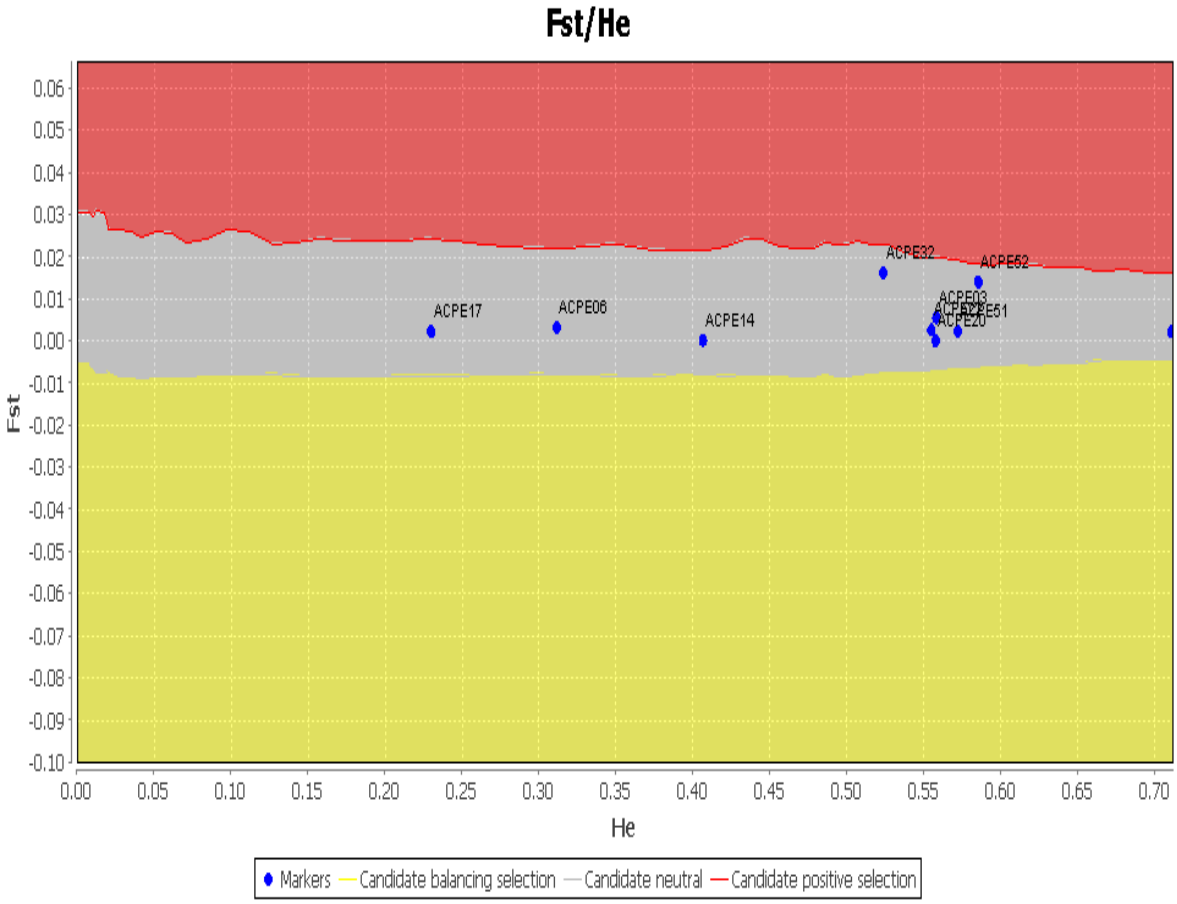


Figure 3.6. Graph showing results of neutrality test run on LOSITAN software. Loci are shown with blue dots. Grey area means that the loci are candidates for neutral selection. Red area means that the loci are candidates for positive selection. Yellow area means that the loci are candidates for balancing selection.

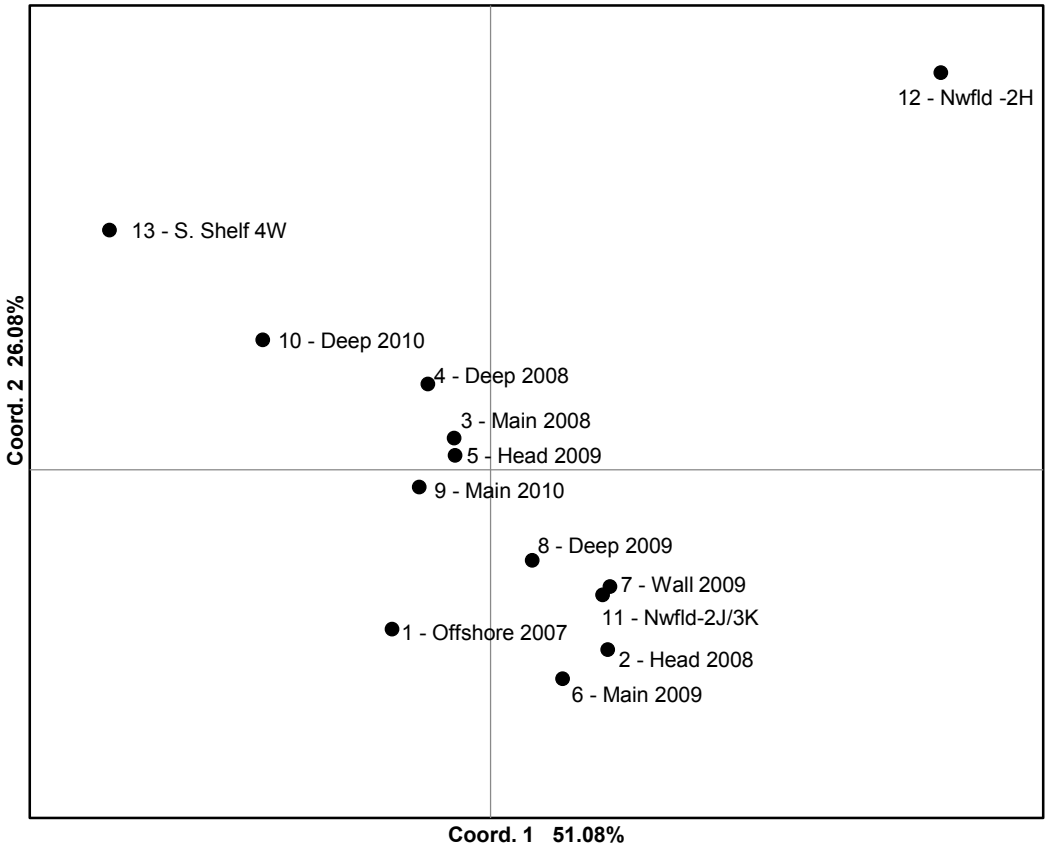


Figure 3.7. Principal Coordinate Analysis (PCoA) for the 13 samples of *Acanthephyra pelagica* along the Atlantic coast of Canada. Obtained with GenAlEx 6.5 Software (Peakall and Smouse, 2006).

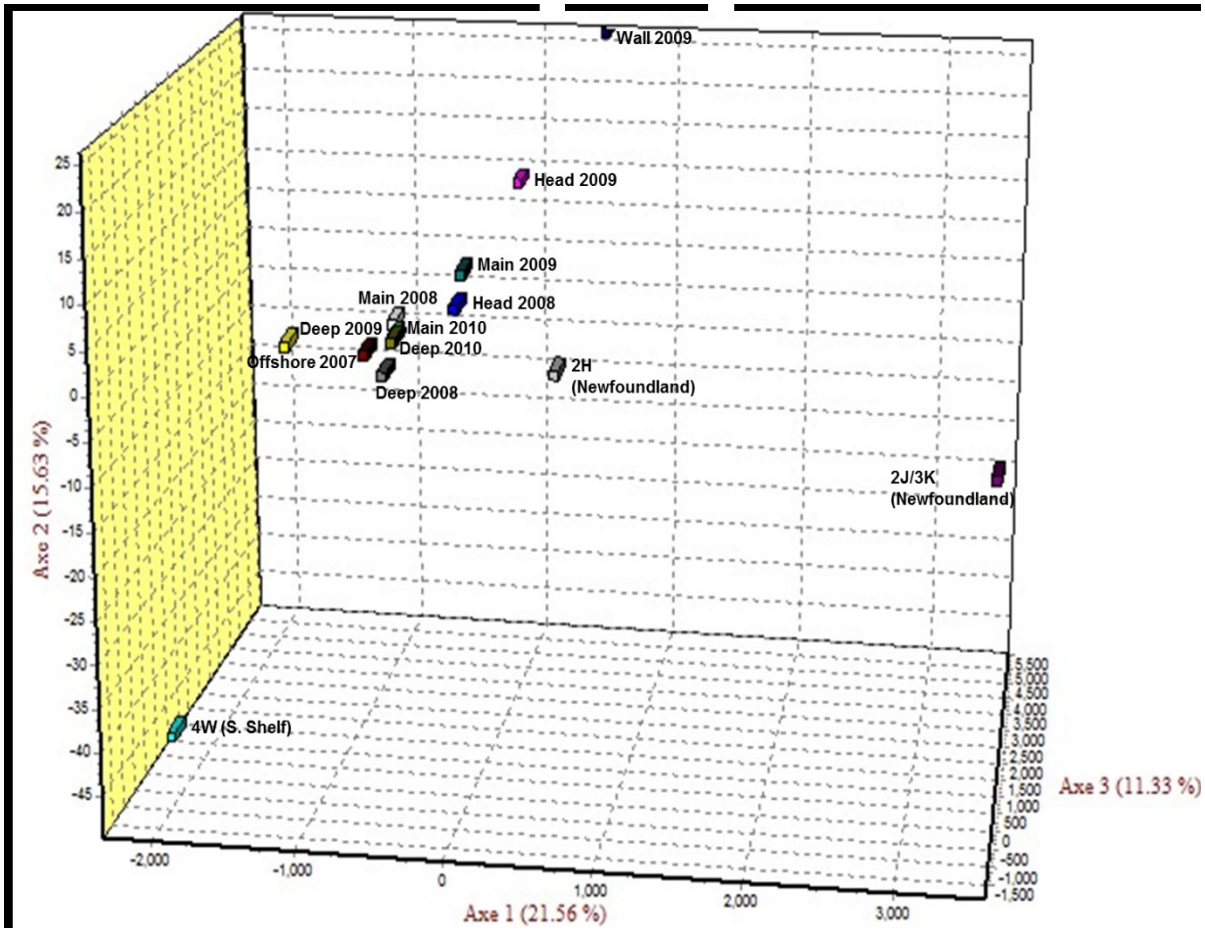


Figure 3.8. Factorial Correspondence Analysis (FCA) plot for the 13 samples of Acanthephyra pelagica along the Atlantic coast of Canada. Obtained with GENETIX 4.05 Software (Belkhir *et al.,* 1996).

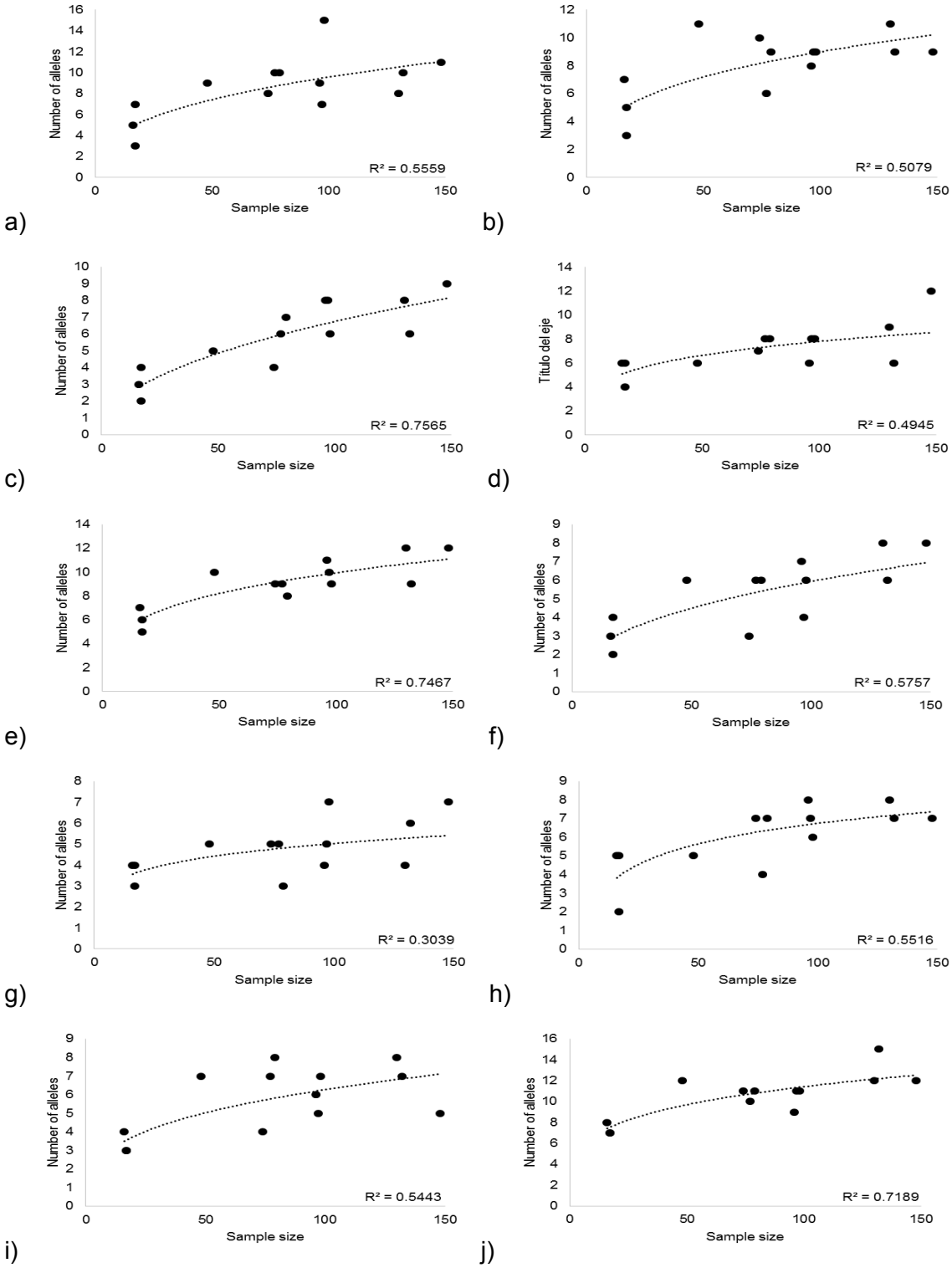


Figure 3.9. Scatterplot showing the relationship between sample size and allelic richness on the locus a) Acpe03, b) Acpe06, c) Acpe14, d) Acpe17, e) Acpe20, f) Acpe22, g) Acpe32, h) Acpe51, i) Acpe52, j) Acpe57. R^2 values are presented on each graph.

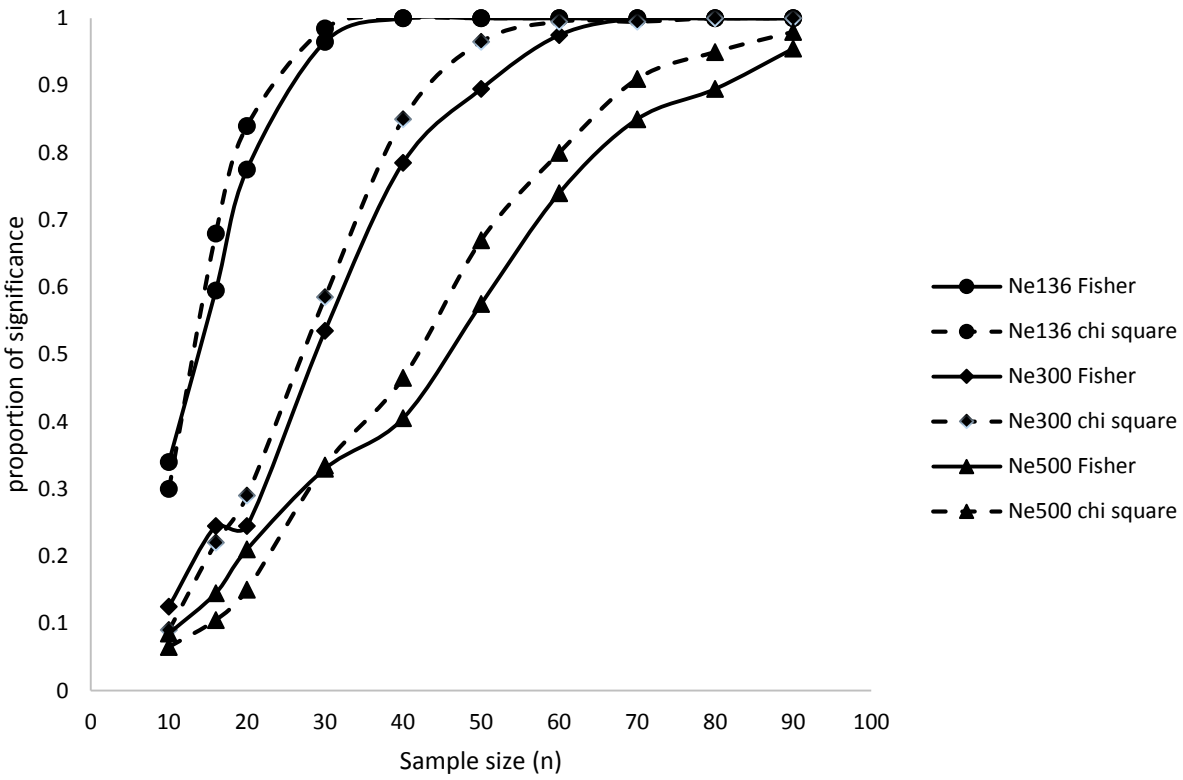


Figure 3.10. Simulation estimates of statistical power to detect differences between samples at increasing sample size. Assuming 13 populations, same samples size (n), 10 microsatellite loci, t=1. N_e=136, 300 and 500. (F_{ST}= 0.0037, 0.0017 and 0.0010 respectively)

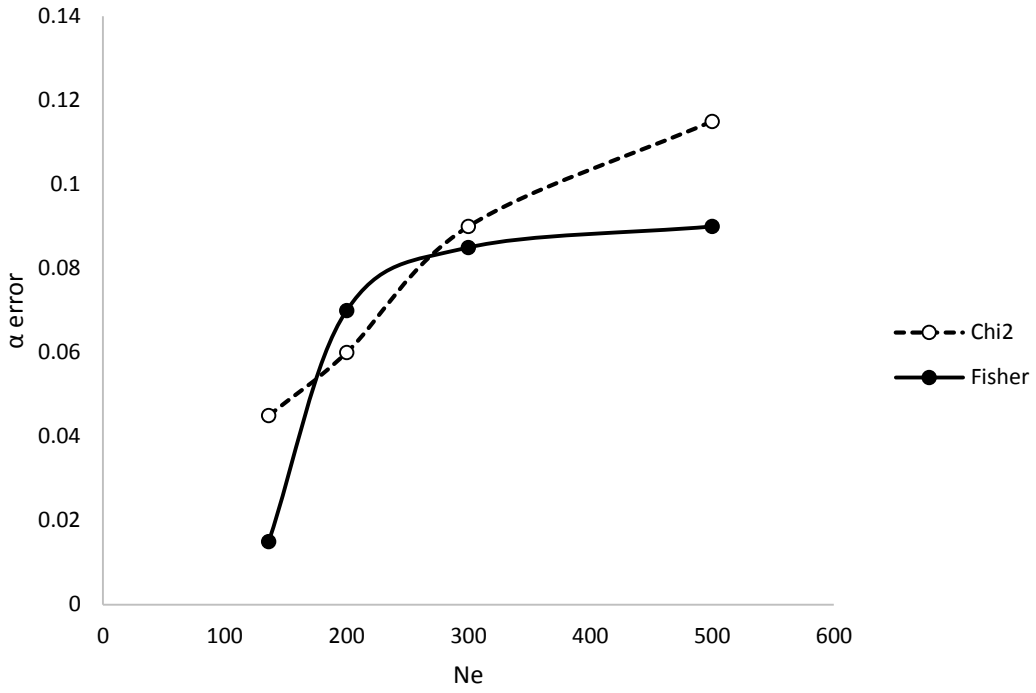
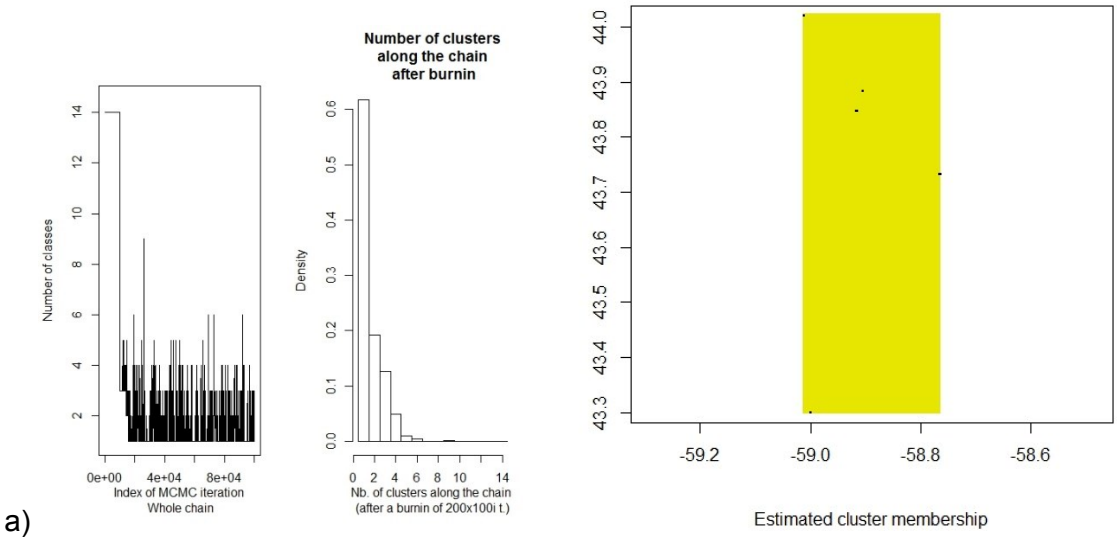
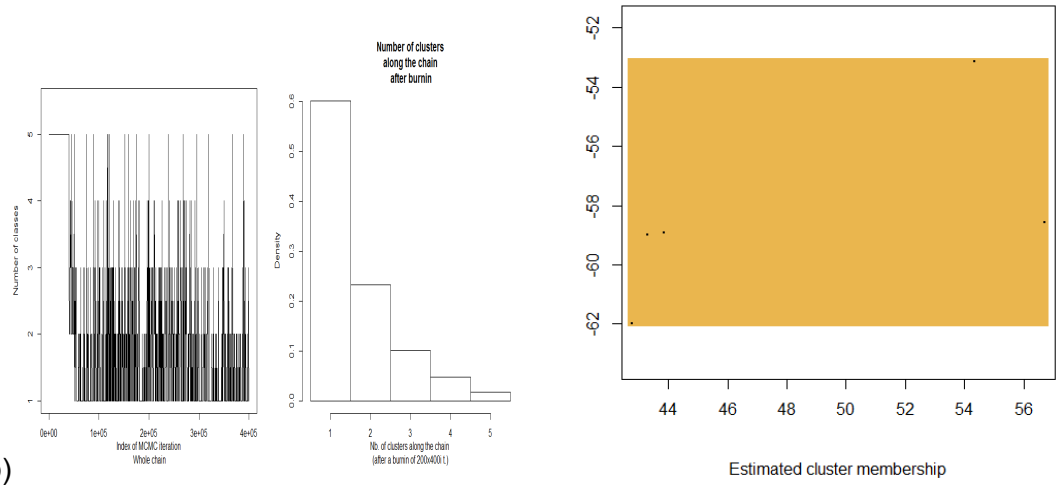


Figure 3.11. Estimation of α error for different effective population size (N_e) of *Acanthephyra pelagica*. Calculated using Chi-square and Fisher approaches. Showing that for a N_e of 136 the probability of rejecting H_O when it is true is under 0.05.



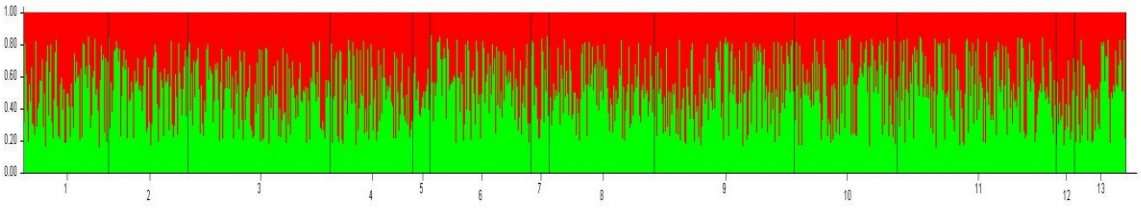
a)



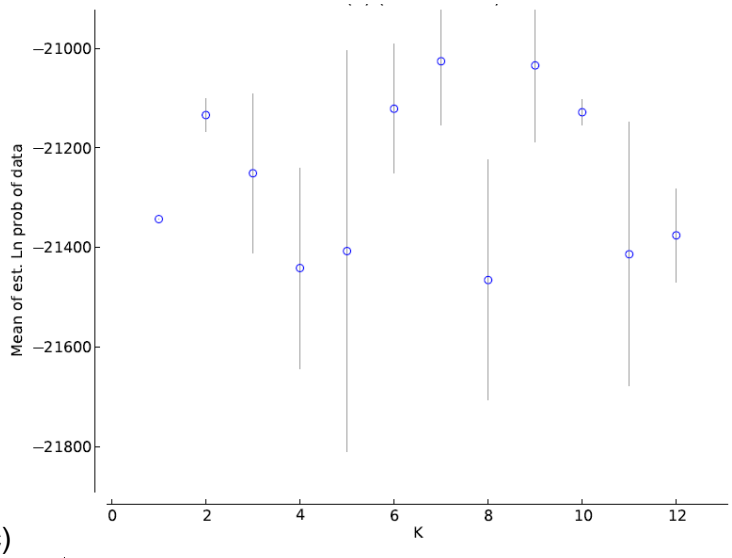
b)

Figure 3.12. Estimation of clusters using a correlated model in Geneland software (Guillot *et al*., 2005). First plots in the left represent the burning period tracing the number of population along the MCMC run. The histograms in the middle shows the probability of the presence of different number of genetic clusters. The plots in the right show the map of posterior probabilities of population membership, black points within each graph represent a sampling point. a) Samples within Sable Gully, b) Samples from Sable Gully and other regions of Atlantic Canada.

a)



b)



c)

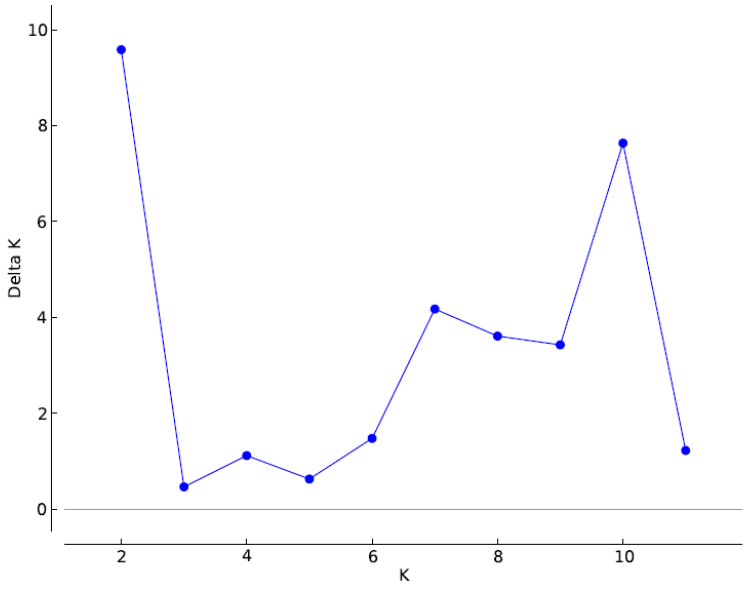


Figure 3.13. Population structure results obtained on Structure 2.3.4 (Pritchard *et al.*, 2000) a) Bar plot for K=2; b) Likelihood plot for the different K tested; c)Delta K plot using Evanno method by Structure Harvester software.

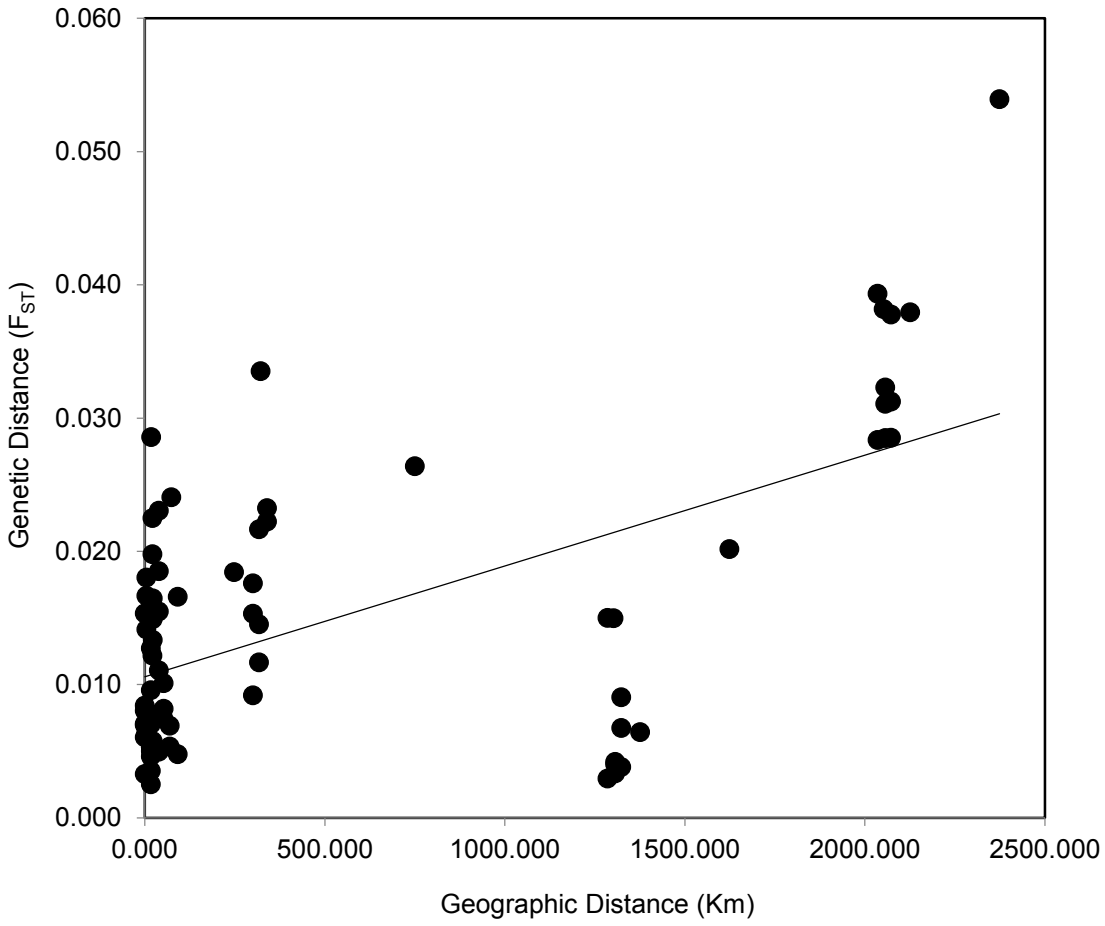


Fig 3.14. The genetic distance in *Acanthephyra pelagica* between pairs of geographical populations (F_{ST}) plotted against the geographical distance between the populations.

Chapter 4: Multiple Paternity

4.1 Introduction

Multiple mating occurs when individuals of one sex mate with more than one individual of the opposite sex (Reynolds, 1996; Neff *et al*., 2000; Neff and Pitcher, 2002), defining mating as fertilization, not just copulation. Studies during the last decades, with the application of polymorphic genetic markers, have shown that multiple mating is widespread in the animal kingdom (Reynolds, 1996; Jennion and Petrie, 2000; Neff and Pitcher, 2002), despite social monogamy found in many species (Mathews, 2007). The existence of multiple mating is relevant to the ecology and evolutionary biology of the species in question, since it affects the strength of natural and sexual selection (Fleming and Gross, 1994; Evans and Magurran, 2000), the effective population sizes (Sugg and Chesser, 1994; Martinez *et al*., 2000), and genetic variability in general.

Mating is not easy to observe directly in nature, therefore, investigating the occurrence of multiple mating in natural populations is often difficult (Yue and Chang, 2010). Inferring the parentage of individuals in a population can assist in the understanding of the mating behaviour and consequently the reproductive strategies of our species of interest. It can be used to examine evidence of sperm competition and whether or not there is evidence of cryptic female choice (Uller and Olsson, 2008; Yue and Chang, 2010).

Multiple mating has both advantages and disadvantages. The disadvantages include an increase of predation risk and enhanced disease transmission (Villesen, 1999; Yue and Chang, 2010). The advantages on the other hand, include ensuring fertilization,

minimizing the probability of genetic incompatibilities, inbreeding and genetic defects that can result from stored sperm, and increasing genetic diversity (Yue and Chang, 2010).

The proportion of broods in a population that exhibits multiple mating is known as the frequency of multiple mating (Neff and Pitcher, 2002). This concept is central for our understanding of the evolution of mating systems and for the conservation of endangered populations (Kelly *et al*., 1999; Zane *et al*., 1999). The degree of multiple mating on the other hand, is the number of individuals genetically contributing to each brood (Neff and Pitcher, 2002). To calculate the number of sires contributing to the brood it is necessary to use a relatively large number of loci, to reduce the probability of not detecting a sire when they share a common genotype (Neff and Pitcher, 2002). The probability of detecting multiple mating (PrDM) depends on the number of loci; the number of alleles and their frequency; the number of offspring analysed; and the number of sires contributing to the brood and their reproductive skew (Neff and Pitcher, 2002).

There are a few studies on mating systems on the class Malacostraca (Salmon, 1983; Berg and Sandifer, 1984), research on the subject has not been as intensive as in other groups. There are diverse mating systems in the order Decapoda. Although most of these systems have not been studied in depth (Bilodeau *et al*., 2005), in general it is known that sperm are not motile and are deposited in spermatophores (Bauer, 1986; Felgenhauer and Abele, 1991). In some species fertilization is internal and in others external (Mathews, 1956; Berg and Sandifer, 1984). In some species sperm can be stored in the spermathecae for months and thus fertilize eggs months after mating (Subramoniam, 1993; Suzuki and Ziegler, 2005). Therefore females can store sperm from different mates. Some species exhibit precopulatory and postcopulatory guarding to prevent multiple mating while in other species multiple mating is the norm. Some species of the Malacostraca group have been reported to present multiple paternity: the crayfish

Orconectes placidus (Walker *et al.*, 2002), the red swamp crayfish *Procambarus clarkii* (Yue *et al.*, 2010), the lobster *Nephrops norvegicus* (Streiff *et al.*, 2004), the lobster *Homarus americanus* (Jones *et al.*, 2003), the crab *Petrolisthes cinctipes* (Toonen, 2004), the snapping shrimp *Alpheus angulosus* (Mathews, 2007), the ghost shrimp *Callichirus islagrande* (Bilodeau *et al*., 2005), the dungeness crab *Metacarcinus magister* (Jensen and Bentzen, 2012), the ectoparasite crab *Dissodactylus primitivus* (Jossart et al,. 2014) and the freshwater shrimp *Caridina ensifera* (Yue and Chang, 2010).

In this thesis Chapter, I examine the evidence for multiple mating in a deep sea species where direct observation is difficult, therefore all the information gathered in this research on *Acanthephyra pelagica* is of great importance. This species exhibits high fecundity with more than 500 eggs laid per female each year in the east Atlantic (Burukovsky and Andreeva, 2010), and according to results of Chapter 3 reproduction appears to occur seasonally. Nevertheless, there is no previous knowledge on the mating system of *A. pelagica.* Here I use polymorphic DNA markers and statistical tools that will allow me to address questions about the mating system, reproductive strategies and determine parentage in this species.

4.2 Methods

4.2.1 Determination of Multiple Paternity

The analysis of multiple paternity proceeded by selecting ovigerous three and four year old females. A piece of muscular tissue was taken from the mother for DNA extraction. The entire egg and a total of 63 eggs per female were used to extract DNA for the offspring (When 63 or more eggs were available). Muscular tissues and eggs were

digested with 400 µg/mL proteinase K (Bio Basic Inc., Markham, ON, Canada) and 300 µL of digestion buffer (10 mM Tris–HCl, pH 8.3, 1 mM EDTA, 400 mM NaCl containing 0.8% sodium dodecyl sulfate (SDS)), for 7 hours at 55°C on a shaker working at 200 rpm. DNA was extracted using glass-milk protocol (Elphinstone *et al.,* 2003).

The loci developed in Chapter 2 together with the allele frequencies found on Chapter 3, were available for use in the paternity analysis. To define which and how many loci to use and the number of eggs to genotype, I utilized the PrDM software (Neff and Pitcher, 2002). This software allowed us to determine the probability of detecting multiple paternity with each locus at different sample sizes. For this analysis I assumed two sires with a skewed contribution to the offspring. The probability was then calculated by combining the loci with higher individual probabilities.

Fragments were amplified for each of the four chosen loci for each mother and her brood. Microsatellite loci were amplified in 7.5 µL total volume containing 1.65 µL of dd H2O, 1 µL of 10x reaction buffer (Bio Basic Inc., Markham, Ontario), 1 µL of 25mM MgSO4 (Bio Basic Inc., Markham, Ontario), 0.1 µL of 1 µM of fluorescently labeled M13 tag, 0.1 µL of 1 µM of un-tailed primer (either forward or reverse), 0.1 µL of 0.1 µM of M13 tailed primer (either forward or reverse), 1 µL of 2.5 µM dNTPs (Bio Basic Inc., Markham Ontario), 0.05 µL of TSG Polymerase (Biobasic Inc.,Markham, Ontario), and 1.3 µL approximately 50 ng/µL of genomic DNA. PCR conditions were as follows: 5 min at 94°C, 35 cycles of denaturation (94°C for 30s), annealing (57 - 65°C for 30s), and extension (72°C for 30s).

To visualize the PCR products, 1 µL of PCR product was mixed with 9 µL of formamide. The mix was loaded on a polyacrylamide gel that was run and imaged on a Li-COR machine (Li-COR Bioscience, Lincoln, Nebraska). The genotypes of each mother and its brood were obtained using SAGA Automated Microsatellite Software 3.3.

With the genotypes I was able to infer paternal genotypes and determine if there is more than one sire contributing to the brood. GERUD 2.0 software (Jones, 2005) was used to confirm multiple paternity. This software aids in the use of data from multiple loci, making full use of multi-locus data to determine the minimum number of males contributing to progeny and to reconstruct the genotypes based on data from polymorphic, codominant markers (Jones, 2005).

4.2.2. Inference of sperm storage

In Chapter 3, I described how carapace length (CL) was measured on each individual to assess the size structure on the Sable Gully population in each year (2007, 2008, 2009 and 2010). This information was used to determine size-age groups and establish their precise boundaries. This information was used to determine the age of each female carrying eggs.

Acanthephyra pelagica females were found to start producing eggs at the age of three, as shown in Chapter 3. Therefore, in our samples only three and four year old females have offspring. For the paternity analyses, eight, three year old and 11, four year old females were chosen out of the samples taken at the Sable Gully. This was done in order to compare the number of potential fathers contributing to the offspring. To discern if older females have more sires, a regression analysis was performed to determine the relation between age and number of sires contributing to the brood. With this information it is possible to infer if there is sperm storage in this species.

4.3 Results

4.3.1 Sample size and loci determination.

The probability of detecting multiple mating (PrDM) was highest for locus Acpe26 and lowest for locus Acpe17 (Table 4.1 and Figure 4.1). The PrDM combining loci with the highest individual probabilities and technical concerns in the lab were taken into consideration in the final choice of loci. I chose to work with four loci: Acpe20, Acpe26, Acpe51, and Acpe57) and 63 offspring individuals as sample size. This combination of variables give a PrDM higher than 95% (0.965) (Table 4.2).

4.3.2 Assessment of multiple paternity

A total of 19 females were genotyped together with their brood. Of these females 11 are four year old and 8 are three year old. The offspring sample size varied according to the availability of eggs. 24 eggs is the smallest sample and 63 is the largest. The development stage varied among broods but was similar among eggs within broods.

Each embryo contained at least one allele per locus from the mother, hence, it is possible to infer that embryos belonged to the mother in which they were found. The GERUD 2.0 software also confirmed that the female holding the embryos was the mother of the offspring. Out of the 19 females/offspring samples, all 19 groups were found to exhibit evidence of multiple paternity, hence, the frequency of multiple mating is 100%. The degree of multiple mating (number of sires) varied from 2 to 4. Eight broods had a minimum of two fathers, seven broods had a minimum of three fathers and four broods had a minimum of four fathers (Table 4.3). In most broods it was not possible to

estimate only one genotype for the sires, instead, different solutions were proposed ranked by likelihood. Therefore, it was not possible to identify the genotype of the father of each individual offspring. This is mainly due to the extensive sharing of genotypes between the mothers and fathers. The contribution of the sires was found to be skewed in all broods (Fig. 4.3).

4.3.3 Inference of sperm storage

No evidence for a relatively high number of sires in broods of older females was found. The regression analysis (Fig. 4.2) shows no relation between female age and number of sires contributing to a brood. Hence, no evidence was found to infer sperm storage from one year to the next in this species.

4.4 Discussion

All 19 females analysed mated with more than one male, as I detected multiple paternity in all 19 broods. These results give insights into the mating behaviour and the existence of multiple mating in wild populations of *A. pelagica*. The main benefit of multiple mating is the increase of genetic diversity in the offspring which raises progeny fitness. Another important reason for multiple mating is to maximize fertilization success of all the eggs since some males may be sterile, males may partition their sperm among females, or there might be a passive loss of sperm in storage organs (Jennions and Petrie, 2000). Although, parentage and mating systems on the crustacean subphylum is still understudied, multiple mating seems to be common in this group. The frequency of

multiple mating within Malacostraca is generally high: 97% in *P. clarkii* (Yue *et al*., 2010), 100% in *C. ensifera* (Yue and Chang, 2010), 40% in *O. placidus* (Walker *et al.*, 2002), 13% in *H. americanus* (Jones *et al.*, 2003), 55% in *N. norvegicus* (Streiff *et al.*, 2004), 80% in *P. cinctipes* (Toonen, 2004), 20% *C. islagrande* (Bilodeau *et al*., 2005), 40% in *M. magister* (Jensen and Bentzen, 2012) 66.7% in *D. primitivus* (Jossart *et al,*. 2014) and 100% in *A. pelagica*. Nevertheless, multiple mating is not a general pattern. Species for which only one father was found include for instance the snapping shrimp *Alpheus angulosus* (Mathews, 2007). The frequency of multiple mating is largely related with the post-copulatory energy investment of the male; multiple mating tends to occur when only females take care of the offspring (Jennions and Petrie, 2000). The behaviour of the males is also important, as they often attempt to prevent other males to reach the female (Jormalainen, 1998). This kind of behaviour has not been observed in marine shrimps (Yue and Chang, 2010), in contrast to other crustaceans free-living shrimp do not exhibit precopulatory mate guarding and they are relatively unaggressive (Correa and Thiel 2003). As previously mentioned, behaviour is hard to observe directly in deep-living species. Considering the high frequency of multiple mating found in this study, however, mate guarding behaviour is unlikely to be present in *A. pelagica*.

It was found that the contribution of the sires to the broods was skewed since one primary sire was assigned to a large proportion of the offspring in each brood (Fig.4.3). Similar patterns were found in other crustaceans: *O. placidus* (Walker, 2002), *C. ensifera* (Yue and Chang, 2010), *P. clarkii* (Yue *et al*., 2010), *M. magister* (Jensen and Bentzen, 2012) and *D. primitivus* (Jossart *et al,*. 2014). The causes for this skew include mating order (Yue et al. 2010) or could be indicating pre-copulatory female choice, where the female may mate several times with a preferred male (Thiel and Correa, 2004), or post-copulatory sperm competition or selection which could happen by sperm digestion or by

sperm sorting and differential use of it (Hasse and Baur, 1995; Yue and Chang, 2010). However it is not possible to determine which of these mechanisms if any, are actually working in this case. On the other hand the presence of a primary sire, found in this study can also be an artifact of males sharing a common genotype, although 4 loci should be enough. An increase in the number loci genotyped would be required to determine if more sires were contributing to a brood and reduce the probability of not detecting a sire.

No evidence was found of a relation between the size/age of the female and the number of sires contributing to the brood. This suggest that there is no evidence that this species presents long term sperm storage. This is somehow an expected result for caridean species, like *A. pelagica,* whose females usually do not present a thelycum (Bauer, 1986). A thelycum is an external seminal receptacle where sperm could be stored. Correa and Thiel (2003) also reviewed the information available reporting that in general, caridean females have no sperm storage structures. The lack of complex structures on the reproductive organs of the caridean group makes sperm storage, highly improbable. Therefore, females need to copulate during each reproductive cycle.

Acanthephyra pelagica is a species with a high fecundity, with a brood size of around 500 eggs (Burukovsky and Andreeva, 2010). Even though this number of eggs was hard to find in females, many of the eggs may have been lost during sampling or sample manipulation. I found that all offspring attached to the females were actually offspring of the assumed mother. Given the degree of development found in some of the broods, I can assume that mothers carry the eggs until hatching. This maternal care is important for offspring survival on the pelagic system. Deep-sea larvae are exposed to fluctuations in food supply and high predation risk during their planktonic phase in the water column. Hence, large and advanced larvae have the necessary reserves to survive and develop

to the juvenile stage through a smaller number of stages, decreasing the time spent in the water column and therefore decreasing mortality risk (King and Butler, 1985; Clarke *et al*., 1991; Ramirez-Llodra, 2002; Baeur, 2004).

Our results in combination with other reports suggests that multiple paternity is a common feature in the Malacostraca class. Finding a skewed contribution of the fathers to the offspring, suggest that pre- and postcopulatory female choice or sperm competition occurred. The high prevalence of multiple paternity is expected to have an impact in male reproductive success. These findings on the mating system of *A. pelagica* are important to understand species behaviour, reproduction and evolution in this species.

Table 4.1. Probability of detecting multiple mating in *Acanthephyra pelagica* for all the loci available for this study at different sample size assuming 2 sires with a skew contribution to the offspring.

Locus	**Offspring Sample Size**				
	10	20	30	40	50
Acpe03	0.113	0.191	0.233	0.256	0.272
Acpe06	0.042	0.069	0.089	0.099	0.104
Acpe14	0.052	0.089	0.110	0.135	0.138
Acpe17	0.012	0.021	0.027	0.032	0.033
Acpe20	0.138	0.225	0.265	0.300	0.315
Acpe22	0.070	0.117	0.142	0.159	0.168
Acpe26	0.504	0.726	0.829	0.880	0.907
Acpe32	0.040	0.069	0.089	0.099	0.108
Acpe43	0.389	0.586	0.689	0.747	0.777
Acpe51	0.140	0.220	0.272	0.296	0.320
Acpe52	0.082	0.134	0.168	0.185	0.199
Acpe57	0.277	0.420	0.503	0.542	0.570

Table 4.2. Probability of detecting multiple mating in *Acanthephyra pelagica* combining the loci with the highest individual probability at different sample sizes. Assuming 2 sires with a skew contribution to the offspring.

Loci	Offspring sample size							
	10	**20**	**30**	**40**	**50**	**60**	**63**	**70**
Acpe26 + Acpe57	0.517	0.739	0.838	0.883	0.909	0.921	0.922	0.927
Acpe26 + Acpe57 + Acpe51	0.545	0.77	0.868	0.913	0.935	0.946	0.948	0.949
Acpe26 + Acpe57 + Acpe51 + Acpe20	0.569	0.796	0.892	0.934	0.953	0.963	0.964	0.966

Table 4.3. Genotypes of the mother and most probable genotype of the fathers of each brood. # Progeny indicate the number of offspring analyzed (if on the side of the mother) and the number of progeny that could be explained for each father.

Mother ID	Years old	Parents	Loci				
			Acpe20	Acpe26	Acpe51	Acpe57	# progeny
1	3	Mother	192/200	242/224	219/227	227/223	62
		Father1	192/200	232/224	227/231	223/239	34
		Father2	192/200	232/242	227/227	227/223	40
		Father3	200/200	232/242	219/235	239/211	3
		Father4	200/204	224/242	227/231	227/227	13
2	3	Mother	200/200	222/238	219/227	223/223	36
		Father1	200/192	238/232	227/227	223/223	19
		Father2	200/192	238/242	227/227	227/231	7
		Father3	200/192	232/222	219/219	227/223	12
3	3	Mother	200/200	232/232	219/227	223/227	40
		Father1	192/200	230/238	227/227	215/215	4
		Father2	192/200	232/234	227/227	215/223	20
		Father3	192/200	224/236	227/231	215/223	6
		Father4	192/200	226/228	227/227	215/223	12
4	4	Mother	220/232	234/234	227/219	223/227	34
		Father1	232/228	238/224	215/227	223/215	25
		Father2	232/220	238/224	215/219	223/215	9
		Father3	228/220	238/234	215/227	223/207	12
5	4	Mother	200/200	226/228	219/227	219/219	22
		Father1	200/196	226/234	219/219	223/211	10
		Father2	200/200	230/224	227/227	223/219	6
		Father3	200/200	230/228	227/235	211/219	10
6	3	Mother	220/228	246/246	227/227	223/227	28
		Father1	228/232	246/224	219/227	227/211	17
		Father2	228/232	246/224	227/231	223/231	19
7	3	Mother	200/200	232/240	227/231	223/199	24
		Father1	200/192	240/240	219/227	223/227	14
		Father2	200/196	232/240	219/227	211/223	17
		Father3	204/192	240/240	219/227	215/215	2
8	4	Mother	200/200	246/246	227/235	227/223	30
		Father1	200/192	246/224	219/227	215/227	26
		Father2	200/200	246/224	219/227	223/219	6
9	3	Mother	208/220	224/242	227/227	219/231	51
		Father1	220/216	242/232	227/219	219/227	46
		Father2	220/220	224/232	227/219	219/227	5
10	3	Mother	200/200	224/226	219/227	223/235	34
		Father1	176/204	228/226	219/231	227/223	8
		Father2	200/192	224/222	219/227	227/223	22
		Father3	200/200	228/228	231/223	223/223	4
11	4	Mother	200/192	246/246	219/227	223/211	48
		Father1	200/192	246/226	227/227	223/223	41
		Father2	200/204	246/224	227/227	223/223	44
12	4	Mother	176/200	224/224	219/227	223/227	63
		Father1	200/204	224/214	227/227	223/235	61
		Father2	200/192	224/224	227/227	223/227	15

Table 4.3 continued

Mother ID	Years old	Parents	Loci				
			Acpe20	Acpe26	Acpe51	Acpe57	# progeny
13	4	Mother	192/200	224/238	219/227	211/223	62
		Father1	192/192	214/218	219/219	223/223	2
		Father2	200/200	224/238	227/227	231/223	60
14	4	Mother	200/192	232/240	219/227	203/223	62
		Father1	200/200	232/240	227/231	223/223	59
		Father2	200/200	240/224	219/227	203/223	41
15	4	Mother	200/200	250/240	227/231	203/223	62
		Father1	200/192	232/240	219/227	203/223	60
		Father2	200/192	232/222	227/231	223/223	28
16	3	Mother	192/200	226/226	219/227	219/227	63
		Father1	200/200	218/226	227/227	223/219	18
		Father2	200/192	218/226	227/227	215/227	40
		Father3	192/204	226/230	227/211	219/231	10
		Father4	204/204	218/218	227/227	215/215	1
17	4	Mother	192/200	226/226	219/227	223/231	61
		Father1	192/184	226/234	227/227	231/227	22
		Father2	200/204	226/216	227/227	223/227	52
		Father3	200/188	226/206	227/231	223/227	45
18	4	Mother	200/200	226/228	227/231	211/223	56
		Father1	192/200	224/228	219/227	223/223	32
		Father2	192/192	232/226	219/227	223/223	12
		Father3	192/192	222/234	219/227	223/223	4
		Father4	192/192	224/226	219/228	199/215	8
19	4	Mother	192/200	232/264	227/227	223/227	63
		Father1	192/204	224/216	227/219	227/211	22
		Father2	200/204	224/232	227/219	223/227	47
		Father3	200/204	216/264	227/219	223/211	7

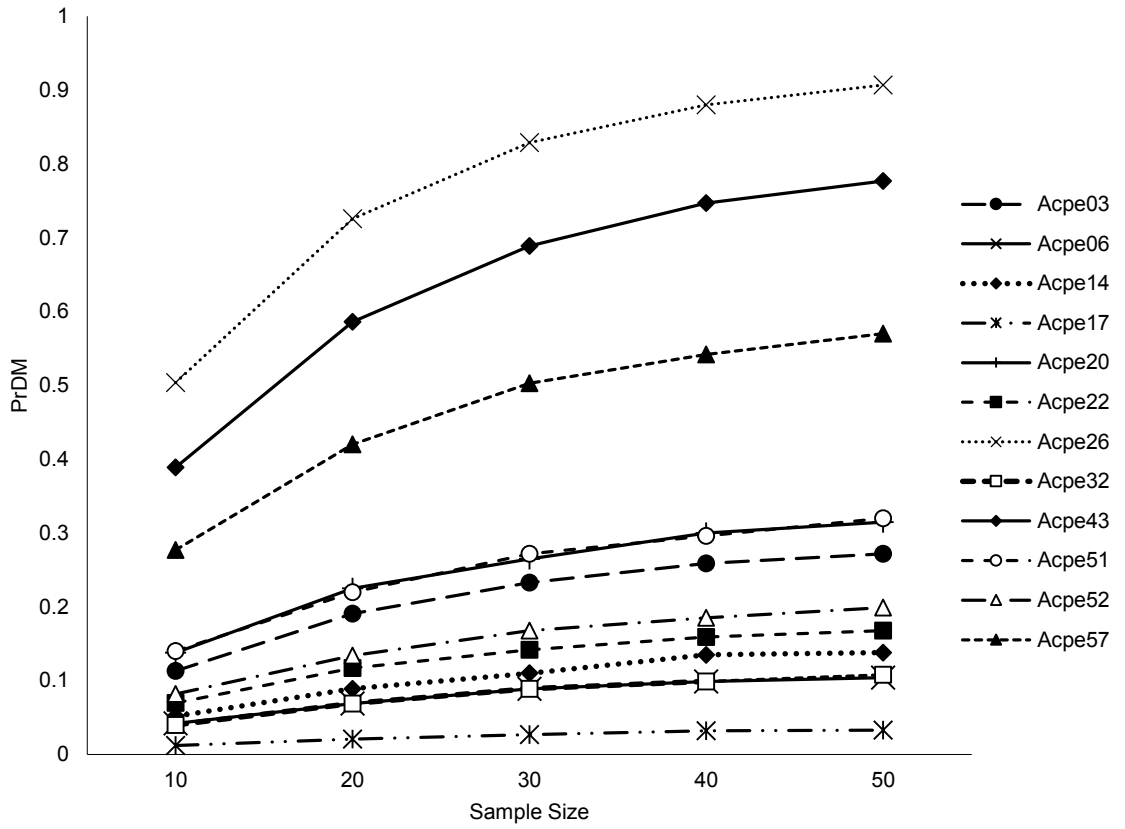


Figure 4.1. Probability of detecting multiple mating in *Acanthephyra pelagica* for all the loci available for this study at different sample size. Assuming two sires with a skew contribution to the offspring.

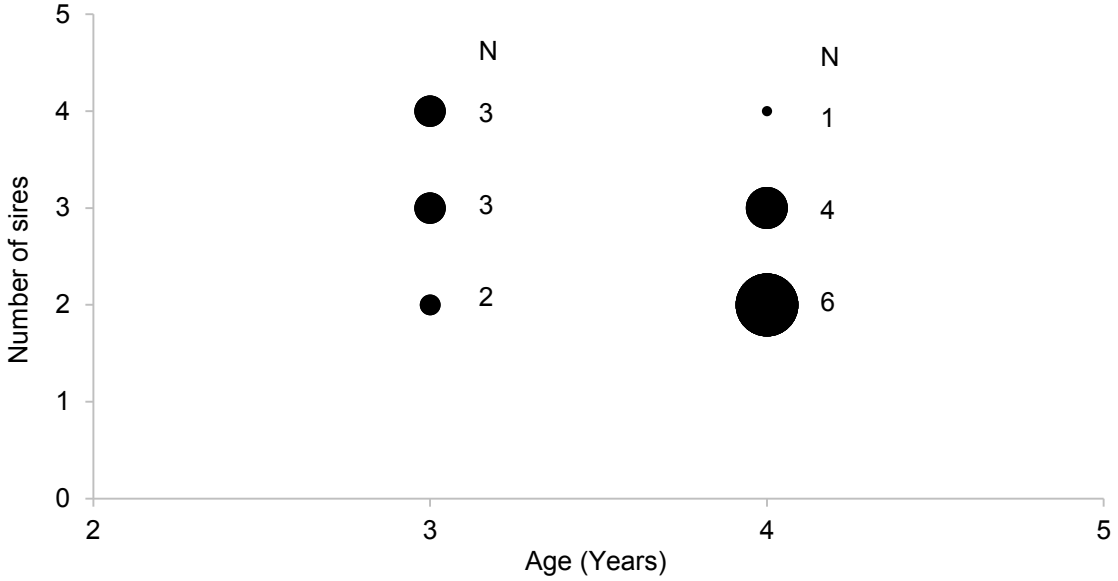


Figure 4.2. Scatterplot showing the relationship between the age of the *Acanthephyra pelagica* females and the number of sires contributing to their offspring.

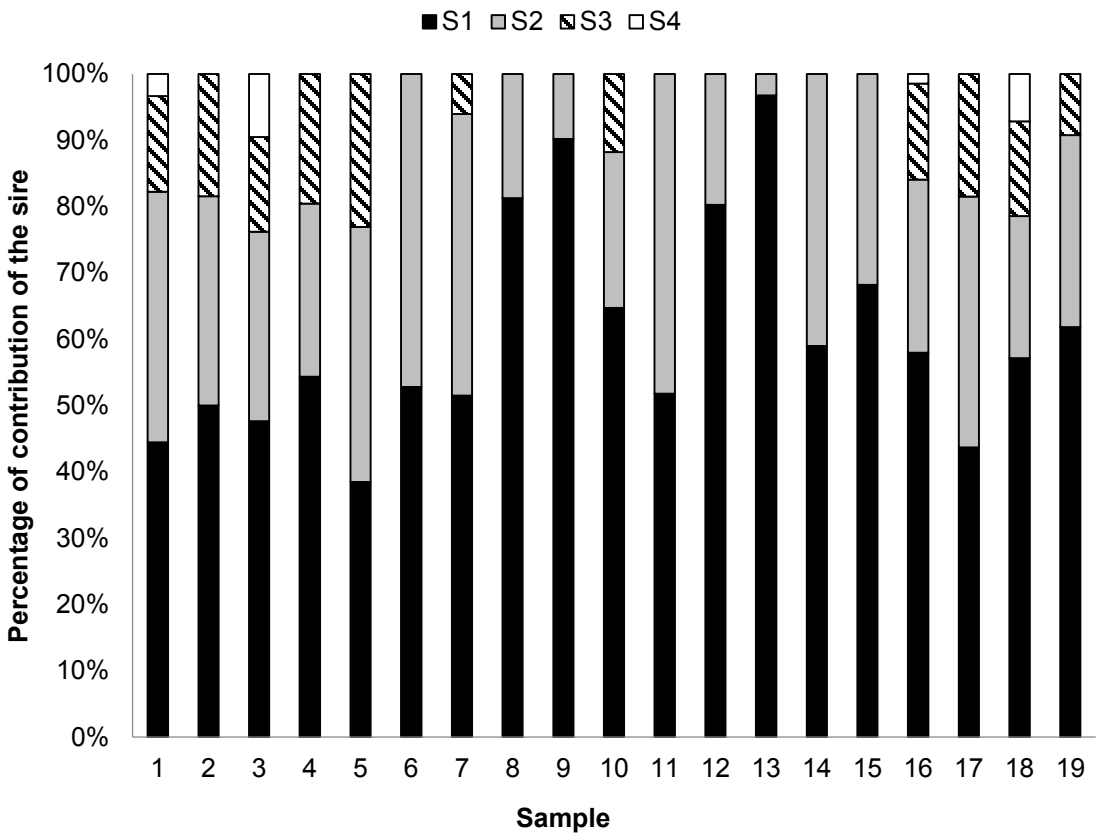


Figure 4.3. Degree of multiple paternity in *Acanthephyra pelagica*. Plot shows the percentage of contribution of the sires to each brood. S1= Sire1; S2= Sire2; S3= Sire3; S4= Sire4.

Chapter 5: Conclusions

Several important results were obtained from this research. The primer design resulted in 26 microsatellite markers, 10 of which are polymorphic and free of null alleles. Few very frequent alleles and many infrequent ones characterized the northwest Atlantic samples. With respect to population structure, a general pattern of panmixia was found with little population structure evidenced in any of the spatial temporal, or cohort groups. However, I did find genetic differentiation when comparing a sample from NAFO Division 4W on the Scotian Shelf (4W) to other samples, particularly those from off Newfoundland where pairwise comparisons were significant even after Bonferroni correction (Fst 0.01 - 0.029) and high for a marine species. These results are also in agreement with IBD results obtained, indicating a general lack of genetic structure at smaller spatial scales, less than 1500 km, but presenting a significant genetic structure at a higher spatial scale, highly correlated to geographic distance and explaining a third of the variance in the data. IBD as a structuring mechanism in this species could be explored in future by targeted sampling over these larger spatial scales.

Reproduction was found to occur once a year, starting at the third year, with a lifespan of 4 years. The paternity analysis revealed that multiple paternity was prevalent in this species, with a variable number of fathers, from 2 to 4, and a skew contribution of the sires to the brood. The presence of multiple mating in *A. pelagica* may not be increasing genetic diversity but it is certainly maintaining the genetic diversity of the population, and also increasing mixing within the population - this is another explanation for the lack of genetic structure in such a wide area. Multiple paternity also increases mixing among cohorts, where a female of one cohort can mate with males of different cohorts, although this mixed cohort could occur even with monogamic reproduction, having multiple mating

decreases the chances of sexual selection for one age over others. Of course this mixing within generations is also influencing the temporal assessment of genetic structure. This may explain why there was no detectable genetic structure when comparing samples from the same locations across the four years.

The effective population size (N_e) was found to be very low for most of the populations, an uncommon result for a marine species. N_e: N_c was below expected values for marine species. It has been suggested that extremely low N_e: N_c ratios may be related to high fecundity and high juvenile mortality, which would generate a high variance in reproductive success depressing N_e (Hedgecock, 1994; Palstra and Ruzzante, 2008). Multiple mating is likely to increase the effective population size due to increase in genetic diversity (Murray, 1964), although it is suggested only in the case of sperm storage occurrence, which is likely not occurring in A. pelagica. Moran and Garcia-Vazquez (1998) maintain that in a small salmon population with few adults, multiple paternity increasing N_e could be the only explanation for the high heterozygosity found. However Karl (2008) argues that this study is not considering the presence of precocious par contributing to the brood, also arguments that because of the skew contribution of the sires to the offspring in MP, there is variance in male reproductive success, therefore, N_e should actually decrease. Poor reproductive success might be the explanation for the low N_e and low N_e/N_c ratio.

This research provides information about the biology and ecology of *A. pelagica*, showing patterns of generally high connectivity throughout the northwest Atlantic. It also showed that the mating system of this species includes multiple paternity. It can be inferred that the biology, reproductive system and behaviour of *A. pelagica,* together with the environment, are producing little genetic differentiation in this species along the Northwest Atlantic.

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APPENDIX A

Appendix A. Indices of genetic variability found in *A. pelagica* for the 13 groups and for the 10 loci. Sample Size (N), No. Alleles (N_A), Effective number of alleles (A_E),Observed Heterozygosity (H_O),Unbiased Expected Heterozygosity (H_E), Fixation Index (F), Allelic Richness (A_R) Private allelic richness (PA_R)

Pop	Locus	N	N_A	A_E	H_O	H_E	F	A_R	PA_R
Offshore	ACPE03	78	10	2.485	0.526	0.601	0.120	5.02	0.29
	ACPE06	79	9	1.722	0.367	0.422	0.124	4.91	0.15
	ACPE14	76	7	1.712	0.289	0.419	0.304	3.87	0.17
	ACPE17	79	8	1.185	0.139	0.157	0.109	2.98	0.14
	ACPE20	79	8	2.147	0.519	0.538	0.028	5.08	0.19
	ACPE22	78	6	2.218	0.551	0.553	-0.004	3.28	0.33
	ACPE32	77	3	1.936	0.532	0.487	-0.101	2.45	0.00
	ACPE51	76	7	2.546	0.697	0.611	-0.149	4.89	0.18
	ACPE52	77	8	2.551	0.364	0.612	0.402	4.92	0.83
	ACPE57	78	11	3.178	0.538	0.690	0.214	7.39	0.31
Head 2008	ACPE03	73	8	2.192	0.370	0.548	0.320	4.21	0.02
	ACPE06	74	10	1.485	0.284	0.329	0.132	4.95	0.10
	ACPE14	73	4	1.529	0.247	0.348	0.287	3.20	0.04
	ACPE17	72	7	1.320	0.236	0.244	0.025	3.69	0.14
	ACPE20	73	9	1.989	0.397	0.501	0.201	5.29	0.02
	ACPE22	72	3	2.085	0.528	0.524	-0.014	2.58	0.00
	ACPE32	74	5	1.860	0.486	0.465	-0.052	3.00	0.15
	ACPE51	72	7	2.636	0.736	0.625	-0.186	4.98	0.06
	ACPE52	73	4	2.320	0.438	0.573	0.230	3.40	0.00
	ACPE57	72	11	3.019	0.514	0.673	0.232	7.03	0.09
Main 2008	ACPE03	131	10	2.323	0.412	0.572	0.276	4.71	0.17
	ACPE06	132	9	1.430	0.273	0.302	0.093	4.37	0.07
	ACPE14	129	6	1.714	0.287	0.418	0.311	3.33	0.10
	ACPE17	128	6	1.184	0.156	0.156	-0.006	2.75	0.13
	ACPE20	130	9	2.091	0.454	0.524	0.130	4.74	0.01
	ACPE22	132	6	2.346	0.583	0.576	-0.017	3.73	0.09
	ACPE32	131	6	2.114	0.420	0.529	0.203	3.14	0.02
	ACPE51	130	7	2.380	0.723	0.582	-0.247	4.16	0.05
	ACPE52	129	7	2.419	0.535	0.589	0.088	3.99	0.17
	ACPE57	131	15	4.162	0.664	0.763	0.126	8.18	0.65
Deep 2008	ACPE03	77	10	2.514	0.455	0.606	0.245	5.33	0.39
	ACPE06	77	6	1.315	0.221	0.241	0.078	3.76	0.00
	ACPE14	75	6	2.023	0.333	0.509	0.341	4.00	0.04
	ACPE17	76	8	1.227	0.197	0.187	-0.065	3.24	0.14
	ACPE20	77	9	2.582	0.532	0.617	0.131	6.06	0.12

Appendix A. continued

Pop	Locus	N	N_A	A_E	H_O	H_E	F	A_R	PA_R
	ACPE22	76	6	2.405	0.526	0.588	0.099	3.71	0.09
	ACPE32	77	5	2.087	0.416	0.524	0.202	3.12	0.02
	ACPE51	76	4	2.320	0.776	0.573	-0.365	3.30	0.00
	ACPE52	76	7	2.431	0.461	0.593	0.218	3.98	0.18
	ACPE57	77	10	3.359	0.662	0.707	0.057	7.02	0.00
Head 2009	ACPE03	16	5	2.016	0.500	0.520	0.008	4.63	0.00
	ACPE06	16	7	1.615	0.313	0.393	0.179	6.36	0.01
	ACPE14	16	3	1.373	0.188	0.280	0.309	2.88	0.00
	ACPE17	16	6	1.395	0.250	0.292	0.117	5.38	0.44
	ACPE20	15	7	2.143	0.667	0.552	-0.250	6.80	0.47
	ACPE22	16	3	2.256	0.563	0.575	-0.011	2.99	0.05
	ACPE32	16	4	2.438	0.375	0.609	0.364	3.87	0.24
	ACPE51	14	5	1.876	0.571	0.484	-0.224	5.00	0.04
	ACPE52	15	4	2.273	0.600	0.579	-0.071	3.87	0.03
	ACPE57	16	8	3.391	0.563	0.728	0.202	7.81	0.00
Main 2009	ACPE03	92	7	2.171	0.446	0.542	0.174	4.22	0.12
	ACPE06	94	9	1.531	0.277	0.349	0.203	4.66	0.07
	ACPE14	94	8	1.776	0.383	0.439	0.124	4.13	0.42
	ACPE17	92	8	1.396	0.207	0.285	0.272	3.87	0.06
	ACPE20	92	10	2.389	0.446	0.585	0.234	5.27	0.02
	ACPE22	92	4	2.174	0.467	0.543	0.135	2.97	0.00
	ACPE32	94	5	1.778	0.383	0.440	0.125	2.68	0.01
	ACPE51	93	7	2.073	0.624	0.520	-0.205	4.03	0.07
	ACPE52	90	5	2.102	0.489	0.527	0.067	3.13	0.00
	ACPE57	93	11	3.573	0.720	0.724	0.000	7.11	0.18
Wall 2009	ACPE03	17	3	1.615	0.118	0.392	0.691	2.97	0.00
	ACPE06	17	5	1.661	0.176	0.410	0.557	4.64	0.05
	ACPE14	17	2	1.486	0.294	0.337	0.101	2.00	0.00
	ACPE17	16	6	1.395	0.313	0.292	-0.103	5.38	0.75
	ACPE20	17	6	2.021	0.412	0.520	0.185	5.44	0.27
	ACPE22	17	2	1.841	0.471	0.471	-0.030	2.00	0.00
	ACPE32	17	3	1.908	0.471	0.490	0.011	2.82	0.74
	ACPE51	17	2	1.895	0.647	0.487	-0.370	2.00	0.00
	ACPE52	17	3	2.232	0.176	0.569	0.680	2.97	0.06
	ACPE57	17	7	3.284	0.647	0.717	0.070	6.44	0.00
Deep 2009	ACPE03	97	15	2.382	0.536	0.583	0.076	6.28	0.86
	ACPE06	97	9	1.488	0.186	0.330	0.434	4.30	0.12
	ACPE14	94	6	1.624	0.287	0.386	0.253	3.49	0.02
	ACPE17	98	8	1.261	0.204	0.208	0.014	3.08	0.14
	ACPE20	98	9	2.065	0.357	0.518	0.308	5.14	0.02

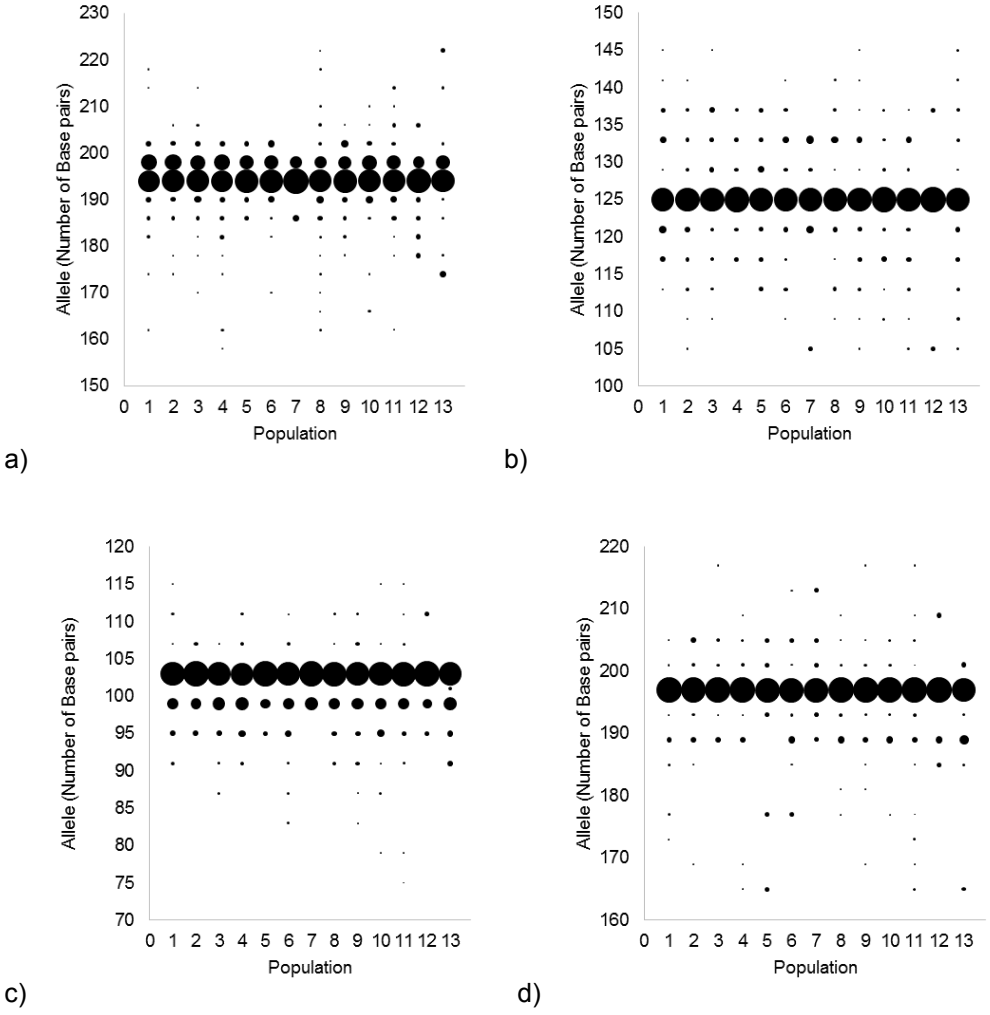
Appendix A. continued

Pop	Locus	N	N_A	A_E	H_O	H_E	F	A_R	PA_R
	ACPE22	96	6	2.095	0.521	0.526	0.004	3.08	0.06
	ACPE32	98	7	2.056	0.469	0.516	0.086	3.24	0.26
	ACPE51	97	6	2.325	0.649	0.573	-0.140	4.34	0.07
	ACPE52	95	7	2.587	0.463	0.617	0.245	4.54	0.23
	ACPE57	96	11	3.076	0.427	0.678	0.367	7.09	0.10
Main 2010	ACPE03	129	8	2.147	0.442	0.536	0.173	4.72	0.02
	ACPE06	130	11	1.451	0.238	0.312	0.233	4.48	0.16
	ACPE14	129	8	1.822	0.326	0.453	0.279	4.20	0.19
	ACPE17	128	9	1.215	0.172	0.177	0.027	3.10	0.30
	ACPE20	128	12	2.189	0.453	0.545	0.166	6.23	0.10
	ACPE22	130	8	2.258	0.515	0.559	0.075	3.64	0.27
	ACPE32	130	4	2.120	0.454	0.530	0.141	3.00	0.00
	ACPE51	128	8	2.391	0.602	0.584	-0.034	4.84	0.21
	ACPE52	128	8	2.382	0.438	0.582	0.246	3.92	0.14
	ACPE57	128	12	3.248	0.727	0.695	-0.050	7.17	0.11
Deep 2010	ACPE03	95	9	2.298	0.358	0.568	0.366	4.53	0.33
	ACPE06	95	8	1.305	0.179	0.235	0.234	3.74	0.06
	ACPE14	96	8	1.733	0.281	0.425	0.335	3.75	0.39
	ACPE17	96	6	1.239	0.167	0.194	0.137	2.92	0.00
	ACPE20	93	11	2.467	0.473	0.598	0.204	5.78	0.25
	ACPE22	94	7	2.316	0.553	0.571	0.026	3.69	0.29
	ACPE32	95	4	2.107	0.537	0.528	-0.022	2.66	0.00
	ACPE51	95	8	2.394	0.716	0.585	-0.229	4.32	0.19
	ACPE52	94	6	2.209	0.447	0.550	0.184	3.29	0.01
	ACPE57	94	9	3.141	0.511	0.685	0.251	6.29	0.00
Nwfld-2J/3K	ACPE03	146	11	2.351	0.452	0.577	0.213	5.22	0.33
	ACPE06	147	9	1.429	0.245	0.301	0.184	4.28	0.02
	ACPE14	146	9	1.621	0.253	0.384	0.338	3.54	0.27
	ACPE17	146	12	1.287	0.192	0.224	0.140	3.75	0.48
	ACPE20	145	12	2.136	0.421	0.534	0.209	5.35	0.10
	ACPE22	148	8	2.296	0.486	0.566	0.138	3.74	0.33
	ACPE32	147	7	1.847	0.388	0.460	0.155	2.81	0.15
	ACPE51	144	7	2.283	0.639	0.564	-0.137	4.36	0.04
	ACPE52	146	5	2.324	0.473	0.572	0.170	3.56	0.00
	ACPE57	147	12	3.451	0.673	0.713	0.052	6.74	0.10
Nwfld -2H	ACPE03	16	7	1.992	0.438	0.514	0.122	6.38	0.42
	ACPE06	17	3	1.127	0.118	0.116	-0.046	2.65	0.05
	ACPE14	17	4	1.441	0.235	0.316	0.232	3.65	0.14
	ACPE17	16	4	1.296	0.250	0.236	-0.094	3.74	0.71

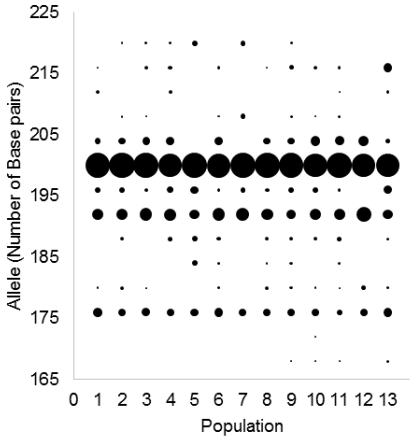
Appendix A. continued

Pop	Locus	N	N_A	A_E	H_O	H_E	F	A_R	PA_R
	ACPE20	17	5	2.592	0.353	0.633	0.425	4.80	0.08
	ACPE22	16	4	2.338	0.750	0.591	-0.311	3.86	0.03
	ACPE32	17	4	1.859	0.353	0.476	0.236	3.82	0.01
	ACPE51	16	5	2.599	0.625	0.635	-0.016	4.86	0.00
	ACPE52	17	3	2.102	0.353	0.540	0.327	3.00	0.00
	ACPE57	17	7	2.861	0.647	0.670	0.005	6.44	0.01
S. Shelf - 4W	ACPE03	48	9	2.247	0.542	0.561	0.024	4.84	0.87
	ACPE06	48	11	1.543	0.313	0.355	0.112	5.57	0.44
	ACPE14	48	5	2.011	0.229	0.508	0.544	4.08	0.52
	ACPE17	48	6	1.551	0.417	0.359	-0.173	3.83	0.05
	ACPE20	46	10	2.827	0.674	0.653	-0.043	6.70	0.36
	ACPE22	47	6	2.409	0.574	0.591	0.018	3.94	0.07
	ACPE32	48	5	2.240	0.646	0.559	-0.167	3.44	0.26
	ACPE51	48	5	2.108	0.688	0.531	-0.308	4.10	0.00
	ACPE52	48	7	2.556	0.479	0.615	0.213	4.67	0.16
	ACPE57	48	12	3.669	0.708	0.735	0.026	8.17	0.51

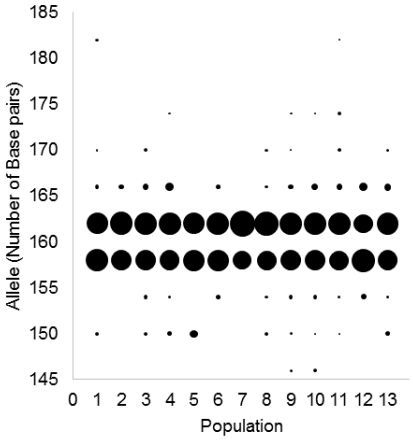
APPENDIX B



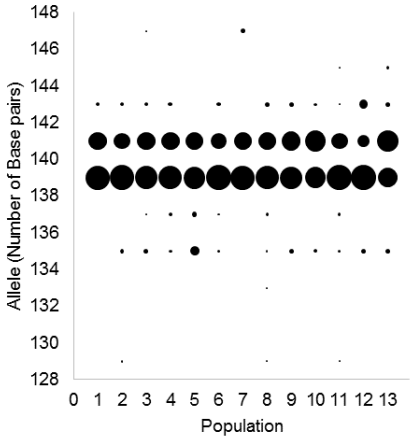
Appendix B.1. Bubble diagram showing the allele frequency distribution on 13 samples of *Acanthephyra pelagica* over the Atlantic coast of Canada. Locus a)Acpe03, b)Acpe06, c)Acpe14, d)Acpe17.



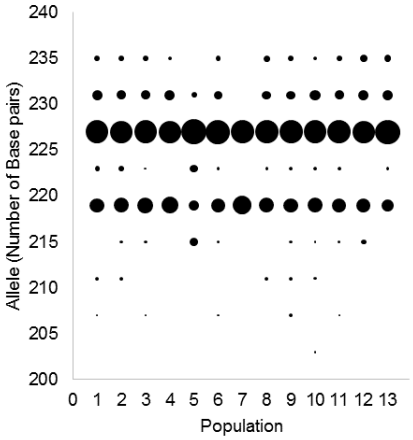
e)



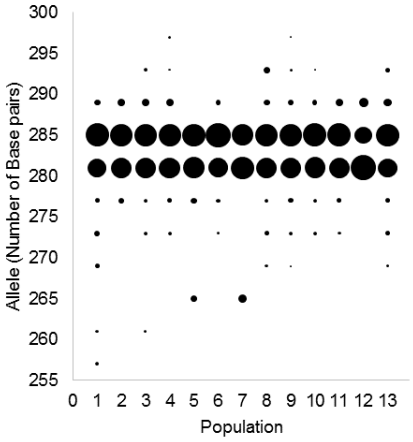
f)



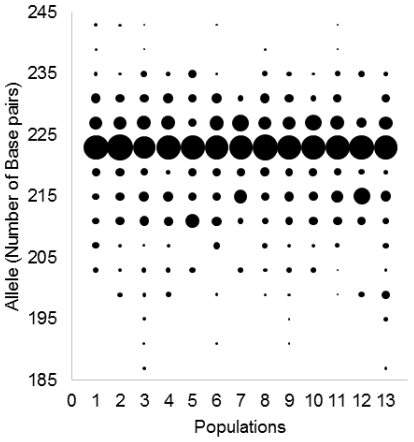
g)



h)



i)



j)

Appendix B.2. Bubble diagram showing the allele frequency distribution on 13 samples of *Acanthephyra pelagica* over the Atlantic coast of Canada. Locus e)Acpe20, f)Acpe22, g) Acpe32, h) Acpe51, i) Acpe52, j) Acpe57.

APPENDIX C

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