Genetic Analysis of Stranded and By-Caught Harp Seals (*Pagophilus groenlandicus*) in the Northwest Atlantic

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Abstract

We are currently witnessing significant climate changes in high latitude ecosystems including many areas of the Arctic. Many high latitude species, like harp seals (*Pagophilus groenlandicus*), will be affected and their populations forced to adapt to changing habitats. The overall fitness and genetic diversity of the population will affect how quickly a population can adapt. This study used microsatellite markers to determine and compare heterozygosity and fitness between stranded and by-caught harp seals along the east coast of the United States. Our study found there is no significant difference in fitness (d^2) between these two groups and that the overall fitness of the population is high. We found that for most markers, stranded seals had a higher mean d^2 and that only one marker showed significant differences between the two groups. Both groups had equal heterozygosities, supporting the idea that the seals come from the same population. These results demonstrate that the increasing strandings and entanglements are due to other factors than fitness or genetic diversity. Climate change is playing a large in role in the survival and adaptation of these species. When compared to ice cover data, there is a correlation between years with light ice cover and high strandings. Future studies should investigate this correlation to see if climate is the driving force behind increases in sightings of harp seals within the United States. Harp seals are a useful indicator species for the changing Arctic climate system and it will be important for managers to understand what they can do to conserve the species and resources of the Arctic ecosystem.

Introduction

We are currently witnessing significant and rapid changes in high latitude ecosystems. Climate variability is affecting sea ice dynamics in sub-Arctic regions of northern and eastern Canada (Johnston et al 2005). Some predictions indicate that circumpolar sea ice cover in the

Arctic may decline as much as 20% by 2050 (e.g. Vinnikov, et al. 1999) with ice-free summers starting as early as 2037 (Wang and Overland 2009). Rapid changes in temperature and ice conditions in high-latitude marine ecosystems pose significant challenges for marine mammals that use sea ice as a platform for breeding and social activity. These species have evolved complex life history strategies that exploit the resources available in these systems (Moore and Huntington 2008, Johnston et al. 2005). In particular, pagophillic, or ice-loving, seals like harp seals (*Pagophilus groenlandicus*), use seasonal sea ice off eastern Canada to give birth to and nurse their pups. Recent research indicates that climate variability has significant effects on the breeding habitats of these seals (e.g. Friedlaender et al. *2010,* Johnston et al. *In Prep*). The availability of habitat can have effects on survivorship of all age classes and reproductive success (Johnston et al. 2005, Lavigne 1988). Changes in ice cover will dictate where species are able to breed and may force breeding to occur in less ideal areas potentially exposing individuals to other threats such as disease and increases in human interactions.

Some species of seals are at risk from climate variability combined with unsustainable anthropogenic mortality (Leaper et al. 2010). Currently over 300,000 harp seals, 10,000 hooded seals, and seals from 4 other species are taken yearly in the Canadian seal fishery (Hammill and Stenson 2007). There has been disagreement regarding the sustainability of some seal hunts (e.g. Johnston et al. 2000) and more sustainable approaches to determining how many seals could be taken a year have been suggested (Leaper et al. 2010, Hammill and Stenson 2010). At present there are no studies assessing the potential effects anthropogenic removals have on the structure of seal populations, or if selection is occurring during these removals, driving genetic change. With the potential combined impacts of climate and

anthropogenic pressure, it will be important to have better control over at least one of these risks to these seals.

Large scale climate shifts and climate variability can cause significant challenges for both population and individuals. Evolutionary adaptation will be important for high latitude species impacted by high levels of climate variability. The fitness of the individual and the overall population will play a role in the adaptability of a species (Frankham 2002). Fitness is tied to genetic variation and a population under pressure from both natural and anthropogenic factors may experience a decrease in genetic variability (Kretzmann et al. 2006, Coltman 1998). Decreased fitness from loss of genetic variability can have not only reproductive effects, but can impact the ability of young to survive when left on their own (Coltman 1998). An individual with decreased fitness may be more likely to strand due to stresses like a higher susceptibility to disease. Data collected from stranding networks along the east coast of the United States have shown the since 1991, over 3,000 harp seals have stranded along the east coast from Maine to North Carolina (Figure 1). By March of 2011, five harp seals had been seen on the coasts of North Carolina and Virginia, more than ever recorded in any previous year, and the entire east coast is seeing an influx in the number of harp seal sightings (Canfield 2011). A decrease in the amount and duration of sea ice in harp seal breeding regions may cause not only juveniles but also adults to have an increased chance of stranding due to being forced into the water sooner after weaning or moulting. Researchers are unsure what is causing this to occur but decreased fitness in combination with climate variability may be contributing factors (Canfield 2011).

Harp seals (*Pagophilus groenlandicus*) are one of the most abundant pinniped species and are distributed across much of the North Atlantic (Lavigne 1988, Riedman 1990). They

breed in two main regions: the Northwest Atlantic off the eastern coast of Canada and the Northeast Atlantic on the West Ice and the White Sea (Lavigne 1988). Within the Northwest Atlantic population there are two main whelping patches, the Front off Newfoundland and Labrador and the Gulf in the Gulf of St. Lawrence (Lavigne 1988, Riedman 1990, Kretzmann 2006). Harp seals complete an annual migration from winter whelping grounds to summer feeding grounds with most individuals moving southward or between populations (Perry et al. 2000). Pups are born from late February until March in the Northwest Atlantic with the total population estimated at around 6 million (Lavigne 1988, Hammill and Stenson 2007). The pups are normally weaned within two weeks of birth and must stay on the ice until they molt their white coat and are able to survive the cold Arctic waters (Lavigne 1988). If a pup is forced into the water before it has fully molted it may not be strong enough to haul back out or hunt for itself. The timing of migration and pupping is strongly tied to ice extent across the entire range (Lavigne 1988). Variability in ice extent and temporal variation in ice cover has lead to unquantified risks for harp seals including increased juvenile mortality and changes in food availability (Johnston et al. 2005).

At present, we know little about the molecular ecology of harp seals in the Northwest Atlantic (Davis et al 2008). Few studies have examined the population genetics of harp seals (e.g. Perry et al. 1996), and little is known about their genetic diversity relative to historical times or across their full range. Sequence data from the cytochrome *b* gene supports the genetic differences seen between Canadian populations and the northeastern Atlantic breeding populations (Perry et al. 2000) and harp seals are currently managed as 2 populations – the Eastern North Atlantic and the Western North Atlantic. Harp seals use distinct and predictable

breeding locations on seasonal sea ice within these two regions – Gulf of St. Lawrence, Front, West Ice, and White Sea. Within the Northwest Atlantic, studies suggest that these whelping patches are not genetically distinct enough to be considered two separate populations, discrepancies between estimates of effective population size and abundance estimates indicate that high removals may have dramatic effects on this population (Perry et al. 2000, Meisfjord et al. 1996). There is significant variability in how climate change is affecting these distinct habitats and subsequently the reproductive success of seals, and further molecular work is required at these fine scales to resolve how this may influence the species (Hammill 2010, Leaper et al. 2009).

Previous molecular studies on harp seals have used mitochondrial markers to look at population structure (Perry et al. 2000, Meisfjord et al. 1996). Finer scale genetic differences may be revealed when populations are assessed using a highly variable nuclear markers like microsatellites (Kretzmann 2006). Microsatellites are simple, tandem repeats that can be polymorphic making them useful for studying population structure and gene flow (Coltman 1996). As a marker, microsatellites can have high variability which makes them robust to tests for heterozygosity (Frankham 2002). Microsatellites as neutral markers can also be used to estimate fitness and population structure based strictly on genetics. Studies have shown that microsatellites can be linked to genes under selection which could impact the fitness of different populations. They have been isolated in many phocid species, like harbor seals and grey seals, and are found to be species specific. Coltman (1996) and Gemmell (1997) have shown that many phocid microsatellite markers have broad utility within phocid seals and species specific markers can amplify polymorphic loci in other pinnipeds. Kretzmann (2006)

used microsatellite markers to examine differences in fitness between surviving and non-surviving stranded juvenile harp seals. Their study determined that microsatellites can be used to find effective measures of fitness in species with high genetic variation and large population sizes.

The goal of this study was to use microsatellite markers to examine genetic diversity, as a proxy for fitness, in two "populations" of harp seals: stranded and by-caught. We used F_{st} and d^2 to determine heterozygosity and fitness within the two populations. We hypothesize that by-caught seals are "healthy" and would have higher fitness when compared to a stranded individuals which would have a lower fitness. This would be explained by stranded seals having decreased genetic diversity in relation to that of by-caught seals.

Methods

Sample Collection

Samples were collected from 146 harp seals that were either found stranded or entangled along the east coast of the United States mainly from Maryland, Maine, and Massachusetts. The samples were recovered by the Maryland Department of Natural Resources, the International Fund for Animal Welfare, and the Northeast Fisheries Science Center between 2001 and 2010. Fifty-three of the samples were from by-caught animals while the remaining ninety-four were stranded individuals. Within the stranded samples, most were found alive or moderately decomposed. All samples received were skin and stored at -20°C in 95% ethanol until analyzed.

DNA Extraction and PCR

DNA was extracted from tissue samples by digestion overnight in a 10% Chelex solution with 0.24 mg of Proteinase K at 60°C. The Proteinase K was denatured for 15 minutes at 100°C. We used 10 microsatellite primer pairs isolated in several species of pinnipeds and found to amplify polymorphic loci in harp seals. The primers were from leopard seals (*Hydruga lepotonyx),* Hl 8 and Hl 15 (Davis et al. 2002), harbor seals (*Phoca vitulina concolour)*, Pvc 9, Pvc 16, and Pvc 19 (Gemmell et al 1997, Coltman et al. 1996), and grey seals (*Halichoerus grypus*), Hg 3.7, Hg 4.2, Hg 6.1, Hg 8.10, and Hg 8.9 (Gemmell et al. 1997). Each forward primer was appended with a T3 tag (ATTAACCCTCACTAAAGGGA).

Polymerase chain reactions (PCR) were carried out in 20 µL reaction volumes. The reaction mix was as follows: 1.2 µL DNA, 1x PCR buffer (20 mM Tris pH 8.8, 50 mM KCl, 0.1% Triton X-100, 0.2 mg/mL BSA NEB purified), 2mM $MgCl_2$, 0.2 mM dNTPs, 0.1 mM forward primer, 0.4 mM reverse primer, 0.4 mM T3 tag labeled with FAM, NED, PET, or VIC fluorescent dye, Taq DNA Polymerase. The PCR profile included an initial denaturation at 94°C for 4 min, followed by 33 cycles of 94°C for 15s, 53°C for 15s, and 72°C for 30s, followed by a final extension at 72°C for 5 min.

Sequencing and Genotyping

PCR products were diluted with 40 µL of autoclaved-nanopure water. For sequencing, 4 µL of diluted PCR product (1 µL per T3 fluorescent tag product: FAM, NED, PET, and VIC) was added to a 9 µL mix including LIZ 500 (MC Labs), 0.0005 mg of sssDNA, and nanopure water and denatured at 94°C for 10 min before being placed on Applied Biosystems ABI 3730x1 automated sequencer.

Genotyping was carried out using GeneMarker software package (SoftGenetics). All runs for a marker were complied together and an allele panel was created in order to score each individual. The individual genotypes were visually checked to avoid any computer errors. Any non-existent, weak (reading below 100), and unreadable genotypes were thrown out and not included in the analysis. After confirmation all genotypes were exported to be used for further analysis.

Analysis

Microsatellite loci were tested for deviations from Hardy-Weinberg equilibrium (HW), for linkage disequilibrium between pairs of loci and for allele frequency differences using GenePop (Web Version 4.0, Raymond and Rousset 1995). For populations that were out of HW for a marker, MicroChecker was used to test for null alleles (Van Oosterhout et al. 2004). A test for population difference, F_{st}, was calculated by running an AMOVA using Arlequin 3.11 (Excoffier et al. 2005). Outlier markers that could be linked to genes under selection were identified using Lositan Selection Workbench (Beaumont and Nicholas 1996, Antao 2008). Lositan evaluates the relationship between F_{st} and heterozygosity and identifies deviations from neutral expectations. The model was run with 10,000 simulations, assuming a stepwise mutation model and a forced 'neutral' mean F_{st}. Structure 2.2 was used to see if there was any evidence of population structure regardless of sampling location (Falush et al. 2003). This program implements a Bayesian clustering approach to estimate population structure without requiring priori designations of population membership. One independent run for K=1-3 were performed using the correlated allele frequencies and admixture models with 300,000

repetitions and a burin of 50,000. The estimated LN probability for the data was compared to determine which K was the best to represent the data.

Allele frequencies were determined using the microsatellite toolkit in Microsoft Excel. The estimate for fitness, mean d^2, was manually calculated in Micrsoft Excel using the equation from Coulson et al. (1998). Mean d^2 is the squared difference in repeat units between two alleles at a locus average over many loci (Coulson et al. 1998). It can provide a better measure of recent inbreeding and population mixing than strict heterozygosity (Coltman et al. 1998). An individual with a low mean d^2 is more likely to be the progeny of related individuals and is more likely to experience inbreeding depression, and an individual with a high mean d^2 may be the progeny of more distantly related individuals (Coltman et al. 1998). The mean d^2 was calculated for each population. An f-test was performed using Microsoft Excel to determine whether populations had equal variances. The t-test for differences in mean d^2 between stranded and by-caught was performed using Microsoft Excel. For the stranded population, we calculated mean d^2 for males and females and performed a t-test to compare the means. We also calculated d^2 of individual loci but no single locus showed significant differences between populations.

Ice Analysis

We assessed the number of harp seal strandings and percent ice cover (February) in the Gulf of St Lawrence to provide an environmental context for stranding rates along the east coast of the United States during 1993 to 2010. The stranding data were compiled from the complete Level A data sets collected for strandings in this region during the study period. Estimates of ice cover were derived from NASA Nimbus-7 Scanning Multi-channel Microwave

Radiometer (SSMR) data (1979-1987) and Defence Meteorological Satellite Program (DMSP) Special Sensor Microwave/Imager (SSM/I) data for the Gulf of St Lawrence as in Friedlander et al. (2010). Ice cover and stranding number anomalies were calculated in relation to the mean of the time series and a linear regression was performed to assess the effects of ice cover on stranding numbers. All statistical analyses of ice and stranding data were conducted using JMP software.

Results

A total of 146 harp seals (94 stranded and 52 by-caught) were genotyped at up to 10 microsatellite loci. Locus Pvc 9 was the least variable with 11 alleles detected in 108 individuals, while locus Hl 15 had the highest variability with 23 alleles detected in 112 individuals. Many alleles were found only in one individual across the range from largest to smallest allele sizes. More rare alleles were found in stranded seals which would be expected because of the larger sample size. For all loci, common alleles were consistent across populations. Allele frequency differences between stranded and by-caught were significant overall ($X^2 = 31.995$, df = 20, P-Value = 0.043). Only one locus (Hg 8.10) showed high significance between population allele frequencies (P-value= 0.003). Within that locus, allele 205 was the most common in by-caught seals, and also high in stranded seals, however they had more alleles total (14 vs. 12) and a broader range of common alleles. When Hg 8.10 was excluded from calculations for allele frequency differences then the overall difference became non-significant ($X^2 = 20.8811$, df = 18, P-Value = 0.29). This shows that the overall significance for frequency difference is due to one allele (Hg 8.10) that when excluded leads to a non-significant outcome.

Tests for linkage disequilibrium found only one pair of loci that were significantly linked (Hl8 & Pvc 19, P=0.002) with all other loci showing non-significant results. Allele frequencies at all 10 loci did not deviate from HW equilibrium (P=0.05-0.98, Table 1), but three loci showed deviations in one population. Two individual loci (Hg 8.9 and Hl8) deviated from HW equilibrium for the by-caught population (P=0.01 and 0.003) and one locus (Pvc 16) deviated for the stranded population (P=0.01). All markers that deviated from HW equilibrium showed evidence for the presence of null alleles. For both linkage disequilibrium and HW equilibrium, multiple tests were run and with a certain number of tests it would be expected that (approx. 5%) of tests to be significant by chance.

Heterozygosity at these 10 markers was high in most individuals and the mean did not differ between the two populations (Table 1). The three loci where one population was out of HW equilibrium (Hg 8.9, Hl 8, and Pvc 16) had the lowest heterozygosities (0.68, 0.72, and 0.66 respectively). This indicates that they were most probably out of HW equilibrium because of a heterozygote deficiency. The heterozygosity estimates for the other loci ranged from 0.76 for Pvc 16 to 0.97 for Pvc 19(Table 1).

The test for population differentiation, F_{ST}, showed non-significant results (F_{st}= 0.0018, p>0.05). The Structure analysis showed no evidence for population structure. These results are consistent with previous studies that found no evidence for distinct genetic populations within the Northwestern Atlantic stock of harp seals (Perry et al. 2000, Meisfjord et al. 1996). Even though microsatellites are considered neutral markers, there is evidence that loci may be linked to genes under selection. Lositan Selection Workbench was used to look for outliers. No markers showed evidence of being an outlier that could be linked to a gene under selection.

Nine of the 10 loci had a higher d^2 estimates in stranded seals with 2 loci being substantially higher (difference of at least 1.5x). For the remaining locus (Hg 8.10), d^2 was the same for both populations. Mean d^2 values ranged from 3.64 to 20 with only one loci (Hg 8.9) showing significant difference between populations (Table 2). The locus, Hg 8.9, has one population out of HW equilibrium which could explain the significance seen. Overall, the mean d^2 for stranded seals was 67.6 and 60.9 for by-caught seals (Figure 2). The difference between population means was not significant (t=-0.777, df=144, 1-tailed P=0.219). Within the stranded population, there was no significant difference found between the mean d^2 values of males and females (t=-0.241, df=75, 1-tailed P=0.405). The same comparison was not done for by-caught animals because sex information was not available.

Discussion

With climates changing, impacts on high latitude ecosystems will begin to increase and species in these systems will have to adapt in order to survive. Our study looked at the differences in overall fitness between stranded and by-caught harp seals. We found that there was no significant difference between the two populations. Stranded seals overall had a higher mean d^2, both as a population and by marker, which was not what we predicted based on the assumption that stranded seals had compromised health due to lower genetic fitness. Within our stranded samples, around 50% were found alive and many were yearlings. Our results are consistent with previous studies using microsatellite makers that looked at stranded seals, both surviving and non-surviving, and found no difference between mean d^2 values (Kretzmann et al. 2006). Stranded and by-caught seals seem to have similar diversity within microsatellite markers which could indicate that seals that strand may be more susceptible to disease due to

differences in other genes such as the major histocompatibility complex (MHC). Variation within the MHC has been associated with disease resistance in other pinnipeds species which could explain why fitness may not show differences between stranded and by-caught seals as hypothesized (Kretzmann et al. 2006, Cammen et al. 2011). Future work is required to assess the extent how MHC genes vary amongst stranded and bycaught seals.

One marker (Hg 8.10) showed a significant difference in allele frequencies between the two populations. This same marker also had equal mean d^2 values for both stranded and by-caught seals. It would be expected that differences in allele frequencies would lead to differences in mean d^2 values. Hg 8.10 was highly heterozygous in both populations which could mean that the alleles represented in each population differed which caused the allele frequency difference but would explain the similarity seen with the other statistics. There were some differences in the distribution of individuals across the alleles and the stranded population had more alleles represented by fewer individuals. Sample size may also play a role as to why frequency differences were seen because the stranded population may have a more representative snapshot of the alleles for this locus. The marker (Hg 8.9) that did show a significant difference in mean d^2 was most likely due to one population being out of HW equilibrium. The populations being out of HW equilibrium probably led to the population-marker combinations having the low heterozygosity values and that the sample sizes were not large enough to account for all alleles in the population.

Our data showed that all of the seals essentially came from the same population. The lack of significant difference between populations would lead to the assumption that as a population, harp seals had high genetic diversity and did not suffer from a bottleneck during

the 1940s through the early 1970s when population numbers fell drastically (Hammill et al. 2007, Leaper et al. 2010). As a population that is subjected to both natural and anthropogenic threats, it is important to know that from a genetic diversity standpoint the population should be able to adapt. High diversity and fitness levels are essential if the population is subjected to a pathogen that could wipe out less fit population (Frankham 2002). Overall our data shows that something other than decreased diversity and low fitness are impacting these seals and causing them to strand.

The seals that are stranding do not seem to be doing so because of low fitness and subsequent health problems. Instead, there may be environmental factors that are driving the variations in strandings on the east coast of the U.S.. Large-scale climate changes are leading to major fluctuations in seasonal sea ice cover off the eastern coast of Canada. There is a positive correlation between NAO phase and ice cover in eastern Canada (Johnston et al. 2005, Friedlaender et al 2010 and in light ice years, there have been drastic decreases in ice cover during the first part of March when harp seals are pupping (Friedlander et al. 2010, Johnston et al. 2005). From 1991 to 1995, ice cover was heavy with a switch to lighter ice years happening in 1996. In 2001, the decrease in ice cover was the most drastic with large areas of eastern Canada having up to a 60% decrease in ice cover (Johnston et al. 2005). Since 1962 thru 2002, the total ice cover for the month of March has been significantly lower when compared to February (Johnston et al. 2005). This has major implications for harp seals that require seasonal ice to pup and molt during the first weeks of March. If there is a decrease in the ice available, seals will be forced to use ice in less ideal areas. Also, if ice is melting sooner in the year then pups could be forced into the water sooner and there could be an increase in juvenile mortality.

Along the east coast of the United States there have been fluctuations in the number of harp seals stranding each year from 1991-2010 (Figure 3). With changes in ice cover it would seem likely that the number of strandings will continue to be high as more seals will be seen further south but there is an interesting relationship occurring between ice cover and the number of strandings in the last years of our dataset (Figure 4). Also if there are more seals being forced to new areas they are going to be increases in interactions between humans and seals both on land and in the water. With increases in stranded seals it will be important for mangers to understand what could be a likely cause and how it can be dealt with.

An explanation for the patterns seen in both populations of seals could be that years with light ice cover are the same as the years when higher numbers of stranded seals are seen. As mentioned previously, 2001 was a very light ice year with early melt. That year coincides with the highest number harp seal strandings. The mid-1990s were heavy ice years and the number of stranded seals follows that same pattern until 1996 when the number of strandings began to increase. For the both the Gulf of St. Lawrence and the Front, 2004 was also a light ice year (Friedlander et al. 2010). In that same year, there were a high number of both by-caught and stranded seals (Figure 3). A similar pattern was seen in 2006 and the reverse was seen in 2003 when ice cover was the highest it had been since 1996 when the shift seemed to have occurred (Friedlander et al. 2010, Johnston et al. 2005). Overall there seems to be a correlation between ice cover and the number of stranded seals seen along the east coast of the United States (Figure 4). At the end of the time period, however, the number of stranded seals seems to drop possibly because fewer seals are found on the traditional breeding grounds, or because the population is in decline (Figure 4). The linear regression shows a strong and significant

negative correlation ($R=0.49$, $p < 0.05$) between percent ice cover and strandings (Figure 5), confirming that years of low ice cover tend to have high stranding rates.

Other species in the Arctic that rely on seasonal sea ice cover are also being impacted (Simmonds et al. 2007, Moore et al. 2008). Some species will be more resilient to changes in ice cover and it will important to know how different species will react (Moore et al. 2008). A species with a similar life history to that of harp seals, hooded seals, exhibits similar stranding patterns (Figure 6). Hooded seals have a smaller population along the east coast of Canada but seem to have similar relationships with ice cover in their whelping areas. Further research is required to assess correlations between strandings and ice cover for pagophillic species like harp and hooded seals as the world warms.

Another issue is whether the demographics of stranded individuals will play a role in the structure of the population. Our stranded seal samples had a sex ratio of 2:1 males to females. The sex ratio may indicate that females are less likely to stray south from breeding areas, especially when ice is low. This could be due to the demands of higher maternal investment and less risky behavior. At present, the Western North Atlantic harp seal population is robust, with little risk from a skewed sex ratio stemming from predominantly male strandings. Since mating occurs in the water, it is hard to determine which males are actually successful in passing along genes (Lavigne 1988) and if fewer males are able to compete for mating opportunities it could potentially lead to a decrease in the fitness of pups because of the low influx of male genes. Also, looking at the distribution of strandings, males seem to travel further. This could be due to relying on ice and temperature as cues for migration and early melting ice could cause them to start migrations sooner and travel further to find food sources.

Another factor that could have major population implications is that most seals that strand are yearlings. Decreased ice cover and early thawing could be a major cause of this because pups are forced into the water sooner and may not be able to fully fend for themselves. Yearlings are also the main target of the seal fishery in Canada ("Overview", Hammill et al. 2007, and Leaper et al. 2001). With higher levels of anthropogenic removal, increased mortality due to early thaws, and increased stranding, there is chance that large numbers of year classes could be removed from a population before reaching breeding age (Hammill et al. 2007, Friedlander et al. 2010). This could have major implications on the growth of the population and the amount of stress the population can stand. Yearlings are still the dominate age class being seen stranding along the coast, but there could be more seals from all age classes being seen. In the coming years, it will become necessary for managers to look at the demographics of stranded seals and see if certain groups are being more impacted and what implications that will have on the population.

Managers will need to begin to create conservation plans that rely on climate change as a major impact on marine species (Simmonds et al. 2007). With the increasing rate of climate change it will be necessary for researchers to understand how different species are adapting and which species will need stricter management. It will be important for future studies to confirm the correlation between ice cover and the number of strandings and to further assess how large scale climate phenomena, like the NAO, are affecting pagophilic seals in the North Atlantic. The available evidence suggests that Arctic ice cover will continue to decrease and it will be crucial to know how those changes will effect the species that rely on the seasonal ice cover for some part of their life history. Abundant high-latitude species, like harp seals, will be

indicators of how the system is changing and what managers can do to conserve the species and resources of the Arctic system.

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Appendix

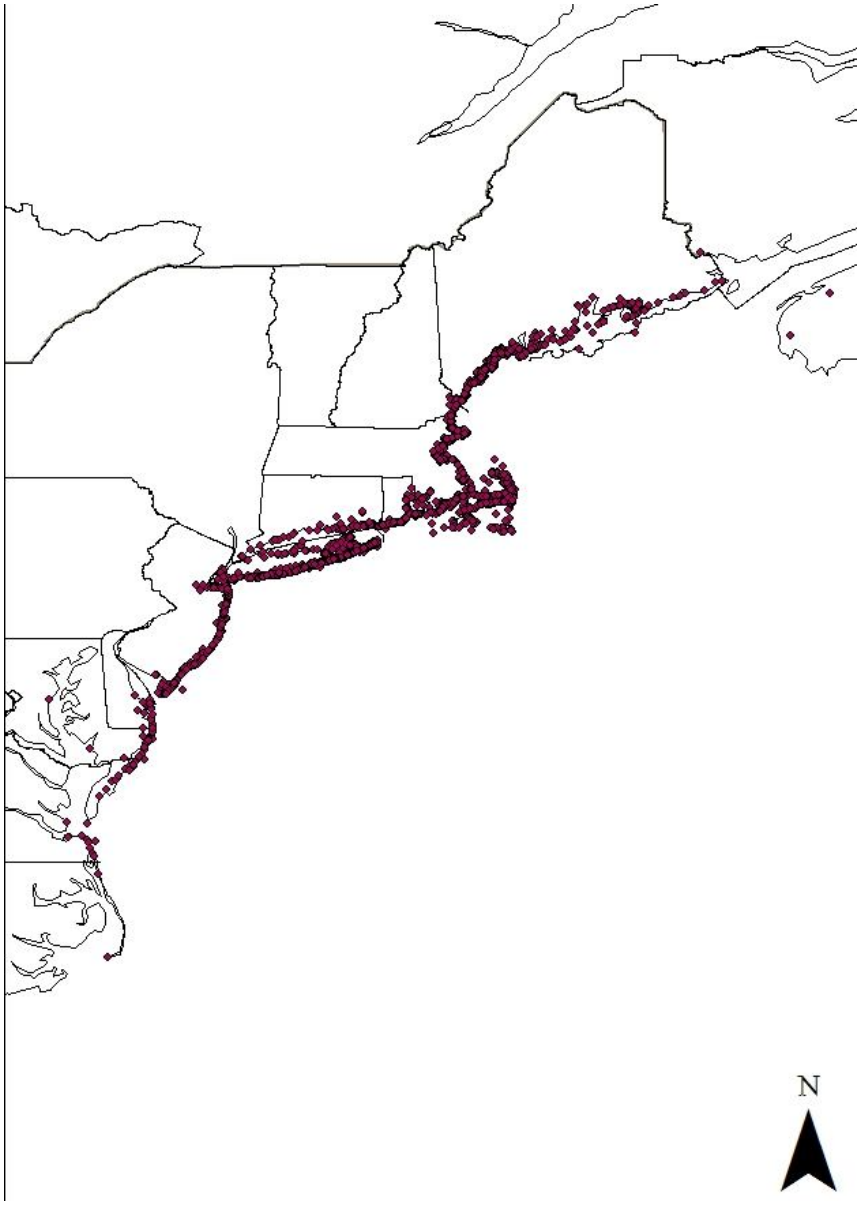

Figure 1. Harp seal strandings along the east coast of the United States: 1991-2010

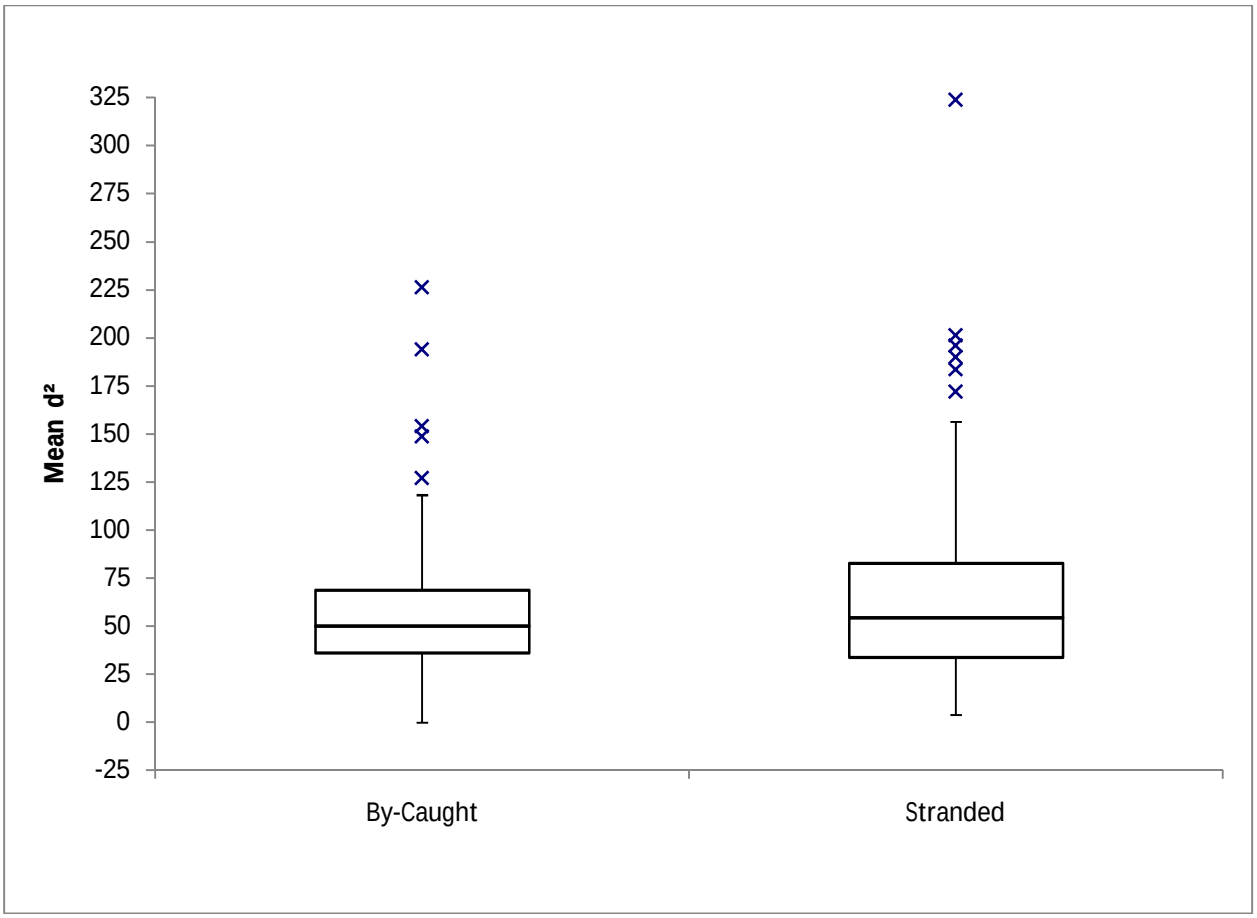

Figure 2. Box and whisker plot showing mean d² by population. X show outliers that fall above 1.5x the top boundary.

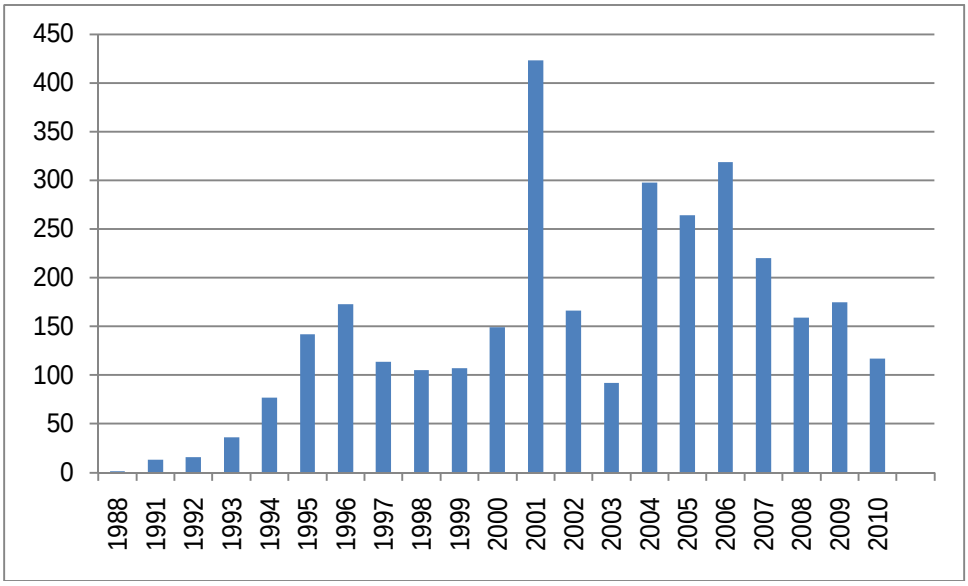

Figure 3. Harp seal strandings along the east coast of the United States- 1991-2010

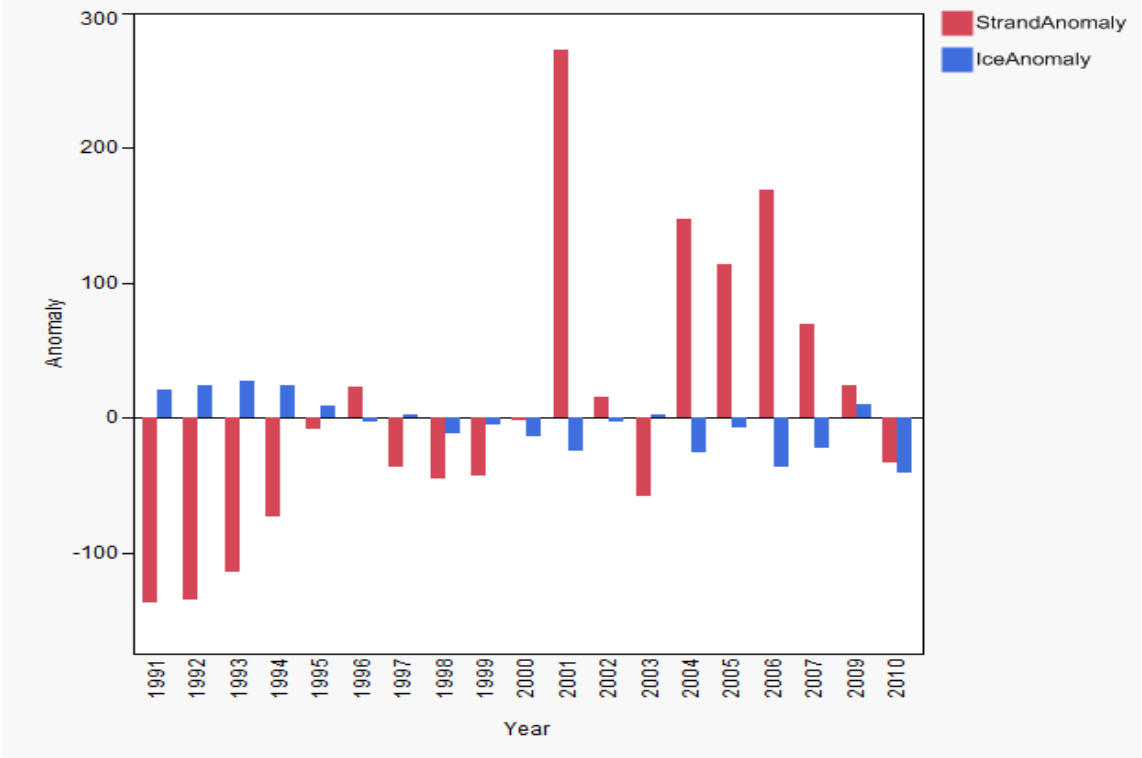

Figure 4. Time series of stranded seal anomaly and ice anomaly from 1991-2010.

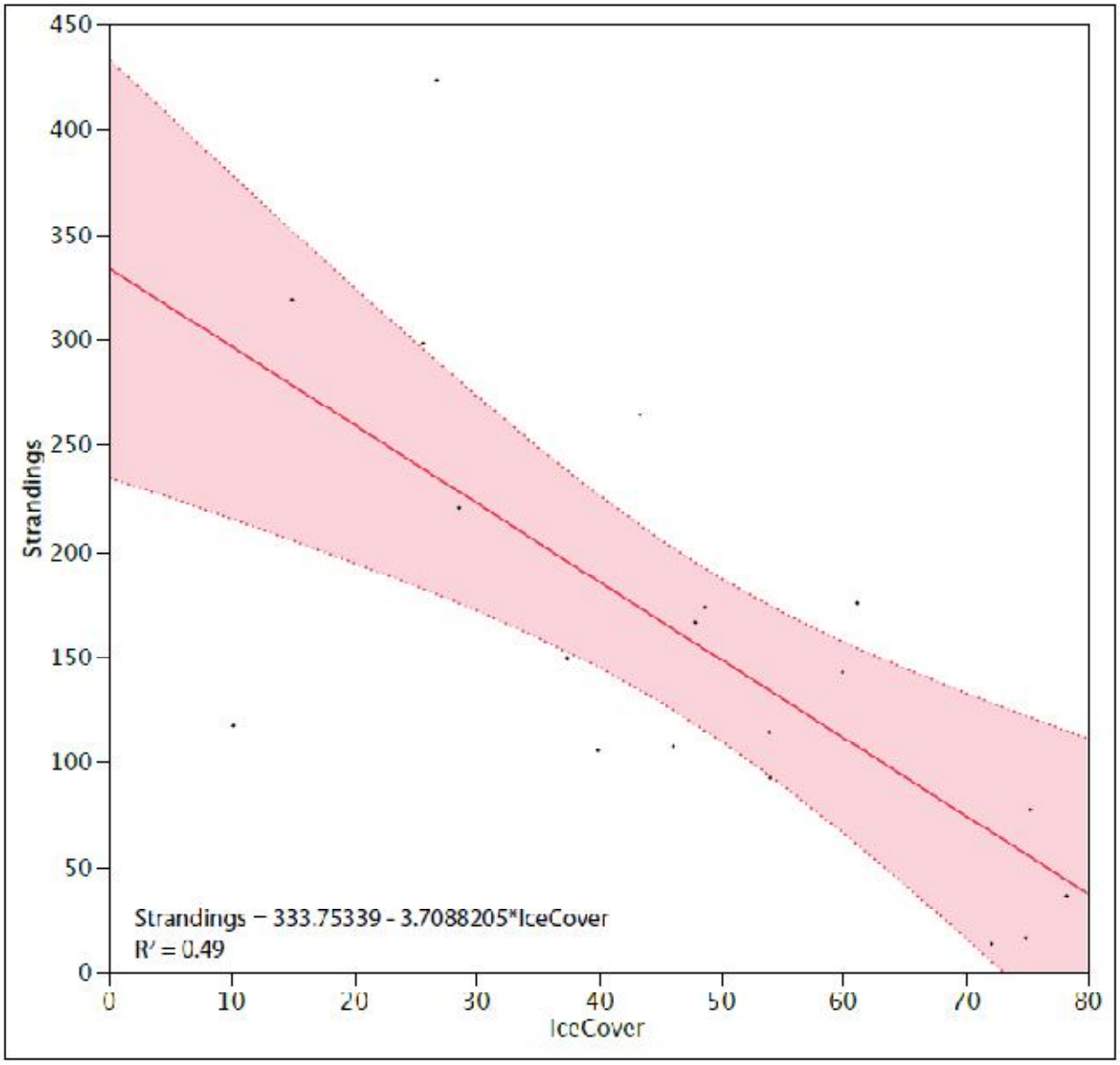

Figure 5. Linear regression of Percent Ice Cover vs. Strandings. Shaded area shows 95% confidence intervals.

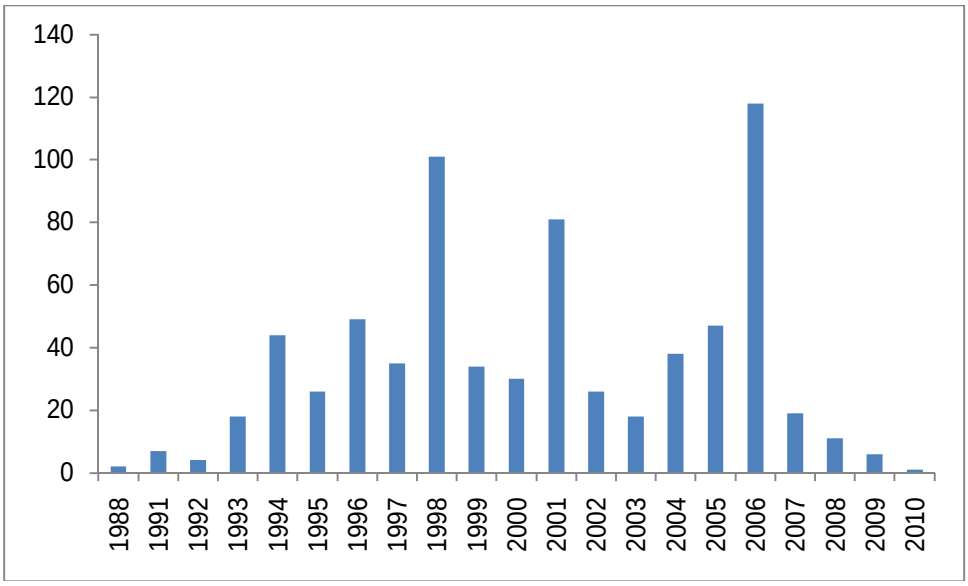

Figure 6. Hooded seal strandings along the eastern coast of the United States – 1991-2010

Table 1. Observed Heterozygosity by Locus and P-value for HW Equilibrium tests. Bolded values represent populations out of HW equilibrium.

	Locus																				
	Hg 3.7		Hg 8.9		Hg 8.10		Pvc 16		Pvc 9		HI 8		Hg 6.1		Hg 4.2		Pvc 19		HI15		Mean H_O
# of alleles	15		21		16		13		11		13		18		14		15		23		
	H_O	P	H_O	P	H_O	P	H_O	P	H_O	P	H_O	P	H_O	P	H_O	P	H_O	P	H_O	P	
Stranded	0.792	0.05	0.783	0.25	0.933	0.89	**0.667**	0.01	0.8	0.43	0.829	0.21	0.864	0.06	0.909	0.66	0.971	0.67	0.887	0.55	0.843
By-caught	0.821	0.29	**0.686**	0.01	0.818	0.71	0.762	0.91	0.82	0.98	**0.722**	0.003	0.848	0.21	0.929	0.53	0.879	0.23	0.927	0.24	0.821

Table 2. Mean d^2 by locus for the two populations. P-values for t-test to compare mean d^2 between populations. Bolded values are significant.

	Mean d^2		
Loci	**Stranded**	**By-caught**	**P-Value**
Hg 3.7	6.01	3.75	0.19
Hg 8.9	9.9	8.94	**0.004**
Hg 8.10	6.27	6.33	0.29
Pvc 16	7.31	3.99	0.32
Pvc 9	4.06	3.64	0.2
Hl 8	7.6	5.6	0.14
Hg 6.1	6.84	5.63	0.24
Hg 4.2	10.46	8.29	0.4
Pvc 19	8.29	6.56	0.45
Hl15	20	14	0.24