**PBL Module 1 – Population Structure, Genetic Variation, and Conservation**

**Report for the Inuit population near Lake Illiyardjuk**

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**Introduction**

Despite 20 marine walruses being introduced to lake Illiyardjuk in 1979, the surrounding population of walruses still hovers around 300. Illiyardjuk walruses (*Odobenus rosmarus fictiousi*) have been shown to have low genetic variability when compared to marine walruses as reported from previous studies. One recent study using mtDNA showed strong differentiation of breeding populations along the five breeding basins/regions of Lake Illiyardjuk: Northern, Kinapek, Hawani, Perpaluktok, and Southern. This report from my company analyzed a late graduate students work who was able to genotype 172 walruses before her passing. The walruses at Lake Illiyardjuk have been isolated for hundreds of generations, and it is apparent that the gene pool is determined by its population size and structure. We can use indirect genetic data to help discern distinct populations of the lake walrus subspecies and infer population connectivity in addition to sex-based dispersal. My company will make recommendations on how to help promote gene flow and assess the walrus populations that may exist at lake Illiyardjuk so that the local Inuits may continue to enjoy a nearby walrus community.

**Methods**

Genetic differentiation (pairwise Fst) between regional walrus samples based on mtDNA variation was given to us to use.

The genetic differentiation using microsatellite data also between the regional walrus samples was compiled using the late Graduate students data formatted using Genalex software, Gen AIEx 6.503.xlam in conjunction with Excel 2016 (Table1). Additional information of the preparation of the samples can be found on the Github link in the supplemental information. The Genalex software and late students data was also used to calculate frequencies of observed heterozygosity (Ho), expected heterozygosity (He), mean observed heterozygosity, fixation index (F), inbreeding coefficient of subpopulations (Fis), and inbreeding coefficient of an individual relative to the total population (Fit), and Nm which is the local population size (N) and average rate of immigration (m). In addition, Genalex generated Nei’s genetic distance and identities. The Genalex data and excel files is provided in the Github link in supplemental information. The regions of walruses and number of individuals (N) is as follows: Northern (N=15), Kinapak (N=20), Hawani (N=79), Perpaluktok (N=43), Southern (N=15). From each decade the number of individuals (N) sampled is as follows: 1980s (N=59), 1990s (N=48), 2000s (N=65).

The software package Structure 2.3.4. was used to help predict the number of populations (K) of walruses at Lake Illiyardjuk. The data inputted into structure was the unassigned population data from the late graduate students work. The information for the project run was 172 individuals, 17 loci, and a “-“ was placed for missing data. The run length Burnin was set to 100, and the number of MCMC reps was set to 50,000. For the job in structure, the K value was set to 6 and the number of iterations was also set to 6. The population Bar Plot and Neighbor Joining tree were taken from Parameter1\_run\_20 (K=4)

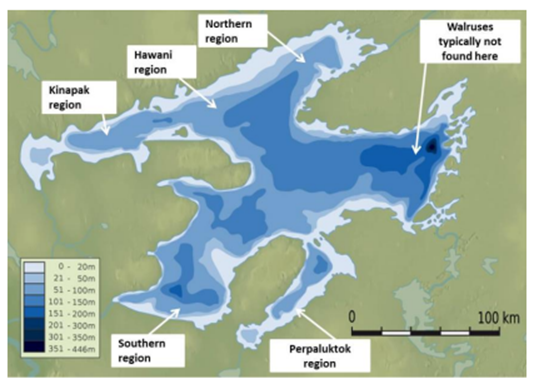
To determine the number of population of walruses, the data from Structure was then compressed into a zip file and fed to the online program Structure Harvester. The graphs produced were analyzed to determine the number of population of walruses surrounding Lake Illiyardjuk.

To examine sex-based dispersal, Genalex was used with the male and female datasets which were modified to reflect the number of populations determined from Structure and Structure Harvester. Estimates of migrants was obtained by using this formula: Nm= ((1/Fst)-1)/4.

**Results and Discussion**

Lake Illiyardjuk is a large lake covering 4,400 km2, the lake was formed during the last glacial period and trapped a subspecies of walrus, the walruses of Lake Illiyarduk (*Odobenus rosmarus fictiousi*) about 10,000 years ago. The glacially formed lake is not a regular shape, it is fragmented with straights that vary in size and irregular depths ranging from the shallowest areas being 0-20m to 351-446m for the deepest. We can see from Figure 1, that the breeding regions are ideally in areas near shore and have depths of around 100m. In addition to becoming an isolated population in the past 10,000 years, the walrus subspecies suffered an anthropogenic bottleneck in the 20th century. We can use genetic methods like the microsatellite data of the late graduate student and the mitochondrial data given to my company to quantify the co-occurrence of immigrants, residents, and use Fst values to asses gene flow.

We can also utilize information about the walruses behavior and context of their environment to assess the impact of dispersal of individuals to local population growth, an aspect that genetic methods alone cannot encapsulate (Lowe & Allendorf, 2010). Data from Genalex for the microsatellite by year data shows that observed heterozygosity has decreased by nearly half from the 1980’s (Ho=0.318) to the 2000’s (Ho=0.184), which significantly lower than the expected heterozygosity for the 2000s (He=0.387). The inbreeding coefficients for the 2000s (Fis=0.52) has also more than doubled since the 1980s (Fis=0.257). As mentioned above, 20 marine walruses were introduced to the lake population in 1979, that appears to not have had a significant effect. The finite population size of the walruses and isolation over hundreds of generations can lead to inbreeding and thus inbreeding depression. Inbreeding and loss of genetic variation can lead to population decline due to reduced fitness. In addition, inbreeding depression is quite often worse in harsh environments, like Northern Canada (Frankham, 1995).



**Figure 1.** Lake Illiyardjuk and its 5 divided breeding regions/basins.

When examining the microsatellite data by location on Genalex, we can make observations about the walrus population, each of the distinct breeding areas individually, and assess. Overall, the mean inbreeding coefficient, Fis, for all regions is 0.18. However, when we start to assess the individual regions, we can see that some of the populations have significant differences between them and that mean Fis alone may lead to an inaccurate conclusion on the inbreeding and dispersal of walruses at Lake Illiyardjuk. The Northern region has the highest observed heterozygosity (Ho=0.34) followed by the Hawani region (Ho=0.32) and then the Southern region (Ho=0.22). The observed heterozygosity in these three regions is very similar to the expected heterozygosity. The Northern region has the lowest inbreeding coefficient (Fis=-0.02/0) followed by the Southern (Fis=0.01) and then the Hawani (Fis=0.06). The lower the inbreeding coefficient means there is less inbreeding taking place. Conversely, the Perpaluktok and Kinapak region share the lowest observed heterozygosity (Ho=0.14). The Kinapak region has the highest inbreeding coefficient (Fis=0.52), and the Perpaluktok region has the second highest inbreeding coefficient (Fis=0.38).

We can see that the Perpaluktok and Kinapak regions, lack proximity to deeper water (101-150m) which the other three regions have (Figure 3). They also have narrow straights that lead to them. If walruses prefer to breed/live near deep water, this feature of the Kinapak and Perpaluktok regions may contribute to a lack of connectivity between the walrus populations. Previous telemetry data has shown that mature walruses are predominantly stationary, yet there is an opportunity for population connectivity when younger walruses disperse to seek mates. It may be concluded that young walruses may be more inclined to choose areas that are easier to access with wider straights and proximal deeper water.

Since there are five breeding areas, it is important to understand the underlying structure and populations of the walruses at Lake Illyyardjuk to better make recommendations on the conservation of the walruses. When we ran the data on Structure and exported it to Structure Harvester, we can look at the L(K) graph and see that the slope asymptotes at four populations (Figure 2). We can also see that the Bar Plot from Harvester for four populations shows that if there are four populations, the individuals are not highly mixed from one another (Figure 3). The Neighbor joining tree from Structure is useful because it can show us visually the relatedness of the four populations, with population 4 (yellow) being most closely related to 1 (red) followed by 3 (blue) and the least related to the other three populations is 2 (green) (Figure 4).

Chart, scatter chart

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**Figure 2.** Number of populations (K) as predicted by Structure Harvester by using the microsatellite DNA data of the walruses at Lake Illiyardjuk.

Timeline

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**Figure 3.** The Bar Plot generated by Structure showing the individuals in their predicted populations as determined by the microsatellite DNA data of the walruses at Lake Illiyardjuk.

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**Figure 4.** Neighbor Joining Tree generated by Structure of the four predicted populations using microsatellite DNA of the walruses at Lake Illiyardjuk.

In subpopulations such as the Illiyardjuk walruses, we can measure the standard divergence at individual loci to asses gene flow, this is referred to at the Fst value. Values of Fst that are low (<0.02) show less genetic divergence and are said to have sufficient gene flow. Fst values that are <0.20 are believed to have enough gene flow to avoid deleterious effects of inbreeding. Values of Fst that are <0.35 can still be adaptively connected, where there is at least one migrant per generation, which is sufficient gen flow to spread adaptive alleles. However, Fst values greater >0.35 show that the population is significantly isolated and there is little to no gene flow between the populations (Lowe & Allendorf, 2010). The mean Fst for all of the regions for each locus is 0.25, indicating that the walrus populations are experiencing inbreeding, but there is enough gene flow occurring to prevent the negative effects of inbreeding. The pairwise microsatellite variation Fst values show that the five regions have predominantly enough gene flow to avoid deleterious effects of inbreeding, in addition, the mean number of migrants, Nm, for all regions for each locus is 2.18. While there is no amount of inbreeding that does not have harmful effects, Nm values as small as one has been observed to be enough to avoid the negative effects of inbreeding and genetic drift (Lowe & Allendorf, 2010). The Northern and Hawani pairwise Fst value for microsatellite variation (Fst 0.03) shows that they are right above the threshold for enough gene flow to maintain similar allele frequencies. Conversely, from a previous study, the pairwise mtDNA comparison of the five regions show that the five breeding areas are highly differentiated (Table 1). This may be caused by females being predominately stationary while males disperse, in this case, a higher Fst value can be expected when using mtDNA to make pairwise Fst comparisons (Prugnolle & De Meeûs, 2002). We can use both the microsatellite and mtDNA data to infer patterns of the different migration patterns of the sexes of walruses since these genetic markers have different modes of inheritance (Prugnolle & De Meeûs, 2002).

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Genetic differentiation (pairwise FST) between regional walrus samples based on microsatellite and mtDNA variation** | | | | | | | |
|  | Nms | Nmt | Northern | Kinapak | Hawani | Perpaluktok | Southern |
| Northern | 15 | 19 | – | 0.454 | 0.392 | 0.597 | 0.446 |
| Kinapak | 20 | 23 | 0.208 | – | 0.344 | 0.698 | 0.389 |
| Hawani | 79 | 47 | 0.033 | 0.221 | – | 0.511 | 0.398 |
| Perpaluktok | 43 | 37 | 0.188 | 0.257 | 0.175 | – | 0.433 |
| Southern | 15 | 19 | 0.143 | 0.241 | 0.120 | 0.195 | – |
|  |

Table 1. Genetic differentiation (pairwise FST) between regional walrus samples based on microsatellite and mtDNA variation. *N*ms = sample size in microsatellite analyses; *N*mt = sample size in mtDNA analyses. Estimates based on mtDNA variation above diagonal, with estimates based on microsatellite variation below.

We can also use the Nei genetic distance and identity values to make assessments on population differentiation. For the Nei distance, a value of zero indicates identical populations. For Nei identity, a value one indicates genetically identical. We can see in Table 2, that the Hawani and Northern breeding grounds are almost identical (Nei identity=0.965), with the Southern area sharing a large identity with the two as well. In similarity with the Fst values, the Kinapak region has the least shared identity with the other regions followed by the Perpaluktok.

Table

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**Table 2.** Pairwise population matrix of Nei genetic distance and Nei genetic identity. The estimates are below the diagonal.

Based on the information above, my company predicts that the Northern and Hawani regions are one population of walruses (Red color, Figures 3&4). The Southern region walruses are a separate population (Yellow color, Figures 3&4) but shares the most gene flow between the Northern and Hawani region. The Kinapak region is the most isolated population (Green color, Figures 3&4), followed by the Perpaluktok (Blue color, Figures 3&4). In addition to determining the number of populations and structure of the walruses at Lake Illiyardjuk, it is important to also look into possible sex-based dispersal. As discussed earlier, walruses are predominately sedentary and may disperse to seek mates. It is apparent from the mtDNA data and microsatellite data that there is a difference in the dispersal of the sexes. We can see that in table 3 that the Fst values show that the populations for both male and female are largely similar, with females having slightly higher Fst values. A lower Fst value can be expected if we believe that males are the sex that is dispersing rather than females (Prugnolle & De Meeûs, 2002)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | |  |  |
| Northern | Kinapak | Perpaluktok | Southern |  |
| 0.000 | 0.201 | 0.181 | 0.108 | Northern |
| 0.250 | 0.000 | 0.257 | 0.230 | Kinapak |
| 0.182 | 0.299 | 0.000 | 0.182 | Perpaluktok |
| 0.146 | 0.317 | 0.217 | 0.000 | Southern |
|  |  |  |  |  |
|  | | | | |

**Table 3.** Pairwise Population Fst Values for females below diagonal. Males are above diagonal in blue. The Northern and Hawani regions have been condensed to one population, the Northern population.

From the Genalex data for males and females with the four populations, we can see that the average number of migrants for males and females was higher in the males with an average of 2.68 migrants versus the females with 1.79 migrants. We can also estimate the number of migrants by using the formula Nm= ((1/Fst)-1)/4 with the Fst values from Table 3 to make a table 4. We can see that the males have more migrants than females, but we are unsure of if it is a statistically significant difference in the number of migrants (Table 4). However, based on the mtDNA data in conjunction with the microsatellite data, these results indicate that there may be sex-based dispersal of the walruses of Lake Illiyardjuk. This affects our conservation efforts because we will have to account for number of males and females in a population and create ways to encourage dispersal of young males to the more isolated breeding areas.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Northern | Kinapak | Perpaluktok | Southern |  |
|  | 1.00 | 1.13 | 2.06 | Northern |
| 0.75 |  | 0.72 | 0.84 | Kinapak |
| 1.12 | 0.59 |  | 1.13 | Perpaluktok |
| 1.46 | 0.54 | 0.90 |  | Southern |

**Table 4.** Estimated number of migrants based on Fst values from Table 3. Males are above diagonal in blue. The Northern and Hawani regions have been condensed to one population, the Northern population.

My company recommends that the local Inuit people of Lake Illiyardjuk encourage the walruses of Lake Illiyardjuk to disperse more between the populations. In particular, moving individuals from the Northern and Southern population to the Kinapak and Perpaluktok. Since walruses are largely stationary, and it may be the males that disperse more, it may be beneficial to capture and relocate young females from one population to another in hopes that she will remain at the new population and breed with the males. If one were to relocate a male walrus, one should possibly do so during mating season so that they can mate before the possibility of the walrus returning to its original population if it decides to disperse.

**Conclusion**

We have used genetic methods to indirectly make observations about the population structure of the walruses at Lake Illiyardjuk. We have found that there may only be four distinct populations of walruses: Northern, Kinapak, Perpaluktok, and Southern. We also established which populations are most connected, the Northern and the Southern. The populations that are the most isolated are the Kinapak and the Perpaluktok. The most isolated regions would benefit the most to increase migrants, however, all populations would benefits from a rotation of individuals across the populations. My company recommends that young walruses be captured and introduced to new populations in hope of them mating and creating gene flow between the populations.

**Works Cited**

Frankham, R. (1995). Conservation genetics. Annual Review of Genetics, 29(1), 305-327.

Lowe, W. H., & Allendorf, F. W. (2010). What can genetics tell us about population connectivity? Molecular Ecology, 19(15), 3038-3051.

Prugnolle, F., & De Meeûs, T. (2002). Inferring sex-biased dispersal from population genetic tools: a review. Heredity, 88(3), 161-165.

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. Science, 236(4803), 787-792.

**Supplementary information**

The link to my GitHub is:

https://github.com/Robertabeard/Evo-Bio-PBL2