

Effects of inbreeding on fitness in the North Atlantic
right whale (*Eubalaena glacialis*)

By
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Abstract

Offspring that are a product of inbreeding often have reduced fitness, known as inbreeding depression. Though our understanding of the impacts of inbreeding depression in wild populations is in its infancy, it is thought that the effects of inbreeding depression are much stronger in the wild than previously suspected. This is due to the cost of inbreeding being higher in wild populations than in captive. The North Atlantic right whale (*Eubalaena glacialis*) is one of the most endangered large whale species and has shown little signs of recovery over the past 70 years. This lack of recovery is due, at least in part, to a reproductive rate that is three-times lower than their known potential. North Atlantic right whales also have extremely low levels of genetic diversity. This project was conducted to assess the degree to which inbreeding could explain the variation seen in the fitness of individuals of this species. Demographic and genetic data measured at 35 microsatellites, made available to us from the North Atlantic Right Whale Consortium, were used to calculate measures of both fitness and inbreeding for individual right whales from 1990-2016. Fitness was measured through the use of the de-lifing method, and the inbreeding coefficient quantified was the internal relatedness calculation. Measures of fitness in survival showed small amounts of variation between years with most individuals surviving. Mean values of fecundity each year showed greater amounts of variation and overall lower levels of fitness. Results from linear regression analyses showed that inbreeding explains little of the variation in both survival and fecundity for this species. This study has provided quantified measures of fitness for each right whale. Further studies should be completed using genomic data to continue examining the potential impacts of inbreeding on fitness in the North Atlantic right whale.

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Introduction

1.1 Inbreeding depression in wild populations

Inbreeding is defined as the mating between relatives (Keller and Waller, 2002), often resulting in a reduction of heterozygosity in offspring (Shields, 1987). A decrease of heterozygosity is often associated with a decrease in fitness, called inbreeding depression (Crnokrak and Roff, 1999). Reduced genetic diversity and the effects of inbreeding have long been known to have negative effects on fitness (Darwin, 1868; Charlesworth and Willis, 2009). Such decreases in fitness are seen in a variety of fitness attributes, such as lower resilience to environmental stress, increased vulnerability to diseases, higher rates of juvenile mortality, and decreases in fecundity (e.g., Ralls et al. 1979). For example, Ralls et al. (1979) found that increased inbreeding within small populations of captive ungulates can lead to decreased rates of survival in juveniles; with 15 out of 16 species showing increased mortality in inbred young. Huisman et al. (2016) also found that inbred red deer females had a 72 percent reduction in lifetime breeding success (LBS), the number of offspring born, and inbred males had a 95 percent reduction. These studies reveal the dramatic influence that inbreeding depression can have on the fitness of both juveniles and adults.

There are two central hypotheses as to why decreased heterozygosity results in fitness declines: the partial dominance hypothesis and the overdominance hypothesis (Wright, 1977). The partial dominance hypothesis suggests that the decrease in heterozygosity unmasks deleterious recessive alleles, resulting in a decrease in fitness. It

is a decrease in heterozygosity that reveals these deleterious recessive alleles, as they are normally hidden in heterozygotes by dominant alleles. Alternatively, the overdominance hypothesis states that heterozygous individuals have higher fitness than homozygous individuals, in general, and therefore a decrease in heterozygosity will result in a decrease in fitness. However, in the case of the overdominance hypothesis the decline in fitness is not attributed to a specific mechanism such as deleterious recessive alleles (Charlesworth, 1987). While evidence for both mechanisms exists, recent studies suggest that the partial dominance hypothesis is the prominent mechanism that leads to inbreeding depression in natural populations (Charlesworth and Willis, 2009).

Populations that are small or decreasing in size are vulnerable to inbreeding depression as mate options decline, causing individuals to become more genetically similar with time. Thus, inbreeding depression is an important area of research in declining populations as it allows us to better understand if, and/or to what degree, genetic factors are limiting population growth (Crnokrak and Roff, 1999). However, we currently have a poor understanding of the impacts of inbreeding depression in the wild because most of our understanding comes from studies of captive and/or laboratory populations (Crnokrak and Roff, 1999, Huisman et al. 2016). While our understanding of how inbreeding depression affects wild populations is in its infancy, it is thought to be much stronger than what is found in captive populations, and stronger than has previously been expected (Crnokrak and Roff, 1999; Kardos et al. 2016). This is due to the cost of

inbreeding being higher in natural environments than in captive ones as conditions can be unpredictable and food supply is not controlled in the wild (Crnokrak and Roff, 1999).

1.2 Inbreeding depression in North Atlantic right whales

One species where inbreeding may be having a large impact is the endangered North Atlantic right whale (*Eubalaena glacialis*). The North Atlantic right whale is one of the most endangered species of large whale (Cooke, 2018). This species is one of three species of right whale, the other two being the southern right whale (*Eubalaena australis*) and the North Pacific right whale (*Eubalaena japonica*) (Rosenbaum et al. 2000). North Atlantic right whales have both low genetic diversity and a reduced reproductive performance, with inbreeding being one hypothesized cause of both these issues (Frasier et al. 2007b; Kraus et al. 2007b). Due to the small and declining population size of this endangered species, it is important to understand the causes behind this reduced reproductive performance and low genetic diversity, to help determine what limits are being placed on recovery and what protective measures can be taken to promote their survival.

In appearance, right whales are black in colour with some having white patches on their ventral surface. Adults average 14 m in length, with females measuring approximately one metre longer than males (Andrews, 1908). They have large heads that make up 25 percent of their body length, with a strongly arched jaw (Andrews, 1908). The size and shape of the head are required to hold the ~250 plates of 2-2.8 m-long baleen that hang from each side of the upper jaw (Kraus and Rolland, 2007). One of the

main identifying features in right whales are the unique callosities on their heads (**Figures 1.1, 1.2**). Callosities are areas of raised and cornified epithelial tissue. Although callosities are dark in colour, they are infested with cyamid crustaceans, also known as “whale lice”, causing them to appear light yellow or cream in colour (Hamilton et al. 2007). Callosities appear when a calf is approximately 7-10 months old and generally remain consistent throughout their life (Kraus et al. 1986). Each whale has its own distinct callosity pattern which allows researchers to accurately identify individuals in the field through the use of photographs which are kept in the *North Atlantic right whale catalog and database* (Kraus et al. 1986, Hamilton et el. 2007, Frasier et al. 2009).

North Atlantic right whales live mainly along the eastern coasts of the United States and Canada, although they have been seen in the Gulf of Mexico and as far northeast as Iceland and Norway (Jacobsen et al. 2004). They generally spend the spring and summer months feeding in and around the Great South Channel, Cape Cod Bay, the Bay of Fundy, and the Roseway Basin (Baumgartner and Mate, 2005; Kenney et al. 2001). Calving primarily occurs between December and March, with females giving birth close to the coasts of Georgia and Florida after an estimated twelve to thirteen month gestation period (Kraus et al. 1986; Best 1994). The age at which females have their first calf varies greatly in this species, however studies have suggested that the average age is approximately eleven years (Kraus et al. 2007a). Time is needed to regain body fat and strength after having a calf, resulting in a time interval of at least three years between calves for an individual female (Kraus et al. 2007a). This three year interval is composed

of a lactation year, a resting year, and a gestation year (Knowlton et al. 1994). A calving interval of longer than three years can be indicative of low food resources (Wade and Schneider, 1992), or more inherent problems, such as inbreeding (Kraus et al. 2007a). Most right whale breeding, feeding, and migrating happens within 80 km of the shoreline making them vulnerable to the industrial activities of humans in the ocean (Kraus and Rolland, 2007).

North Atlantic right whales received their name from being known as the “right” whale to kill by whalers due to the fact that they are relatively slow, have a coastal distribution, have among the thickest layers of blubber of all whale species, which results in high yields of oil and causes the whales to float when dead making transportation easier, and due to their long baleen plates. It was believed for an extended time that sixteenth-century Basque whaling in the Strait of Belle Isle was a major cause for population declines in this species (Gaskin, 1991). However, it is now known that the large number of whales previously estimated as being killed during this time (12,000-15,000) were not in fact right whales, but rather were the closely related bowhead whale (*Balaena mysticetus*; Rastogi et al. 2006; McLeod et al. 2008). Therefore, previous estimates of the impact of Basque whaling on this species were inaccurate and overestimated (Rastogi et al. 2006; McLeod et al. 2008). While studies have revealed that right whales were not the primary species hunted by the Basques, right whales were hunted by American whalers along the east coast of the United States from the mid seventeenth century to the early twentieth century, with the most intense hunting

happening in the 1700's (Reeves et al. 1999). To prevent further hunting of this species, the League of Nations granted them international protection in 1935. This was then followed by the International Whaling Commission placing a ban on all right whale hunting throughout the world (Kraus and Rolland, 2007).

Although North Atlantic right whales have been protected since 1935, recovery has been extremely slow. Specifically, they have increased at a rate of ~2%, whereas right whale populations in the southern hemisphere are increasing at rates of 6-7% (e.g., Corkeron et al. 2018). Moreover, abundance has been declining since 2010, with a current estimate of ~410 individuals (Pace et al. 2017). There are two main causes behind why this species is recovering at a lower rate than expected, and lower than those of other right whale populations: high anthropogenic mortality rates and low reproductive success.

Anthropogenic mortalities are primarily caused by entanglement in fishing gear (Knowlton et al. 2012) and ship strikes (Kraus et al. 2005). Knowlton et al. (2012) found that between the years 1980 and 2009, 83% of individuals had been entangled at least once in their lifetime and 59% were entangled more than once. These are alarmingly high rates of entanglement and the injuries they cause can reduce fitness or, in some cases, be fatal, in addition to undoubtedly causing extreme amounts of pain and suffering.

While these anthropogenic mortalities are of great concern with regard to the diminishing population size, the reduced reproductive performance is also a major limiting factor. For example, no calves were born to the entire species this past year (2017-2018). North Atlantic right whale reproductive patterns appear to be quite varied as

some females reproduce consistently every three years while others have never had a calf or have longer interval periods between births (Knowlton et al. 1994). Overall, this species is reproducing at a rate three times lower than what we would expect to see with three year birth intervals (Frasier et al. 2007a). This expected reproductive rate is considered reasonable based on the growth rates seen in the South Atlantic right whale populations, which are congruent with the average female having a calf approximately once every three years (Best et al. 2001, Cooke et al. 2001).

North Atlantic right whales not only have low reproductive performance, but also have extremely low genetic variability. When mitochondrial DNA (Malik et al. 2000), microsatellite markers (Waldick et al. 2002), and minisatellite markers (Schaeff et al. 1997) were studied, all analyses revealed low diversity. A genetic bottleneck was hypothesized as an explanation for this, resulting from the periods of American whaling (Knowlton et al. 1994). However no genetic bottleneck was found indicative of the eighteenth to twentieth-century reductions in population abundance (Waldick et al. 2002). These results, combined with the recent revision of the impact of Basque whaling on this species (Rastogi et al. 2004; McLeod et al. 2008), suggest that it was already at a relatively small size prior to the onset of industrial American whaling. One implication of this interpretation is that at least some of the factors that are keeping this species at a relatively small size may have been acting for a long time, and are therefore not solely reflective of human activities.

Studies looking at the correlation between genetic variance and breeding success in both southern and North Atlantic right whales have shown that mating between genetically similar individuals appears to be unsuccessful (Schaeff et al. 1997) and that surviving calves have higher levels of genetic diversity than the expected values produced by random mating (Frasier et al. 2013). While it is unlikely that inbreeding alone is the cause of the low reproductive success, these data suggest the possibility of a strong correlation between inbreeding and low reproductive success in North Atlantic right whales (Kraus et al. 2007a). Both the low genetic variation and the reduced reproductive performance are signs that inbreeding depression is likely impacting this species to some degree, however accurate measurements of both inbreeding and individual fitness have yet to be quantified in right whales and therefore the actual impacts remain unknown.

1.3 Measures of Inbreeding

The standard way to measure inbreeding coefficients (F) in populations has historically been through the use of pedigree analysis by estimating how much of the genome was identical by descent (IBD) (Charlesworth and Willis, 2009). However, this is not a feasible method for wild populations, as accurate and in-depth knowledge regarding pedigrees is needed, yet is often unknown. Determining inbreeding coefficients using pedigree analysis is also inadequate as individuals of the same degree of inbreeding can have varying levels of homozygosity; and it is this homozygosity, rather than the inbreeding coefficient itself, that is the cause of inbreeding depression. This lack of knowledge and continued use of pedigree analysis results in inaccurate estimates of

inbreeding in individuals within wild populations (Kardos et al. 2016). With continued developments in genetics, it is now possible to obtain a more accurate assessment of individual inbreeding depression using nuclear DNA (Szulkin et al. 2010).

Microsatellites are sequences of non-coding DNA made up of short, one-to-five base pair segments which are repeated multiple times in tandem (Litt and Luty, 1989). These nuclear DNA microsatellites are useful markers for assessing relatedness within a population as they are inherited from both the mother and father, unlike mitochondrial DNA which is inherited only through the maternal lineage. The genotypes obtained with the analysis of microsatellites can be used to infer parentage, relatedness, individual identity, and to estimate aspects of genetic variation such as heterozygosity, therefore enabling inbreeding coefficient estimation (Coulson et al. 1998).

1.4 Quantifying Fitness

Traditionally, lifetime fitness measurements have been used to calculate an individual's fitness. These methods estimate the fitness of individuals by quantifying their reproductive success across their entire lifetime, called lifetime breeding success (LBR) (Charlesworth, 1994; Coulson et al. 2006). While this measurement has been used to successfully learn about several aspects of fitness, it also suffers from two major downfalls (Grafen, 1988). First, for most wildlife population studies there are not enough data available to properly quantify life-long measures (Grafen, 1988). Lifetime fitness measurements are particularly difficult to estimate in long-lived species, such as right whales. While the life expectancy of right whales is currently unknown, the current

estimate is approximately 70 years (Hamilton et al. 1998). However, it is possible that they may live upwards of 100 years as the life expectancy of the closely related bowhead whale is 200 years (George et al. 1999). Consequently, it is possible for right whales to outlive the researchers studying them, making it extremely difficult to collect and measure individual fitness for an entire lifetime. The second reason is a lack of correction for how ecological and environmental variation may impact the performance of an individual (Coulson et al. 2003). For example, the ability of an individual to live through a time of population decline is far more impressive than an individual's ability to live through a time of population growth. Therefore, external factors driving the growth and/or decline of a population at a given time should be considered when measuring fitness, as this gives a more accurate estimation of an individual's intrinsic fitness. Both the difficulties in obtaining data and the lack of correction for environmental and ecological variation are significant issues that can affect the way fitness is quantified in the wild, and, furthermore, in assessments of what factors are influencing fitness.

To address these issues, Coulson et al. (2006) developed a method to properly quantify an individual's fitness; called the 'de-lifing' method. This method calculates each individual's relative contribution to population growth for each year, based on survival and fitness components. The de-lifing method accounts for both ecological and environmental variation as it takes the entire population growth into consideration at the specific time when an individual's contribution is calculated. De-lifing also limits the amounts of data needed, depending on the length of time step used, and life-long

measurements of performance are no longer required. In addition, the de-lifing method can be used to separately quantify an individual's contribution to fecundity and survival, both of which are key components of fitness, but which may be influenced by different factors. This ability to separate survival and fecundity provides researchers with much more specific and accurate results of fitness. Further details of this calculation are provided in the methods section (section 2.3).

1.5 Objectives

For this study I conducted analyses on previously collected data. I used the photo-identification and demographic data for survival and reproduction, which have been collected over the past thirty nine years and made available through the North Atlantic Right Whale Consortium (NARWC; Hamilton et al. 2007; "North Atlantic Right Whale Consortium", n.d.), to quantify fitness components for the 724 individuals within the population using the de-lifing method. These analyses aim to produce estimates of the overall fitness of each individual, as well as estimates of the specific survival and reproductive components of fitness. Additionally, this will provide quantified data regarding the variation seen in reproductive success between females, which has yet to be done.

To estimate individual inbreeding coefficients, I used previously collected genetic data, also made available through the NARWC, based on 35 microsatellite loci for 469 individuals; which is approximately 80% of the population (Brown et al. 1991; Schaeff et al. 1993, Frasier et al. 2007a). These data were then be used to test the associations

between inbreeding and fecundity in females and inbreeding and survival in both males and females.

It is expected that if inbreeding depression is prevalent in the North Atlantic right whale, there will be strong negative correlations between inbreeding coefficients and fecundity. While it is also likely that a negative correlation between inbreeding and survival exists, it is possible that this result may be masked by the high anthropogenic mortalities present in this species, which are likely causing most of the mortalities, and presumably have little to do with inbreeding. The results of the analyses will indicate the effects inbreeding depression is having on recovery, as well as provide possible explanations to the variation observed in the low reproductive success of the North Atlantic right whale.

Methods

2.1 Obtaining Survival, Reproductive Output, and Relatedness Data Through Photo Identification and Genetic Analysis

Quantifying patterns of fitness for North Atlantic right whales requires survival and reproductive histories for a substantial number of individuals (Grabowski and Porto, 2017). For right whales, survival and reproductive output data are collected using photo-identification. Specifically, photographs are taken by numerous individuals and organizations and sent to the New England right whale research team, who curate the photo-identification data and make it available through the North Atlantic Right Whale Consortium (NARWC) (“North Atlantic Right Whale Consortium”, n.d). The NARWC was formed in 1986 for the purpose of sharing data with various government, academic, and conservation organizations (“North Atlantic Right Whale Consortium”, n.d.; Pettis et al. 2018). The NARWC maintains the photo identification data for every sighting of each right whale. Photo-identification data have been collected year-round for over 40 years (Hamilton et al. 2007) and deposited in the *North Atlantic Right Whale Catalog* and an associated sightings database, which are part of the NARWC, that houses sighting records and photographs from 1935 to the present (Hamilton et al. 2007).

North Atlantic right whales can be identified as individuals through these photographs by the callosity patterns on their heads (**Figures 1.1, 1.2**) that differ among individuals, and which remain stable throughout the lifetime of each individual (e.g., Kraus et al. 1986). As a result, researchers are able to obtain individual-based data from

photographs, including migration and association patterns, reproductive histories, and abundance estimates (e.g., Hamilton et al. 2007).

Data obtained from the photo records regarding mortality and offspring numbers are of particular relevance to this study, as these data are needed to quantify individual fitness. For measurements of mortality, individuals are recorded as dead if the carcass is found and can be identified as a specific individual. However, most deaths are not observed. To account for this, the NARWC has developed a “presumed dead” criterion, where any whale that has not been seen for six years is presumed dead in the sixth year without a sighting (Knowlton et al. 1994). This is based on data showing that >99.5% of whales are seen within a six-year time frame, and therefore it is unlikely that an individual would be alive, yet not seen, for an entire six years (Hamilton et al. 2007).

For births, calves nurse for approximately eight months, during which time they remain in close association with their mothers (Hamilton et al. 1995). Due to this close association over a long period of time, it is relatively easy to identify which females have reproduced in each year based on the photo-identification data. However, males play no role in raising the offspring, and both sexes in this species are highly promiscuous, making it impossible to identify paternity based on field data. Instead, extensive genetic analyses are required to identify fathers (Frasier et al. 2006; Frasier et al. 2007a), and although these data have led to interesting findings (e.g., Frasier et al. 2013), the paternity data are not complete enough to be used here to assess individual reproductive success.

The photo-identification data were used in this study to obtain mortality data for males and females, and reproductive data for females only. I obtained these data sets, ranging from 1980-2016, from the NARWC. However, only data from 1990-2016 were used because the calving grounds were not surveyed consistently prior to 1990, which could result in misleading findings on reproductive success in the earlier years (Brown et al. 2007).

In addition to photo-identification data, most right whale research field teams have been collecting small skin samples for genetic analyses since the late 1980s (Brown et al. 1991). Skin biopsy samples are collected from free-swimming whales using a cross-bow with a modified arrow, specific for skin sample collection (Brown et al. 1991). While collection of genetic data is most frequently completed using these skin samples, tissue samples can also be obtained from whale carcasses and fecal samples and used for DNA analyses (Best et al. 2005). Furthermore, DNA has been collected from historic whale bones, enabling us to have a greater understanding of past right whale lineages (Rastogi et al. 2004; McLeod et al. 2008).

Genetic samples have been used to create profiles for individual right whales starting in the early 1990s (Brown et al. 1991), and genetic profiles are now available for ~80% of all living photo-identified individuals. This collaborative collection of genetic data has been used in a variety of ways including identifying deceased whales, examining habitat use patterns, linking mothers to calves, and measuring genetic variance (e.g. Schaeff et al. 1997; Malik et al. 2000). The North Atlantic right whale genetic databank is

maintained jointly by Trent University and Saint Mary's University, both members of the NARWC, which houses data received from a number of contributors and partners. The genetic profiles include molecular sex determination, sequencing a portion of the mitochondrial control region haplotypes, and genotype analysis at 35 microsatellite loci (Frasier et al. 2007a). This extensive collection of genetic data combined with the information gleaned from photo-identification has enabled researchers to trace lineages and examine relatedness between individuals (Frasier et al. 2007b). The genetic data, like the photo-identification data, are housed and maintained by members of the NARWC. I obtained available genotypes, based on the 35 microsatellite loci, from the NARWC and used this data to quantify inbreeding coefficients for the 469 individuals, 216 females and 253 males, in the population from 1990-2016.

2.2 Quantifying Inbreeding

Several methods for calculating heterozygosity for multiple genetic markers have been developed to use in heterozygosity-fitness correlation (HFC) studies (e.g. Frère et al. 2010; Küpper et al. 2010). The most frequently used estimates for heterozygosity are the proportion of heterozygous loci in an individual (PHt), standardized heterozygosity (Hs) (Coltman et al. 1999), internal relatedness (IR) (Amos et al. 2001), and homozygosity by locus (HL) (Aparicio et al. 2006).

PHt is measured by taking the number of heterozygous loci and dividing it by the number of genotyped loci. While PHt is a very simple way to calculate heterozygosity, it is not always the best estimate for studies with a small number of genetic markers, when

allele frequencies differ across loci, or when individuals are not typed at the same loci, as these would cause estimates to be measured across different genetic markers (Coltman et al. 1999; Aparicio et al. 2006). To correct for this problem, Coltman et al. (1999) standardized the individual proportions of heterozygous loci by dividing the proportion of heterozygous typed loci by the mean heterozygosity of typed loci. This standardization guaranteed all individuals were measured on the same scale (Coltman et al. 1999). This standardized version of P_{Ht} was denoted H_s. While H_s corrected the main problem found with P_{Ht}, it does not weigh alleles based on frequency and therefore assumes a linear relationship between heterozygosity at a locus and the number of alleles, which could underestimate heterozygosity if the relationship is exponential (Aparicio et al. 2006).

IR (Amos et al. 2001) estimates homozygosity and is measured with the following equation:

Equation 2.1: Internal Relatedness

$$IR = (2H - \sum f_i) / (2N - \sum f_i)$$

where H is the number of homozygous loci, N is the total number of loci, and f_i is the frequency at which the i th allele appears in the genome (Amos et al. 2001). The IR estimate weighs rare alleles more heavily than common alleles in the sample (Amos et al. 2001). Results from IR range from negative one to positive one, with negative values suggesting the individual is more heterozygous, or outbred, and positive values suggesting higher levels of homozygosity, or inbreeding (Amos et al. 2001). This estimate is useful in populations with high levels of inbreeding, however it overestimates

homozygosity in open populations where immigration may introduce rare alleles to the population (Aparicio et al. 2006).

HL (Aparicio et al. 2006) is calculated by the following equation:

Equation 2.2: Homozygosity by Locus

$$HL = \sum E_h / (\sum E_h + \sum E_j)$$

where E_h is the expected heterozygosity of an individual's loci in homozygosity and E_j is the expected heterozygosity of an individual's loci in heterozygosity. This estimate corrects for the problem of overestimating homozygosity in IR by weighing the contribution of each locus instead of each allele, which results in the most informative loci, those with higher allelic variability, having the highest weight (Aparicio et al. 2006).

Aparicio et al. (2006) completed a simulation study to compare the performance of PHt, IR, and HL under various conditions. They found that while PHt was a simpler measurement, it was limited in its ability to use datasets with variation in the number of genetic markers used. HL was determined to correlate better with both genome-wide homozygosity and inbreeding, making it a better estimator for measuring heterozygosity in populations that are often affected by migration or other processes that introduce new alleles to the population. IR performed the best for populations that exhibited high levels of inbreeding and was a better predictor in populations with low expected heterozygosities (Aparicio et al. 2006). IR is more efficient in populations with high levels of inbreeding because the presence of a homozygous rare allele at the locus of an

individual within a closed population suggests the individual may be more inbred compared to individuals homozygous for more common alleles. Therefore, IR's use of direct allelic frequencies and its ability to weigh rare alleles more heavily make it the best predictor for populations with high inbreeding (Aparicio et al. 2006).

Due to low numbers in the North Atlantic right whale population, there is little to no introduction of new alleles from migration or other mechanisms. Thus, there is little benefit to using HL for heterozygosity estimates in this study (Aparicio et al. 2006). North Atlantic right whales also have extremely low genetic diversity and inbreeding is suspected to be present in the population (Malik et al. 2000, Waldick et al. 2002, Schaeff et al. 1997). Therefore, it was determined that IR (Equation 2.1) would be the best estimate of homozygosity in individual right whales.

2.3 Use of De-lifing Method to Quantify Individual Fitness

The de-lifing method, developed by Coulson et al. (2006), enables an accurate estimation of individual fitness. The calculation for fitness can be divided into two components: survival and fecundity. By using the survival and reproductive data obtained from the NARWC, I quantified the contribution of both individual males and females to survival and individual females to reproduction using the calculations provided by Coulson et al. (2006). An example of the results of the calculations can be found in **Table 1** which include hypothetical data for one year. The survival component is calculated using the survival data with the following equation:

Equation 2.3: Survival

$$\frac{s_{t(i)} - \bar{s}_t}{N_t - 1}$$

where $s_{t(i)}$ is the binary variable shown in column “Alive”, indicating whether (1) or not (0) an individual survived that year; \bar{s}_t is the mean of $s_{t(i)}$ using the whole population survival data for year t , and N_t is the total population size for year t . The fecundity component is the same as the survival component, however it utilizes birth data instead of survival. The fecundity component equation is:

Equation 2.4: Fecundity

$$\frac{f_{t(i)} - \bar{f}_t}{N_t - 1}$$

where $f_{t(i)}$ is the number of offspring an individual gives birth to in year t , \bar{f}_t is the mean of $f_{t(i)}$ using the whole population data for year t , and N_t is the whole population size for year t . The survival (Equation 2.3) and fecundity (Equation 2.4) components can be added together to calculate an individual’s contribution to overall population growth for a given year, denoted as $p_{t(i)}$:

Equation 2.5: Fitness

$$p_{t(i)} = \frac{s_{t(i)} - \bar{s}_t}{N_t - 1} + \frac{f_{t(i)} - \bar{f}_t}{N_t - 1}$$

Both the survival and fecundity components were calculated for every individual right whale for each year from 1990-2016 using Equation 2.3 and Equation 2.4 to give the individual's contribution to population growth in the respective component.

2.4 Fitness and Inbreeding Analyses

My analyses began with calculations of fitness for all individuals known to be alive at some point between 1990-2016, a sample size of 724 individuals with 301 females and 424 males. Survival data from the NARWC for male and female whales were used to assign binary variables, zero and one, to each individual in each year according to survival (1) or death (0) from year t to year $t+1$, as seen in column two of **Table 1**. The mean value of survival for each year was then calculated and the survival component of each individual in each year was determined using Equation 2.3.

The reproductive data from the NARWC was used to assign binary variables to each individual for each year depending on if they had a calf (1) or not (0). Paternity data is not available, so all males received a value of 0. Binary variables were used in both survival and fecundity measures as female right whales cannot have more than one calf a year (Kraus et al. 2007a). Mean values of offspring for each year were calculated and used in the fecundity equation (Equation 2.4) for each individual in every year. Data regarding only females who had been adult for 10 or more years (111 females) from the reproduction calculations were then selected for comparisons with inbreeding coefficients. All calculations were completed using the program R (R Core Team, 2018, version 3.5.1) via RStudio (RStudio Team, 2016, version 1.1.463).

Next, the equation for IR (Equation 2.1) was used to estimate inbreeding for each individual whale alive at some point from 1990-2016 that also had genotype data available from the NARWC (469 individuals). These analyses were completed using the Genhet package in R (R Core Team, 2018, version 3.5.1) created by Coulon (2010). The results, ranging from -1 to 1, represent each individual's inbreeding coefficient.

Finally, I conducted linear regression analyses across three different models for both survival and fecundity. To analyze the correlations seen with survival contributions, one model created was between survival and years alive, one between survival and internal relatedness, and one with both years alive and internal relatedness as well as the interaction between those. The linear regression models using fecundity contributions were created in the same way as the survival data with one model between fecundity and years adult, one between fecundity and internal relatedness, and one with both years adult and internal relatedness as well as the interaction between those. To measure which model represented the best fit for the data, the Akaike Information Criterion (AIC; Akaike, 1987) was used. This method measures the relative expected performance of a series of models, with the lowest measure showing the best fit (Akaike, 1987). In this study, our parameters for survival were internal relatedness and years alive, and for fecundity the parameters were internal relatedness and years adult.

Results

3.1 Fitness: Survival Component

The total number of individuals alive in each year from 1990-2016 showed fairly steady growth in the population (**Figure 2**). Slight declines occurred only in 1995, 1997, 2015, and 2016. This population growth is reflected in the mean values of survival each year, which remained close to one, indicating that the majority of individuals survived each year (**Figure 3**). The lowest mean value of survival was 0.965 in year 1997, suggesting that 3.5% of the population died that year, and the highest was 0.993 in 1992, suggesting that 99.3% of the population survived. While yearly values did not change a significant amount (**Figure 3**), variation did occur in the data from year to year with the largest difference being -0.028 between 1992 and 1993 (**Figure 4**).

The total survival contribution for each individual over the 26 year period varied between individuals, with the majority having survival values over zero (**Figure 5**). The greater number of positive survival values compared to negative survival values reflects the previous data in **Figures 2** and **3**, indicating that most individuals survived from year to year. The lowest individual contribution to survival was -0.0033 and the highest individual contribution to survival was 0.0014.

3.2 Fitness: Fecundity Component

The total number of births varied greatly across the years; ranging from only one calf born in 2000 to 39 calves born in 2009 (**Figure 6**). The mean female fecundity value each year (**Figures 7, 8**) follows a similar pattern as **Figure 6**, with variation between years.

Measures of mean female fecundity each year remain low, varying between the lowest value of 0.007 in 2000 and the highest value of 0.211 in 2001. **Figure 7** shows variation in fecundity is greater compared to variation seen in survival (**Figure 3**).

The majority of females had negative contributions, while a small number of females contributed positively (**Figure 9**). A female would have a negative contribution if they did not have a calf, or had a small number of offspring, during the 26 year period of study or if they died early on in the study without having a calf. The total female fecundity contribution values are consistent with **Figure 7**, showing overly low levels of fecundity in the population, with a select few individuals regularly having calves.

3.3 Inbreeding Coefficients

Of the 724 whales in our original photo-identification data only 469 had genetic data available to calculate internal relatedness. Large amounts of variation occurred in the inbreeding coefficients across the 469 individuals. Values ranged from -0.463, being the most outbred, to 0.573, being the most inbred, with a mean value of -0.002 (**Table 2**). In order to compare values of internal relatedness with fecundity, the inbreeding data were filtered for females who were adult for 10 years or more from 1990-2016. Of the 469 individuals with genetic data, 216 were females, and 111 of those females had been adult for over 10 years.

3.4 Analyses Between Inbreeding and Fitness

Survival contributions for the 469 individuals with available genetic data were compared to both the number of years individuals were alive (**Figure 10**) and individual measures

of internal relatedness (**Figure 11**). **Figure 10** shows a strong positive correlation between survival and years alive for both males and females, and **Figure 11** shows a very slight negative correlation between survival and internal relatedness. Fecundity contributions for the 111 adult females were then compared to the number of years the females had been adult between 1990-2016 (**Figure 12**) and the individual measures of internal relatedness (**Figure 13**). Individuals that remained alive during the entire 26 year period of study or survived during a year of population decline had higher measures of fitness, whereas individuals with lower levels of fitness died early in the study period or did not survive during a year of population growth. Therefore, the positive correlation between the number of years alive and measures of contribution to survival is expected.

The Akaike Information Criterion (AIC), used to compare the models, shows the model between survival and years alive has the lowest AIC value of -5273.55 (**Table 3**). Out of the three models, survival and internal relatedness had the highest AIC score of -4917.89, meaning that internal relatedness does not explain much of the variation in survival relative to years alive.

There was a strong positive correlation between fecundity and the number of surviving years as an adult (**Figure 12**), comparable to that seen in **Figure 10**. This is also to be expected as individuals who are adult for a longer amount of time between 1990-2016 have more opportunities to reproduce than others. **Figure 13**, similar to **Figure 11**, shows a slight negative correlation between fecundity contributions and internal relatedness.

While the negative correlation seen in **Figure 13** is slightly stronger than **Figure 11**, it is a fairly weak correlation overall.

The AIC values show the model between fecundity and number of years being an adult had the lowest AIC score of -940.38 (**Table 4**). Out of the three models, fecundity and internal relatedness had the highest AIC score of -900.17, meaning that internal relatedness does not explain much of the variation occurring in fecundity relative to years adult.

Discussion

The values of inbreeding were highly variable, however none exceeded 0.573 (Table 2). It is possible that no higher levels of inbreeding were found due to fertilizations and pregnancies in right whales being less successful between gametes that are genetically similar (Frasier et al. 2013). Therefore any mating between whales that would produce an individual with higher levels of inbreeding would likely not be successful. When comparing measures of expected IR to observed measures of IR across North Atlantic right whale calves, Frasier et al. (2013) found the mean observed IR value of -0.0272 to be significantly lower than that of the mean expected IR value (95% CI = -0.0250-0.0412, $P < 0.019$). Therefore, although in this study the number of individuals we would expect to see over the observed value of 0.573 is unknown, it can be suspected based on the data from Frasier et al. (2013) that the genetic variability measured in this species is still higher than what one would expect from random mating and that is why values over 0.573 were not seen.

Our results show that the measures of inbreeding explain little of the variation seen in fitness for both survival and fecundity. Although slight negative correlations exist in both cases, the variation around these estimates, and their shallow slope, indicate that inbreeding explains little, if any, variance in either fitness measure. There are three possible explanations as to why this trend is seen in the data. One being that inbreeding depression is not impacting the reproductive success of this population and that there are, in fact, other influencing factors. A second is that a relationship between inbreeding and

fitness is not seen due to the study being done in one population rather than across different populations, and a third is that the use of 35 microsatellites is too low resolution to properly quantify measures of inbreeding in this species.

There are five main potential factors that could be influencing the reproductive success in the North Atlantic right whale: environmental contaminants that disrupt endocrine function, body condition/nutrition, genetics, infectious disease, and marine biotoxins (Reeves et al. 2001). If the lack of relationship between inbreeding and fitness seen in my results is due to inbreeding depression not playing a primary role in reproductive success, it is possible that one of the other four factors beyond genetics could be an explanation. Rolland et al. (2016) measured the health of North Atlantic right whales from 1980 to 2008 and found that declines in health coincided with declines in the number of calves born from 1998 to 2000. Considering that the five main factors previously listed would all contribute to the overall health of a right whale, it is possible that these factors, or the interactions between them, are influencing fitness more significantly than genetic factors alone. However further studies would need to be done in order to find which factor has the greatest affect on fitness.

Another possible explanation is that inbreeding is impacting the fitness of this species, however it was not shown in the trend of the data due to the fact that only the North Atlantic right whale population was examined. If all individuals in the population have high levels of homozygosity and are similarly affected by it, the trend line would show little to no relationship. Previous studies have shown that both the genetic diversity

and the calving rates of the North Atlantic right whale are substantially lower than that of Southern hemisphere right whales (Schaeff et al. 1997; Corkeron et al. 2018). Therefore it is possible that if inbreeding and fitness were measured across different populations of right whales, it may show a negative correlation between levels of homozygosity and fitness, with the North Atlantic right whale being considerably more affected by inbreeding depression than other populations.

A final possible reason for the weak correlations found between inbreeding and fitness is that measures of inbreeding were based off of 35 microsatellite markers. Recent uses of genomic data, such as tens of thousands of single nucleotide polymorphisms (SNP's), in studies of inbreeding depression have shown that measures of inbreeding using genomic data are much more precise and accurate than measures calculated based on microsatellites (e.g., Hoffman et al. 2014; Kardos et al. 2016). For example, when comparing numbers of inbred individuals in a study of oilfield mice, estimates based on 27 microsatellite loci were ten times less than that of the number based on ~13,000-15,000 SNP's. (Hoffman et al. 2014). This same study also showed that the estimated effects of inbreeding on the ability of harbour seals to fight off lungworm infections increased nearly five times when using >14,000 SNP's compared to 27 microsatellite loci (Hoffman et al. 2014). Therefore, although 35 microsatellites is a substantial amount of genetic data for a wild population, it is possible that the genetic data used in this study does not provide high enough resolution to quantify inbreeding with adequate precision and accuracy for identifying fitness effects. Further studies should be

conducted based on genomic data to continue testing associations between inbreeding and fitness, which would either confirm my findings or result in more precise measures for the effects of inbreeding depression.

An important result of this study is that we now have quantified measures of fitness for each right whale between 1990-2016, as well as a method to continue analyzing fitness levels in the future. This is extremely useful as it will enable researchers to continue examining patterns in both survival and reproduction and to more accurately assess rates of recovery. These measures of fitness also reveal which individuals are performing well and which individuals are not. This information can then be used to look more specifically at the varying conditions surrounding those individuals to assess the possible contributing factors affecting fitness. Moreover, as North Atlantic right whales are one of the most endangered large whale species, having the ability to measure and track fitness values each year is important in improving conservation efforts.

The methods used in this study, particularly the de-lifing method, are useful in analyzing and quantifying measures of fitness in wild populations in order to assess effects of inbreeding depression. As our knowledge in whether, and to what degree, inbreeding depression impacts wild populations is in its early stages, studies similar to this one, using various other wild species, would be helpful in contributing to our increased understanding around this topic. This has the potential to then also increase our knowledge in whether genetic factors are limiting population growth in other endangered

or declining wild populations and what conservation efforts can be taken to help protect them.



Figure 1.1: Shipboard photograph of male whale 1227. Note the visible white callosity pattern. Photo taken in 2006 by the New England Aquarium.



Figure 1.2: Aerial photo of whale 1245, a female, born in 1982. Photo taken in 2005 by the New England Aquarium. Image comparison shows difference in callosity patterns between individuals.

Table 1: Example calculations for contribution to population growth through de-lifing method. The first column identifies which individuals are present in the population in the given year. The second column shows which individuals survived using a binary number system, with 1 being used if the individual survived from year t to year $t+1$ and 0 being used if the individual did not survive. The third column measures the number of offspring an individual has in year t , only including the offspring that survive to year $t+1$. The fourth and fifth column are the survival and fecundity components of fitness, using the survival and offspring data respectively. Column six is the overall contribution to population growth.

ID	Alive $s_{t(i)}$	Offspring $f_{t(i)}$	Survival component $\frac{s_{t(i)} - \bar{s}_t}{N_t - 1}$	Fecundity component $\frac{f_{t(i)} - \bar{f}_t}{N_t - 1}$	Contribution to population growth $P_{t(i)}$
1000	1	1	0.0357	-0.0179	0.0179
1001	1	2	0.0357	0.1250	0.1607
1002	1	0	0.0357	-0.1607	-0.1250
1003	0	0	-0.1071	-0.1607	-0.2679
1004	1	3	0.0357	0.2679	0.3036
1005	0	1	-0.1071	-0.0179	-0.1250
1006	1	0	0.0357	-0.1607	-0.1250
1007	1	2	0.0357	0.1250	0.1607
Totals	6	9	0	0	0
Means	0.75 (\bar{s}_t)	1.125 (\bar{f}_t)	0	0	0

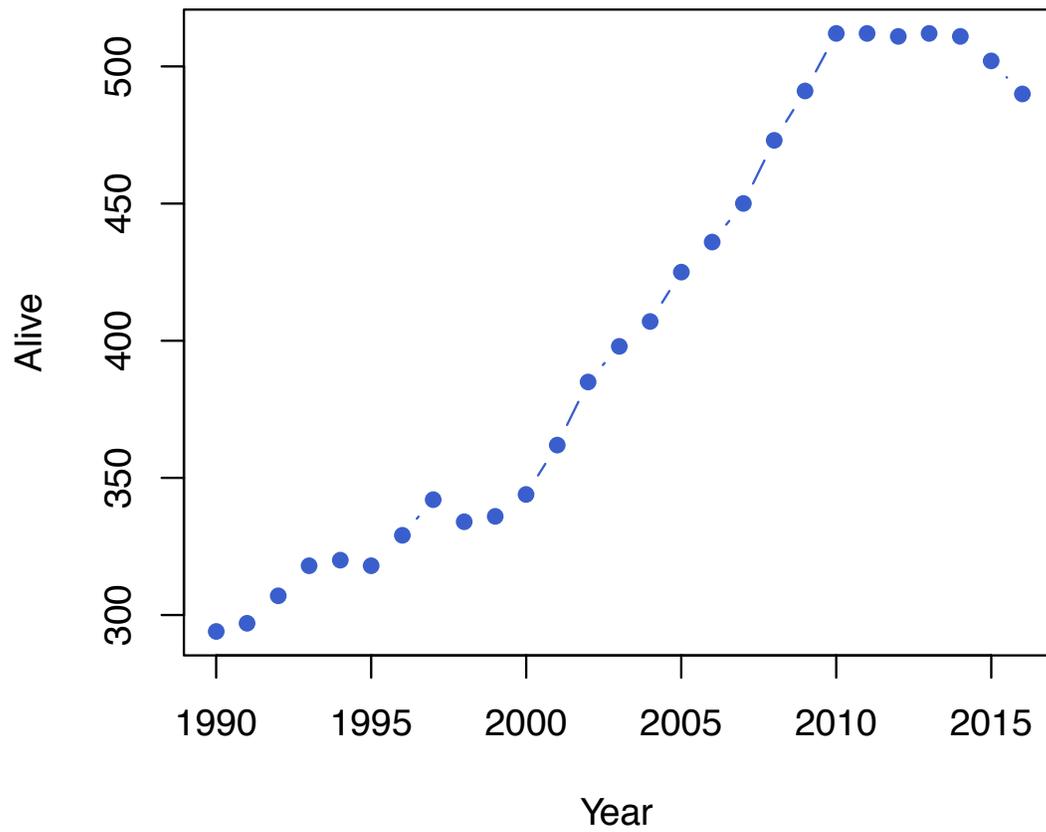


Figure 2: Number of North Atlantic right whales alive each year from 1990-2016.

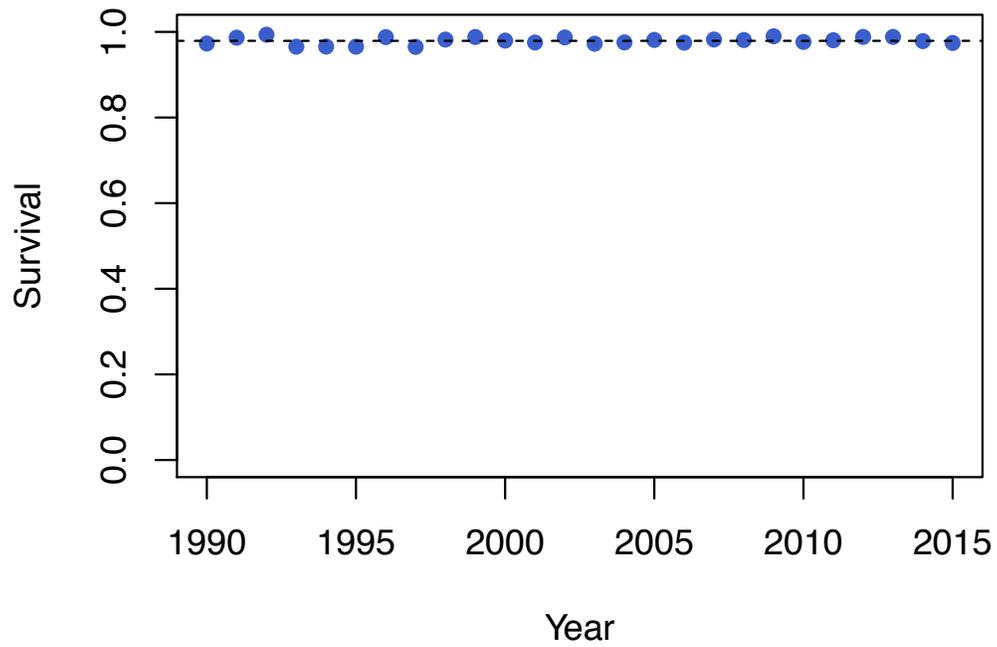


Figure 3: Mean values of survival in the population each year from 1990-2016. A measure of one indicates every individual survived and zero indicates every individual died that year. The dashed line is the mean of all years (0.979).

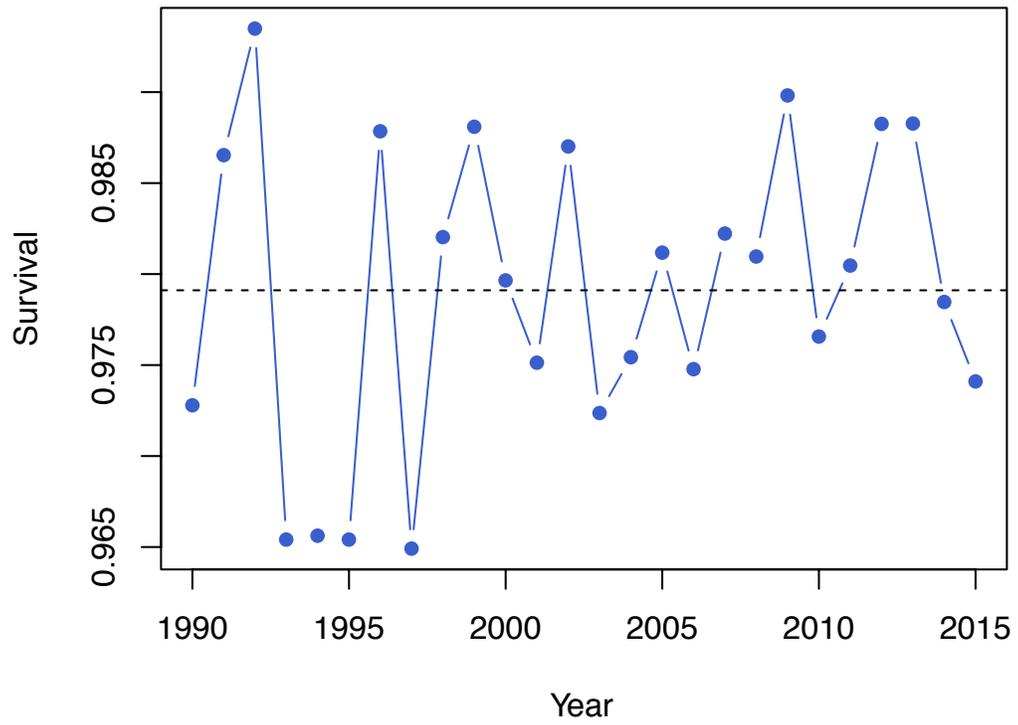


Figure 4: A zoomed in image of mean survival values each year from 1990-2016. This shows the variation occurring in survival from year to year. The dashed line is the mean of all years (0.979).

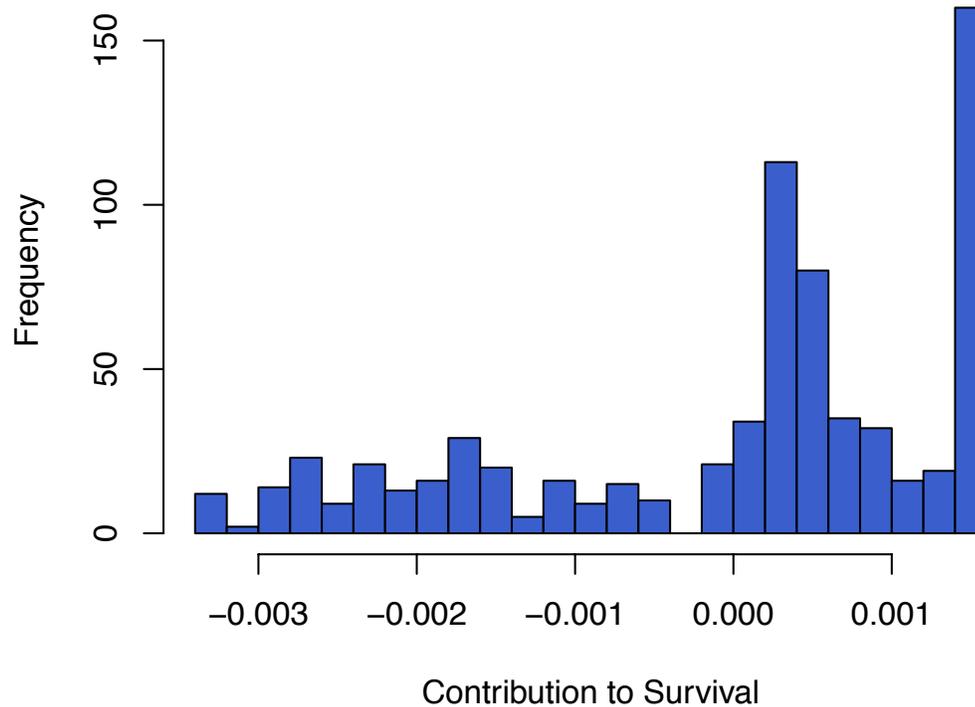


Figure 5: Total contributions to survival for each individual in the population between 1990-2016 (724 individuals).

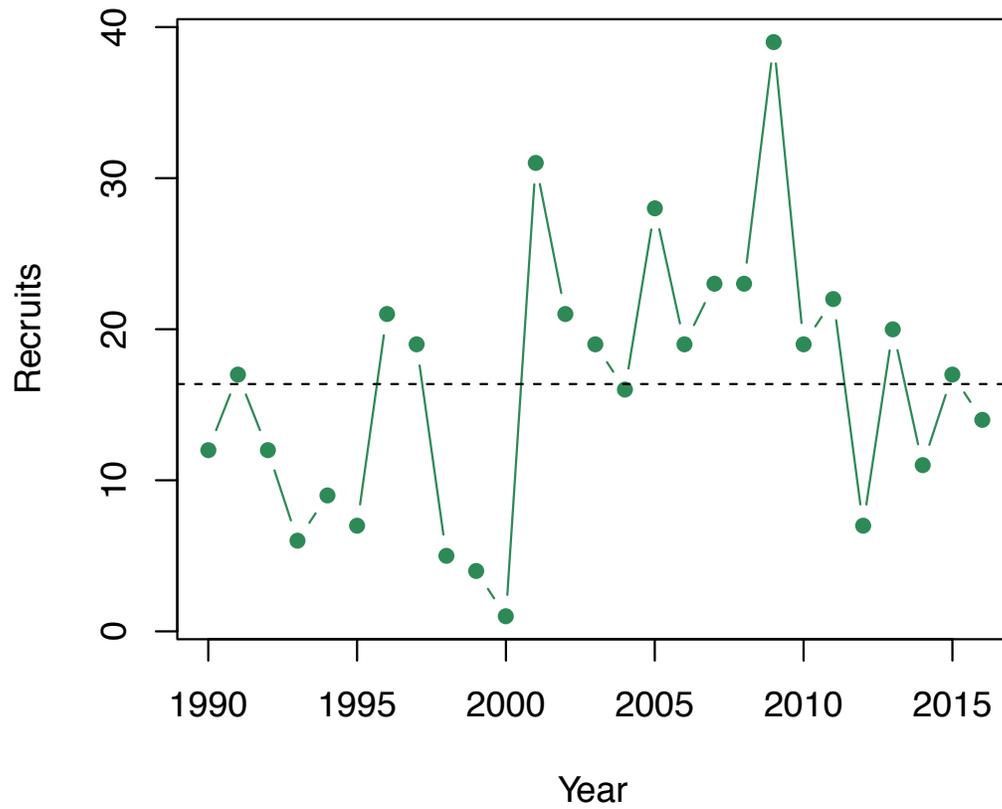


Figure 6: Total number of calves born each year from 1990-2016. The dashed line represents the mean number of calves born (16.37) over the 26 year period.

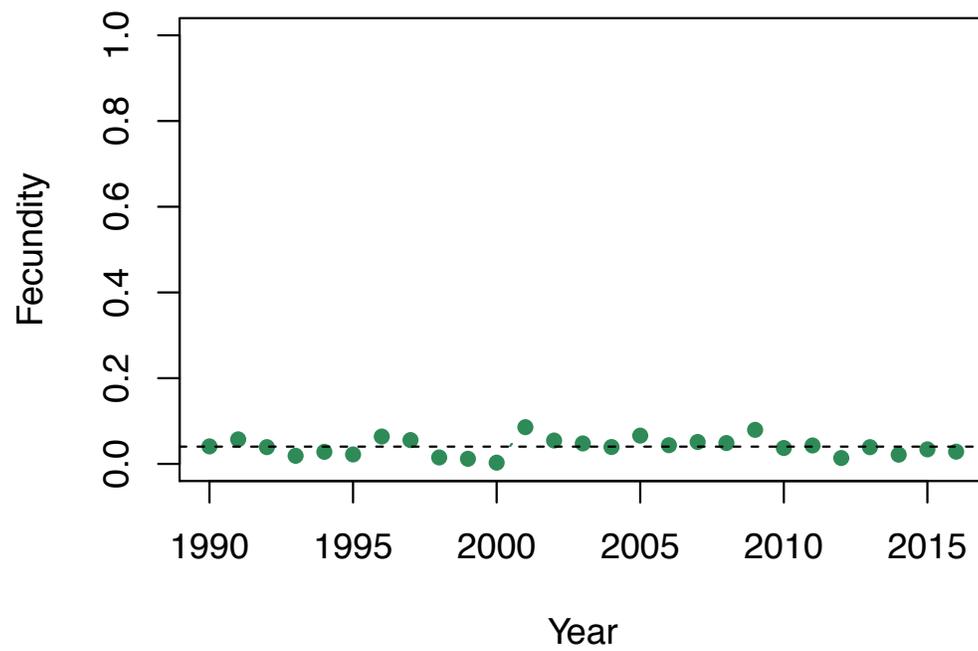


Figure 7: Mean values of fecundity in females each year from 1990-2016. A measure of one indicating every female had a calf and zero indicating no females reproduced. The dashed line is the total mean of all years (0.101).

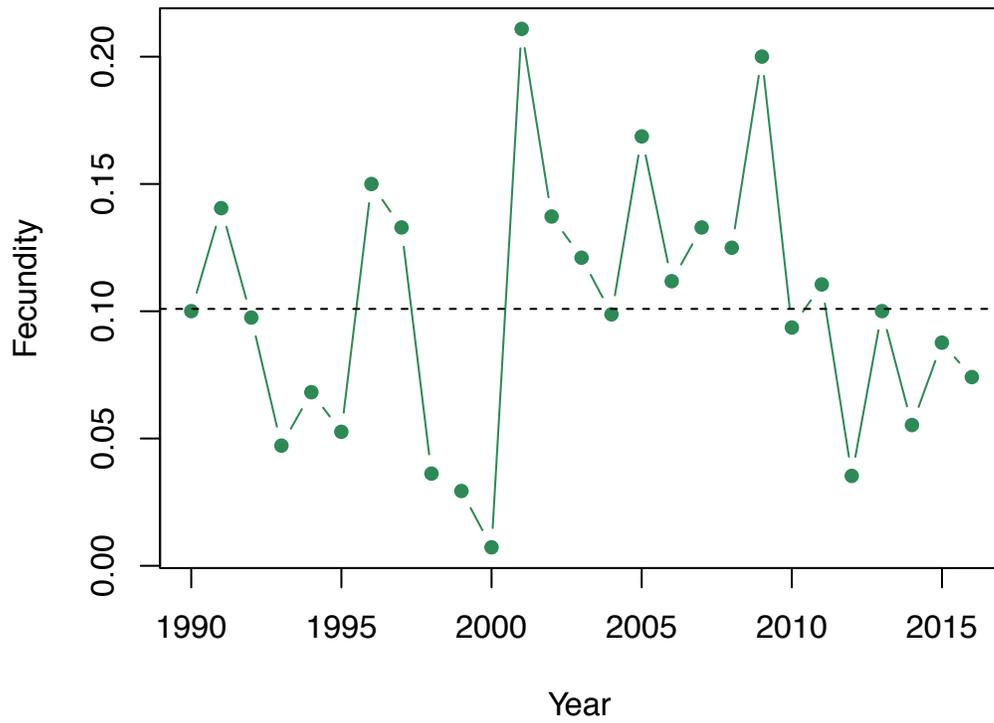


Figure 8: A zoomed in image of mean fecundity values for females each year from 1990-2016. This shows the variation in fecundity occurring from year to year. The dashed line is the total mean of all years (0.101).

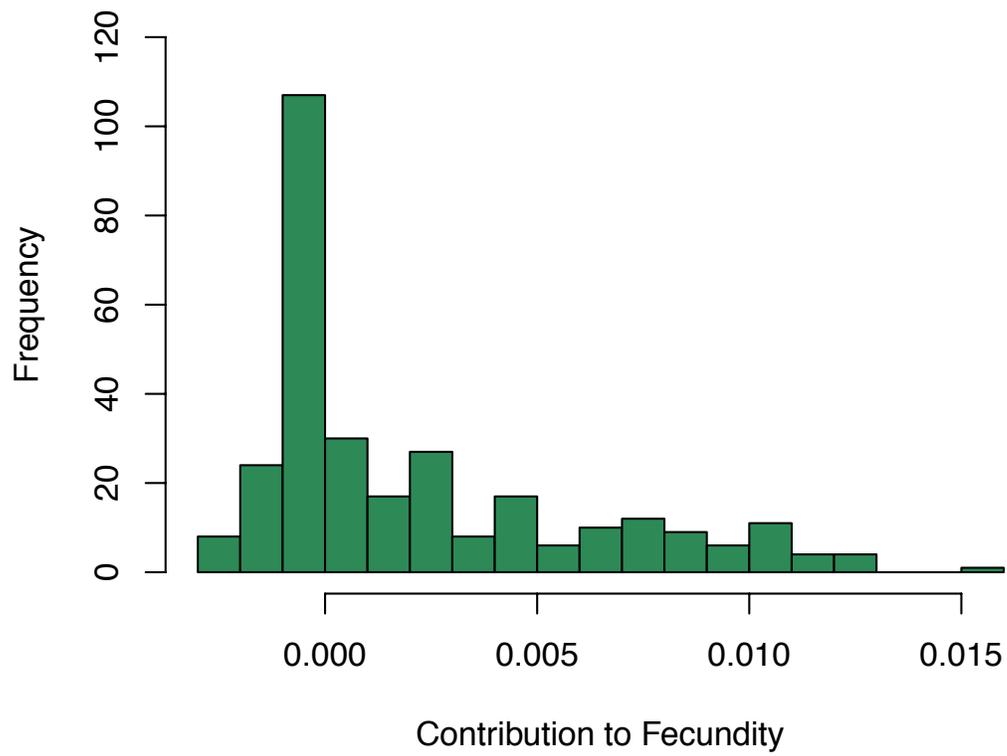


Figure 9: Total contributions to fecundity for each female in the population between 1990-2016 (301 individuals).

Table 2: Maximum, minimum, and mean measures of internal relatedness in the population.

	Internal Relatedness
Most Outbred/Lowest	-0.463
Most Inbred/Highest	0.573
Mean	-0.002

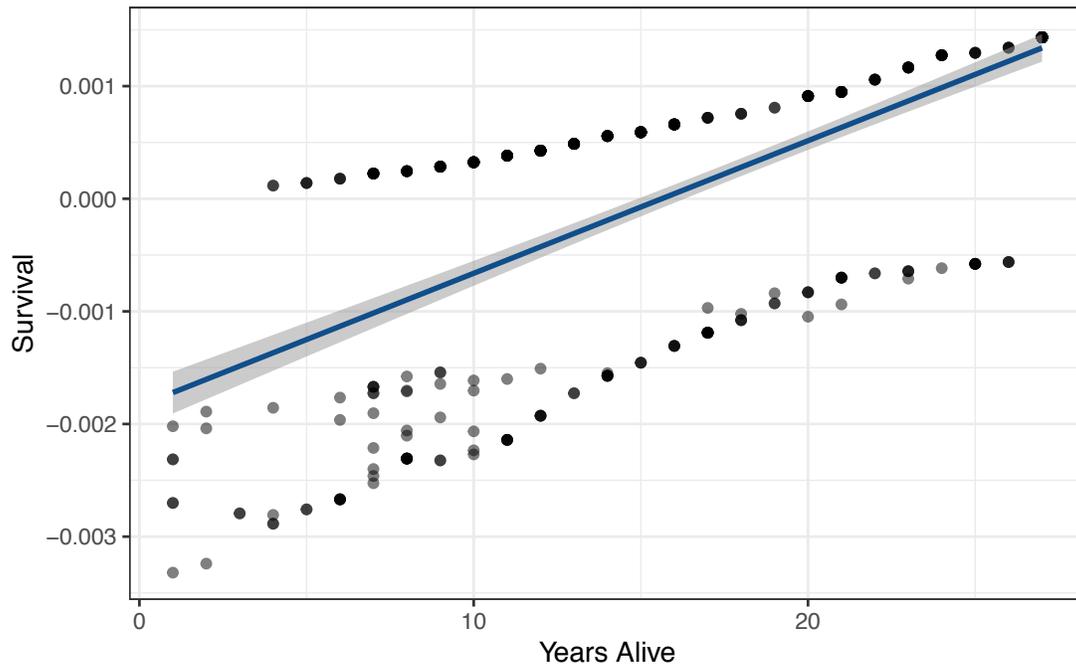


Figure 10: Linear regression between survival contribution and years alive of the 469 individuals with available genetic data. The blue line is the trend line for the data with the grey shading representing the 95% confidence interval.

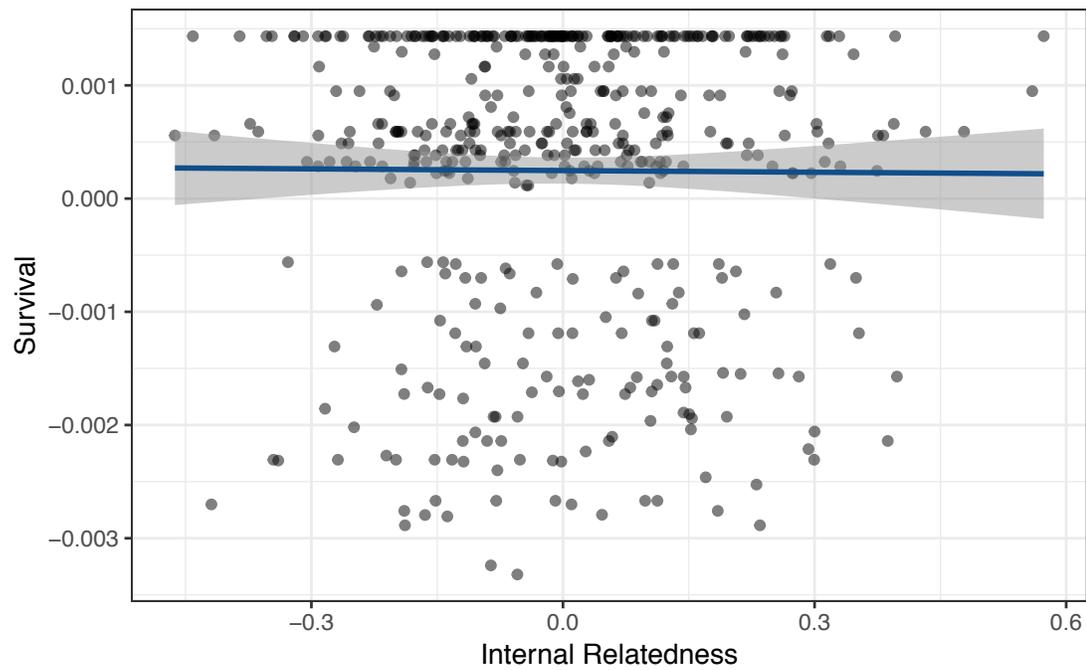


Figure 11: Linear regression between survival contribution and internal relatedness of the 469 individuals with available genetic data. The blue line is the trend line for the data with the grey shading representing the 95% confidence interval.

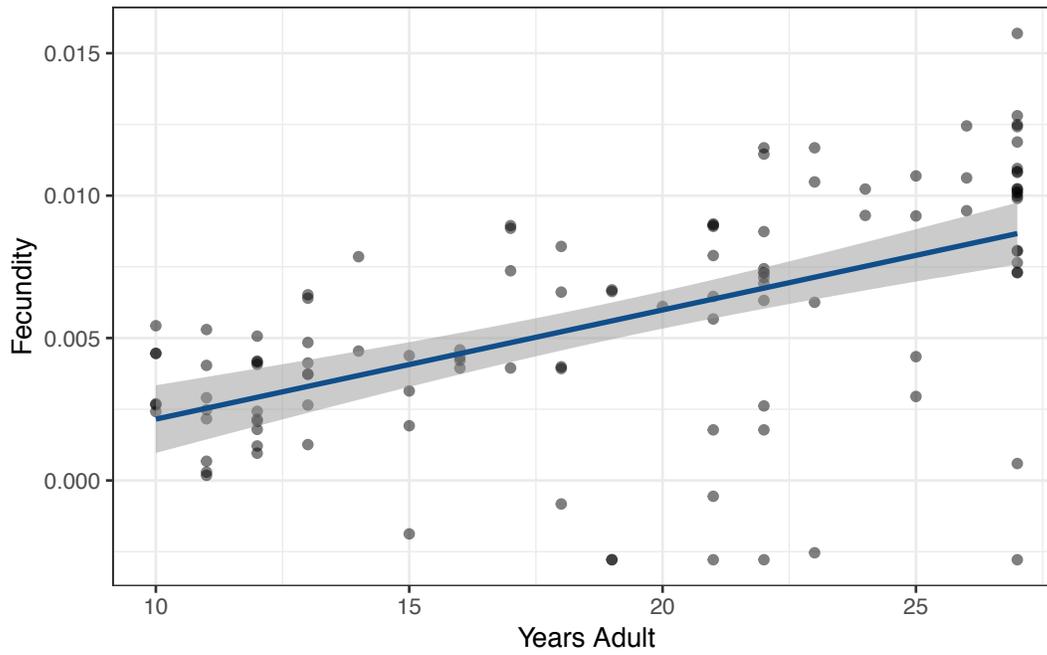


Figure 12: Linear regression between fecundity contribution and years adult of the 111 adult females with available genetic data. The blue line is the trend line for the data with the grey shading representing the 95% confidence interval.

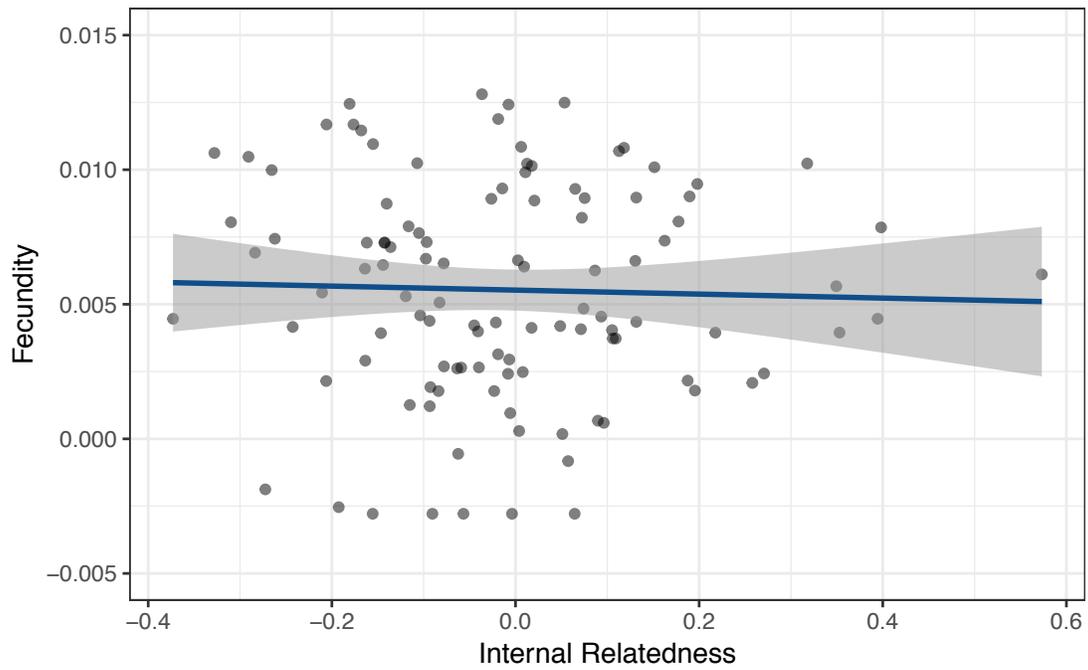


Figure 13: Linear regression between fecundity contribution and internal relatedness of the 111 adult females with available genetic data. The blue line is the trend line for the data with the grey shading representing the 95% confidence interval.

Table 3: Akaike Information Criterion (AIC) of linear regression models using survival contribution data. First model tests linear regression between survival contribution, internal relatedness, and years alive. Second model tests between survival contribution and years alive. Third model tests survival contribution and internal relatedness. Degrees of freedom are measured for each model and the one with the lowest AIC value is the best fit.

Model	df	AIC
Model IR and years alive	5	-5270.700
Model years alive	3	-5273.545
Model IR	3	-4917.891

Table 4: Akaike Information Criterion (AIC) of linear regression models using fecundity contribution data. First model tests linear regression between fecundity contribution, internal relatedness, and years adult. Second model tests between fecundity contribution and years adult. Third model tests fecundity contribution and internal relatedness. Degrees of freedom are measured for each model and the one with the lowest AIC value is the best fit.

Model	df	AIC
Model IR and years adult	5	-936.6777
Model years adult	3	-940.3819
Model IR	3	-900.1685

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