

Patterns of male reproductive success in a highly promiscuous whale species: the endangered North Atlantic right whale

T. R. FRASIER,* P. K. HAMILTON,† M. W. BROWN,† L. A. CONGER,† A. R. KNOWLTON,† M. K. MARX,† C. K. SLAY,† S. D. KRAUS† and B. N. WHITE*

*Natural Resources DNA Profiling and Forensic Centre, DNA Building, Trent University, 2140 East Bank Drive, Peterborough, ON, Canada K9J 7B8, †New England Aquarium, Edgerton Research Laboratory, Central Wharf, Boston, MA 02110, USA

Abstract

Parentage analyses of baleen whales are rare, and although mating systems have been hypothesized for some species, little data on realized male reproductive success are available and the patterns of male reproductive success have remained elusive for most species. Here we combine over 20 years of photo-identification data with high-resolution genetic data for the majority of individual North Atlantic right whales to assess paternity in this endangered species. There was significant skew in male reproductive success compared to what would be expected if mating was random ($P < 0.001$). The difference was due to an excess of males assigned zero paternities, a deficiency of males assigned one paternity, and an excess of males assigned as fathers for multiple calves. The variance in male reproductive success was high relative to other aquatically mating marine mammals, but was low relative to mammals where the mating system is based on resource- and/or mate-defence polygyny. These results are consistent with previous data suggesting that the right whale mating system represents one of the most intense examples of sperm competition in mammals, but that sperm competition on its own does not allow for the same degree of polygyny as systems where males can control access to resources and/or mates. The age distribution of assigned fathers was significantly biased towards older males ($P < 0.05$), with males not obtaining their first paternity until ~15 years of age, which is almost twice the average age of first fertilization in females (8 years), suggesting that mate competition is preventing younger males from reproducing. The uneven distribution of paternities results in a lower effective population size in this species that already has one of the lowest reported levels of genetic diversity, which may further inhibit reproductive success through mate incompatibility of genetically similar individuals.

Keywords: endangered species, mating systems, paternity, polygyny, reproductive success, right whale

Received 28 April 2007; revision received 15 August 2007; accepted 28 August 2007

Introduction

The integration of genetic and long-term field data for parentage analyses of wildlife populations is now widespread, providing methods to assess realized reproductive success and investigate aspects of reproductive biology that neither approach could address independently. The first

studies implementing this approach often found large discrepancies between behavioural and genetic estimates of parentage (e.g. Quinn *et al.* 1987; Gibbs *et al.* 1990), and such a result is now common (e.g. Hughes 1998; Worthington Wilmer *et al.* 1999). These studies have led to a new understanding of mating systems and strategies, and the role of natural and sexual selection on the evolution of behaviour, morphology, and physiology (Coltman *et al.* 1999a; Preston *et al.* 2003). Additionally, in the case of small and/or endangered populations, paternity information and

Correspondence, Timothy R. Frasier, Fax: 705-748-1132, E-mail: timothyfrasier@trentu.ca

the resulting data on specific mating pairs reveal otherwise hidden clues into the factors driving variation in individual reproductive success and survival (e.g. Amos *et al.* 2001a, b), and provide insights into the factors influencing recovery (e.g. Amos & Balmford 2001; Vilà *et al.* 2003).

Despite this utility, parentage analyses in baleen whales are rare, likely due to the difficulty in sampling a reasonable percentage of mother–calf pairs as well as potential fathers in these dispersed and wide-ranging species. Although general patterns in male reproductive success have been hypothesized for some species based on interpretation of observed behaviour (e.g. Clapham 2000; Kraus & Hatch 2001), and/or physiological data on male reproductive organs (Brownell & Ralls 1986), little data on individual male reproductive success are available to test these hypotheses. As a result, the details of realized male reproductive success have remained elusive for most baleen whale species.

The one exception to this pattern is the humpback whale (*Megaptera novaeangliae*). Various hypotheses regarding the mating system of this species have been proposed (Clapham 1996), and multiple male mating tactics have been observed, including direct competition between males (Pack *et al.* 1998), and the ‘escorting’ of females (Clapham 1996). Additionally, the intriguing songs of the humpback whale have been hypothesized to form the basis for female choice, with the song being an indicator of male fitness (Tyack 1981). However, more recent data suggest that the songs may function in male social ordering rather than to attract females (Darling & Bérubé 2001). The recent application of paternity analyses to two humpback whale populations gave similar results, and suggested that there is surprisingly little skew in reproductive success between males, resulting in a large male effective population size (N_e) (Nielsen *et al.* 2001; Cerchio *et al.* 2005). This result was unexpected due to the polygynous social mating system of humpback whales and the strong male-biased skew in the operational sex ratio during the mating season. The interpretation is that males using different mating tactics have approximately equal reproductive success, with no one tactic resulting in exceptionally higher reproductive success (Cerchio *et al.* 2005). These studies indicate that the mating system of humpback whales is more complex than previously thought, and add to the growing amount of evidence suggesting that multiple successful mating tactics are more prevalent in wild populations than have previously been assumed (e.g. Hughes 1998; Worthington Wilmer *et al.* 1999).

The mating system of right whales (genus *Eubalaena*) has been of longstanding interest due to the extreme physiological adaptations and behavioural patterns associated with mating that have evolved in these species. Right whales grow to lengths of over 17 m, attain weights of ~74 000 kg, have penises that reach lengths of ~2.3 m, and have testicles that have a combined weight of ~972 kg (Brownell & Ralls 1986). These represent the largest testes of any mammal

(Atkinson 2002), and the highest testes weight-to-body weight ratio and one of the highest penis length-to-body length percentages of all baleen whales (Brownell & Ralls 1986). Moreover, this combined testes weight is vastly larger than expected based on models developed to assess the relationship between testes weight and body weight in other mammalian species (Harcourt *et al.* 1981; Kenagy & Trombulak 1986; Ginsberg & Rubenstein 1990), with the difference between observed and expected testes weight being larger than has been reported for any other mammalian species (e.g. Kenagy & Trombulak 1986). Under the assumption that sperm competition selects for large testes and increased penis length (Ginsberg & Huck 1989; Gomendio *et al.* 1998), these data suggest that the right whale mating system represents the most intense example of sperm competition in any whale species, and perhaps in any mammal.

Additionally, the mating behaviour of right whales is also suggestive of a mating system based on intense sperm competition. Apparent mating groups are called surface active groups (SAGs), and usually consist of one female (the ‘focal’ female) and anywhere from two to over 40 males competing for the positions closest to her side (Payne & Dorsey 1983; Kraus & Hatch 2001). It appears that females use a specific call to attract males into these groups (Parks 2003), and once a SAG has formed, the female remains inverted at the surface, making copulation difficult. During this time males aggressively compete for positions closest to the female, with those males in the closest proximity being able to copulate when she rolls over to breathe (Kraus & Hatch 2001). Through this behaviour, female right whales incite competition between males. It is hypothesized that by invoking this competition females can selectively mate with males of the highest fitness, representing those males that have the strength and stamina to remain in close proximity for long periods of time (Kraus & Hatch 2001).

Documented SAGs last an average of 1 h (Kraus & Hatch 2001), but can be much longer. Females roll over to breathe an average of once every minute (Kraus & Hatch 2001), and if copulation takes place each time she rolls over (as appears to be the case), then in the average SAG, a female will have copulated 60 times, frequently with different males. Additionally, a case has been documented when a female copulated with two males simultaneously (Mate *et al.* 2005). Thus, the female reproductive tract likely represents an arena of intense competition between sperm from multiple males. Combined, these data suggest that the mating system of right whales involves both precopulatory physical competition between males for mating opportunities, as well as postcopulatory competition in the form of sperm competition. However, there are currently no studies that have assessed if this prominent social mating system is also representative of the genetic mating system in this species (e.g. Hughes 1998), and it is currently unknown how such

intense mate competition would be reflected in the patterns of male reproductive success. Given the endangered status of all right whale species, and their role as perhaps the most extreme example of sperm competition in any mammalian species, there are both practical and theoretical incentives for assessing realized reproductive success to identify the reproductive and genetic consequences of such a mating system.

The North Atlantic right whale (*Eubalaena glacialis*) is arguably the world's most endangered baleen whale, with only 300–350 individuals representing the entire species (IWC 2001). Moreover, despite international protection for over 70 years, and research and conservation actions for over 25 years, there have been little-to-no signs of recovery (IWC 2001), and some studies even suggest that numbers have been declining for over a decade (Caswell *et al.* 1999; Fujiwara & Caswell 2001). Thus, there is great concern regarding the viability of this species, with current research focused primarily on identifying what factors are limiting recovery and developing conservation solutions to mitigate those factors.

Anthropogenic mortality due to ship strikes and entanglement in fishing gear is unquestionably one of primary factors limiting recovery, and reducing these mortalities is the focus of the majority of conservation initiatives (Johnson *et al.* 2007; Knowlton & Brown 2007; Moore *et al.* 2007). However, in addition to being plagued by a high rate of anthropogenic mortality, this species also suffers from a compromised reproductive potential. Female North Atlantic right whales are capable of giving birth once every 3 years (Knowlton *et al.* 1994; Kraus *et al.* 2001). The current population size estimate is 300–350 individuals, the sex ratio is ~50:50 (Brown *et al.* 1994), and it is estimated that 60% of the females are adults (Hamilton *et al.* 1998), resulting in ~90–105 adult females. Therefore, since females are capable of giving birth every 3 years, it is expected that ~30–35 calves should be born per year. This expectation also seems appropriate based on data from South Atlantic right whale populations (*E. australis*), whose growth rates are consistent with the average female giving birth approximately once every 3 years (Best *et al.* 2001; Cooke *et al.* 2001). However, despite a slight increase in calf numbers in recent years (Kraus *et al.* 2007), the average number of calves born per year has remained relatively steady at ~13/year (Kraus *et al.* 2001, 2007), indicating that the reproductive performance of this species is three times lower than expected. The individual-based evidence for this reduced reproductive performance is that there is a high percentage of adult females who have markedly low reproductive success; representing those females that have either never had a documented calf or those having extremely long interbirth intervals. The factors resulting in this reduced reproductive rate are unknown, but hypotheses include intrinsic factors such as the low level of genetic variability and/or inbreed-

ing, and extrinsic factors such as reduced habitat quality (e.g. nutritional stress and biotoxins), disease, and pollution (e.g. Kraus *et al.* 2007). Due to concerns regarding the influence of this reduced reproductive performance on species recovery, obtaining a better understanding of the reproductive biology of the North Atlantic right whale, including the factors influencing reproductive success, is a high priority.

Here we combine over 20 years of photo-identification data with high-resolution genetic data for the majority of individual North Atlantic right whales (278 individuals including 87 mother–calf pairs and 116 candidate males) to assess paternity in this species. These data are used to test hypotheses of realized male reproductive success based on expectations from behavioural and/or physiological data. Specifically, we test the hypotheses that the patterns and variance in male reproductive success, and age of male first fertilization, are different than expected in a random mating system but consistent with the expectations based on the current view of mate competition. These data allow for estimation of the skew in male reproductive success, and provide insights into how this social mating system influences patterns of male reproductive success and what the subsequent implications are for the reproductive biology and effective population size of this species.

Methods

Photo-identification

Individual North Atlantic right whales were identified based on natural markings, primarily callosity patterns on the head, but also including any other body pigmentation or scarring patterns. Callosity and pigmentation patterns are stable and individual-specific characteristics of right whales that provide reliable methods for individual identification and have formed the basis for right whale research worldwide (Payne *et al.* 1983; Kraus *et al.* 1986a; Best 1990). Systematic photo-identification surveys of North Atlantic right whales have been ongoing since 1979 (Brown *et al.* 2007), and take place almost year-round and throughout the whales' known range, resulting in data on the movement patterns (e.g. Brown *et al.* 2001), visual health status (Pettis *et al.* 2004), and behaviour (e.g. Kraus & Hatch 2001) of individual whales throughout the seasons, as well as throughout their lifetimes. Photo-identification data from 1980 through 2001 were used for these analyses.

Right whale calves are not weaned until after approximately 12 months (Hamilton *et al.* 1995), and mother–offspring relationships were determined behaviourally based on these stable associations between mothers and their calves (Knowlton *et al.* 1994; Kraus *et al.* 2001). The ages of individuals were determined directly based on the known year of birth, and individuals were considered to be of

unknown age if they were added to the catalogue as adults or juveniles, and therefore the year of birth was unknown. Gender was determined using both field and molecular techniques. In the field, gender was determined based on direct observation of the genital area (Payne & Dorsey 1983), and/or, for females, based on the stable association with a calf (Knowlton *et al.* 1994).

Sample collection and genetic profiling

Skin samples were collected from individual right whales using a crossbow with a modified bolt and tip as described in Brown *et al.* (1991). DNA was extracted and gender was determined for all samples as described in Shaw *et al.* (2003). All samples were genotyped at 35 microsatellite loci using the multiplex PCR protocols described in Frasier *et al.* (2006). Twenty-eight of these 35 loci were used in subsequent paternity analyses: two loci were removed due to high estimated frequencies of null alleles, and five were removed due to significant signs of linkage disequilibrium (Table S1, Supplementary material). The estimated variability for each of the 28 loci used in the analyses, and the details of why the seven loci were removed, are provided in Table S1, Supplementary material. Overall, the average allelic diversity (A), observed heterozygosity (H_O), expected heterozygosity (H_E , Nei 1978), and polymorphic information content (PIC, Botstein *et al.* 1980) for the 28 loci are 3.64, 0.32, 0.32, and 0.289, respectively (see Table S1 for details). These low estimates of genetic variability are comparable to those found in other endangered species and/or populations where genetic factors are allegedly influencing reproduction and/or recovery, such as the cheetah (*Acinonyx jubatus*) ($A = 3.4$, $H_O = 0.39$; Menotti-Raymond & O'Brien 1995), and the Texas State Bison Herd (*Bison bison*) ($A = 2.54$, $H_O = 0.38$; Halbert *et al.* 2004).

Paternity analysis

Paternity analyses were conducted independently for each year of the study (1980–2001) to account for a changing pool of candidate males and the maturation of calves from earlier years into parents in subsequent years. The analyses were then based on the sampled mother–calf pairs and the candidate males available for each year. Males were considered as candidates if they were either of unknown age or at least 5 years old, and were alive in the year prior to when the calf was born. Although females become sexually mature at approximately 8 years of age (Kraus *et al.* 2001), and this is also often assumed for males (e.g. Hamilton *et al.* 1998), it is not actually known at what age males become sexually mature. Therefore, to prevent the erroneous exclusion of younger males as candidate fathers, males were considered candidates if they were at least 5 years old in the year of

fertilization. This approach should be conservative (e.g. estimating that there are more candidate males than are actually available), as males in other baleen whale species for which there are data appear to reach sexual maturity at a similar age as females (e.g. Clapham 2000).

Paternity analyses for each year were conducted using three different methods: (i) the exclusion approach, (ii) the likelihood approach implemented by CERVUS version 2.0 (Marshall *et al.* 1998), and (iii) the randomization approach of NEWPAT version 5 (Worthington Wilmer *et al.* 1999). The exclusion approach is the method used for paternity testing in human forensic cases, and is also frequently used for paternity analyses of wildlife populations (e.g. Vigilant *et al.* 2001). With the exclusion approach, the genotypes of mother–offspring dyads are compared and the paternal alleles inherited by the offspring are identified. The genetic profiles of all candidate males are then compared with the inferred paternal alleles, and all males whose genotypes are not consistent with the putative mother–offspring–father relationship are excluded as potential fathers. Paternity is assigned when only one male remains nonexcluded as the father (e.g. when all other males are excluded based on a mismatch with the mother–offspring dyad at one or more loci). In this scenario, the offspring must share one allele at each locus with the mother, and share the other allele at each locus with the nonexcluded male. Assigned fathers could not mismatch the mother–offspring dyad at any locus. Paternity was considered unresolved if more than one male was nonexcluded.

In situations where a large number of loci are used and a high percentage of the candidate males are sampled, paternities assigned through the exclusionary approach are likely to represent the true fathers. However, this method could result in a downward bias in assigned paternities due to cases where there are multiple nonexcluded males (Chakraborty *et al.* 1988), or if some true fathers are excluded due to mismatches resulting from mutations or genotyping errors (Hoffman & Amos 2005). To account for these potential sources of bias, two other methods of paternity assignment were also used; those implemented by the programs CERVUS and NEWPAT.

The program CERVUS (Marshall *et al.* 1998) uses a likelihood-based method of paternity assignment based on the difference in the likelihood between the two most likely males (Δ). The Δ -value is calculated for the two most likely candidate males for each offspring and simulations are then conducted to estimate the critical values of Δ associated with assigning paternity with a particular level of statistical confidence (Marshall *et al.* 1998). Using this approach, paternity analyses were conducted for right whale calves using strict (95%) and relaxed (80%) levels of statistical confidence in paternity. Simulations were based on 10 000 cycles, a genotyping error rate of 0.010, and allowing individuals to be missing data for up to three of

the 28 loci. The number of candidate males and proportion of sampled candidates varied accordingly for each year.

The program NEWPAT takes a slightly different approach to paternity assignment and is based on a randomization process. Briefly, males are identified whose genotypes are consistent with the putative mother–offspring–father relationships. Once such a relationship is found, a file of ‘pseudomales’ is generated from the allele frequencies. The pseudomales with relatedness values equal to or higher than that between the candidate male and the offspring are then tested against the mother–offspring genotypes to yield the probability that such a male would be consistent with the mother–offspring–father relationship by chance alone in the data set (Worthington Wilmer *et al.* 1999). For the right whale paternity assessment, analyses were conducted allowing no mismatches (strict), and allowing one mismatch (relaxed) between the putative father and the mother–offspring dyad. A file of pseudomales 1000 times larger than the sampled candidate male data set was generated for each test, the acceptable probability of null matches was set at 0.03, and a maximum of three unscored loci were allowed. Paternity was assigned if (i) only one male was consistent with the putative mother–offspring–father relationships under the chosen criteria and the probability of finding such a match by chance was < 0.2 , or (ii) if multiple males met the mother–offspring father criteria, paternity was assigned if only one male had a probability of finding such a match by chance of < 0.2 .

Patterns of paternity in nonsampled fathers

To estimate how many males may have fathered those calves for which all sampled males could be excluded as fathers, the paternal relatedness of these calves was estimated using the program DADSHARE (www.zoo.cam.ac.uk/zoostaff/amos/). The program begins by comparing the genetic profiles of the known mother–offspring pairs to infer the paternal portion of the profile. A relatedness matrix of these inferred profiles is then calculated using the method of Queller & Goodnight (1989). A dendrogram is developed based on the relatedness matrix that indicates which offspring are consistent with having the same father. Additionally, the relatedness values associated with each node are indicated.

To simulate a few males fathering *all* offspring, DADSHARE performs Monte Carlo simulations to generate randomized data sets where one male fathers all the offspring, and where 2, 3, 4, and 5 fathers share all offspring equally. To simulate each male fathering *only a few* offspring, Monte Carlo simulations were performed where all fathers have one, two, three, four, and five offspring each. The average r -value (and associated standard deviations) of the external nodes generated from the simulations then provided a reference

with which the observed average r -value of the external nodes can be compared.

Test of random mating

To test the hypothesis that the distribution of sampled males assigned as fathers for zero, one, or more offspring is consistent with random mating, a randomization approach similar to that implemented by Cerchio *et al.* (2005) was used. These randomizations generated the expected distribution of paternities if all candidate males had an equal probability of fathering calves within each year.

To make the simulation results directly comparable with the results from the paternity analyses, the same data were used for both types of analyses. Specifically, the simulations were based on the sampled candidate males that were used in each year of the paternity analysis, and the number of mother–calf pairs for which paternities were assigned for each year. To simulate the patterns of paternity expected under random mating, these data were used in a four-step process. For the first year of the analyses, the candidate males were randomized (step 1). Fathers for the number of offspring born in that year for which paternities were assigned were then selected (with replacement) from the randomized males (step 2). A list of the males who were assigned paternity was recorded (step 3). The simulations then moved on to the next year, choosing fathers from the list of candidate males, for the number of offspring that had paternities assigned, for that year (step 4). This process was repeated for each year of the study (1980–2001). After the simulations were conducted for all years, the number of calves fathered by each male was summed across all years to generate the expected number of males assigned zero, one, two, three, or more offspring in this data set if mating is random (e.g. all candidate males have an equal probability of fathering offspring). This process (of simulating each year from 1980 to 2001) was repeated 1000 times to generate the mean expected number of sampled males assigned paternity to zero, one, or more offspring under random mating, and the associated standard deviations.

Age of male first fertilization

To test if adult males of all ages have an equal probability of fathering offspring, a simulation/randomization approach similar to that described above was used. However, testing this hypothesis involved generating expected distributions of males of each age assigned paternity in this data set if all candidate males have an equal probability of fathering offspring regardless of age. These simulations were based on the candidate males used in the paternity analyses and the mother–offspring dyads for which fathers were assigned for each year.

Table 1 Paternities assigned for right whale calves. Included are the year, number of sampled mother–calf (M–C) pairs, the number of sampled candidate males used for analyses in each year, and the number of paternities assigned by each method. There were no sampled mother–calf pairs for 1980, 1999, or 2000. Note that the ‘total’ number of sampled candidate males is not additive from the column because many males were candidates in multiple years

Year	No. of sampled M–C pairs	No. of sampled candidate males	Exclusion	Paternities assigned			
				CERVUS (strict)	CERVUS (relaxed)	NEWPAT (strict)	NEWPAT (relaxed)
1981	3	27	1	1	1	1	1
1982	4	39	2	2	2	2	2
1983	2	47	1	1	2	1	1
1984	7	54	3	1	4	3	3
1985	2	56	2	2	2	2	2
1986	3	62	1	1	1	1	2
1987	4	69	2	2	2	2	2
1988	2	75	2	2	2	2	2
1989	9	84	3	3	5	3	6
1990	3	89	0	0	0	0	0
1991	4	92	2	1	3	2	2
1992	6	95	3	1	4	3	4
1993	2	100	1	1	1	1	1
1994	1	105	0	0	0	0	0
1995	3	106	2	2	2	2	2
1996	9	108	6	5	7	5	6
1997	7	109	4	3	5	4	5
1998	3	115	2	2	2	2	2
2001	13	118	7	6	9	7	7
Total	87	128	44	36	54	43	50

For each year, mother–calf pairs were generated that represented the number of mother–calf pairs for which paternity was assigned. The candidate males for that year were randomized, and males were selected (with replacement) as fathers for these offspring. The age of each assigned father was recorded, and this process was repeated for each year of the study. The number of fathers of each age (or of unknown age) was counted across all years to generate the expected age distribution of fathers in this data set if all adult males have an equal probability of fathering offspring, regardless of age. This process was repeated 1000 times to generate mean values for the expected age distribution of fathers, and the associated standard deviations.

Results

Genetic profiling

A total of 278 individuals were genotyped at these 28 loci, including 127 females and 151 males, representing: ~63% of all whales identified through 2001, and ~74% of all catalogued whales alive in 2001, 35% of all mother–calf pairs, and 69% of all candidate males (males of unknown age or over the age of five). This large percentage of sampled

individuals makes the North Atlantic right whale one of the most comprehensively sampled species. Despite the low levels of genetic variability found in this species, the use of a large number of loci has resulted in a high level of resolution, with an estimated probability of identity (P_{ID} , Paetkau & Strobeck 1994) of 5.92×10^{-11} . Additionally, because there are relatives within the data set, we also calculated the more conservative probability of identity among siblings (P_{ID-Sib} , Evett & Weir 1998). This estimate was also low (3.61×10^{-5}), indicating that within this species of ~350 individuals these loci provide adequate resolution to confidently distinguish between the identity of siblings.

Paternity

From 1980 to 2001, 87 mother–calf pairs were sampled and used for the paternity analyses. The fewest paternities (36) were assigned using the 95% confidence level of CERVUS, and the most paternities (54) were assigned with the 80% confidence level of CERVUS (Table 1). The other methods (exclusion, and using both the strict and relaxed criteria with NEWPAT) resulted in intermediate numbers of assigned paternities. The paternities assigned using the exclusionary approach and the strict criteria with NEWPAT were almost

identical (Table 1). The only difference was one male assigned a paternity in 1996 by the exclusionary method who was not assigned paternity under the selected criteria for NEWPAT; although he was the only male consistent with the putative mother–offspring–father relationship, the probability of finding such a match by chance was estimated at > 0.2 .

Comparing the results from the exclusionary approach and the strict criteria of CERVUS and NEWPAT shows that the majority of differences are due to males being assigned paternity by exclusionary methods and NEWPAT, who had low likelihood scores as calculated by CERVUS, resulting in fewer males being assigned under the strict criteria of CERVUS. Comparing the relaxed criteria of NEWPAT and CERVUS shows that the primary differences between paternity assignments were the number of allowed mismatches. While males in NEWPAT were only considered potential fathers if they had up to one mismatch with the mother–calf dyad, such a restriction was not made in CERVUS, and therefore a male could mismatch at any number of loci and still be assigned paternity if he had a Δ score larger than the critical value determined for the 80% confidence criteria. This difference is illustrated by the fact that the average number of mismatches for males assigned paternity using the relaxed criteria for CERVUS who were not assigned by the relaxed criteria of NEWPAT was 2.6 (range 1–4).

To subsequently assess patterns of male reproductive success, the paternities assigned in the two most extreme scenarios were used: the strict criteria of CERVUS assigning the fewest paternities, and the relaxed criteria of CERVUS assigning the most. The rationale for this approach is that the true patterns of male reproductive success will likely lie between those estimated based on these two extremes, and therefore the range of possibilities should be captured using this method.

Random mating

At the 95% confidence level, 26 males were assigned paternity for 36 calves, resulting in an average of 1.38 calves/male. A similar average was found at the 80% confidence level, where 36 males were assigned paternity for 54 calves, for an average of 1.5 calves/male. The simulations implemented by DADSHARE suggest that the pattern is also similar for those calves for which all sampled males were excluded as fathers. The observed average paternal relatedness values for calves with unsampled fathers (0.43 and 0.38 under the 95% and 80% criteria, respectively) fell in-between the range of expected values if each successful male fathered one or two offspring each (Fig. 1). These data suggest that the average number of calves per successful unsampled male is also between one and two, and therefore that the patterns of male reproductive success are similar for sampled and nonsampled fathers.

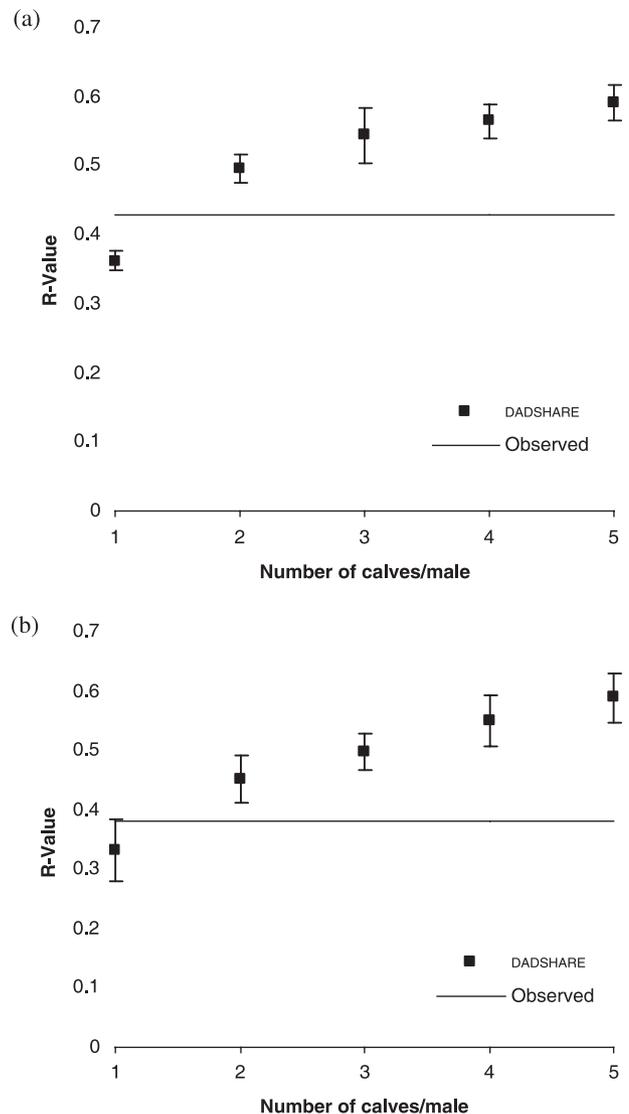


Fig. 1 Paternal relatedness for calves with unsampled fathers. The simulations implemented by DADSHARE provide the mean paternal relatedness values (r value) expected if each successful male fathers 1, 2, 3, 4, or 5 calves each and the associated standard deviations. Plotted are these expected values based on the paternity information from the strict (a), or relaxed (b) criteria, and the standard deviations for these expected values. The dashed line represents the observed average paternal relatedness value for each scenario. In both cases, the observed value falls between those expected if each successful male fathers one or two calves each.

The expected distribution of males identified as fathers for zero, one, or more calves under random mating (as generated by the randomization/simulation processes) fit a Poisson distribution well, as expected (G -tests; both criteria $P > 0.1$). However, the observed distributions differed significantly from these expectations (G -tests; both criteria $P < 0.001$). In both cases, this was due to an excess of males

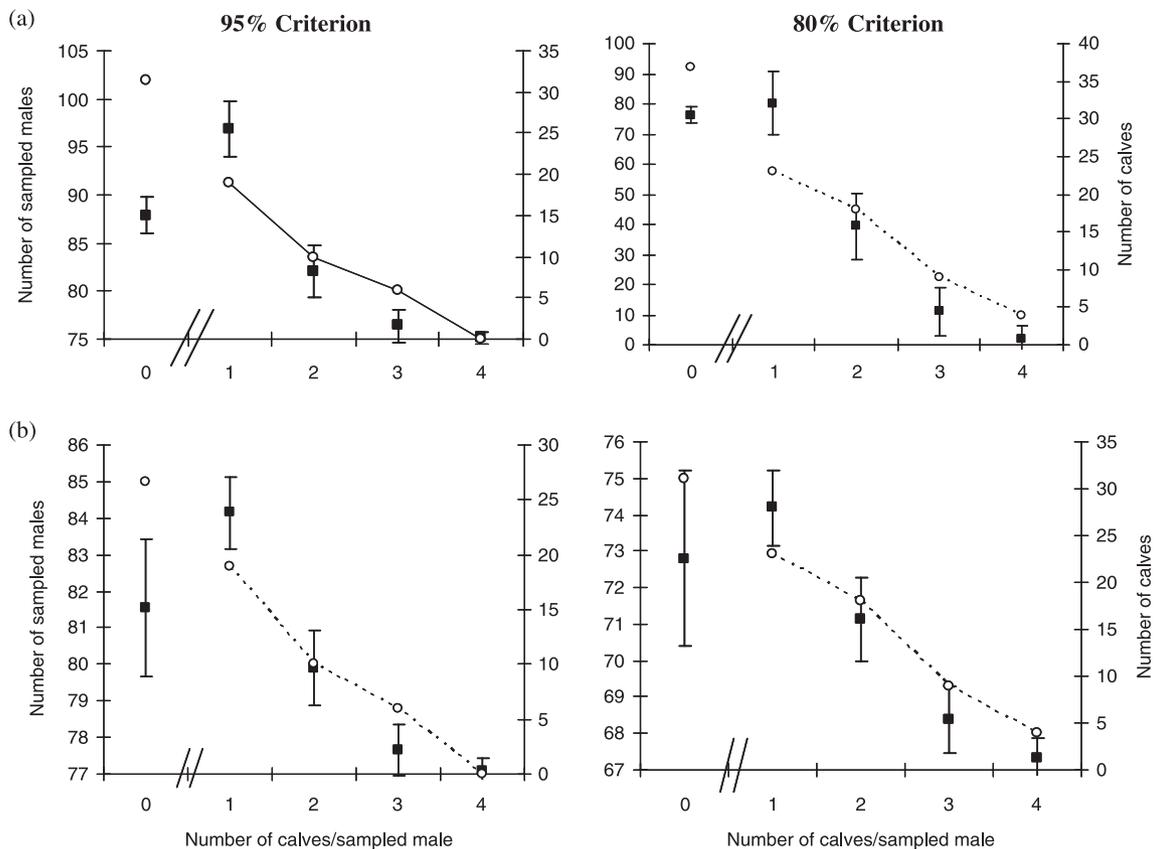


Fig. 2 Male reproductive success in the North Atlantic right whale. For males not identified as fathers for any offspring, the graph shows the observed (open circles) and expected (black squares) number of sampled males not identified as fathering any sampled calves. For sampled males assigned as fathers for one or more offspring, the graph shows the number of calves born to males fathering one or more offspring under the hypothesis of random mating. The mean expected values under random mating and standard deviations are based on the randomization/simulation procedures and the observed paternity distributions are based on the strict (95%) and the relaxed (80%) criteria with CERVUS. Graphs based on (a) including all males ages five and over in the simulations, and (b) including only those males ages 10 and over in the simulations.

not assigned any paternities, a deficiency of males fathering just one offspring, and an excess of males fathering multiple calves than expected (Fig. 2a). These data reject the hypothesis that mating is random in this species, and show that there is more skew in male reproductive success than expected. This skew results in the observed variance in the reproductive success of males assigned one or more paternities being over 1.5 times higher than that expected under random mating based on the randomization/simulation procedures (95% criteria, observed variance = 0.41, expected = 0.23; 80% criteria, observed variance = 0.6, expected = 0.24).

Candidate males used for the random mating simulations represented those males ages 5 years and over, and represent exactly the same males as those used in the paternity analyses. However, if males are not sexually mature until approximately the age of females (e.g. ~8 years), then these analyses would be biased, and include a large number of 'candidate' males who could not really reproduce,

and thus result in the large number of males not assigned any paternities. To address this issue, the random mating simulations were also conducted using just those males ages 10 years or older (or of unknown age). The results are similar (Fig. 2b), and the difference between the observed and expected distribution of paternities among males is still significantly different ($P < 0.05$ for both criteria).

Age of first fertilization for males

No males under the age of 10 years (in the year of fertilization) were assigned paternities using any method of paternity assignment. This result is in agreement with the general assumption that males do not reach sexual maturity at an earlier age than females. Therefore, for the randomization/simulation procedures that tested the hypothesis that all adult males (regardless of age) have an equal probability of fathering offspring, males were

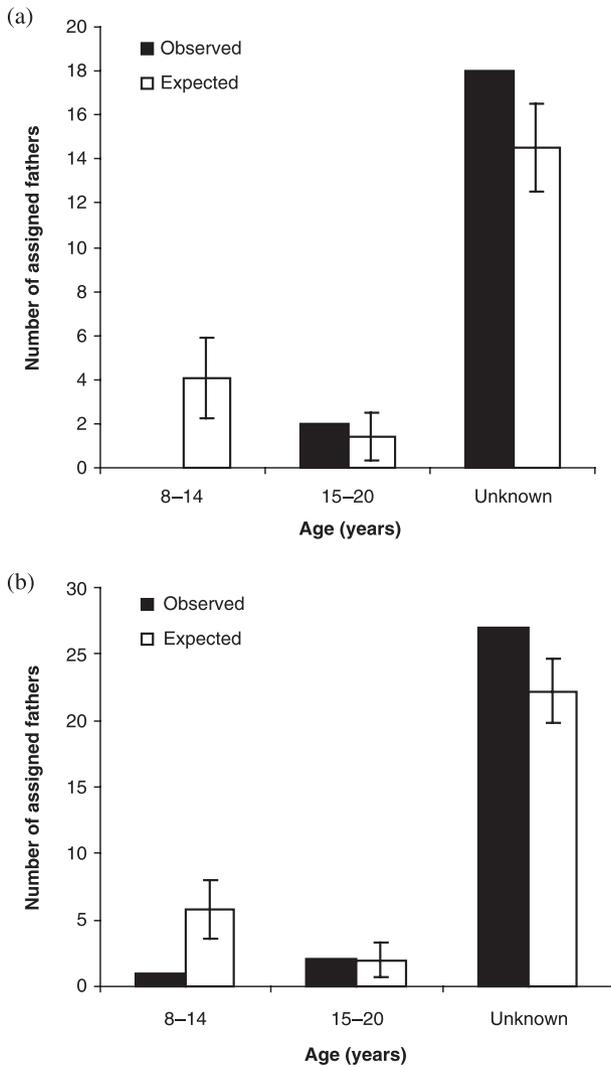


Fig. 3 Age distribution of males assigned paternities. The dashed lines represent the observed number of assigned fathers in the age ranges of 8–14 years, 15–20 years, or of unknown age. The boxes represent the mean expected number (and associated standard deviations) of assigned fathers in each age range if all adult candidate males have an equal probability of fathering offspring regardless of age, as generated by the randomization/simulation procedures. Data illustrated are based on the paternity assignments using the strict (a), and relaxed (b) criteria of CERVUS.

considered candidates if they were at least 8 years old in the year of fertilization, based on the estimated age of sexual maturity for females (Kraus *et al.* 2001). Under this assumption, the first year that males of known age would be included as candidate fathers would be 1989. These would represent those males born in 1980 who became sexually mature at the age of eight in 1988, and then were potential fathers for the calves born in 1989. Males of known age make up only a small proportion of the candidate males in 1989, but the number of known age candidate males has been slowly

increasing throughout this study. Therefore, to test this hypothesis, the randomization/simulation analyses were based on the data from the last decade of the study period (1992–2001).

Using the strict criteria for paternity assignment, only three males of known age were assigned paternities during this time period (as well as throughout the entire study), one was 17, and the other two were 15 years old in the year of fertilization. This pattern is significantly different than expected if all adult candidate males had an equal probability of fertilization regardless of age (G -test, $P < 0.025$), with fewer males ages 8–14 years, and more males of unknown age fathering offspring than expected (Fig. 3a). Using the relaxed criteria, four males of known age were assigned paternities, the three from the strict criteria, as well as one male who was 10 years old in the year of fertilization. This pattern is also significantly different than expected (G -test, $P < 0.05$), with fewer males age 8–14 years, and more males of unknown age assigned paternities than expected (Fig. 3b). It is noteworthy that the 10-year-old male assigned paternity under the relaxed criteria mismatches the putative mother–offspring–father relationship at three loci, and was not assigned paternity using any other method, and is therefore not likely the true father. Removing this individual from the analysis increases the difference between the observed and expected patterns of paternity (data not shown).

Sighting histories of individual whales can be used to gain further information on the age of assigned fathers who were of unknown age. For this analysis, the males assigned paternity using the 95% criteria with CERVUS, and with the exclusionary approach were used. Males assigned paternity based on the 80% CERVUS criteria were not used here because many of the males assigned using this approach who were not assigned by the exclusionary method mismatched the putative mother–offspring–father relationships at multiple loci, and were therefore not likely the true fathers. In situations such as this, where individual reproductive success is being evaluated, it is desirable to use the more stringent criteria because random false exclusion of true fathers will reduce samples size but will not bias results, whereas including incorrectly assigned fathers will bias these results (e.g. Cerchio *et al.* 2005).

The majority (74%) of unknown age males assigned paternity from 1992 to 2001 based on either the 95% CERVUS or exclusionary criteria have sighting histories going back to the first few years of the study (1980–1981), and were not seen as calves, suggesting that they were born before regular surveys began and are therefore older than any male assigned paternity of known age. Combined, these data suggest that paternity is biased towards older males, and that right whales do not obtain their first paternity until over the age of ~15 years, almost twice the average age of first fertilization (8 years) in females (Kraus *et al.* 2001).

Mate fidelity

The paternities assigned using the 95% confidence criteria in CERVUS and/or the exclusionary methods were also used to assess the potential for mate fidelity in this species, based on an analysis of individuals identified as parents of multiple calves. With the 95% criteria, six females had fathers assigned for more than one of their offspring, and eight males were identified as fathering multiple calves. This resulted in 24 different pairwise comparisons of offspring who share at least one parent and for which both parents were identified. With the exclusionary method, nine females had fathers assigned for more than one of their offspring, and 11 males were identified as fathering multiple calves. This resulted in 33 pairwise comparisons of offspring who share at least one parent and for which both parents were identified. In only one case (the same case under both criteria) did two offspring share both parents, and in-between these events, the female involved had an offspring with a different male. These data are in agreement with expectations based behavioural and morphological data (as well as the paternity data presented above) suggesting that right whales do not exhibit mate fidelity.

Discussion

Paternity analyses

It is noteworthy that there was very close agreement between the different paternity assignment methods used in this study. The method of exclusion resulted in 44 paternity assignments. With the 80% confidence level criterion of CERVUS, 54 paternities were assigned. However, ~20% of these are expected to represent incorrect males being assigned as fathers (Slate *et al.* 2000). Accounting for these results is an estimate of 43.2 correct paternities, which is almost exactly the number of paternities assigned under the exclusion criterion. The 95% confidence level criterion of CERVUS assigned paternity to 36 calves; however, this approach is known to not assign paternity to some true fathers (those with common alleles) due to low Δ scores, even if they do not mismatch the mother-offspring dyad at any loci (Marshall *et al.* 1998; Slate *et al.* 2000). To estimate the expected percentage of sampled true fathers that were excluded using this criterion in the data set, the paternity simulation function of CERVUS was used. The genotyping error rate estimated for these data is ~0.001, based on genotype comparisons of individuals that have been sampled multiple times ($N = 101$ duplicate sampling events) and comparisons of known mother-calf pairs ($N = 92$) (Frasier 2005). With this error rate and the allele frequencies in this population, the simulations indicate that 18% of the true fathers that are sampled would not be assigned paternities under the 95% criterion. Put another way, the 36 assigned

fathers likely represent only 82% of the true sampled fathers. Accounting for this bias results in an expectation of 43.9 true sampled fathers in our data set. Again, this result matches what was obtained using the exclusionary method.

The strict criterion using the method of NEWPAT resulted in 43 paternity assignments, which again is almost identical to those assigned based on exclusion. However, allowing one mismatch in NEWPAT (relaxed criterion) resulted in the assignment of 50 fathers, indicating that with the exclusionary method, six males were excluded as fathers based on a mismatch at a single locus. Although these may represent true fathers that mismatch due to genotyping errors or mutations, two lines of data suggest that this is not the case and that these males were correctly excluded. First, the estimated genotyping error rate is ~0.001, and therefore it is highly unlikely that six genotyping errors would be observed in a sample of this size. Second, the convergence of all four of the other methods on an estimate of 44 sampled fathers is indicative of the true number of sampled fathers being approximately 44. Combined, these data suggest that these males were correctly excluded, even if it was based on a mismatch at only a single locus.

The close agreement between the paternity assignment methods, after accounting for the known biases associated with each criterion, suggests that the paternities assigned using the exclusionary method are likely the best representation of the actual paternities. These data also confirm that the subsequent tests we performed based on the two extreme criteria for paternity assignment do encapsulate what are likely the true patterns of paternity in this species.

Implications for population size

One of the first results that stands out in this study is the surprising lack of identified fathers given the percentage of sampled males in this species. If 69% of all candidate males have been sampled, then it is expected that fathers for ~69% of all calves should be sampled. However, fathers were only assigned for 51% of the calves, with all males being excluded as the fathers for the remaining 49% (based on the method of exclusion). This represents significantly fewer paternities than expected (G -test, $P < 0.001$). This trend is similar throughout all years of the study (data not shown), indicating that it is not just the result of a lower percentage of males being sampled in the early years. The two potential explanations for this finding are that: (i) the small percentage of nonsampled males are particularly successful, and account for all of these paternities; or (ii) that the population size is larger than is currently estimated, and therefore there are more nonsampled males in this species than currently estimated. The results of the DADSHARE analyses indicate that the patterns of paternity in nonsampled males is similar to that for sampled males, rejecting the hypothesis that the nonsampled males are

particularly successful, and suggesting instead that the population size is larger than is currently assumed.

In North Atlantic right whales, the photo-identification data suggest that the majority of individuals have been identified, and that the direct count of individuals is an accurate census of population size (Clapham *et al.* 1999). Therefore, this genetic inference of whales has significant implications, suggesting that the number of males (and thus the total population size) may be larger than is currently estimated. Similar results have been found in previous studies of other marine mammals, where surprisingly few paternities were assigned despite (presumably) exhaustive sampling of the population (Coltman *et al.* 1998; Worthington Wilmer *et al.* 1999). Given the weight of the implications of this finding on current estimates of population size and assumptions regarding the known distribution for such an endangered species, this aspect of the data will be examined in more rigorous detail than is appropriate here, and will be reported in future studies.

Male reproductive success

The patterns of paternity indicate that there is significantly more skew in male reproductive success than is expected if mating is random, with a significant excess of males not assigned any paternities, a deficiency of males fathering one calf, and an excess of males fathering multiple calves. This skew results in the variance in the reproductive success of fathers being over 1.5 times higher than expected if mating was random. High variance in male reproductive success is expected in polygynous species, and these data suggest that the presumed social mating system of this species, based on both pre- and postcopulatory mate competition, is indeed representative of the genetic mating system. To provide more context for interpretation, these results were compared to other systems where comparable data on male reproductive success have been reported. To make the data comparable across studies, the standardized variance (SV = variance/mean) calculation was used (e.g. Coltman *et al.* 1998), which was either reported directly in the other studies, or estimated from data presented in those studies.

The data on variance in male reproductive success show a clear and expected trend, with known highly polygynous populations having high variance, and populations thought to be only slightly polygynous having a low variance (Fig. 4a). The surprising outlier in this distribution is the horseshoe bat (*Rhinolophus ferrumequinum*), which despite showing reversed sexual dimorphism (females are larger than males), has a higher variance in male reproductive success than even the red deer of Rum (*Cervus elaphus*), which is frequently the textbook example of a highly polygynous mating system (e.g. Feldhamer *et al.* 1999). Historically, it has been generalized that those populations with a standardized variance greater than one are poly-

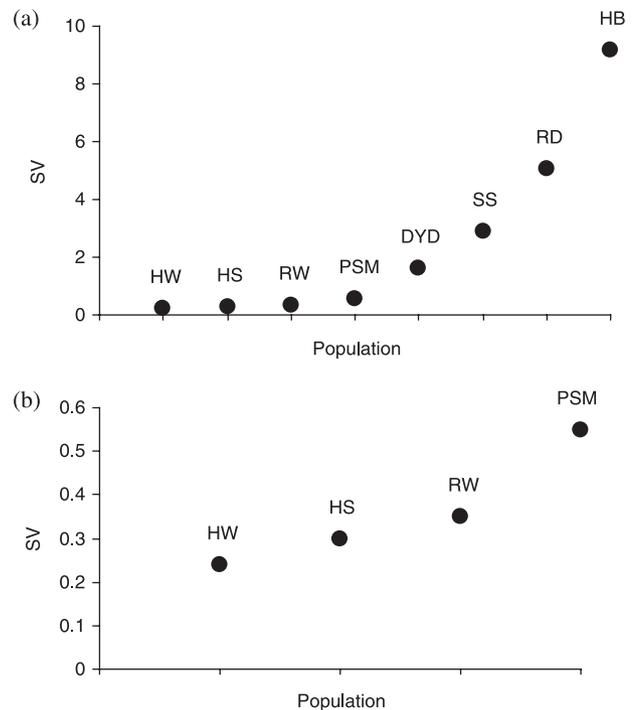


Fig. 4 Standardized variance (SV = variance/mean) in male reproductive success across a range of species. Included are data on the horseshoe bat (HB, *Rhinolophus ferrumequinum*, Rossiter *et al.* 2006), soay sheep (SS, *Ovis aries*, Pemberton *et al.* 2004), red deer (RD, *Cervus elaphus*, Clutton-Brock *et al.* 1982), dasyurid (DYD, *Antechinus stuartii*, Holleley *et al.* 2006), brushtail possum (PSM, *Trichosurus vulpecula*, Taylor *et al.* 2000), harbour seals (HS, *Phoca vitulina*, Hayes *et al.* 2006), humpback whales (HW, *Megaptera novaeangliae*, Cerchio *et al.* 2005), and right whales (RW, *Eubalaena glacialis*, this study). (a) Values for all populations considered (b) close-up of populations whose SV is less than one. In all cases, SV is based only on individuals that have had one or more offspring (e.g. the 'zero paternities' class of males is excluded) due to uncertainty in the size of the potential male gene pool in some studies. In cases where multiple estimates were available (based on strict and relaxed paternity assignment criteria, for example) the average SV value was used.

gynous, and those with a SV less than one are monogamous (Boness *et al.* 1993). This is clearly an oversimplification, as all of the populations examined here are considered to be polygynous; however, another pattern is apparent in the graph based on the degree to which males can control access to mates and/or resources. Theoretically, it is expected that those populations where males can control access to females or resources will have higher variance in reproductive success than those where males cannot (Emlen & Oring 1977). Such a pattern can be seen in Fig. 4, where the SV value appears to be related to the degree to which males can control access to mates. For example, although both soay sheep (*Ovis aries*) and red deer have male dominance hierarchies, red deer harems are relatively stable whereas

soay sheep females are fairly promiscuous (Pemberton *et al.* 1992; Coltman *et al.* 1999b). Thus, red deer males have more control over access to mates, and as a result have a higher variance in male reproductive success.

Similarly, those populations where males have little-to-no opportunity to control access to resources or mates have the lowest SV values (Fig. 4a). Indeed, the three populations with the lowest SV values [harbour seals (*Phoca vitulina*), humpback whales (*Megaptera novaeangliae*), and North Atlantic right whales] are all aquatically mating marine mammals. The environmental potential for polygyny (EPP, Emlen & Oring 1977) is very low for aquatically mating marine mammals, due to a lack of clustered resources to which access can be controlled, and the ability to move in three dimensions limits the ability of males to control access to mates. Thus, in these systems, males must rely almost exclusively on other tactics (e.g. sperm competition) to maximize reproductive success, which clearly do not allow the same degree of polygyny as mate- or resource-defence strategies.

These data on male reproductive success are consistent with the idea of intense sperm competition in right whales. With sperm competition, it is expected that male reproductive success will be correlated with those characteristics involved in the production and delivery of sperm (e.g. testes size and penis length). Males will vary in these traits, resulting in an increased variance in male reproductive success over that expected if mating is random. The fact that the standardized variance in male reproductive success in North Atlantic right whales is higher than that expected if mating is random, and the highest value obtained for all tested aquatically mating marine mammals (including the humpback whale) (Fig. 4b), suggests that sperm competition is indeed resulting in the relatively high SV, and is more intense in this species than in the other marine mammals examined.

The data on testes weight suggest that sperm competition is more intense in right whales than any other mammal. However, the variance in male right whale reproductive success is among the lowest reported. These two perspectives can be reconciled by considering the context in which sperm competition acts in different species. For the majority of mammals, males are able to control access to resources or mates to some extent (e.g. Daly & Wilson 1983). In many of these cases, other reproductive strategies, including sperm competition, may also be influencing reproductive success (e.g. Preston *et al.* 2003; Holleley *et al.* 2006). However, resource- or mate-defence polygyny results in a much higher variance in male reproductive success than sperm competition (Fig. 4). Because the degree to which selection can act on a trait is related to its variance (Fisher 1958; Arnold & Wade 1984), then in these systems selection is expected to be stronger on traits involved in resource- or mate-defence, than on traits involved in sperm competition.

However, in aquatically mating marine mammals, and baleen whales in particular, sperm competition may be the primary (or only) strategy for males to increase their reproductive success. Therefore, sperm competition could be very intense in this species, but still result in low variance in reproductive success relative to those systems where males also compete via resource- or mate-defence. Thus, right whales may indeed represent one of the most intense cases of sperm competition in mammals, resulting in a high variance in reproductive success relative to other marine mammals, but low relative to mammals in general.

One of the primary factors that influence the ability of males to control access to mates is the synchrony of ovulation in females (Emlen & Oring 1977). In humpback whales, there is asynchrony in both female migration times to the mating grounds and ovulation (Clapham 1996). This asynchrony increases the potential for male reproductive skew and competition, by providing an environment where it is possible for one male to fertilize multiple females within a breeding season. However, females are also spread out on the mating grounds, reducing the number of potential mates that one male could conceivably have. Unfortunately, little is known regarding the distribution of female North Atlantic right whales in the mating grounds, or the length and synchrony of estrous. The location of the mating grounds is yet to be identified, and therefore, there are no data regarding female spatial distribution or behaviour during the breeding season. Additionally, there is currently no information available regarding the timing and duration of estrous. Although right whales have a distinct 'calving season' during the winter months, there is wide variation around calving dates, suggesting that births take place over a span of several months (Kraus *et al.* 1986b). These data suggest that female ovulation may also be asynchronous, resulting in the potential for males to compete for multiple mates within a season. This assumption is consistent with the extreme size of right whale testes, which would presumably require strong selection pressure based on sperm competition, and is suggestive of a relatively high potential for polygyny.

Out of all of the paternities assigned, there was only one case where a male fathered more than one calf in a single year. This male (individual 1818) fathered two calves in 2001, a year when 31 calves were born, which is the largest calf year yet recorded for this species. Moreover, although more males than expected fathered multiple calves, the average intercalving interval was 5.7 years, which is not suggestive of particular males having periods of heightened reproductive success. If males formed dominance hierarchies, it would be expected that specific males would have particularly high reproductive success in a clustered period of time, corresponding to their rein at a high rank. However, the clear lack of this pattern supports the hypothesis that the mating system of right whales is not based on dominance

hierarchies. Additionally, only one case was found throughout the 20 years where the same pair mated. These data suggest that full-siblings are rare in this species and, similar to humpback whales (Clapham & Palsbøll 1997), show that right whales are promiscuous and do not show fidelity to specific mates.

Reproductive success and age

In many studies of paternity, age has a significant influence on male reproductive success. This is usually due to older males having larger traits for male–male competition (e.g. horns, Coltman *et al.* 2002), or higher ranks in dominance hierarchies (Widdig *et al.* 2004). A similar pattern was also found for North Atlantic right whales, where paternity was biased towards older males, with most males not obtaining their first paternity until they reach an age that is almost twice the average age in first fertilization in females (~15 years in males vs. ~8 years in females). Due to the fact that in mammals lower energetic requirements are necessary for males to become sexually mature than females (Daly & Wilson 1983), it is likely that males are physiologically capable of becoming sexually mature at a similar age (if not earlier) than females, suggesting that a processes such as mate competition is preventing younger males from reproducing. This result also suggests that there are no other mating strategies available for males, such as younger males ‘sneaking’ paternities, as is found in some other species (e.g. Coltman *et al.* 1999b). However, in right whales, there are no clear external characteristics that would make males more successful with age.

Right whale growth rates slow well before individuals reach the estimated age of sexual maturity (Whitehead & Payne 1981), and studies to estimate the age of individuals based on length and/or length proportions have not had success in ageing whales over the age of 10 years due to the similarity in these measurements of whales ages 10 years and older (Sironi *et al.* in press). Therefore, size does not appear to be related to the increased reproductive success of older males. Additionally, right whales do not have traits for male–male competition that would change with age. It has previously been hypothesized that the callosities on the heads of right whales are used in male–male competition (Payne & Dorsey 1983), but this hypothesis has yet to be rigorously tested. Moreover, callosity patterns are stable throughout the lifetime of the whales, which is what makes them useful for individual identification, and therefore they would not be related to older males being more successful, even if they are used in male–male competition. Thus, there is a lack of external characteristics that seem reasonable as candidate traits for driving the differential reproductive success of older males. Finally, there is no evidence of a dominance hierarchy in right whales, even in apparent mating groups (Kraus *et al.* 2001), and given the

lack of defensible resources and lack of control over female movements, it seems unlikely that a hierarchy exists.

Two reasonable hypotheses that are based on ‘internal’ characteristics are learned skill in surface active groups, and increased testes development in older males. For North Atlantic right whales, SAGs are seen most frequently during the summer and autumn months, when the whales are in feeding habitats. Based on an estimated 12-month gestation period (Best 1994), and calves being born in the winter, these apparent mating groups are not occurring at the right time for fertilization to occur. This has raised the question as to why whales would spend a large amount of time and energy engaged in this activity during a time when feeding should be the highest priority, and when this behaviour is not resulting in fertilizations (Kraus *et al.* 2007). One of the current hypotheses is that this behaviour represents ‘practice’, where males are developing the skills and stamina necessary to obtain fertilizations during the mating season (Kraus *et al.* 2001). It is possible that it takes years for males to become competitive in SAGs, which could result in the differential reproductive success of older males. Parks *et al.* (2005) described a ‘gunshot’ sound that only adult males are known to make. These sounds are produced in SAGs or while males are alone or in pairs. The authors hypothesized that these sounds could serve as advertisement for females or serve an agonistic signal to other males. The fact that only adult males make this sound adds further weight to the hypothesis that males may learn certain behaviours with age that allow them to compete for access to females. A second hypothesis is that the large testes are still developing even after males have reached a large body size. Thus, the testes may not be fully developed and/or productive in younger males, which could result in males not being reproductively successful until an older age than expected based on external body size. Preliminary work on androgen hormones show that levels are vastly higher in adult males than in juveniles (Rolland *et al.* 2005), which makes this hypothesis feasible; however, more data are needed to assess and compare hormone levels in males of specific ages.

There are no other baleen whale species for which direct data are available to estimate the age at which males gain their first fertilization. For these other species indirect data have been used to estimate the age of sexual maturity. For example, in humpback whales the involvement of males in ‘breeding-related’ behaviour has been used to estimate that males reach sexual maturity at a similar age as females (Clapham 1992). However, the data presented here suggest that this criterion may be misleading. The fact that right whale males of all ages participate in SAGs (Kraus & Hatch 2001; Best *et al.* 2003), and that the bias in the age distribution of successful males is unrelated to any identified external characteristics (e.g. size), suggest that for baleen whale species, the realized reproductive biology of

males may be quite different than would be expected based on indirect data such as size and/or behaviour.

Conclusion

The understanding of reproductive success and the reproductive problem in the North Atlantic right whale have previously been based solely on data from females, with nothing known about male reproductive success. The analyses presented here increases our understanding of reproduction by adding data from the male perspective. Specifically, these data show that the patterns of male reproductive success are significantly different than expected if mating is random, but are consistent with the behavioural and physiological data suggesting promiscuity in both sexes and strong pre- and postcopulatory competition between males. This system results in a high variance in male reproductive success for an aquatically mating marine mammal, but still a relatively low variance for mammals in general. The competition between males also results in a bias in the age distribution of successful males, with reproduction being limited to only older males. From an evolutionary perspective, this system likely leads to increased reproductive success of males with better competitive skills and/or characteristics and reduced reproductive success of males that are less well-equipped for such competition. However, from a conservation genetics standpoint, this system reduces the number of reproductively successful males, and therefore the effective population size (N_e), which in turn increases the rate at which genetic diversity is lost in small populations such as this one.

In addition to its small population size, the North Atlantic right whale also has extremely low levels of genetic diversity. Indeed, where comparable data are available, this species has lower diversity than the cheetah (*Acinonyx jubatus*) (Frasier *et al.* 2007), which has historically been one of the most public examples of a species with low genetic diversity and the consequences thereof, although that understanding has been largely revised (e.g. Caughley 1994). One of the primary hypotheses for the long-term reduced reproductive potential of the North Atlantic right whale is this low level of genetic diversity and/or inbreeding (Frasier *et al.* 2007). Specifically, it is hypothesized that the low genetic diversity is resulting in the genetic similarity and incompatibility of a large portion of mating pairs, which in turn is resulting in a high rate of spontaneous abortion and reduced reproductive success. In order to test this hypothesis, it is necessary to have genetic profiles for mating pairs and their offspring (*sensu* Ober *et al.* 1998). Therefore, in addition to addressing the issue of the mating system and male reproductive success in this species, this study has also identified specific mating pairs that will be used to test the hypothesis that genetic factors are influencing reproductive success and recovery in the North Atlantic right whale.

Acknowledgements

We thank B. McLeod, R. Rolland, C. Kyle, and three anonymous reviewers for comments on the manuscript. Access to the data used throughout this study was kindly provided by the North Atlantic Right Whale Consortium. This work has been supported by The Penzance Foundation through the Woods Hole Oceanographic Institution (WHOI) Ocean Life Institute Right Whale Initiative, the Natural Sciences and Engineering Research Council of Canada, World Wildlife Fund Canada, National Marine Fisheries Service, National Fish and Wildlife Foundation, Department of Fisheries and Oceans Canada, and the Canadian Whale Institute.

References

- Amos W, Balmford A (2001) When does conservation genetics matter? *Heredity*, **87**, 257–265.
- Amos W, Worthington Wilmer J, Kokko H (2001a) Do female grey seals select genetically diverse mates? *Animal Behaviour*, **62**, 157–164.
- Amos W, Worthington Wilmer J, Fullard K *et al.* (2001b) The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **268**, 2021–2027.
- Arnold SJ, Wade MJ (1984) On the measurement of natural and sexual selection: theory. *Evolution*, **38**, 709–719.
- Atkinson S (2002) Male reproductive systems. In: *Encyclopedia of Marine Mammals* (eds Perrin WF, Würsig B, Thewissen JGM), pp. 700–704. Academic Press, San Diego.
- Best PB (1990) Natural markings and their use in determining calving intervals in right whales off South Africa. *South African Journal of Zoology*, **25**, 114–123.
- Best PB (1994) Seasonality of reproduction and the length of gestation in southern right whales *Eubalaena australis*. *Journal of Zoology*, **232**, 175–189.
- Best PB, Brandão A, Butterworth DS (2001) Demographic parameters of southern right whales off South Africa. *Journal of Cetacean Research and Management (Special Issue)*, **2**, 161–170.
- Best PB, Schaeff CM, Reeb D, Palsbøll PJ (2003) Composition and possible function of social groupings of southern right whales in South African waters. *Behaviour*, **140**, 1469–1494.
- Boness DJ, Bowen WD, Francis JM (1993) Implications of DNA fingerprinting for mating systems and reproductive strategies of pinnipeds. *Symposium of the Zoological Society of London*, **66**, 61–93.
- Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, **32**, 314–331.
- Brown MW, Brault S, Hamilton PK *et al.* (2001) Sighting heterogeneity of right whales in the western North Atlantic: 1980–1992. *Journal of Cetacean Research and Management (Special Issue)*, **2**, 245–250.
- Brown MW, Kraus SD, Gaskin DE (1991) Reaction of North Atlantic right whales (*Eubalaena glacialis*) to skin biopsy sampling for genetic and pollutant analysis. *Report of the International Whaling Commission, Special Issue*, **13**, 81–89.
- Brown MW, Kraus SD, Gaskin DE, White BN (1994) Sexual composition and analysis of reproductive females in the North Atlantic right whale, *Eubalaena glacialis*, population. *Marine Mammal Science*, **10**, 252–265.

- Brown MW, Kraus SD, Slay CK, Garrison LP (2007) Surveying for discovery, science, and management. In: *The Urban Whale: North Atlantic Right Whales at the Crossroads* (eds Kraus SD, Rolland RM), pp. 105–137. Harvard University Press, Cambridge, Massachusetts.
- Brownell RL Jr, Ralls K (1986) Potential for sperm competition in baleen whales. *Report of the International Whaling Commission, Special Issue*, **8**, 97–112.
- Caswell H, Fujiwara M, Brault S (1999) Declining survival probability threatens the North Atlantic right whale. *Proceedings of the National Academy of Sciences, USA*, **96**, 3308–3313.
- Caughley G (1994) Directions in conservation biology. *Journal of Animal Ecology*, **63**, 215–244.
- Cerchio S, Jacobsen JK, Cholewiak DM, Falcone EA, Merriwether DA (2005) Paternity in humpback whales, *Megaptera novaeangliae*: assessing polygyny and skew in male reproductive success. *Animal Behaviour*, **70**, 267–277.
- Chakraborty R, Meagher TR, Smouse PE (1988) Parentage analysis with genetic markers in natural populations. I. The expected proportion of offspring with unambiguous paternity. *Genetics*, **118**, 527–536.
- Clapham PJ (1992) Age at attainment of sexual maturity in humpback whales, *Megaptera novaeangliae*. *Canadian Journal of Zoology*, **70**, 1470–1472.
- Clapham PJ (1996) The social and reproductive biology of humpback whales: an ecological perspective. *Mammal Review*, **26**, 27–49.
- Clapham PJ (2000) The humpback whale: seasonal feeding and breeding in a baleen whale. In: *Cetacean Societies: Field Studies of Dolphins and Whales* (eds Mann J, Connor RC, Tyack PL, Whitehead H), pp. 173–196. University of Chicago Press, Chicago.
- Clapham PJ, Palsbøll PJ (1997) Molecular analysis of paternity shows promiscuous mating in female humpback whales (*Megaptera novaeangliae*, Borowski). *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **264**, 95–98.
- Clapham PJ, Young SB, Brownell RL Jr (1999) Baleen whales: conservation issues and status of the most endangered populations. *Mammal Review*, **29**, 35–60.
- Clutton-Brock TH, Guinness FE, Albon SD (1982) *Red Deer: Behavior and Ecology of Two Sexes*. University of Chicago Press, Chicago.
- Coltman DW, Bancroft DR, Robertson A, Smith JA, Clutton-Brock TH, Pemberton JM (1999b) Male reproductive success in a promiscuous mammal: behavioural estimates compared with genetic paternity. *Molecular Ecology*, **8**, 1199–1209.
- Coltman DW, Bowen WD, Wright JM (1998) Male mating success in an aquatically mating pinniped, the harbour seal (*Phoca vitulina*), assessed by microsatellite DNA markers. *Molecular Ecology*, **7**, 627–638.
- Coltman DW, Festa-Bianchet M, Jorgenson JT, Strobeck C (2002) Age-dependent sexual selection in bighorn rams. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **269**, 165–172.
- Coltman DW, Smith JA, Bancroft DR *et al.* (1999a) Density-dependent variation in lifetime breeding success and natural and sexual selection in Soay rams. *American Naturalist*, **154**, 730–746.
- Cooke JG, Rowntree VJ, Payne RS (2001) Estimates of demographic parameters for southern right whales (*Eubalaena australis*) observed off Península Valdés. *Argentina. Journal of Cetacean Research and Management (Special Issue)*, **2**, 125–132.
- Daly M, Wilson M (1983) *Sex, Evolution, and Behavior*, 2nd edn. PWS Publishers, Belmont, California.
- Darling JD, Bérubé M (2001) Interactions of singing humpback whales with other males. *Marine Mammal Science*, **17**, 570–584.
- Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. *Science*, **197**, 215–223.
- Evvett IW, Weir BS (1998) *Interpreting DNA Evidence*. Sinaur Associates, Sunderland, Massachusetts.
- Feldhamer GA, Drickamer LC, Vessey SH, Merritt JF (1999) *Mammalogy: Adaptation, Diversity, and Ecology*. McGraw-Hill, Boston, Massachusetts.
- Fisher RA (1958) *The Genetical Theory of Natural Selection*. Oxford University Press, Oxford, UK.
- Frasier TR (2005) *Integrating genetic and photo-identification data to assess reproductive success in the North Atlantic right whale (Eubalaena glacialis)*. PhD Thesis, McMaster University, Hamilton, Ontario.
- Frasier TR, McLeod BA, Gillett RM, Brown MW, White BN (2007) Right whales past and present as revealed by their genes. In: *The Urban Whale: North Atlantic Right Whales at the Crossroads* (eds Kraus SD, Rolland RM), pp. 200–231. Harvard University Press, Cambridge, Massachusetts.
- Frasier TR, Rastogi T, Brown MW, Hamilton PK, Kraus SD, White BN (2006) Characterization of tetranucleotide microsatellite loci and development and validation of multiplex reactions for the study of right whale species (genus *Eubalaena*). *Molecular Ecology Notes*, **6**, 1025–1029.
- Fujiwara M, Caswell H (2001) Demography of the endangered North Atlantic right whale. *Nature*, **414**, 537–541.9.
- Gibbs HL, Weatherhead PJ, Boag PT, White BN, Tabak LM, Hoysak DJ (1990) Realized reproductive success of polygynous red-winged blackbirds revealed by DNA markers. *Science*, **250**, 1394–1397.
- Ginsberg JR, Huck UW (1989) Sperm competition in mammals. *Trends in Ecology & Evolution*, **4**, 74–79.
- Ginsberg JR, Rubenstein DI (1990) Sperm competition and variation in zebra mating behavior. *Behavioral Ecology and Sociobiology*, **26**, 427–434.
- Gomendio M, Harcourt AH, Roldán ERS (1998) Sperm competition in mammals. In: *Sperm Competition and Sexual Selection* (eds Birkhead TR, Møller AP), pp. 667–755. Academic Press, San Diego, California.
- Halbert ND, Raudsepp T, Chowdhary BP, Derr JN (2004) Conservation genetic analysis of the Texas State Bison Herd. *Journal of Mammalogy*, **85**, 924–931.
- Hamilton PK, Knowlton AR, Marx MK, Kraus SD (1998) Age structure and longevity in North Atlantic right whales *Eubalaena glacialis* and their relation to reproduction. *Marine Ecology Progress Series*, **171**, 285–292.
- Hamilton PK, Marx MK, Kraus SD (1995) Weaning in North Atlantic right whales. *Marine Mammal Science*, **11**, 386–390.
- Harcourt AH, Harvey PH, Larson SG, Short RV (1981) Testis weight, body weight and breeding system in primates. *Nature*, **293**, 55–57.
- Hayes SA, Pearse DE, Costa DP, Harvey JT, Le Boeuf BJ, Garza JC (2006) Mating systems and reproductive success in eastern Pacific harbour seals. *Molecular Ecology*, **15**, 3023–3034.
- Hoffman JI, Amos W (2005) Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Molecular Ecology*, **14**, 599–612.
- Holleley CE, Dickman CR, Crowther MS, Oldroyd BP (2006) Size breeds success: multiple paternity, multivariate selection and male semelparity in a small marsupial, *Antechinus stuartii*. *Molecular Ecology*, **15**, 3439–3448.

- Hughes CR (1998) Integrating molecular techniques with field methods in studies of social behavior: a revolution results. *Ecology*, **79**, 383–399.
- International Whaling Commission (2001) Report of the workshop on the status and trends of western North Atlantic right whales. *Journal of Cetacean Research and Management (Special Issue)*, **2**, 61–87.
- Johnson AJ, Kraus SD, Kenney JF, Mayo CA (2007) The entangled lives of right whales and fishermen: can they coexist?. In: *The Urban Whale: North Atlantic Right Whales at the Crossroads* (eds Kraus SD, Rolland RM), pp. 380–408. Harvard University Press, Cambridge, Massachusetts.
- Kenagy GJ, Trombulak SC (1986) Size and function of mammalian testes in relation to body size. *Journal of Mammalogy*, **67**, 1–22.
- Knowlton AR, Brown MW (2007) Running the gauntlet: right whales and vessel strikes. In: *The Urban Whale: North Atlantic Right Whales at the Crossroads* (eds Kraus SD, Rolland RM), pp. 409–435. Harvard University Press, Cambridge, Massachusetts.
- Knowlton AR, Kraus SD, Kenney RD (1994) Reproduction in North Atlantic right whales (*Eubalaena glacialis*). *Canadian Journal of Zoology*, **72**, 1297–1305.
- Kraus SD, Hamilton PK, Kenney RD, Knowlton AR, Slay CK (2001) Reproductive parameters of the North Atlantic right whale. *Journal of Cetacean Research and Management (Special Issue)*, **2**, 231–236.
- Kraus SD, Hatch JJ (2001) Mating strategies in the North Atlantic right whale (*Eubalaena glacialis*). *Journal of Cetacean Research and Management (Special Issue)*, **2**, 237–244.
- Kraus SD, Moore KE, Price CA *et al.* (1986a) The use of photographs to identify individual North Atlantic right whales (*Eubalaena glacialis*). *Report of the International Whaling Commission, Special Issue*, **10**, 145–151.
- Kraus SD, Pace RM III, Frasier TR (2007) High investment, low return: the strange case of reproduction in *Eubalaena glacialis*. In: *The Urban Whale: North Atlantic Right Whales at the Crossroads* (eds Kraus SD, Rolland RM), pp. 172–199. Harvard University Press, Cambridge, Massachusetts.
- Kraus SD, Prescott JH, Knowlton AR, Stone GS (1986b) Migration and calving of right whales (*Eubalaena glacialis*) in the western North Atlantic. *Report of the International Whaling Commission, Special Issue*, **10**, 139–144.
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.
- Mate B, Duley P, Lagerquist B, Wenzel F, Stimpert A, Clapham P (2005) Observations of a female North Atlantic right whale (*Eubalaena glacialis*) in simultaneous copulation with two males: supporting evidence for sperm competition. *Aquatic Mammals*, **31**, 157–160.
- Menotti-Raymond M, O'Brien SJ (1995) Hypervariable genomic variation to reconstruct the natural history of populations: lessons from the big cats. *Electrophoresis*, **16**, 1771–1774.
- Moore MJ, McLellan WA, Daoust P-Y, Bonde RK, Knowlton AR (2007) Right whale mortality: a message from the dead to the living. In: *The Urban Whale: North Atlantic Right Whales at the Crossroads* (eds Kraus SD, Rolland RM), pp. 358–379. Harvard University Press, Cambridge, Massachusetts.
- Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.
- Nielsen R, Mattila DK, Clapham PJ, Palsbøll PJ (2001) Statistical approaches to paternity analysis in natural populations and applications to the North Atlantic humpback whale. *Genetics*, **157**, 1673–1682.
- Ober C, Hyslop T, Elias S, Wiekamp LR, Hauck WW (1998) Human leukocyte antigen matching and fetal loss: results of a 10-year prospective study. *Human Reproduction*, **13**, 33–38.
- Pack AA, Salden DR, Ferrari MJ *et al.* (1998) Male humpback whale dies in competitive group. *Marine Mammal Science*, **14**, 861–873.
- Paetkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology*, **3**, 487–495.
- Parks SE (2003) Response of North Atlantic right whales (*Eubalaena glacialis*) to playback of calls recorded from surface active groups in both the North and South Atlantic. *Marine Mammal Science*, **19**, 563–580.
- Parks SE, Hamilton PK, Kraus SD, Tyack PL (2005) The gunshot sound produced by male North Atlantic right whales (*Eubalaena glacialis*) and its potential function in reproductive advertisement. *Marine Mammal Science*, **21**, 458–475.
- Payne R, Brazier O, Dorsey EM, Perkins JS, Rowntree VJ, Titus A (1983) External features in Southern right whales (*Eubalaena australis*) and their use in identifying individuals. In: *Communication and Behaviour of Whales* (ed. Payne R), pp. 371–445. AAAS Selected Symposium 76. Westview Press, Boulder, Colorado.
- Payne R, Dorsey EM (1983) Sexual dimorphism and aggressive use of callosities in right whales (*Eubalaena australis*). In: *Communication and Behaviour of Whales* (ed. Payne R), pp. 295–329. AAAS Selected Symposium 76. Westview Press, Boulder.
- Pemberton JM, Albon SD, Guinness FE, Clutton-Brock TH, Dover GA (1992) Behavioral estimates of male mating success tested by DNA fingerprinting in a polygynous mammal. *Behavioral Ecology*, **3**, 66–75.
- Pemberton JM, Coltman DW, Smith JA, Bancroft DR (2004) Mating patterns and male breeding success. In: *Soay Sheep: Dynamics and Selection in an Island Population* (eds Clutton-Brock T, Pemberton J), pp. 166–189. Cambridge University Press, Cambridge, UK.
- Pettis HM, Rolland RM, Hamilton PK, Brault S, Knowlton AR, Kraus SD (2004) Visual health assessment of North Atlantic right whales (*Eubalaena glacialis*) using photographs. *Canadian Journal of Zoology*, **82**, 8–19.
- Preston BT, Stevenson IR, Pemberton JM, Coltman DW, Wilson K (2003) Overt and covert competition in a promiscuous mammal: the importance of weaponry and testes size to male reproductive success. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **270**, 633–640.
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.
- Quinn TW, Quinn JS, Cooke F, White BN (1987) DNA marker analysis detects multiple maternity and paternity in single broods of the lesser snow goose. *Nature*, **326**, 392–394.
- Rolland RM, Hunt KE, Kraus SD, Wasser SK (2005) Assessing reproductive status of right whale (*Eubalaena glacialis*) using fecal hormone metabolites. *General and Comparative Endocrinology*, **142**, 308–317.
- Rossiter SJ, Ransome RD, Faulkes CG, Dawson DA, Jones G (2006) Long-term paternity skew and the opportunity for sexual selection in a mammal with reversed sexual size dimorphism. *Molecular Ecology*, **15**, 3035–3043.
- Shaw CN, Wilson PJ, White BN (2003) A reliable method of gender determination for mammals. *Journal of Mammalogy*, **84**, 123–128.

- Sironi M, Kraus SD, Nordheim EV, Rowntree VJ, Snowdon CT (2005) Age estimation of North Atlantic right whales (*Eubalaena glacialis*) by allometric measurements on photographs. Paper SC/57/BRG7 presented to the International Whaling Commission Scientific Committee, June 2005. 14pp.
- Slate J, Marshall T, Pemberton J (2000) A retrospective assessment of the accuracy of the paternity inference program CERVUS. *Molecular Ecology*, **9**, 801–808.
- Taylor AC, Cowan PE, Fricke BL, Cooper DW (2000) Genetic analysis of the mating system of the common brushtail possum (*Trichosurus vulpecula*) in New Zealand farmland. *Molecular Ecology*, **9**, 869–879.
- Tyack PL (1981) Interactions between singing Hawaiian humpback whales and conspecifics nearby. *Behavioral Ecology and Sociobiology*, **8**, 105–116.
- Vigilant L, Hofreiter M, Seidel H, Boesch C (2001) Paternity and relatedness in wild chimpanzee communities. *Proceedings of the National Academy of Sciences, USA*, **98**, 12890–12895.
- Vilà C, Sundqvist A-K, Flagstad Ø *et al.* (2003) Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proceedings of the Royal Society of London, Series B*, **270**, 91–97.
- Whitehead H, Payne R (1981) New techniques for assessing populations of right whales without killing them. In: *Mammals in the Sea. Vol. III. FAO Fisheries Serial No. 5.* (eds Clark JG, Goodman J, Soave GA) pp. 189–209. United Nations Food and Agriculture Organization, Rome.
- Widdig A, Bercovitch FB, Streich WJ, Saueremann U, Nürnberg P, Krawczak M (2004) A longitudinal analysis of reproductive skew in male rhesus macaques. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **271**, 819–826.
- Worthington Wilmer J, Allen PJ, Pomeroy PP, Twiss SD, Amos W (1999) Where have all the fathers gone? An extensive microsatellite analysis of paternity in the grey seal (*Halichoerus grypus*). *Molecular Ecology*, **8**, 1417–1429.

This study combined data from two ongoing long-term aspects of North Atlantic right whale research; the photo-identification/sightings database, and the genetic database. All authors played a key role in one or more of the following ways: photo-identification fieldwork and analyses, sample collection, and genetic analyses. The overall goals of these projects are to identify the factors preventing the recovery of this endangered species, to identify appropriate conservation/management measures, and to provide the data necessary to conduct these analyses and monitor/assess trends through time.

Supplementary material

The following supplementary material is available for this article:

Table S1 Variability of the 35 loci at which all North Atlantic right whale samples were genotyped.

This material is available as part of the online article from:
<http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-294X.2007.03570.x>
 (This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.