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Changes in age-related sexual selection in a humpback whale population recovering from exploitation

Highlights

- Age structure of male humpback whales 50 years post-whaling shows signs of recovery
- Shifting population age structure impacted male mating behaviors and reproduction
- As the population recovered, age-related sexual selection pressure grew
- Recovery and past exploitation shape current population dynamics and reproduction

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In brief

Using epigenetic aging, Eichenberger et al. showed that the age structure of male humpback whales shifted over 19 years, indicating signs of recovery from past exploitation. Concurrently, there was a shift in age-related mating behaviors corresponding with older males being more successful in reproducing than younger ones.

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Changes in age-related sexual selection in a humpback whale population recovering from exploitation

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SUMMARY

Whaling, one of the most extensive and prolonged global commercial hunting operations, pushed many large whales to the brink of extinction. To understand how evolutionary processes may be directly impacted by the consequences of exploitation, we assessed age-dependent population dynamics, reproductive tactics, and reproductive success of 485 male humpback whales (*Megaptera novaeangliae*) from a recovering breeding ground in New Caledonia. Over 19 years (2000–2018), the population age structure switched from a left-skewed, rapidly increasing low-density population toward a more evenly distributed age structure when abundance was higher in later years. Concurrent with the change in population age structure across time, there was a shift in age-related male mating tactics. Most strikingly, during years of higher abundance, the key reproductive tactics of singing and escorting were over-represented by older age categories when compared with the underlying age structure, suggesting age- and/or density-related tactic choice in males. This behavioral shift corresponded with an increase in the relative reproductive success of the oldest males during periods of higher abundance compared with lower abundance. Overall, our work suggests that at lower population densities, young males were less constrained by age-related restrictions on reproductive success. A reduction of age-related sexual selection could have acted to buffer genetic diversity loss during a time of lower abundance, when this was most critical. Integrating these changes in the pattern of sexual selection with population dynamics provides critical insights into the evolutionary consequences of exploitation and recovery in long-lived species.

INTRODUCTION

Sexual selection is one of the central forces in evolution and explains a wide diversity of morphological and behavioral adaptations.[1] It arises from fitness differences associated with non-random success in the competition for access to gametes.[2] However, human activities can drive evolutionary change that often opposes natural and/or sexual selection. This can change the fitness landscape, allowing otherwise suboptimal phenotypes to increase in frequency.[3–5] For example, the evolutionary response to long-term hunting not only results in substantial changes in population abundance but may further cause changes in somatic growth rates and reproductive parameters (i.e., age at maturity and birth interval) and thereby the productivity of individuals and populations.[6,7]

Reproduction in male mammals is age- or size-dependent, with mating success typically increasing with age and then declining with senescence.[8] The age or experience of an individual can influence its competitive ability and, in turn, reproductive success. For example, in bighorn sheep (*Ovis canadensis*), male mating success increased with age and, in older rams (>7 years), horn length.[9] However, mating success was not solely restricted to the oldest males, as younger, smaller rams achieved mating success through alternative mating tactics that are less dependent on body and horn size.[9] These alternative mating tactics may have evolved to overcome differences in mating competitiveness as individuals incorporate information about their ability or hierarchy status to adopt a mating tactic that maximizes their fitness.[10] Unrestricted hunting of bighorn trophy rams decreased the proportion of older rams in the population. Consequently,

Table 1. Description of the recorded behavioral contexts of male humpback whales

Behavioral context	Group type	Description of behavior
Singer	usually a lone male	a male producing long bouts of complex song (Herman[26]); the underlying function(s) of humpback whale song and its role within the mating system are not entirely known; however, in humpback whales, song is displayed solely by males and mainly during the breeding season, and consequently, singing is recognized as a male mating behavior (Herman[26])
Solitary escort	dyad of a single male with a female or a mother with a calf	males often escort a single female (with or without her newborn calf) to form a pair; it is unknown whether a male's defense and escorting of the female results in copulation or instead reflects mate guarding following earlier copulation (Clapham[34])
Principal escort	competitive group	male humpback whales often engage in agonistic fights to gain or maintain the privileged position closest to the female (nuclear animal) in so-called competitive groups (Tyack et al.,[27] Clapham et al.[28]); the male holding the position closest to the nuclear animal is termed principal escort; these temporary group formations of three or more adults in humpback whales are thought to be a form of direct male-male competition over mating access (Eichenberger et al.[35])
Challenger	competitive group	a challenger is a male actively challenging the principal escort for his position closest to the female within the competitive group (Tyack et al.[27])
Secondary escort	competitive group	any other males within the competitive group that are actively following the female of the group are termed secondary escorts (Tyack et al.[27])
Male dyad	group of two males	two males in close proximity to each other, showing no signs of aggression, and neither is producing song
Solitary	solitary individual	in this study, since we only focused on males, all solitary individuals are male

Groups of two individuals were differentiated into "solitary escort" (male-female dyad) or "male dyad" (male-male dyad) based on the individual's genetically identified sex, or for females, based on the constant and close association of mother and calf. Groups of two for which only one or neither individual was successfully sexed could not be differentiated and were thus excluded. See also Tables S1, S2, and S8. Note: for more detailed information on humpback whale song or male reproductive tactics, we direct readers to a recent review of baleen whale songs (Clark and Garland[36]) and reproductive tactics in baleen whales (Eichenberger et al.[35]).

previously less competitive younger males obtained a larger proportion of mates compared with young males in unexploited populations. This led to increased sexual selection on the body weight and horn length of younger males,[11] demonstrating that exploitation-driven demographic shifts can modify mating competition and success in a terrestrial mammal across age distributions.

There has been little research on the evolutionary consequences of whaling. This is despite whaling being one of the most dramatic and well-documented examples of human exploitation of wild animal populations.[12] Many baleen whale populations declined to 1% of their pre-exploitation size, among them the Southern Hemisphere humpback whale (*Megaptera novaeangliae*) populations.[12,13] Many humpback whale populations have increased in population size since the cessation of illegal whaling in the 1970s.[14,15] However, the degree of recovery is heterogeneous, and several populations remain at low levels relative to historical abundance, including the Oceania breeding metapopulation.[16] The age structure of several populations of humpback whales was found to be strongly left-skewed,[17,18] akin to that of populations under size-selective hunting (e.g., bighorn sheep). However, while most whaling operations are known to have preferentially killed large whales, the most recent and intensive period of whaling on humpback whales in the South Pacific (including Oceania) was an illegal, indiscriminate Soviet whaling operation.[19] Given the level of exploitation that led many populations to a complete collapse,[20] the impact of any size-selection that may have occurred during whaling would be minimal when compared with the large reduction in overall population size. Thus, the left-skewed age structure of humpback whales is unlikely to be a simple remnant of size-selective whaling but more likely the predicted result of rapid population growth in response to lower competition for resources post-whaling. Today, even with increases in population size, the long life spans of humpback whales (maximum ca. 90 years[21,22]) and the only recent cessation of whaling (50 years ago) mean that reaching stable age distributions (i.e., one that includes the oldest age classes of 50 years plus) could still be several decades away.[23] Nevertheless, despite the intensity of their past exploitation, some humpback whale populations have since successfully recovered,[24,25] likely owing to the species' ability to rapidly breed and forage across trophic levels.

The consequences of the demographic changes resulting from exploitation and subsequent population growth may have further impacted the behavior of humpback whales on the breeding grounds. Male humpback whales have a variety of reproductive tactics. They sing one of the most complex acoustic displays in the animal kingdom[26] and are also readily observed to physically compete over a single female within competitive groups, often with high levels of aggression between males[27,28] (Table 1). Further, males show individual plasticity in these tactics, as they have been found to switch from singing

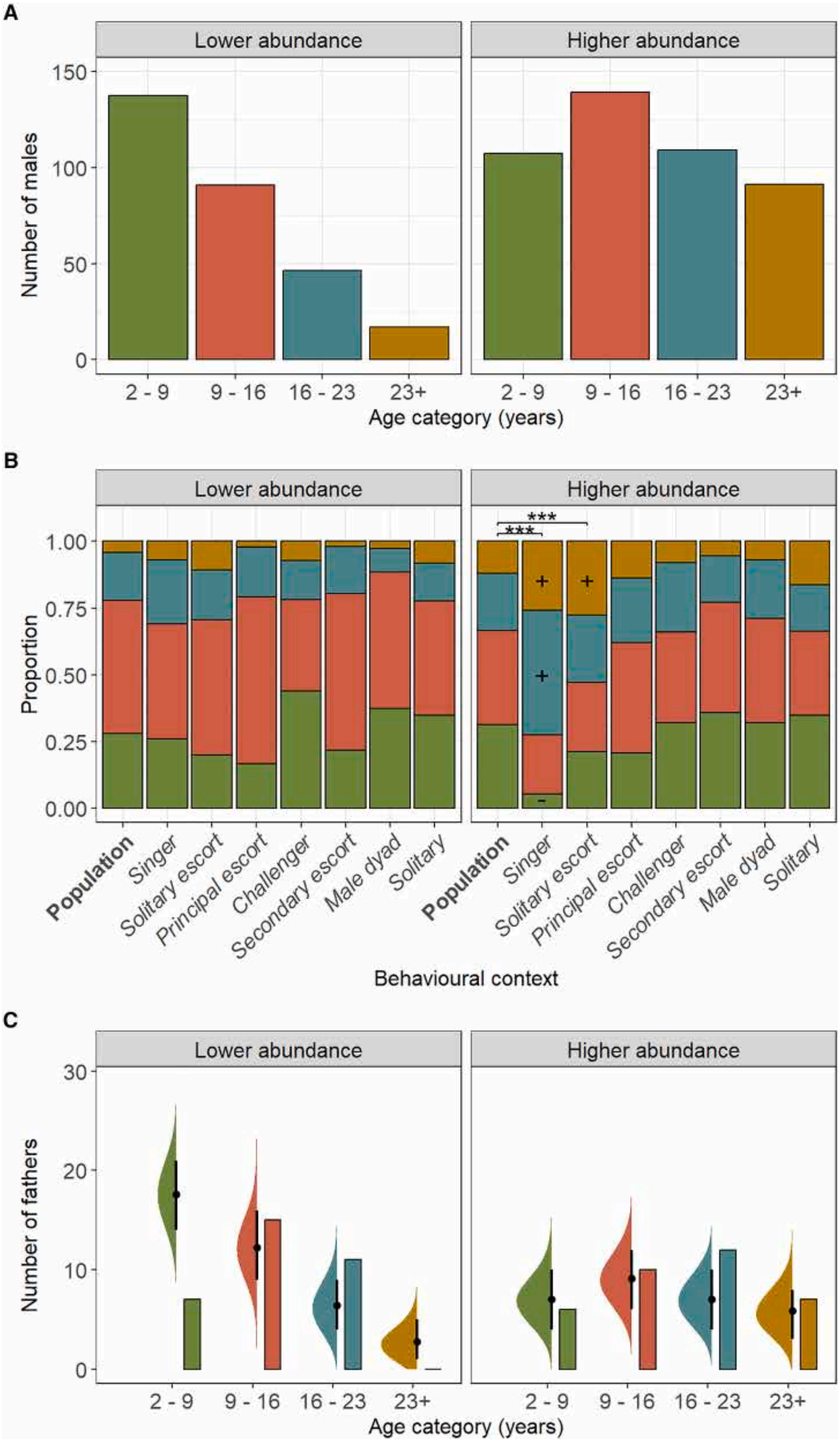

Figure 1. Age distribution of males and fathers in the lower and higher abundance periods

(A) The mean number of males ($N = 485$) in each of the four age categories (2–9, 9–16, 16–23, and ≥23 years) in the population in the two time periods: lower abundance (2000–2008) and higher abundance (2009–2018).

(legend continued on next page)

to physical competition with increasing numbers of other male competitors.[29,30] Combined with the male-biased sex ratio observed on humpback whale breeding grounds (1.5:1, male:female),[31] sexual selection theory would predict intense competition among males, leading to high male reproductive skew and strong sexual selection.[1,10] However, contrary to these predictions, male reproductive skew was found to be low in the New Caledonian breeding population,[32] consistent with data from other exploited humpback whale populations.[33] Both the occurrence of alternative mating tactics and a left-skewed (young and recovering) population age structure could explain the observed low reproductive skew and thus mild polygyny in this population.[33] However, it remains unclear how demographic processes resulting from commercial whaling have shaped patterns of sexual selection in male humpback whales through time and how this impacts current population recovery and reproductive success.

The role of sexual selection in population recovery is a key question, as, despite their history of exploitation, humpback whales have maintained high levels of genetic diversity through the whaling-induced demographic bottleneck.[37] This has been attributed to the fact that the species underwent a short bottleneck relative to generation span, the time frame during which genetic diversity is lost. This allowed much pre-whaling molecular variation to persist through the population nadir. However, sexual selection and patterns of reproduction could also have played a role in the maintenance of genetic diversity and population recovery in this species, as a high reproductive skew is expected to decrease genetic diversity during a bottleneck.

Here, we used epigenetic age estimates[17,38] of 485 male humpback whales sampled off New Caledonia, representing ~24% of the male population,[32] to assess age-dependent reproductive tactics and reproductive success relative to population demography over 19 years (2000–2018). The estimated abundance of humpback whales in New Caledonia showed an anomalous increase from 562 whales (95% confidence interval [CI] = 351–772) in 2008 to 1,291 whales (CI = 945–1,637) in 2009.[39] This is consistent with an overall increase in the number of males that used the study area from 379 males (CI = 158–600) around the start of our study period (1995–2001)[40] to 2,084 males (CI = 1,761–2,407) over the following two decades (2000–2018),[32] as well as an increase in the group encounter rate in our study region over 23 years (1995–2017).[41] This sudden increase in population size within 1 year and the overall increase in the number of males on the breeding ground were likely due to a combination of population growth, immigration from the neighboring East Australian population,[42] and/or high reproductive capacity.[31,43] Considering that our research in a constant area has resulted in estimates of abundance that have more than doubled, it is reasonable to assume that a whale's probability of encountering conspecifics in this breeding aggregation has increased concurrently (i.e., increased density). To assess age-dependent reproductive tactics and reproductive success, first, we quantified the male age structure across time by dividing the study period into either side of the reported increase in abundance (after 2008),[39] representing periods of lower and higher abundance, and presumably lower and higher density, respectively. Second, we examined the role of age in seven behavioral contexts, including established key reproductive tactics. Finally, using previously published paternity assignments (n = 66 fathers),[32] we investigated whether a male's chance of siring offspring changed with age. This work aims to provide critical insights into how sexual selection varies over a demographic bottleneck in a species with a complex suite of male reproductive behaviors, with implications for how such dramatic changes may impact other exploited mammals.

RESULTS

Population age structure reflects population increase after exploitation

We used the previously developed humpback epigenetic aging model[17] to estimate the year of birth for 485 male humpback whales from the New Caledonian breeding ground. This allowed us to estimate the population's age structure over two time periods: 2000–2008 and 2009–2018, representing times of lower and higher abundance of whales on the breeding ground. The age structure in the period of lower abundance was significantly different from the age structure during higher abundance (Fisher's exact test [FET]: $p < 0.001$; Figure 1A). Initially, the population age structure was predominantly composed of young individuals in the first two age categories (2–9 and 9–16 years), but in the higher abundance period, the male age structure became more evenly distributed (Figure 1A).

Age categories of males undertaking key reproductive tactics shift through time

From the initial 485 identified males, 439 aged 2 years or older were sighted in a total of 1,717 observations in 1,047 different groups between 2000 and 2018. Of those, 372 males engaged in one of the seven behavioral contexts of interest in 1,016 observations of 707 different groups (Table 1). Individual males frequently engaged in more than one behavioral context or reproductive tactic even within a single breeding season. Observations of fathers (n = 21 males) of 23 paternity assignments (two males sired twice) in the year they sired offspring showed that 70% (n = 16) were in a competitive group, with 30% (n = 7) as the principal escort (for 57%, the social role within the competitive group was unclear); 35% (n = 8) were solitary escorts, with 26% (n = 6) escorting a single female and 9% (n = 2) escorting a mother with calf; and 26% (n = 6) were singing (Tables S1 and S2). One father was observed as the solitary escort of the mother of his inferred offspring (Table S1). Only one father was not observed singing, escorting, or in a competitive group in

(B) Observed age distribution of males engaging in different behavioral contexts (N = 372) compared with the age distribution of the underlying population (i.e., expected distribution; $^{*}p < 0.05$, $^{***}p < 0.001$). Age categories that were significantly ($p < 0.05$) over-represented (+) or under-represented (−) in relation to the underlying population age structure are indicated with the respective symbol.

(C) Distribution of the observed number of fathers (bars; N = 68 paternity assignments) compared with the simulated number of fathers based on the underlying population age structure (half-violins with interquartile range and mean).

See also Figures S1, S2, and S4, Table S8, and Data S1 and S2.

Table 2. Chi-squared and post-hoc analysis of the age distribution of males engaging in different behavioral contexts within the two time periods

Context	Time period (abundance)	X^2 (df = 3)	p_{adj}	Cramer's V	2–9 years	9–16 years	16–23 years	≥23 years
Singer	lower	3.02	0.495	0.13	1.000	0.835	0.835	0.835
	higher	41.74	**<0.001**	0.49	***<0.001***	0.088	**<0.001**	**0.005**
Solitary escort	lower	9.69	0.100	0.21	0.585	1.000	1.000	0.094
	higher	33.87	**<0.001**	0.30	0.081	0.095	0.671	**<0.001**
Principal escort	lower	4.13	0.495	0.17	0.389	0.389	1.000	1.000
	higher	3.03	0.495	0.13	0.714	1.000	1.000	1.000
Challenger	lower	7.15	0.222	0.24	0.193	0.193	1.000	0.857
	higher	1.17	0.761	0.09	1.000	1.000	1.000	1.000
Secondary escort	lower	2.07	0.600	0.12	1.000	1.000	1.000	1.000
	higher	6.43	0.222	0.14	0.664	0.664	0.664	0.344
Male dyad	lower	6.37	0.222	0.16	0.354	1.000	0.354	1.000
	higher	3.14	0.495	0.09	1.000	1.000	1.000	0.780
Solitary	lower	3.69	0.495	0.16	0.881	0.881	1.000	0.881
	higher	2.73	0.507	0.10	0.970	0.970	0.970	0.970

The male age structure of each behavioral context was compared with the underlying age structure of the population within each time period (lower abundance: 2000–2008; higher abundance: 2009–2018). Significant p values, after BH correction, are highlighted in bold. Underscored and italic p values indicate a significant over- or under-representation of certain age categories compared with the underlying population age structure, respectively. See also Figure S4, Tables S1, S2, and S8, and Data S1 and S2.

the year he sired. Given the consistent engagement of fathers in these behavioral contexts overall and relative to the rest of the male population (Table S2), we conclude that (1) singing, (2) solitary escorting (especially of a female without calf), and (3) competing in a competitive group (especially as the principal escort) are behavioral contexts that result in potential mating opportunities and, thus, are considered key reproductive tactics. Note that 26% of fathers (n = 6) were observed to have engaged in more than one key reproductive tactic in the year they sired offspring (Table S1).

In the two time periods of lower and higher abundance, we assessed the age structure of males observed in all seven behavioral contexts but focused on the identified key mating behaviors. During the period of lower abundance, the age structure of singers and solitary escorts was not significantly different from the underlying age structure (X^2 $p \geq 0.05$; Table 2, Figure 1B). During the period of higher abundance, the age structure of singers and solitary escorts was significantly different from the underlying age structure (X^2 $p < 0.001$ for both). Post hoc analysis showed that the older males were over-represented as singers (16–23 years, $p < 0.001$; ≥23 years, $p = 0.005$) and solitary escorts (≥23 years, $p < 0.001$), while younger, likely immature, males (2–9 years) were under-represented as singers ($p < 0.001$; Figure 1B, Table 1). For solitary escorts, this age-related pattern was driven by solitary escorts of females without calves, and thus likely of higher reproductive potential,[44] compared with escorts of mothers with calves (Figure S1). The age structure of principal escorts was not significantly different from the underlying age structure in either time period (Table 2; $p > 0.05$).

The age structure of males observed undertaking behaviors less likely to result in mating, such as non-primary males in a competitive group (challenger or secondary escort), those in male dyads, and males observed alone (solitary), was not significantly different from the underlying age structure in either time period (Table 2; $p > 0.05$).

Age-related differences in male reproductive success

A total of 56 fathers siring 68 offspring[32] were successfully aged and available for further analysis (Figure 2). The mean age of males siring their first and second sampled offspring was 15 and 17 years, respectively (Figure 2). The age distribution of fathers between time periods was significantly different (FET: $p = 0.04$). In the lower abundance period, males between 9 and 23 years sired offspring at a higher rate than expected from the underlying age structure (9–16 years: observed > expected in 79% of simulations; 16–23 years: 96% of simulations). However, no father above the age of 23 years was identified in the lower abundance period, which was unexpectedly low based on the underlying age structure (0% of simulations; Figure 1C, Table 3).

In the higher abundance period, there was a "rebalancing" toward paternities reflecting the underlying age structure, with older males (≥16 years) more likely to sire offspring (16–23 years: observed > expected in 97% of simulations; ≥23 years: 63%). As a result, the rate at which younger males (9–16 years) sired offspring declined to a likelihood close to random (58%; Figure 1C). In both time periods, the youngest age category (2–9 years) was less successful in siring offspring than expected based on the population age structure, consistent with these males most likely being immature.

Estimates of male reproductive skew during the lower (1.22) and higher abundance periods (1.13) were similar. Further, the number of fathers siring one or two offspring did not significantly differ (FET: $p = 0.489$) during lower (one or two offspring: 21/6) and higher abundance (27/4).

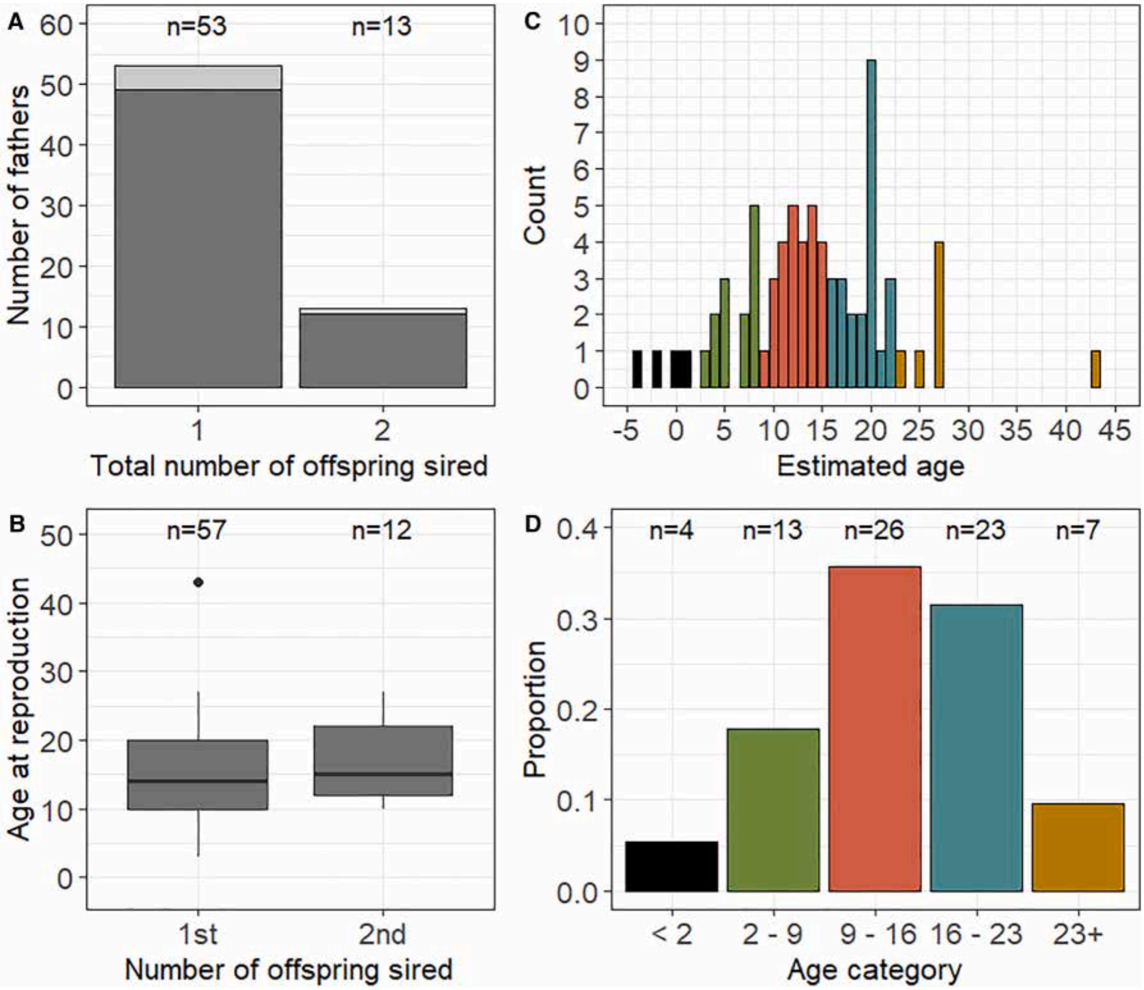

Figure 2. Age at male reproduction

(A) The number of fathers with (dark gray) and without (light gray) age estimates that sired one or two offspring.

(B) The mean age at reproduction of males siring their first and second sampled offspring.

(C) The raw estimated age of males in the year they sired offspring. Four fathers were estimated to be less than 2 years old in the year they sired offspring and were thus excluded from all further analyses.

(D) The proportion of fathers in each of the four age categories (green: 2–9 years, red: 9–16 years, blue: 16–23 years, and yellow: ≥23 years). Males below the age of 2 years (calves and yearlings; in black) were excluded from the analysis.

See also Tables S1 and S8 and Data S1 and S2.

DISCUSSION

Our epigenetic aging of 485 individual male humpback whales revealed a shift in the population age structure from a left-skewed (i.e., younger), rapidly increasing low-abundance population toward a more evenly distributed male age structure due to a higher proportion of older males. This shift in the age structure, combined with a higher population abundance in the second half of the study, indicates signs of recovery of the New Caledonian breeding population. This shift in the male age structure has had profound effects on the age distribution of fathers and patterns of sexual selection. We theorize that the low number of older males during the period of lower abundance resulted in less age-related sexual selection pressure on younger males. As the population age structure became less skewed due to the greater proportion of older males, we hypothesize that sexual selection pressure increased, resulting from age-related reproductive success. Considering the optimistic outlook for humpback whales, we can expect their population age structure to continue shifting toward older males. The observed shift in age structure in this study, 50 years post-whaling, likely relates to increasing competition for resources as abundance increased throughout the 19-year-long study period. Considering the chronology of events that have impacted humpback whales, it ultimately is their history of human exploitation that has directly or indirectly shifted their population age structure, reproductive tactics, and patterns of reproductive success. Thus, our observed trends in humpback whale population dynamics, behavior, and sexual selection are a consequence of their past exploitation and ongoing recovery. Integrating these population dynamics with changes in the

Table 3. Simulation analysis of reproductive success by age and time period

Time period	Age category (years)	Observed # fathers	Avg. simulated # fathers	Obs. > sim. (%)
Lower abundance	2–9	7	18	0.00
	9–16	15	12	78.51
	16–23	11	6	95.62
	≥23	0	3	0.00
Higher abundance	2–9	6	7	25.92
	9–16	10	9	57.52
	16–23	12	7	97.22
	≥23	7	6	63.14

The observed number of fathers within each time period (lower abundance: 2000–2008; higher abundance: 2009–2018) for each age category is compared with the average (avg.) simulated number of fathers. The final column shows the percentage of simulations in which the observed number of fathers (Obs.) was larger than the simulated number of fathers (Sim.). See also Figure S4, Table S8, and Data S1 and S2.

pattern of sexual selection provides critical baseline information on the salience of mating displays and highlights the potential evolutionary consequences of exploitation.

Population age structure reveals signs of recovery

Despite commercial whaling ending in 1963 in the Southern Hemisphere, illegal Soviet whaling continued into the 1970s.[14,15] This drove the Oceanian humpback population to fewer than 200 whales in the 1970s, and it only slowly recovered thereafter.[45] The relative scarcity of older whales and the strong left-skewed population age structure we demonstrated are consistent with this history of population exploitation and growth. Furthermore, the mean age of 21 years in the final year of data collection (2020) is in line with other aging studies from the region (i.e., only 15% of males were over 30 years old, and less than 1.5% were older than 40 years; Figure 3).[17,18] The population age structure became less skewed due to a greater proportion of older males in later years, suggesting either internal recruitment or immigration fueling the growth of the older age categories (Figure S2), signaling the ongoing process of population recovery, either locally or regionally. Considering that the long lifespan of many great whales has been masked by the demographic disruptions of whaling,[23] we can expect the proportion of older males (>23 years) in the population to continue increasing over time. Our study demonstrates the broad utility of the previously developed humpback epigenetic aging model[17] to different populations around the globe. Here, we updated the humpback epigenetic aging model with data from individuals of known age collected from our study population (Figure 4). As this technique has become more widely applied, evidence suggests that epigenetic clocks can be refined with data from related populations and species to develop aging assays for populations that are more challenging to study.[46–48] This highlights the potential of epigenetic aging as a tool for researching life history trends, population dynamics, and conservation status of wild populations (see supplemental information).

Age- and density-related reproductive tactics and success

This study explores the changes in mating tactics with both male age and shifting population dynamics, highlighting how both

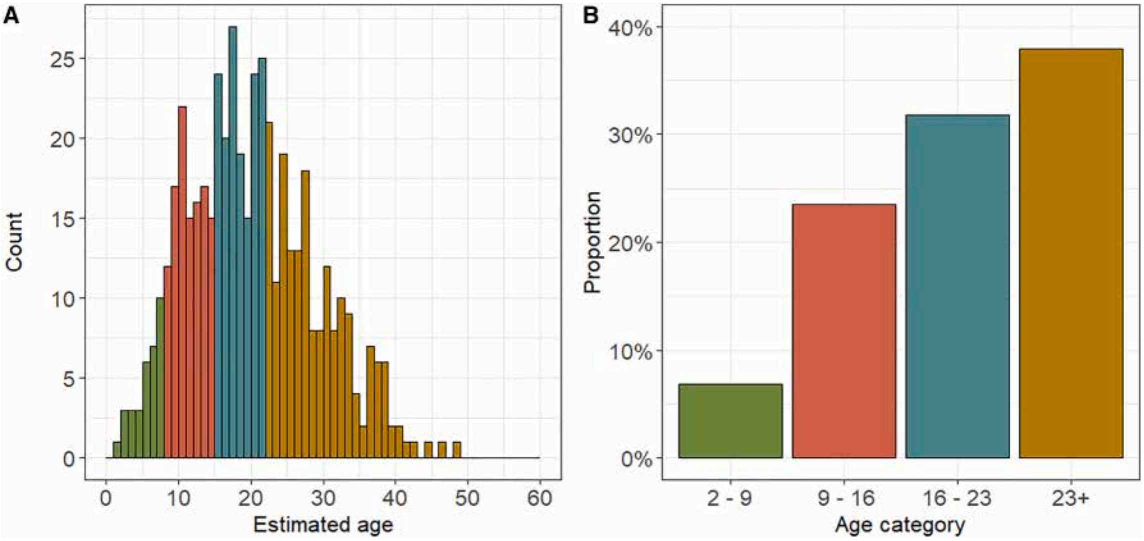

Figure 3. Age structure of the New Caledonian male humpback whale population in the last year of the study period (2020)

(A) Distribution of the age estimates of the sampled male population in the year 2020. The population showed a mean age of 20.6 years, and the oldest sampled male was estimated to be 49 years old. The different colored bars indicate the different age categories, also shown in the right panel.

(B) The proportion of aged males in each of the four age categories (green: 2–9 years, red: 9–16 years, blue: 16–23 years, and yellow: ≥23 years). Despite most males (37.9%) being in the oldest age category (≥23 years), only 15% (*N* = 73 males) were over 30 years old, and less than 1.5% (*N* = 7 males) were older than 40 years, indicating the population was still relatively young given the humpback whale lifespan (ca. 90 years).[21] Males below the age of 2 years (calves and yearlings) were excluded from all further analyses.

See also Data S1 and S2.

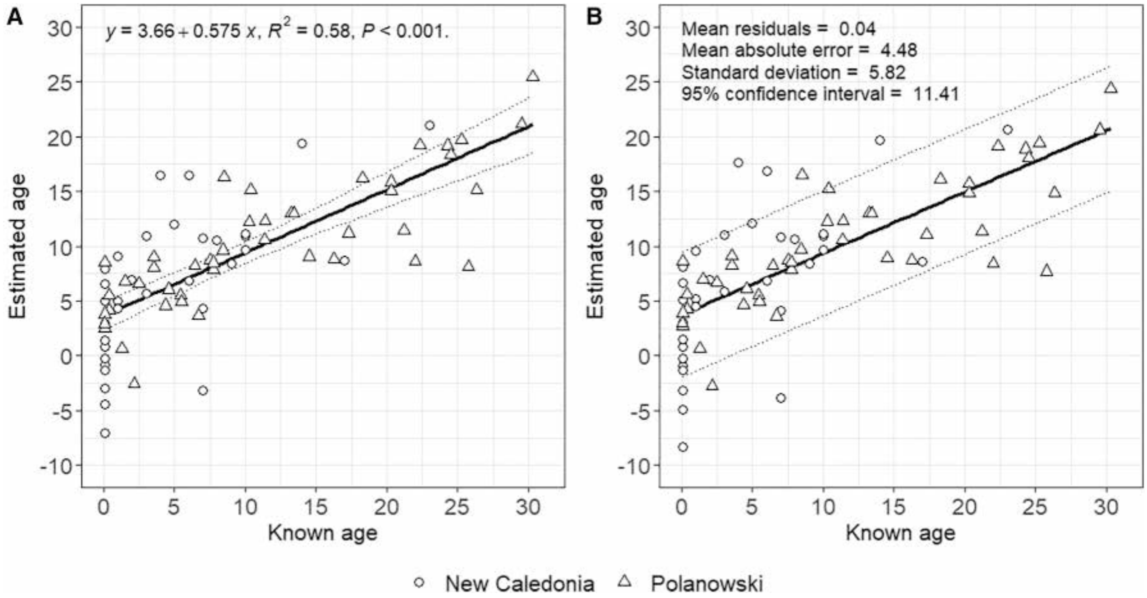

Figure 4. Accuracy and precision of the aging model using the combined calibration data

(A) Multiple linear regression for estimated ages of 78 samples from 68 whales from measurements of CpG methylation at two CpG sites (TET_C and GRIA2).

(B) Results of the leave-one-out cross-validation (LOOCV) analysis. The estimated age of every sample in the calibration data, when the model is based on the other 77 samples, is plotted against the known age.

In both plots, the 95% CI of the regression (dotted line) is shown. The calibration samples from the New Caledonian and Polanowski et al.[17] (Gulf of Maine, USA, and Australia) datasets are indicated with circles and triangles, respectively.

See also Figure S3 and Data S1 and S2.

ontogeny and broader social factors influence mating decisions. Mating behaviors are often age- and/or size-dependent, with individuals displaying mating tactics around the time, or even slightly before, they reach sexual maturity.[49] By investigating the behavioral context of males who successfully sired calves, we confirmed that the behaviors of singing, solitary escorting, and competing in competitive groups were correlated with successful reproduction. While inferences on the causation remain out of reach in the absence of any direct observations of mating in this species, our results provide valuable, indirect evidence to support the notion of classifying these behaviors as male reproductive tactics in humpback whales.

The ontogeny of reproductive tactics is not fully understood in humpback whales. Overall, we suggest that young male humpback whales attempt to engage in mating behaviors from an early age, yet relatively few are able to successfully reproduce. Males of all ages regularly participated in competitive groups. We hypothesize that these younger males participate in competitive groups to learn and practice until they become experienced enough to successfully defend a female from other male competitors, similar to how young northern elephant seals (*Mirounga angustirostris*) learn about their physical and social environment before attempting to reproduce within their fiercely competitive mating system.[50] The same may apply to singing, as previous studies on the Hawaiian breeding grounds estimated that 15% of singers were sexually immature males, likely practicing their song.[51] A male's age, or stage of development, might thus influence how likely he is to employ a certain reproductive tactic and/or how likely a tactic renders him successful in siring offspring. Similarly, using age as a proxy for body size, larger, older males may show increased competitive ability or be preferred by mature females.[34,35,52]

Our work contributes to the growing body of evidence that animals need to learn to hone reproductive tactics, with examples from species including elephants, birds, and other cetaceans. Young female African elephants (*Loxodonta africana*) gradually learn the estrus-associated visual signals important to their strategy of mate choice from their female kin.[53] Male juvenile Java sparrows (*Lonchura oryzivora*) repeatedly practice their courtship dance well before sexual maturation.[54] By doing so, they increased their motor performance and so potentially improved their future reproductive success.[54] Similarly, young male North Atlantic right whales (*Eubalaena glacialis*) seem to hone their mating tactics by participating in social groups called surface active groups, although they have never been observed copulating with a female.[49,55]

However, male reproductive tactics are also likely to be influenced by the social context and population density.[30] For example, the reproductive effort of yearlings in male reindeer (*Rangifer tarandus*) was lower as the sex ratio of the population became closer to even and intrasexual competition was higher, suggesting that yearling males took a more active role when older males were absent.[56] Similarly, yearling males in the lekking black grouse (*Tetrao tetrix*) were more likely to mate when the age structure was left-skewed and population density was increasing, as they presumably had better access to females due to the presence of fewer older males.[57] In humpback whales, male reproductive tactics on their migration route along East Australia shifted toward more males engaging in physical competition over singing as density increased over time.[29]

Changes in age structure are inextricably tied to population dynamics and the expected processes of recovery from past exploitation. We are unable to completely tease apart each of their effects on the reproductive behavior and success of male humpback whales separately in this study. However, neither are they expected to act in isolation in nature. Thus, it is important to consider both changes in population dynamics (e.g., density and age structure), combined and in relation to each other, when interpreting the observed changes in male reproductive tactics and success. Within any given breeding aggregation, an increase in population abundance, as observed on the humpback whale breeding ground in New Caledonia, ultimately results in a male's increased probability of encountering other male competitors and/or female mates (i.e., increased density). In the current study, singing became over-represented in older age classes (>16 years) in the higher abundance period, thus suggesting it is both an age-related (this study) and a density-related display.[29] Singing may aid a male in finding or attracting a female mate, especially at low density; however, once male density increases to a certain threshold, these benefits may be outweighed by the costs of attracting an increasing number of male competitors,[30] unless the singer himself has a higher chance of being competitively superior (e.g., older, larger, and/or more experienced).

Reproductive success by age category varied significantly between the two periods of lower and higher abundance. In particular, the increasing proportion of older males as singers and solitary escorts correlated with increased reproductive success of the oldest males in the higher abundance period compared with the lower abundance period. However, they were less successful at siring offspring than their predominant engagement in key mating behaviors would predict. Further, some males of the youngest age category (2–9 years) sired offspring, albeit at a lower rate than expected based on their relative abundance in the population. In male eastern gray kangaroos (*Macropus giganteus*), dominance status was under strong sexual selection, yet male reproductive skew was low, as many dominant males sired no offspring, while several subordinate males successfully reproduced.[58] The observation of young humpback whale fathers in both time periods (this study) and the documented low male reproductive skew in New Caledonian humpback whales[32] suggest that while age appears to be an important determinant of male reproductive success, other traits also likely play a role. Future work should investigate additional traits that may affect reproductive success via male-male competition and female mate choice.

Evolutionary implications of changes in reproductive patterns due to exploitation

Our results suggest that the evolutionary consequences of whaling may be more far-reaching than solely a decrease in abundance, but that in some species, there could be mechanisms that buffer some of the impacts. With most of our knowledge on baleen whales coming from the post-whaling era, we have limited historical knowledge on their reproductive behaviors and lifespan, and little is known about the demographic conditions and population dynamics under which these behaviors originally evolved (e.g., humpback whale song). Today, many previously exploited populations of baleen whales show signs of recovery, and the stabilization of their populations at or near carrying capacity may shift baselines once again, offering new insights into their lifespan,[23] reproductive strategies, and the underlying functions of their complex behaviors.

Population density can have strong effects on selective pressures and influence evolutionary processes that shape mating systems, including switch points between alternate mating tactics and mate encounter rates.[59] Following a concomitant increase in density, the number of encounters between mates and the number of competitors, individuals can become more selective in their choice of mates. Consequently, as density increases, reproductive success is expected to become higher in individuals with favorable traits or phenotypes, with a corresponding increase in sexual selection. This hypothesis could at least partially explain our findings, as age-related patterns in male reproductive tactics (singing and solitary escorts) and success were only evident in the period of higher abundance, once the age structure became less skewed toward younger males and density was presumably higher.

In the period of lower abundance during which the age structure was left-skewed and density was presumed to be lower, the encounter rate of individuals, especially older males (>23 years), may have been too low to result in strong age-related sexual selection, and the age-related competitive advantage of older males was overwhelmed by females being more likely to encounter younger males. In pre-exploitation populations of bighorn sheep, larger horn size was correlated with increased mating success in older rams; however, younger or smaller rams achieved mating success through alternative mating tactics that were less dependent on horn length and body size.[9] Unrestricted selective hunting for rams with large horns decreased the number of competitors in the population and correlated with a decrease in horn length over time.[60] This resulted in young male rams being released from the suppression of older rams and allowed them to obtain an increasing proportion of mates.[11] At the same time, young rams experienced increased competition among themselves and increased sexual selection on horn length and body mass.[11] Similarly, the left-skewed male age structure of humpback whales post-exploitation likely released young males from age-related sexual selection pressure, thus allowing them to sire a larger proportion of offspring. Consequently, male-male competition and sexual selection among the young may have increased compared with pre-exploitation populations of humpback whales.

Interactions between mating systems and population density are two-way,[59] and a change in patterns of sexual selection also has implications for population genetic diversity. Age-related reproductive success reduces the effective population size and increases the chance of inbreeding and genetic diversity loss, particularly in small populations.[61] Therefore, the shift toward less age-biased patterns of reproductive success could, in theory, increase effective population size and promote the maintenance of genetic diversity during the recovery from a population bottleneck. The North Atlantic right whale is one of the most endangered large whales. North Atlantic right whales show little signs of recovery, and their levels of genetic variability are among the lowest reported for a wild population.[62] A multitude of factors likely led to the stark contrast between the level of recovery of North Atlantic right whales and humpback whales.

For example, the extended and ongoing human-induced mortality of North Atlantic right whales from vessel strikes and entanglement in commercial fishing gear results in a much longer and more severe bottleneck compared with humpback whales, and consequently, increased loss of genetic diversity.[63,64] Reproduction in North Atlantic right whales was biased toward older males, with males not obtaining their first paternity until ~15 years of age,[61] contrasting our findings of relaxed age-related reproductive success in New Caledonian male humpback whales during lower abundance. Our current understanding of the evolutionary consequences of human-induced bottlenecks on the intertwined effects of population dynamics and sexual selection in affected populations of baleen whales is limited. In humpback whales, a relaxation of age-related sexual selection at lower densities could have facilitated a larger effective population size and acted to buffer genetic diversity loss during a time of lower abundance, when this was most critical.

Conclusion

In summary, epigenetic aging of males from the historically exploited New Caledonian humpback whale population revealed a shift from a left-skewed toward a more evenly distributed age structure over 19 years. The shift in the male age structure had profound effects on the age distribution of fathers and patterns of sexual selection. Older males were more often observed to engage in the key reproductive tactics of singing and solitary escorting and were more successful in siring offspring during times of higher abundance than lower abundance. Younger males sired more calves than expected at times of lower abundance, which we interpret to reflect lower age-related sexual selection pressure. Our results support theoretical expectations that reproductive tactics and sexual selection can be age- and density-dependent, with implications for the maintenance of genetic diversity through a population bottleneck. Differences in patterns of reproductive success across the study highlight the importance of accounting for changes in the underlying population age structure across time, especially in previously exploited populations. Long-term monitoring is thus crucial to assess population dynamics and how they intertwine with sexual selection and its consequences on the recovery and genetic diversity of exploited wild populations.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Franca Eichenberger (franca.eichenberger@outlook.com).

Materials availability

This study did not generate new, unique reagents.

Data and code availability

- Raw methylation levels and derived age estimates reported in this study are provided in the Data S1. The calibration data from Polanowski et al.[17] can be requested from Simon Jarman (simon.jarman@curtin.edu.au).
- All original R code is available in this paper's Data S2 and is publicly available at doi.org/10.6084/m9.figshare.31037917 as of the date of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization: E.C.G., E.L.C., and C.G.; formal analysis: F.E.; investigation: F.E., E.L.C., E.C.G., C.G., and L.R.; methodology: F.E., E.L.C., and S.J.; data collection: C.G., F.E., E.C.G., and J.R.; data curation: C.G., E.C.G., and D.J.S.; funding acquisition: E.C.G. and C.G.; project administration: E.C.G.; resources: C.G. and E.C.G.; supervision: E.C.G., E.L.C., C.G., and L.R.; writing—original draft: F.E.; writing—review & editing: F.E., E.C.G., E.L.C., C.G., L.R., S.J., D.J.S., and J.R.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Megaptera novaeangliae (humpback whale)	New Caledonia, Oceania, South Pacific	Dr. Claire Garrigue, IRD, Opération Cétacés (https://operationcetaces.wordpress.com)
Deposited data		
Raw methylation levels and age estimates	This paper	Data S1
Calibration data	Polanowski et al.[17]	https://doi.org/10.1111/1755-0998.12247
Paternity assignments	Eichenberger et al.[32]	https://doi.org/10.1098/rsos.241424
Software and algorithms		
R	R Development Core Team[65]	http://www.r-project.org
Other		
Humpback Epigenetic Age Assay (HEAA)	Polanowski et al.[17]	https://doi.org/10.1111/1755-0998.12247

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

This study used photo-identification, behavioral, paternity, and epigenetic ageing data from humpback whales (*Megaptera novaeangliae*) on their winter breeding ground in New Caledonia, South Pacific, as part of a long-term population monitoring project since 1995. Permits for the use of animals for scientific purposes were granted by the New Caledonian government and the Provinces Sud, North, and Isles to C.G. The University of St Andrews granted animal ethics approval.

METHOD DETAILS

Study population and data collection

The New Caledonian breeding population, recognized by the International Whaling Commission as BSE2, is one of seven breeding stocks in the Southern Hemisphere.[20] Located in the western South Pacific, the New Caledonia population is part of the IUCN Red List Endangered Oceanian breeding metapopulation,[16,66,67] from which it is, however, genetically distinct.[68] The most recent New Caledonian abundance estimate (male only) was 2,084 (95% CI = 1,761 – 2,407, 1995 – 2019).[32] During annual field surveys in the austral winter, samples for genetic analysis and photographs for identification were collected from 1995 to 2020.[32] Survey effort was focused on the South Lagoon (22°43′ S, 166°90′ E) and was largely constant across the study period.[32,41] Whales were carefully approached to be photographed and their behavior recorded (Table 1) along with any changes in group composition during focal follows, following published methodology.[41,69] Skin samples were collected from adults, juveniles, and calves either using a crossbow with a specially adapted bolt,[70] or a small dart fired from a modified veterinary rifle.[71] On some occasions, sloughed skin from individuals engaging in surface-active behaviors was also collected.[72] Skin samples were stored in 70% ethanol at -20°C. Individual humpback whales were identified using fluke photos[73] and/or microsatellite genotypes.[40] DNA profiles, including genetically identified sex and 15 nuclear microsatellite loci, of 1,606 individuals (962 males and 640 females) were previously constructed and used in a paternity analysis that inferred 66 fathers of 79 offspring sired between 1995 and 2018 on the same breeding ground.[32]

Humpback ageing model: Calibration and assessment

Molecular age biomarkers measure age-related modifications to DNA or RNA to estimate an individual's chronological age.[74] Some of these age-dependent modifications occur in the epigenome, such as the DNA methylation of specific CpG sites.[17] In an analysis of whales of known age, Polanowski et al.[17] screened 37 CpG sites in eight different humpback whale genes for a relationship with age. They identified seven CpG sites on three different genes (CDKN2A, TET2, GRIA2) with a strong methylation-age relationship and developed an ageing model for the estimation of age in humpback whales.[17] Their humpback epigenetic ageing model is an example of an "epigenetic clock"[75].

Genomic DNA from a total of 535 samples ($N = 485$ individual males) was analyzed using the humpback epigenetic ageing model developed by Polanowski et al.[17] DNA samples were treated with sodium bisulphite to convert unmethylated cytosines to the RNA base uracil, allowing the differentiation and detection of unmethylated versus methylated cytosines. Cytosine methylation levels were measured at eight CpG sites in three different humpback whale genes (CDKN2A, TET2, GRIA2; Table S3) previously identified in Polanowski et al.[17] Pyrosequencing was performed on a PyroMark Q24 system (Qiagen) following the methods described in Polanowski et al.[17]

All analyses were performed using R Statistical Software (v4.0.4[65]; Data S2). Using samples of known age (calibration data; Table S4), we calculated which of the CpG sites on each of the three genes showed the strongest age-methylation correlation to create a regression model to estimate unknown ages. From the eight measured CpG sites, two sites (CDK2NA_A and CDK2NA_C) showed a different age-methylation correlation (based on a Fisher's z-Test using the *cocor* R package[76]) across the two calibration datasets (one including Gulf of Maine, USA and East Australia humpback whales from Polanowski et al.[17] (n = 45, Pol) and one using known age individuals from the focal population of New Caledonia (n = 33, NC)) (Table S5; Figure S3A), and were thus removed from further analyses, allowing the combining of the two calibration datasets (n = 78). Of the six remaining CpG sites, four showed a significant age-methylation relationship in the combined calibration dataset (TET_A, TET_B, TET_C, and GRIA2; Table S5). These were integrated in three different combinations of CpG sites on separate gene regions in the multiple linear regression models (Table S6). The model with the best AIC score contained the two CpG sites: TET_C and GRIA2 (Figure S3B).

Seven individuals in the calibration dataset were represented by multiple samples. The Cook's Distance and hat values (leverage) associated with these data (were tested using the *stats* package) indicated that no one sample had a large influence on the model parameter estimates (Table S7), and thus, all samples were retained for model calibration.

The accuracy of the final ageing model was assessed from the multiple linear regression of the methylation at both CpG sites and the known age of individuals in the calibration data (Figure 4A). The regression R^2 of 0.58 ($p < 0.001$) indicated that although most of the proportion of the variation in methylation can be explained by age, there were additional factors affecting methylation levels at the selected CpG sites. The y-intercept and gradient ($y = 3.66 + 0.575\ x$) of the multiple regression showed that while young whales will have their age slightly overestimated, the age of older males will be slightly underestimated. The precision of the final ageing model was derived from the Leave One Out Cross Validation (LOOCV with k = 1; *caret* package[77]; Figure 4B) and estimated to be 4.48 years based on the mean absolute error (MAE) between known and estimated age in the LOOCV.

Age estimation of New Caledonian humpback whales

Age estimates of the 535 samples (N = 485 males) collected between 1996 and 2020 ranged from -9.5 to 37.2 years and were centered around 11.1 years (Figure S4; Table S4). Several samples (n = 20) yielded negative age estimates (Figure S4B) and were set to zero before calculating the estimated year of birth of individuals. For individuals with multiple samples of unknown age (n = 91 samples from 41 individuals), the mean estimated year of birth was calculated. From the estimated year of birth, the estimated age of individuals was calculated for any year within the study period. Finally, given the precision of our humpback epigenetic ageing model was ~4.5 years and the oldest known age New Caledonian male was 23 years, ages were binned into four categories (2 – 9 years, 9 – 16 years, 16 – 23 years, 23+ years) of right-open intervals (e.g., $2 \leq x < 9$) that were also biologically relevant given the suggested mean age of sexual maturity is 9 – 11 years.[78]

Samples were selected for the ageing analysis based on inferred paternities and behavioral contexts (singing, escorting, pair, etc.; Table 1) and thus had the potential to be age biased. To ensure that we had an unbiased representative sample age structure of males, a randomly selected subset was taken across the entire male data set. The age distribution of our behaviorally selected samples (N = 396 males) did not significantly differ from the age distribution of the randomly selected subset of samples (N = 89 males) (*t*-test, $t = -0.943$, $df = 123.96$, $p = 0.347$). Our dataset of aged males was thus considered as representative of the New Caledonian male breeding population as possible, given the underlying data collection it was derived from.

Implementation of epigenetic ageing in baleen whales

Emerging technologies for measuring DNA methylation are increasing the potential for developing biomarkers for estimating chronological age for a wider range of species.[74,79] Here, we adapted a previously developed epigenetic ageing model for humpback whales by Polanowski et al.[17] to the New Caledonian humpback whale breeding population by calibrating it with additional samples of individuals of known age collected at our study population.

Our New Caledonian humpback whale epigenetic ageing model showed an accuracy of 4.45 years, less than 5% of the estimated maximum lifespan of humpback whales (ca. 90 years; earplug laminations [22], see also Gabriele et al.[21]; molecular biomarker,[80] see also: Carroll et al.[81]), and performed similarly well to other cetacean epigenetic ageing studies.[82–85] In relation to the longevity of humpback whales, we were able to estimate the age of New Caledonian humpback whales with good accuracy. However, the accuracy of our age estimates was lower compared to several other methylations clocks.[46,86] The performance of epigenetic ageing models is influenced by several factors, such as 1) the number of screened and identified CpG sites,[87] 2) the sample size and age distribution of the calibration dataset,[88] and 3) other biological and/or external factors affecting the variation in measured methylation levels.[82,88,89]

Previous work by Polanowski et al.[17] for humpback whales identified eight age-related CpG sites. However, using our combined calibration dataset (Polanowski and NC), only two CpG sites were retained in our final ageing model. Including additional age-associated CpG sites would thus likely increase the accuracy of our age estimates. Although screening a large number of CpG sites (> 30,000) undoubtedly improves the accuracy of epigenetic age estimates,[46,48,84,86,89] it also renders the development of epigenetic ageing models computationally, statistically, and financially more challenging.

We extended the calibration dataset of Polanowski et al.[17] (Pol: 45 males) with additional samples of individuals of known age from the New Caledonian population (NC: 23 males). While the sample size of our combined calibration dataset (N = 68 males) was close to the recommended minimum of 70 individuals,[88] the age range (ca. 0 – 30 years) only covered about a third of a humpback whale's estimated lifespan. Our calibration dataset was further highly skewed towards younger individuals, with an overall mean known age of only 8.6 years. A more balanced age distribution and a wider age range in the calibration data would likely improve the accuracy of our ageing model. The sampling of old known-age individuals in wild populations, especially in previously exploited populations of long-lived species such as the humpback whale, is extremely challenging. In the New Caledonian population, less than 1.5% of aged males were estimated to be older than 40 years old in 2020 (Figure 4).

Using the combined calibration dataset resulted in a different and smaller set of CpG sites for our final ageing model (TET_C + GRIA2) compared to the original model by Polanowski et al.[17] (CDKN2A_A + TET_C + GRIA2). This further resulted in a slight reduction of accuracy, precision, and coefficient of determination (R^2) in our New Caledonian model compared to the original humpback ageing model. Our adapted ageing model explained about 60% (Figure 3A) of the observed variation in DNA methylation levels at selected CpG sites, thus indicating that other age-unrelated factors are also at play. Biological and environmental factors, such as sun exposure,[90] dietary changes,[91] early life stress,[92] and chemical pollutants, can affect methylation patterns over time,[93,94] which can lead to differences between species or even populations. Different methylation patterns between two populations of Atlantic fin whales (*Balaenoptera physalus*) were suggested to be the result of genetic differences and/or dissimilar environments affecting these populations.[82] Sampling a large number of individuals of known age, and especially older individuals, is extremely challenging in wild populations, and especially in species with a long lifespan. Implementing data from related populations and species to widen the age range or increase the number of individuals of known age offers a way to generate broad epigenetic clocks for species that are more challenging to sample.[46–48] However, possible differences in age-methylation relationships at CpG sites across different species and/or populations require further investigation and should not be ignored. Applying epigenetic clocks across species or populations, as well as the pooling of calibration datasets, should be thoroughly assessed as we have done here, and resulting age estimates interpreted with these caveats in mind. Our study provides valuable insights into the implications and application of epigenetic ageing, a promising tool in improving our understanding of the life history trends and population dynamics of wild populations.

QUANTIFICATION AND STATISTICAL ANALYSIS

Population age structure through time

The study period was divided on either side of a reported increase in abundance after 2008,[39] representing time periods of lower abundance (2000 – 2008) and higher abundance (2009 – 2018). We then tested whether the mean number of males in each age category differed between the two time periods using a Fisher's Exact test (FET, *stats* package). For this, we derived the population age structure from the estimated ages of all sampled males for each year of the study period and then calculated the mean number of males in each age category within each time period.

The role of age in male reproductive behaviors

We recorded seven behavioral contexts of male humpback whales commonly observed on the breeding ground, including different behaviors (e.g., singing, competing, escorting) and social roles (i.e., within competitive groups) (Table 1). In humpback whales, the two primary reproductive tactics are suggested to be singing and successfully outcompeting other males in a competitive group [34] (Table 1). However, mating has never been directly observed in this species, and the specific behaviors that lead to each successful paternity are unknown. Here, we first investigated potential links between male behaviors and successful reproduction by assessing all observations of inferred fathers in the year they sired a calf (Table S1). Behavioral contexts fathers consistently engaged in were considered most likely to result in successful matings and thus recognized as key reproductive tactics (Table S2). Then, for each of the seven behavioral contexts (Table 1) and each time period, we compared the proportion of males in each age category to the underlying age distribution of the observed male population using a Chi-squared test (*stats* package). The underlying population age distribution and the age structure of each behavioral context were derived from all observations of aged males within each time period to ensure the analysis on the role of age in male behavior is relative to the age distribution of males that visited the study area during that time. We complemented the Chi-squared tests with Cramer's V to quantify the strength of association between behavioral context and age category (*rcompanion* package[95]). Additionally, the Benjamin-Hochberg correction (*stats* package) and a post hoc analysis (*chisq.posthoc.test* package[96]) based on the Chi-squared residuals[97] were performed to identify which age category was driving the difference from the underlying age distribution.

Age-related differences in reproductive success

From the 66 fathers of the 79 paternity assignments derived in Eichenberger et al.,[32] a total of 59 fathers of 71 offspring sired from 2000 – 2018 were successfully aged. Of these, three fathers were unrealistically estimated to have sired offspring before the age of 2 years and thus excluded (Figure 2), leaving a total of 56 fathers of 68 calves available for further analyses (Table S8). First, we tested whether the age structure of fathers changed across time by comparing the number of males in each age category between the two time periods (2000 – 2008 and 2009 – 2018) using a Fisher's exact test (FET). The expected age distribution of fathers was generated based on the underlying male population age structure using a simulation approach: (i) for each year (2000 – 2018), males for the number of inferred fathers in a given year were selected (with replacement) from the sampled male population; (ii) the number of simulated fathers in each age category was then summed within each of the two time windows; (iii) this process was repeated 10,000 times to generate the expected age distribution of fathers assuming age-independent, random mating. Third, we estimated male reproductive skew (number of offspring per successful male) in each time window to assess changes in reproductive skew throughout the study period using an FET.