Close-kin methods to estimate census size and effective population size

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Running head: Close-kin, N, and N_e

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January 2021

Abstract

The last two decades have witnessed rapid developments and increasing interest in use of (1) genetic methods to estimate effective population size (N_e) and (2) close-kin mark-recapture (CKMR) methods to estimate abundance based on the incidence of close relatives. Whereas N_e-estimation methods have been applied to a wide range of taxa, all CKMR applications to date have been for aquatic species. These two fields of inquiry have developed largely independently, and this is unfortunate because deepest insights can be gained by joint evaluation of eco-evolutionary processes. In this synthesis, we outline factors (life-history traits; experimental design; stochasticity; ancillary information) that should be considered in identifying good candidate species and determining sampling/analytical regimes that can produce meaningful estimates. We show that the N_e/N ratio and the probability of a close-kin match both depend on a vector of parental weights that specify relative probabilities that different individuals will produce offspring. Although age-specific vital rates are central to both methodologies, for CKMR they are nuisance parameters that can bias abundance estimates unless properly accounted for, whereas they represent the signals of genetic drift that N_e estimation methods depend on. The most robust implementations of CKMR incorporate information from both parent-offspring pairs and siblings into a single, overarching framework, within which all demographic parameters can be jointly estimated in a way that allows coherent statements about uncertainty. Coordinating N_e and CKMR estimation methods using the same or overlapping datasets would facilitate joint evaluation of both the ecological and evolutionary consequences of abundance.

Key words: siblings, parentage analysis, reproductive skew, mark-recapture, genetics

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1 | INTRODUCTION

Quantifying abundance is central to all aspects of organismal biology, with two metrics being particularly important. The number of individual (census size, *N*) informs a wide range of studies in basic and applied ecology, including population growth rates, competition and predation, immigration and emigration, behavior, extinction risk, and ecosystem function (Krebs 2009). The evolutionary analogue of census size is effective population size (N_e), which determines rates of genetic drift, inbreeding, and loss of genetic variability and mediates the effectiveness of natural selection (Charlesworth 2009). N_e is usually smaller than *N* and sometimes much smaller (Frankham 1995; Hauser and Carvalho 2008; Palstra and Fraser 2012), which means that some abundant populations could nevertheless experience rapid genetic change—a scenario that has been proposed for many marine species with huge populations and high fecundity (Hedgecock and Pudovkin 2011).

Both key population-size metrics are challenging to estimate in nature. Many animal species are vagile, cryptic, or rare, making detection and enumeration difficult (Seber 1986; Taylor and Wade 2000; Holmes 2001; Doak et al. 2005), and this is particularly true in the marine realm (**"**Managing fisheries is hard: it's like managing a forest, in which the trees are invisible and keep moving around;" J. Shepard; http://jgshepherd.com/thoughts/)**.** Effective population size is defined in terms of key demographic parameters (mean and variance in offspring number; Crow and Denniston 1988, Caballero 1994), but collecting these data often entails formidable logistical challenges, especially for long-lived species. As a consequence, indirect genetic methods have been used to estimate N_e since the early 1970s (Krimbas and Tsakas 1971). Similarly, mark-recapture (MR) methods to estimate abundance have been a cornerstone of wildlife ecology for the past half century (Jolly 1965; Seber 1982; Pace et al. 2017). For species that are difficult to capture and/or physically tag, naturally occurring genetic markers can be used to identify "recaptures" of the same individual from passively-collected samples such as feces or hair. For example, Kendall et al. (2008) used genotypes derived from hair samples to obtain a robust estimate of abundance of a threatened population of grizzly bears (*Ursus arctos*) in Glacier National Park, USA, and Buckworth et al. (2012) used 'genetagging' to estimate harvest rate and catchability in Spanish mackerel (*Scomberomorus commerson*) from northern Australia.

More recently, researchers have begun to capitalize on the realization that, if naturally-occurring genetic marks are used, it is not necessary to capture individuals more than once, and the single "capture" can simply involve collecting DNA from a dead individual. Close kin can also be used in a MR framework because individuals "mark" their relatives with shared genes (Skaug 2001). The two kinship categories that have been used to date with close-kin mark-recapture (CKMR) are parent-offspring pairs (POPs) and siblings. Successful application of CKMR methods to a number of fish species (Rawding et al. 2014; Bravington et al. 2016a; Hillary et al. 2018; Bradford et al. 2018; Ruzzante et al. 2019; Bravington et al. 2019), together with refinements in statistical methodology (Bravington et al. 2016b; Skaug 2017; Conn et al. 2020), have generated a great deal of interest in using this approach more broadly to study marine, freshwater, and terrestrial taxa (e.g., Stewart et al. 2018; Oleksiak and Rajora 2020). The basic principles of CKMR have also been used to place an upper bound on the pre-Columbian human population size in the Caribbean (Fernandes et al. 2020).

Considerable advances in the estimation of effective size have also been made over the past ~15 years. The AgeNe model (Waples et al. 2011) allows one to estimate N_e in species with overlapping generations, based on age-specific vital rates. Some additional factors that can influence N_e and the N_e/N ratio have also been examined, including persistent individual differences (Lee et al. 2011; 2020), density dependence (Myhre et al. 2016), and skip breeding and constrained litter size (Waples and Antao 2014). Prior to the mid-2000s, most genetic estimates of N_e used the temporal method (Nei and Tajima 1981; Waples 1989), which requires at least two samples spaced in time. Most recent applications, however, use one of two single-sample methods (Palstra and Fraser 2012): a bias-adjusted method based on linkage disequilibrium (LD; Waples 2006) or a method based on the frequencies of siblings (Wang 2009), and these methods have been widely applied to marine, freshwater, and terrestrial species. Precision and bias of both single-sample methods has been extensively evaluated using simulated data (Waples and Do 2010; Tallmon et al. 2010; England et al. 2011; Gilbert and Whitlock 2015; Wang 2016), as have effects of age structure on LD estimates (Robinson and Moyer 2013; Waples et al. 2014).

Although these recent advances in methods for estimating N and N_e have occurred simultaneously, they have also occurred largely independently. That is unfortunate, as CKMR estimates of abundance and related quantities depend on the same key demographic parameters that are needed to calculate N_e. Furthermore, genotypes for individuals sampled for CKMR can be also be used to generate indirect genetic estimates of effective size, as was recently demonstrated for southern bluefin tuna, *Thunnus maccoyii* (Waples et al. 2018a).

In this synthesis, we consider in some detail the close relationship between CKMR-based methods to estimate abundance and related quantities and demographic and genetic methods to estimate effective size. Recent work by Akita (2020a,b) has made a good start in this direction for some specialized scenarios, and here we provide a more general treatment. Although POPs-based CKMR has been successfully applied to semelparous species (Rawding et al. 2014), most species are iteroparous with overlapping generations, and that is our focus here. We outline various factors (biology of the focal species; experimental design; stochasticity; availability of ancillary information) that should be considered in determining whether a focal species is a good candidate for study and, if so, what kind of sampling/analytical regime would be required to produce meaningful estimates of abundance and other demographic parameters. Factors affecting performance of the methods ares illustrated with worked examples. We clarify under what condition the incidence of siblings provides information about adult abundance and when it provides information about effective size. We show that the N_e/N ratio and the probability of a close-kin match both depend on a vector describing the relative probabilities different individuals have of producing offspring. We also show that, although age-specific vital rates are central to both types of analyses, they have different consequences for performance of CKMR estimates than they do for estimates of effective size.

Our overall goal is to provide a reference document that potential users of these methods can consult in planning experimental design and subsequent analyses. Because a full understanding of the evolutionary ecology of natural populations requires being able to quantify both abundance and effective size, it is important to maximize the synergistic effects of the two types of analyses. All CKMR applications published to date are for aquatic species, so we will

draw heavily on those examples; however, CKMR methods can also potentially be applied to terrestrial species, just as have genetic methods to estimate effective size.

2 | EVOLUTIONARY DEMOGRAPHY

2.1 | Vital rates and reproduction

General features of population demography that underpin models used here are described below (see Table 1 for notation and definitions of terms). The focal population is assumed to be isolated and iteroparous, with separate sexes. Time periods, assumed to be years, are discrete and age is indexed by x. Reproduction follows the seasonal birth-pulse model (Caswell 2001). At age x, individuals produce on average b_x offspring and then survive to age x+1 with probability s_x. Both b_x and s_x can differ with age and sex. First reproduction occurs at age α (age at maturity) and maximum age is ω. Often, offspring are not enumerated until they reach a certain age, at which point they are considered "recruits." We designate z as age at recruitment ($1 \leq z \leq \alpha$) and scale b_x to production of offspring that survive to age z. Cumulative survival from age z through age x is $l_x = \prod_{i=z}^{x} s_{i-1}$, with l_z=1. Letting N_x be the number of individuals of age x alive at any given time, common definitions of census size include total abundance: $N_T = \sum N_{x,x=0,\omega}$; number of post-recruits: $N_R = \sum N_{x,x=z,\omega}$; number of adults: $N_A = \sum N_{x,x=\alpha,\omega}$. Except as noted we focus on N_A.

Each year, all adults have the potential to breed and produce offspring according to a generalized Wright-Fisher (WF) model of reproduction. Let k_i be the reproductive output (RO = number of offspring) produced by the ith parent, and let $\bar{k}$ and V_k be the mean and variance in offspring number across N_A potential parents. In the classical WF model of random reproductive success, each adult has an equal and independent probability of being the parent of each offspring; this produces a multinomial distribution of offspring number, with $E(V_k) = \bar{k}(N_A-1)/N_A$, which is closely approximated by the Poisson variance $V_k = \bar{k}$. The generalized model (Waples 2020) differs in allowing adults to have unequal probabilities of parenting a given offspring. Define a vector of relative parental weights, $\boldsymbol{w}$, where w_i is the relative probability that adult i will be the parent of a given offspring. These relative weights can be converted to a vector of absolute (standardized) parental weights, $\boldsymbol{W}$, where $W_i = w_i/\Sigma w_i$ and $\Sigma W_i = 1$. If all weights are equal, we recover the original WF model, with $E(V_k) \approx \bar{k}$; in real populations, however, different individuals generally will have different parentage probabilities, which leads to overdispersed variance in reproductive success ($V_k > \bar{k}$) relative to the random expectation. This in turn has important consequences for both N_e and CKMR.

Within a single year of reproduction, two components contribute to variation in individual weights: (1) an among-age effect, which arises from systematic changes in fecundity with age; and (2) a within-age effect, which reflects difference in expected reproductive success of individuals of the same age and sex. The among-age effect is determined by the b_x vector from a life table. The within-age effect depends on the relationship between an individual's expected reproductive success and the mean for its age and sex. Thus, the relative parental

weight for individual i of age x can be written as $w_i = b_x + \delta_i$, where δ_i is the deviation from the age-specific mean. A positive δ means that the individual is expected to be above average for its age and sex, while a negative δ indicates the reverse. If all δ_i=0, the within-age effect disappears, and the expected age-specific variance in reproductive success equals the mean ($E[V_{k(x)}] \approx b_x$). Empirical data for $V_{k(x)}$ are rarely published, but there are many reasons why individuals of the same age and sex might not have identical expectations of reproductive success. Some might have established breeding territories or acquired harems and others not; some might have phenotypes that favor them in sexual selection. In many species, fecundity depends more on size than age, in which case variation in w_i for same-age, same-sex individuals will be correlated with variance in size-at-age, leading to $V_{k(x)} > b_x$ (see Waples et al. 2018a for an example involving southern bluefin tuna). The magnitude of the within-age effect is quantified by the age-specific index $\phi_x = V_{k(x)}/b_x$ (Waples et al. 2011; Waples 2016a,b).

Finally, in iteroparous species individual weights can vary across years, for two major reasons: (1) if b_x varies with age; (2) if an individual's condition factor changes, which in many species depends on whether it has reproduced in a recent year (see Section 3.2.2).

2.2 | Effective population size

Close-kin methods provide information about abundance in the recent past, so contemporary (rather than long-term) effective size is our focus. Mean and variance in offspring number are the major factors that determine effective size. For seasonal reproduction in an age-structured species, the most relevant effective-size metric is the effective number of breeders per year (N_b) because it relates directly to the annual number of adults (N_A). N_b can be calculated using the standard discrete-generation formula for inbreeding effective size (Crow and Denniston 1988; Caballero 1994):

$$N_b = \frac{\bar{k}N_A - 1}{\bar{k} - 1 + {V_k}/{\bar{k}}} \quad . \tag{1}$$

With separate sexes, N_b can be calculated separately for males and females and an overall effective size obtained using Wright's (1938) sex-ratio adjustment. Hereafter, for simplicity we focus on census size and effective size for females, but exactly analogous equations apply to males. Table 2 illustrates how the ratio N_b/N_A changes as a function of the two factors that contribute to annual V_k: variation in fecundity with age, and ϕ_x that exceeds the random expectation of 1. Equation 1 can be reformulated in terms of the parental weights described above. Let $CV_W^2 = \sigma_W^2/\bar{W}^2$ be the squared coefficient of variation (CV) of the individual weights (which is the same whether calculated using raw or standardized parental weights). Then, the effective number of female breeders per year is just (Waples 2020):

$$N_{b(f)} \approx \frac{N_f}{1 + CV_W^2}, \text{ so } \frac{N_b}{N_f} \approx \frac{1}{1 + CV_W^2} \quad . \tag{2}$$

Although N_b relates directly to annual abundance estimated by CKMR, virtually all evolutionary theory depends on effective size per generation (N_e) rather than N_b per year. Calculating N_e in iteroparous species is complicated by the necessity of integrating information across multiple episodes of reproduction. Hill (1972) provided the most general solution:

$$N_e \approx \frac{4N_z T}{V_{k\bullet}+2} \quad , \qquad (3)$$

where $T=\Sigma x l_x b_x / \Sigma l_x b_x$ is generation length and $V_{k\bullet}$ is the variance in lifetime number of offspring produced by the N_z individuals in a cohort. The program *AgeNe* (Waples et al. 2011) calculates $V_{k\bullet}$ using the species' age-specific vital rates (s_x, b_x, ϕ_x), under the assumptions (adopted from Hill 1972 and Felsenstein 1971) that population size is constant and survival and reproduction are independent over time. Demographic N_e also can be converted from an estimate of N_b based on the N_b/N_e ratio, which largely depends on 2-3 life history traits (Waples et al. 2013).

Consequences of some potential violations of the independence assumption have been evaluated. If some individuals are consistently above or below average (for their age and sex) at producing offspring (that is, if their δ_i values are positively correlated over time), these persistent individual differences increase lifetime $V_{k\bullet}$ and reduce N_e per generation (Lee et al. 2011, 2020). Females (and sometimes males) of many animal species (including birds, fish, and large mammals) often skip one or more breeding seasons after reproducing (Shaw and Levin 2013), which leads to a complex pattern of negative and positive temporal correlations in realized reproductive success, depending on the time lag. Skip breeding generally reduces N_b per season (because not all adults reproduce each year) but increases N_e per generation (because it limits opportunities of some individuals to dominate lifetime reproductive success). These effects are more pronounced for species with high fecundity and high juvenile mortality (Waples and Antao 2014).

3 | PROPERTIES OF ESTIMATORS OF CENSUS AND EFFECTIVE SIZE

3.1 | Traditional mark-recapture

The traditional mark-recapture method involves three elements:
Capture and mark a number (m_1) of individuals;
Release them to mix with the general population;
Collect a second sample of m_2 individuals; those marked are the "recaptures" (R).
In the simplest version of MR, if a long list of assumptions is met the estimate of abundance is simply $\hat{N} = m_1/P$, where $P = R/m_2$ is the fraction of the second sample that is marked. This leads to

$$\hat{N} = Y/R, \qquad (4)$$

where $Y = m_1 m_2$ is the number of potential comparisons of individuals in the two samples, and the denominator is the number of matches. In practice, a complex statistical framework for conducting MR analyses has been developed to account for various factors that complicate interpretation, including (but not limited to) mortality between tagging and recapture; heterogeneity in capture probabilities; and immigration and emigration between marking and recapturing (reviewed by Pollock 2000; Lindberg 2012).

3.2. | Close-kin mark-recapture

CKMR is a framework for estimating demographic parameters, including but not restricted to abundance, based on pairwise comparisons of individuals to find close kin. The probability that one particular pair of samples will turn out to be a POP, say, depends both on individual-level covariates (e.g., birthdate: a parent's birthdate must be earlier than that of its offspring) and on population-level demographics (e.g., adult abundance). Except in trivial cases, several demographic parameters (not just abundance) must be specified to compute the probabilities of finding a close-kin match. Consequently, CKMR does *not* normally reduce to a single closed-form expression for estimating N. Rather, CKMR is used to build a log-likelihood incorporating outcomes of each pairwise comparison; the log-likelihood function depends on the unknown parameters, including abundance, and parameter estimates are found by maximizing the log-likelihood (or a Bayesian version thereof; Bravington et al. 2016b). Very often, samples will be taken from multiple cohorts, and it becomes necessary to model a time-series of abundance via age-structured population dynamics, as in many fish stock assessments (Quinn and Deriso 1999).

A typical CKMR analysis involves several types of comparison. For POPs, juveniles from several cohorts are compared to adults of different sizes and sexes, sampled at different times; similarly, individuals from many different cohorts often must be compared to search for siblings. The optimal way to estimate parameters is to handle all types of pairwise comparison inside a single population-dynamics model that can a) keep track of adult demographics, and b) estimate parameters. Except in very simple cases, estimates of abundance and other demographic variables (and their variances) have to be found numerically. There is no requirement in CKMR to assume stable populations; indeed, incorrectly making that assumption generally leads to bias (Skaug 2001). Unless the sex ratio is even and vital rates are the same in both sexes, the distribution of genetic "marks" passed on to their offspring by male and female parents will be different. In general, therefore, it is best to make sex-specific CKMR estimates.

3.2.1 | Parent-offspring pairs

Notwithstanding the difficulty in developing a general closed formula for CKMR, one key metric is central to all CKMR analyses because it describes the probability that any given pair of individuals will produce a close-kin match: the relative reproductive output (*RRO*) of different potential parents. For POPs, consider the probability [$P(i \rightarrow j)$] that female i is the mother of offspring j. A general expression for this probability is (Bravington et al. 2016b):

$$P(i \rightarrow j) = k_{i,tj} / TRO_{tj} = RRO_i, \qquad (5)$$

where $k_{i,tj} = RO_{i,tj}$ is the number of offspring by female i in the year of j's birth and $TRO_{tj} = \Sigma k_{i,tj}$ is the total number of offspring by all females in that year. To estimate abundance, it is necessary to combine these probabilities across all sampled offspring and potential parents. POPs provide information about N_f in the year the offspring were born, so a time-series of abundance estimates can be obtained using data from multiple cohorts of offspring.

Because actual reproductive output is rarely known, CKMR generally deals with expected relative reproductive output, *ERRO*, based on expectations for different classes of parents (Bravington et al. 2016b). This concept provides a direct link to traditional MR and to

the parental weighting scheme described above, where parental weights are calculated as $\boldsymbol{W}$=E(RO)/TRO, so for female i, $ERRO_i$) = W_i.

CKMR estimates based on POPs are greatly simplified by the fact that each offspring has exactly one mother. This means that variation in offspring number does not, by itself, cause bias (but, as discussed below, it can if there is unmodeled correlation between reproductive output and sampling probability). For illustration, consider a simple example of a single, closed population with N_f adult females, equiprobable samples of S_p adults and S_o offspring, and no mortality between reproduction and sampling of adults. Also assume that parentage assignments are made without error and that all $Y = S_p \times S_o$ pairwise comparisons are made to search for POPs. Consider two extreme scenarios: 1) All parents produced exactly the same number of offspring; and 2) A single female produced all the offspring. In Scenario 1, each comparison has probability $1/N_f$ of producing a POP, so the expected number of POPs (E(R_{POP})) is $Y*(1/N_f) = S_p*S_o/N_f$. In Scenario 2, only two outcomes are possible: if the prolific female is one of the adults sampled, she matches to each of the offspring, and $R_{POP} = S_o$; if that female is not sampled, there are no matches and R_{POP}=0. The first outcome occurs with probability S_p/N_f, so overall E(R_{POP})=S_o*S_p/N_f, exactly as in Scenario 1.

This simple example would lead to an estimator of adult female census size, directly analogous to a standard Lincoln-Petersen MR estimate (Seber 1982), as

$$\widehat{N}_f = Y/R_{POP} \quad , \qquad (6)$$

which has the same general form as Equation 4. Because this result is not sensitive to variation in offspring number, CKMR estimates based on POPs are not confounded by a signal related to N_e or N_b.

It is important to recognize, however, that the probability that any comparison will produce a parent-offspring match does depend on how potential parents and offspring are sampled. In particular, non-reproductive individuals in a given year cannot produce POPs, so including such individuals in a search for matches will reduce P(POP). For example, if the sample of potential parents includes immature individuals, $\widehat{N}_f$ will apply to the population segment that is sampled rather than just adults (see Table 3). If skip breeding occurs and only reproductively active individuals are subject to sampling, $\widehat{N}_f$ will not reflect the fraction of the adults that are not breeding that year (Figure 1). The extent to which these factors represent problems in any particular application depends on the experimental design and the population segments for which estimates are needed.

Furthermore, variation in offspring number can affect CKMR estimates based on POPs in two important ways. First, precision of POPs-based estimates is inversely related to variation in reproductive success. If one or a few parents dominate reproduction, the number of matches will vary widely depending on whether the prolific parent(s) are sampled. Second, if reproductive success is skewed and the probability that an adult will be sampled is correlated with its *RRO,* the abundance estimate will be biased unless this correlation is accounted for (see Table 4).

3.2.2 | Siblings

CKMR for siblings is more complicated because (1) the parents are not observed directly, and (2) the probability of a sibling match depends heavily on the extent to which reproductive output varies both within and across years. Because only cross-cohort sibling comparisons are suitable for estimating abundance (see below), and because full siblings from different cohorts will be rare in large, randomly mating populations, CKMR analyses generally focus on finding half-sibling pairs (HSPs).

Consider two ordered time periods, with t2>t1. The probability that two randomly selected offspring, one from time t1 and one from t2, have the same female parent is (from Equation 3.9 in Bravington et al. 2016b):

$$P(MHSP) = \sum_{i=1}^{N_{f1}} \left[\frac{k_{i,t1}}{TRO_{t1}} \times \frac{k_{i,t2}}{TRO_{t2}}\right] = \sum_{i=1}^{N_{f1}} \left[RRO_{i,t1} \times RRO_{i,t2}\right] \quad . \tag{7}$$

Notation in Equation 7 follows that in Equation 5 except for the subscripts t1 and t2 to indicate time periods. In a sibling-only design, it is necessary to sum across all possible females that could have been alive when potential siblings were born. Females that mature after t1 cannot produce a sibling match for offspring born in t1, so the summation is Equation 7 is taken over the N_{f1} females that were sexually mature at time t1.

Equation 7 does not generally lead to a closed-form estimator of adult abundance, unless the species' biology allows a number of simplifying assumptions (see Hillary et al. 2018 for an example involving the white shark, *Carcharodon carcharias*, which also required estimating adult mortality from the data). However, some heuristic insights into factors that influence $P(MHSP)$ can be achieved if the equation is reformulated as follows (see Supporting Information for details):

$$P(MHSP) = mean(RRO_{t2*}) + N_{f1}[cov(RRO_{i,t1}, RRO_{i,t2*})] \ , \tag{8}$$

where the "*" in RRO_{t2*} indicates that this vector only includes data for the subset of t2 females that were mature at time t1. Equation 8 is a simple linear regression, with an intercept = $mean(RRO_{t2*})$, a slope = N_{f1}, and the independent variable being the covariance in individual *RRO* across time. It might appear from Equation 8 that the slope represents a signal of female population size at time t1, but that it misleading: the denominator of the population covariance is N_{f1}, so that signal cancels out, leaving a term that is proportional to $cov(RRO_{i,t1}, RRO_{i,t2*})$. Below we consider some insights regarding sibling-based CKMR that can be gained from closer inspection of the intercept and covariance terms from Equation 8. Two general cases can be identified.

Case 1. Offspring are from the same cohort ($t_1 = t_2$).

In this case, only a single time period is involved, so $RRO_{i,t1} = RRO_{i,t2} = RRO_i$, with mean($RRO_i$) = $1/N_f$. Noting that the covariance of a random variable with itself is its variance, Equation 8 becomes

$$P(MHSP) = N_f[(1/N_f)^2 + var(RRO_i)] = 1/N_f + N_f[var(RRO_i)]. \tag{9}$$

Equation 9 shows that, when offspring from the same cohort are compared, the probability of finding a sibling match increases as a function of the realized variance in offspring number. This variance is the primary signal for N_b, so siblings from the same cohort provide information related to effective size and not directly about adult abundance. For this reason, CKMR estimates using siblings generally attempt to exclude within-cohort comparisons (Bravington et al. 2016b, 2020; Hillary et al. 2018).

Case 2. Offspring are from different cohorts ($t_1 \neq t_2$).

If the covariance term in Equation 8 is 0, $P(MHSP)$ simplifies to $mean(RRO_{t2*})$, which indicates that the main signal from cross-cohort siblings relates to abundance at the time the younger sibling was born. However, $cov(RRO_{i,t1}, RRO_{1,t2*}) = 0$ only occurs under two unlikely conditions: (1) $var(RRO_{i,t1}) = 0$ and/or $var(RRO_{i,t2}) = 0$; or (2) $RRO_{i,t1}$ and $RRO_{i,t2}$ jointly vary in such a way that the N_{f1} terms that comprise $cov(RRO_{i,t1}, RRO_{1,t2*})$ magically sum to exactly 0. Even if all parents have the same expected fecundity, the probability that all females will produce exactly the same actual number of offspring is vanishingly small for any population of appreciable size. The second condition is also highly unlikely to occur by chance, although it certainly might be the case that the deviation from zero covariance is small enough to be of little practical importance.

The most important factors that can affect the two terms in Equation 8 are the following:

Mortality. If an adult female dies between t1 and t2, it can't be a parent in time t2, so $RRO_{i,t2} = 0$. These null parents are included in the summation in Equation 7 and will reduce $mean(RRO_{t2*})$ and therefore $P(MHSP)$ unless this mortality is accounted for (see Table 5).

Changes in fecundity with age. In addition to including these null parents, $mean(RRO_{t2*})$ differs from mean *RRO* for all N_{f2} adult females at time t2 because it does not include reproductive output by newly recruited adult females. If mortality t1-t2 is accounted for, then the partial $mean(RRO_{t2*})$ should on average equal overall $mean(RRO_{t2})$, provided that reproductive success of individuals surviving from t1 is representative of overall female reproductive success in time t2. However, individuals that survived from t1 will on average be older than newly-recruited females; therefore, if fecundity changes with age (as is common in many species, especially fish and other poikilotherms with indeterminate growth), this will affect $P(MHSP)$ unless it also is accounted for.

Persistent individual differences. If within-age individual deviations from mean fecundity (the δ_i defined in Section 2.1) are non-zero and positively correlated over time, such that some individuals are consistently above or below average at producing offspring, this will (all else being equal) lead to positive $cov(RRO_{i,t1}, RRO_{1,t2*})$ and a higher proportion of siblings, which in turn will downwardly bias abundance estimates, unless accounted for. Body size is one factor that can easily lead to positive temporal correlations in reproductive success. Fecundity often depends on size more directly than on age, and an individual that is large (or small) for its age at time 1 is also likely to be an outlier in subsequent years. Whether an individual has secured a territory is another factor that could produce persistent individual differences.

Skip breeding. Skip breeding leads to a complex pattern of $cov(RRO_{i,t1}, RRO_{1,t2*})$ that can vary in both magnitude and sign, depending on the species' life history and the time interval for cross-cohort comparisons (Figure 1). For example, consider a species for which females

generally skip y years after reproducing before doing so again. Comparisons of offspring born $<y$ years apart will produce few if any sibling matches, while comparisons exactly y years apart will produce sibling matches at a rate determined not by female population size as a whole, but by the $\approx N_f/y$ females that actually reproduce each year. In a study that includes a sufficient number of offspring cohorts, it is possible that these factors will more or less cancel each other, such that the overall fraction of sibling matches reflects overall abundance. The extent to which this is true, however, should be evaluated by a quantitative analysis. It should be possible to develop an adjustment for skip breeding using the vector (θ_t) of the probability that an individual will reproduce in the current year, given that it last reproduced t years before (Shaw and Levin 2013). For female loggerhead turtles (*Caretta caretta*), θ_t = [0.025, 0.443, 0.634, 0.743, 1] for t=1,5 (Waples and Antao 2014), indicating a substantial effect of prior reproduction lasting four years. In contrast, for female black bears (*Ursus americanus*) in Michigan θ_t = [0.035, 0.94, 1] for t=1,3 (Waples et al. 2018b), indicating a faster recovery.

Simulated data illustrate some of these effects (see Supporting Information and Figures S1 and S2). Major take-home messages can be summarized as follows:

- Even with equal parental weights, random variation in realized *RO* creates random variation in sibling probabilities, but mean($cov(RRO_{i,t1}, RRO_{1,t2*})$ ≈ 0 and Equations 7 and 8 are unbiassed for *P*(*MHSP*);
- When parental weights are unequal but not correlated over time, Equations 7 and 8 are still unbiased but the variance among replicates is higher;
- Parental weights that are unequal and positively correlated over time lead to positive ($cov(RRO_{i,t1}, RRO_{1,t2*})$ and elevated *P*(*MHSP*);
- Changes in fecundity with age can increase *P*(*MHSP*).

These results show that the net effects of multiple factors can be complex. Therefore, except in the simplest cases, sibling probabilities should be calculated in an integrated likelihood framework, using conditional expectations for individual reproductive success over time and taking into account all relevant covariates (Bravington et al. 2016b; Hillary et al. 2018).

3.3 | Effective population size

Because demographic data required to calculate N_b or N_e directly are difficult to obtain, genetic data are widely used to provide indirect estimates. The sibship method developed by Wang (2009) fits nicely into the MR and CKMR frameworks. Wang's model considers three types of relationship in the one-generation pedigree: full siblings (FS), half siblings (HS), and unrelated (U), which nevertheless can be related through previous generations. In a random sample of m progeny, $Y = m(m\text{-}1)/2 \approx m^2/2$ pairwise comparisons are possible, and expected proportions of siblings are simple functions of N_e (Wang 2009):

$$E[Q_{HS} + 2Q_{FS}] = \frac{4}{N_e}, \qquad (10)$$

where $Q_{HS} = R_{HSP}/Y$ is the fraction of pairs that are half siblings (MHSP and PHSP combined) and $Q_{FS} = R_{FSP}/Y$ is the fraction that are full siblings. Equation 10 ignores the (generally small) correction for departures from Hardy-Weinberg proportions proposed by Wang (2009). Rearrangement leads to the estimator of effective size:

$$\widehat{N}_e = \frac{4Y}{R_{HSP}+2R_{FSP}} \quad , \tag{11}$$

which is of the same general form as Equations 4 for traditional MR. When applied to offspring from a single cohort, Equation 11 provides an estimate of N_b. Wang et al. (2010) described an extension of this sibship method to estimate N_e when generations overlap, but their model assumes that $\phi_x = 1$ for each age and sex so is of somewhat limited general applicability. Linkage disequilibrium (LD) quantifies associations of alleles at different gene loci. Random LD is generated every generation by mating among a finite number of parents, and that provides the basis for estimating effective size. Loci on the same chromosome ("linked") provide information about historical N_e, so unlinked markers are most suitable for estimating contemporary N_e. For unlinked loci, N_e can be estimated from r^2 (a measure of LD) and sample size (S = number of individuals) (Hill 1981):

$$\widehat{N}_e \approx \frac{1}{3(r^2-1/S)}. \tag{12}$$

The program LDNe (Waples and Do 2008) adjusts for bias in Equation 12 caused by ignoring second-order terms in $1/S$ and $1/N_e$.

When applied to offspring from a single cohort, the LD method primarily reflects the signal from parental N_b, with some influence from N_e in previous generations (Waples 2005; Waples et al. 2014). When applied to mixed-age samples, LDNe generally underestimates generational N_e by 10-40% (Waples et al. 2014). Blower et al. (2019) developed simulation software that can help researchers interested in estimating effective size in age-structured species using the LD method to determine sample sizes of individuals and loci that should produce desired levels of precision.

3.4 | Spatial structure and sampling

Spatial structure, where reproduction and/or sampling is concentrated in certain geographic locations, will *not* bias CKMR estimates if either of the following conditions are met (Conn et al. 2020): (1) offspring (siblings analyses) or parents and offspring (POPs analyses) mix thoroughly before sampling occurs; or (2) sampling is equiprobable, meaning that every individual has an equal chance of being sampled. To illustrate the first criterion, if reproduction is panmictic in area A, where adults are sampled, and offspring randomly migrate to areas B and C, sampling in just one of those areas still produces a random sample of all offspring (see Bravington et al. 2016 for an empirical example involving southern bluefin tuna).

If the second criterion (and other CKMR assumptions) are met, the resulting abundance estimate will be unbiased, but it will reflect the total number of adults. If spatially-restricted reproduction is persistent and there are independent or semi-independent populations or stocks for which separate abundance estimates are desired, the naive CKMR estimate of a single overall abundance might be unsatisfactory. If estimates are available for key parameters describing the degree of reproductive connectivity and distributional overlap, it might be possible to develop an analytical framework to develop separate abundance estimates for different areas (Davies et al.

2017). Furthermore, spatial patterns in the distribution of close-kin can provide insights into population subdivision and connectivity (Økland et al. 2009; Wang 2014; Feutry et al. 2017).

More generally, problems can occur if neither of the above criteria are met—that is, when mixing is incomplete and sampling is spatially biased (Conn et al. 2020). It is crucial that sampling events be independent, with the key proviso being that whether an individual is sampled should have no effect on the probability that one of its close kin is also sampled. If this criterion is satisfied, conditioned on covariates associated with the sampled individuals, then expected values of *RRO* are multiplicative, which makes the CKMR analyses tractable. Joint sampling of close kin in the same spatio-temporal stratum at higher frequencies than would occur by chance will increase the probability of close-kin matches; this potential source of bias can be reduced by appropriate filtering of the data (as Bravington et al. 2020 did with the school shark, *Galeorhinus galeus*). Another important sampling consideration was noted above: selective sampling of adults based on age (or size) will bias POP-based CKMR estimates if fecundity also varies with age/size and these correlations are not accounted for (Table 4). Fortunately, if population stratification is strong enough to substantially affect CKMR estimates, this should be apparent from even a fairly coarse preliminary sampling design (Conn et al. 2020).

Sampling considerations for single-sample genetic estimators are similar to those for CKMR analyses involving siblings. Without independent information, it is not possible to reliably compensate for a non-random sample by excluding some or all putative siblings, although some approaches can reduce bias (Waples and Anderson 2017). Biases to genetic estimates of N_b from undetected population structure should be similar to those for CKMR-based estimates of abundance. If the focal population is part of a metapopulation but sampling is local, the LD method primarily estimates local N_b unless migration is very high in genetic terms (>5-10%; Waples and England 2011; Gilbert and Whitlock 2015).

3.5 | Precision and bias

Meaningful CKMR estimates require (1) genetic data robust enough to reliably identify close-kin pairs; and (2) sufficient sampling effort to produce the desired level of precision. We have assumed that all close-kin matches are correctly identified, which implies that criterion (1) has been met (see Bravington et al. 2016b for a summary of basic principles of kin identification). In practice, some level of uncertainty is associated with both genotyping and kin identification, so pedigree-reconstruction models should account for potential errors and evaluate their consequences.

CKMR methods are categorical (each pairwise comparison is either a POP/HSP or not), so only integer numbers of close-kin matches are possible (Figure 2). [In theory, probabilistic kin assignments could be used, as they commonly are for calling genotypes in genomics-scale datasets (Korneliussen et al. 2014), but this would cause hideously daunting consequences for likelihood calculation—M. Bravington, pers com. January 2021.] As is the case with traditional MR, the most important factor affecting precision is how many matches ("recoveries") are found, which in turn depends on the scale of sampling compared to population size. The goal should be to find enough close-kin matches that $\hat{N}_A$ is robust to random sampling error, as well as to limited errors in pedigree reconstruction. A crude approximation to a minimum CV for $\hat{N}_A$ from MR or CKMR is $1/\sqrt{R}$, where R is the number of close-kin matches (Bravington et al.

2016b), so R=50 translates into a CV of about 15%. As a rule of thumb, Bravington et al. (2016b) suggest that in a short-term, POPs-only analysis, this level of precision can be obtained with total (juvenile + adult) samples of approximately $10\sqrt{N}$, and similar considerations apply for HSPs. For example, a sample of 500 offspring, equally split among two cohorts, would provide ample precision for a HSP analysis if N were about 10^3 ($500>10\sqrt{1000}=316$), but the same sampling effort would be woefully inadequate for a population with $N \approx 10^6$ ($500<<10\sqrt{10^6}=10^4$; Figure 2).

Population size presents interesting tradeoffs for experimental design of CKMR. The above rule-of-thumb indicates that to achieve comparable levels of precision, it is necessary to sample a larger fraction of small populations, which can be logistically challenging and perhaps risky or impossible when the species is legally protected. On the other hand, in large populations actual relatives will be rare, but vast numbers of individuals must be compared to find the needle-in-the-haystack matches, so even a very stringent Type I error rate (tiny α) could produce many false positives. How many markers are required to limit false positives to a manageable level, yet still provide adequate power to identify true close kin? True POPs share on average half their genes and are likely to be confused only with full siblings, which can be distinguished based on experimental design, or because POPs must share at least one allele at each locus. Therefore, robust parentage assignments can generally be made using relatively modest numbers of loci (Rawding et al. 2014; Bravington et al. 2016a; Ruzzante et al. 2019). In contrast, reliably distinguishing HSPs from unrelated or more distantly-related individuals (e.g., cousins) is challenging and generally requires at least 10^3 SNPs. Evaluations of expected power should consider non-independence of markers on the same chromosome, which creates pseudoreplication that reduces the information content of large genomic studies for pedigree inference (Thompson 2013; Waples et al. 2020).

Other major factors that affect precision include model complexity and uncertainty in measuring covariates. Precision is inversely related to the number of parameters that must be estimated, which in turn depends on the species' biology and the sampling regime. Often, key covariates (especially age and size) can only be estimated with uncertainty, and this blurs the probability calculations and reduces the information content. Effects of these factors on precision can be reduced to some extent by careful attention to experimental design.

Traditional MR estimates are only asymptotically unbiased for large samples, so an adjustment that reduces small-sample bias (Chapman 1951) is commonly used. However, sample sizes that are small enough for the bias correction to be important will not produce CKMR estimates with sufficient precision for most practical application. For simplicity, we ignore small-sample bias here. As discussed elsewhere, the most important consideration regarding potential bias in CKMR is failure to properly account for all relevant covariates. If survival rates of males and females differ, adult sex ratio can be skewed even when the primary ratio is 1:1. Skewed sex ratios do not bias CKMR estimates, provided female and male abundance are estimated separately, which can be done based on maternal inheritance of mitochondrial DNA (Bravington et al. 2016b).

The same factors that affect precision of sibling-based CKMR estimates apply to Wang's sibship method for estimating effective size, with one exception: CKMR analyses should exclude same-cohort comparisons, while Wang's method is restricted to single cohorts. Like Jack Sprat and his wife, the two methods carve up the data into non-overlapping layers.

For a multi-year sampling scheme based on siblings, it is possible to estimate relative precision for estimating N and N_b (Supporting Information). If <5 years of samples are collected, precision to estimate N_b can be greater, especially if annual survival is relatively low, but in longer studies precision for CKMR generally will be higher (Figure S3).

Unlike CKMR and Wang's sibship method, the LD method is based on a continuous variable (r^2) and provides continuously-distributed estimate of N_e and N_b. For up to a few hundred markers, the LD method and Wang's sibship method provide roughly comparable precision (Waples and Do 2010; Wang 2016). Once reliable sibling detection has been achieved, use of more genetic markers does not further increase precision. In contrast, the number of pairwise comparisons that can be used to estimate r^2 increases with the square of the number of loci, so in theory precision of the LD method could become exceedingly high with genomics-scale datasets. In practice, precision is constrained by lack of independence of the locus pairs, such that performance does not improve much above a few thousand SNPs (Waples et al. 2020). Fortunately for the analysis of large populations, marginal benefits of adding more loci are greater for populations with large N_e.

4 | DISCUSSION

The analyses above show that CKMR estimates of abundance and genetic methods to estimate effective population size share a dependence on the core features of population demography: means and variances of age-specific vital rates and covariances in realized reproductive success over time. We have also shown that effective size and the N_e/N ratio can be predicted based on a vector of parental weights, $\boldsymbol{W}$, that is mathematically equivalent to the *ERRO* concept that underpins CKMR. A CKMR study will generate some or all of the demographic data needed to calculate effective size directly, and genotypes for the sampled individuals provide an opportunity to apply indirect genetic methods for estimating N_e or N_b. Nevertheless, demographic parameters and other covariates influence CKMR and effective size estimators in different ways. The most important points related to practical application of these methods can be summarized as follows (see also Tables 6 and 7).

4.1 | Close-kin mark-recapture

1) Probabilities of close-kin matches depend on the relative reproductive output (RRO) of different classes of parents, and these probabilities are affected by many aspects of the focal species' biology and experimental design. Because actual *RRO* is seldom known, CKMR deals with expected *RRO*, or *ERRO*. Unbiased estimates of key demographic parameters, including abundance, can be obtained by properly incorporating effects of relevant covariates into the calculation of *ERRO*. Failure to include an important covariate in *ERRO* calculations, or incorrectly specifying the estimation model, are the most important potential sources of bias for CKMR, and these are analogous to the "no unmodeled heterogeneity of capture probability" problem in traditional MR models.

2) When properly formulated, CKMR produces estimates of a population's total reproductive output (*TRO*) at one or more time periods. If other factors that can affect *TRO* can be accounted for, the component related to adult abundance can be estimated separately. In some

cases, however (for example, in many fisheries stock assessments), *TRO* rather than the number of adults is a more relevant parameter.

3) The probability of a parent-offspring match can be affected by skip breeding or sampling selectivity based on any fecundity-associated covariate (e.g., size, age). Sibling probabilities are particularly sensitive to vital rates, as robust estimates require information about both age-specific survival and fecundity, as well as the incidence of skip breeding. For both POPs and HSPs, the degree to which *ERRO* differs among individuals and the sampling intensity jointly determine the degree to which precision is reduced compared to the naive model in which all recaptures are equiprobable.

4) It is not necessary to separately estimate all covariates that might affect CKMR estimates of abundance (although doing so can be of interest; Conn et al. 2020), but it is necessary to *account for* these effects, which often can be done using same data by incorporating the relevant information into calculation of *ERRO*. For example, patterns of skip breeding can be evaluated using both POPs and HSPs by examining lag times between repeated offspring assignments to the same parent (Bravington et al. 2016a, 2020). Information on changes in fecundity with age can be obtained using a combination of POPs and a method for ageing (Bravington et al. 2016a); however, selectivity (if any) and mortality are confounded in POPs-only analyses. HSPs can resolve the ambiguity between fecundity, selectivity, and mortality in POPs analyses and be used to account for adult mortality (Hillary et al. 2018), which in turn is necessary for unbiased HSP estimates of abundance. By themselves HSPs are not very useful for estimating abundance unless one has independent information about age-specific vital rates or can assume they are constant for adults (as in Hillary et al. 2018). However, because of these features of complementarity, a combination of POPs and HSPs generally will provide the most robust framework for CKMR analyses, especially when fecundity varies with age.

5) In most cases it will be necessary to have some means of ageing individuals, so that (a) individuals can be sorted into offspring and potential parents for POP analyses; (b) offspring can be sorted into cohorts for HSP analyses; and/or (c) changes in fecundity with age can be accounted for, as necessary. When ageing is unreliable, a CKMR approach still might be feasible but would require the likelihood framework to integrate across plausible ranges for various ageing scenarios, which would increase the level of uncertainty associated with resulting estimates (see Hillary et al. 2018 and Bravington et al. 2020 for examples).

6) Except for some specialized applications (e.g., annual estimates for semelparous species; see Rawding et al. 2014), robust estimates using CKMR will generally require collection of data from several different years or cohorts. This also facilitates estimation of key demographic parameters such as skip breeding, adult mortality, and age-specific fecundity.

7) Incorporating all the CKMR analyses into a single estimation framework obviates the need to make simplifying assumptions about stationarity that often are not realistic for natural populations. Any CKMR analysis will involve multiple sources of uncertainty, and including them all within a single likelihood or Bayesian framework is the most effective way to produce meaningful statements about robustness of the estimates.

4.2 | Effective population size

1) Many factors that are nuisance parameters for CKMR abundance estimates are part of the genetic drift signal and hence create no problems for genetic methods that estimate effective

size. This applies to changes in survival and fecundity with age, skewed sex ratio and skewed offspring distribution, and skip breeding. Persistent differences in reproductive success don't affect estimates of N_b; their effects on genetic drift should be detectable in genetic estimates of N_e. Although detailed life-history and age-structure information for the population as a whole is necessary for demographic estimates of N_e or N_b, ageing of individuals is primarily important for sorting offspring into single cohorts.

2) Single-sample genetic methods estimate the effective number of breeders that produced the sampled offspring (Waples 2005; Wang 2009). Researchers should keep in mind that, depending on the experimental design and the biology of the target species, this might not represent the entire adult population.

3) To the extent that using many SNP loci will lessen the Type I error problem in identifying false siblings, genomics-scale datasets can help improve performance of the sibship method for the analysis of large populations. To a somewhat greater extent, the LD method can take advantage of increased precision afforded by genomics datasets, but marginal benefits decrease rapidly after a few thousand loci are used.

4) Despite the many close parallels, the typical framework for estimating effective size differs in an important way from CKMR: stationarity. Hill's (1972) formula for N_e when generations overlap (Equation 3) assumes constant population size and stable age structure. Unless N is very small, Hill's method is robust to random demographic variation (Engen et al. 2005a; Waples et al. 2011). However, allowing for environmental stochasticity requires a different formulation (Engen et al. 2005b), and even this becomes problematical under strong density dependence, when vital rates vary over time and generation length is not well defined (Myhre et al. 2016). In estimating effective size in age-structured populations, therefore, it is typically assumed that generational N_e is constant (e.g., Jorde and Ryman 1995; Waples et al. 2011).

Fortunately, the same limitation does not apply to seasonal estimates of effective size. Because Wang's sibship method considers only a one-generation pedigree, for iteroparous species it can be used to produce separate estimates of N_b each year. The standard LD method assumes that markers are not physically linked, in which case LD from previous generations rapidly breaks down, leaving a signal dominated by N_e or N_b of the parents that produced the sample (Waples 2005). Datasets including linked loci can be used to estimate contemporary effective size if (1) comparisons are restricted to loci on different chromosomes (which can be done in NeEstimator, Do et al. 2014), or (2) resulting bias due to linkage is adjusted using a method proposed by Waples et al. (2016). Tightly linked markers on the same chromosome contain information about N_e in the more distant past. If detailed information about genomic structure is available, this information can be leveraged to provide a temporal series of $\hat{N}_e$ estimates. This has been done with humans (Tenesa et al. 2007), red drum (*Sciaenops ocellatus*; Hollenbeck et al. 2016), and Atlantic salmon (*Salmo salar*; Lehnert et al. 2019). Single-sample genetic estimators can be used to provide a continuous time-series of annual estimates of N_b, which are generally done independently each year (Ruzzante et al. 2016; Whitely et al. 2015; Waples et al. 2018a,b). In theory, it should be possible to develop an analytical framework analogous to that used by CKMR that jointly considers multiple years of data to estimate

temporal trajectories of N_b and other population parameters, but we are not aware of any attempts to do that.

4.3 | Concluding remarks

Understanding and being able to predict the ratio of effective size to census size has been a goal for many years (Frankham 1995; Nunney 1995; Kalinowski and Waples 2002; Palstra and Fraser 2012): Because N_e is difficult to measure in the wild, inferences about evolutionary processes in a population can be made based on knowledge of the N_e/N ratio and an estimate of N. However, this goal has proved elusive, for two major reasons. First, even within a population, the ratio can vary over time due to density dependence, leading to a negative correlation between N_e/N and N due to genetic compensation (Ardren and Kapuscinski 2003; Saarinen et al. 2010; Myhre et al. 2016). Second, genetically-based estimates of N_e/N range over several orders of magnitude, primarily because of many "tiny" estimates ($<10^{-3}$) for marine species, attributed to 'sweepstakes" reproductive success (Hedgecock 1994; Hedrick 2005; Hauser and Carvalho 2008).

Because census and effective population size (respectively) determine the ecological and evolutionary consequences of abundance, the richest insights can be gained by using a combination of methods (and overlapping datasets) to estimate both parameters. This can be done using a combination of CKMR methods to estimate N and genetic and demographic methods to estimate N_e. Akita (2020a) illustrated hypothetically how this might be done using simulated data. For a real-world example, Waples et al. (2018a) used the same genetic and demographic data reported by Bravington et al. (2016a) in their CKMR study of southern bluefin tuna to place a lower bound of about $3x10^4$ on the genetic estimate of N_e and an upper bound of about $1.5x10^6$ on the demographic estimate of N_e (Figure 3). The range of N_e/N ratios that were most consistent with the combined data was about ..1 to 0.5. This ruled out any substantial effect of sweepstakes reproductive success for southern bluefin tuna, even though the species' longevity and high fecundity are similar to several marine species for which "tiny" estimates of N_e/N have been reported (Hedgecock and Pudovkin 2011).

Acknowledgments

Mark Bravington contributed a great deal to our understanding of the material presented here. We thank Eric Anderson, Peter Grewe, Daniel Ruzzante, and Hans Skaug for useful discussions. Mark Bravington, Jann Martinsohn, Todd Seamons, and Antonella Zanzi provided insightful comments on an earlier version of the manuscript. RW's work on this project was supported by an International Science Fellowship from the National Marine Fisheries Service and by a Froelich Fellowship from CSIRO. The authors declare no conflict of interest. This manuscript did not generate new empirical data.

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Table 1. Definitions and notation used in this study.

x	Age (in years)
z	Age at recruitment, when individuals are first inventoried
α	Age at maturity
ω	Maximum age
s_x	Probability of surviving from age x to age x+1
l_x	Cumulative probability of surviving from age at recruitment to age x
N_z	Total number of offspring produced per time period that survive to age z
N_x	Number of individuals of age x alive at any given time; $N_x = N_z l_x$
N_A (N_f, N_m)	Number of adults (females, males) with age≥α alive at any given time
N_e	Effective population size per generation
N_b	Effective number of breeders in one year
$\hat{N}_A, \hat{N}_e, \hat{N}_b$	Estimates of census size and effective size
S	Number of individuals sampled
Y	Number of pairwise comparisons to search for close-kin matches
POP	Parent-offspring pair
HSP	Half-sibling pair
R	Number of close-kin matches identified (R_{POPS} or R_{HSPS}), which correspond to 'recoveries' in mark-recapture models
k_i	Number of offspring produced in one time period by parent i
$\bar{k}$	Mean k_i for all parents in one time period
V_k	Variance of k_i for all parents in one time period
b_x	Mean k_i for parents of age x in one time period
δ_i	Difference between expected number of offspring for individual i and the mean for that age and sex
B_x	Total number of offspring produced by all parents of age x in one time period: $B_x = N_x b_x$
$V_{k(x)}$	Variance of k_i for adults of age x in one time period
ϕ_x	Ratio of variance to mean reproductive success in one time period for adults of age x; $\phi_x = V_{k(x)}/b_x$
$V_{k\bullet}$	Variance in lifetime number of offspring produced by the individuals in a cohort
RO	Reproductive output, measured in terms of number of offspring produced. In one time period, $RO_i = k_i$ for individual i
TRO	Total reproductive output of all parents in one time period; $E(TRO) = \sum_{x=z}^{\omega} B_x$
RRO	Relative reproductive output of an individual in one time period, compared to total reproductive output of all parents; $RRO_i = RO_i/TRO$.
$ERRO$	Expected relative reproductive output, based on a population's vital rates. For an individual of age x, $ERRO_x = E(RO_x)/E(TRO) = b_x/\sum_{x=z}^{\omega} B_x$.
w_i	Relative probability that adult i will be the parent of a given offspring
W_i	$W_i = w_i/\Sigma w_i = ERRO_i$ = absolute probability that adult i will be the parent of a given offspring
CV_W^2	Squared coefficient of variation of the parental weights; $CV_W^2 = \sigma_W^2/\bar{W}^2$

Table 2. Influence of two components of variation in annual reproductive success on the effective number of breeders per year (N_b) and the ratio of N_b to the number of adults (N_A). The modeled population has 10 age classes and a total of N_A = 1040. Among-age effects depend on whether fecundity changes with age, and within-age effects are quantified by the ratio $\phi_x = V_{k(x)}/b_x$. $\bar{k}$ and V_k are the mean and variance in offspring number among all N_A adults reproducing each year. N_b was calculated from Equation 1 using $\bar{k}$ and V_k from the program AgeNe (Waples et al. 2011); "Constant" fecundity scenarios used the vital rates in Table 3 and "Increasing" fecundity scenarios used the vital rates in Table 4. Vital rates were the same in males and females, and ϕ was the same for each age.

Fecundity	ϕ	$\bar{k}$	V_k	N_b	N_b/N_A
Constant	1	1.92	1.92	1040	1.00
Constant	2	1.92	3.85	684	0.66
Constant	5	1.92	9.61	337	0.32
Constant	10	1.92	19.23	183	0.18
Increasing	1	1.92	3.37	747	0.72
Increasing	2	1.92	5.29	544	0.52
Increasing	5	1.92	11.06	299	0.29
Increasing	10	1.92	20.68	171	0.16

Table 3. Hypothetical life table for a species that matures at age 3, has constant survival and fecundity, and has a maximum lifespan of 10 years. The life table assumes constant production of N_1 = 1000 female offspring per time period that survive to age 1. s_x = probability of survival from age x to $x+1$; l_x = cumulative survival through age x; $N_x = l_x N_1$ = number of females alive at age x; b_x = fecundity (mean number of female offspring that survive to age 1) at age x; $B_x = N_x b_x$ = total production of female offspring by individuals of age x; $TRO = \sum_{x=1}^{10} B_x$ = total reproductive output of all females in one time period; $RRO_x = b_x/TRO$ = relative reproductive output of an individual of age x. Random samples of 100 potential parents and 100 progeny are taken and all possible comparisons are made to identify maternal parent-offspring pairs (POPs). The expected number of POPs by age of mother and across all ages (last two columns) depends on whether the sample of potential mothers is drawn only from mature (age 3+) individuals or from the entire population. Within each age class, $E(R_{POPx})$ = (number of comparisons)*(probability of a POP)*(fraction of comparisons involving a parent of age x) = $100*100*RRO_x*N_x/\Sigma N_x$.

							Parent sampling	
							Age 3+	Age 1+
Age(x)	s_x	l_x	N_x	b_x	B_x	RRO_x	$E(R_{POPx})$	$E(R_{POPx})$
1	0.5	1	1000	0	0	0	0	0
2	0.5	0.5	500	0	0	0	0	0
3	0.8	0.250	250	0.962	240	0.00096	2.31	0.95
4	0.8	0.200	200	0.962	192	0.00096	1.85	0.76
5	0.8	0.160	160	0.962	154	0.00096	1.48	0.61
6	0.8	0.128	128	0.962	123	0.00096	1.18	0.48
7	0.8	0.102	102	0.962	98	0.00096	0.95	0.39
8	0.8	0.082	82	0.962	79	0.00096	0.76	0.31
9	0.8	0.066	66	0.962	63	0.00096	0.61	0.25
10	0	0.052	52	0.962	50	0.00096	0.48	0.20
		ΣN_x	2540	TRO	1000	$\Sigma E(R_{POP})$	9.61	3.94
		$\Sigma N_x(3+)$	1040					

Table 4. As in Table 3, but fecundity increases with age, and sampling potential mothers (age 3+ only) follows two scenarios. If sampling is non-selective (each adult female is equally likely to be sampled), the distribution of POPs by maternal age differs from that in Table 1 (being skewed toward older mothers) but $\Sigma E(R_{POP})$ is the same, leading to the same estimate of N_f. In the second scenario sampling is selective, with older/larger females more likely to be sampled. For selective sampling, P_{samp} = relative probability of a female of age x being sampled, age-specific $\text{Effort}_x = N_x P_{samp}$, and $E(R_{POPx}) = 100*100*RRO_x*\text{Effort}_x/\Sigma\text{Effort}_x$. If sampling is selective and age-specific selectivity is positively correlated with fecundity, $\Sigma E(R_{POPx})$ is higher and $\widehat{N}_f$ will be lower unless selectivity is accounted for.

Age(x)	s_x	l_x	N_x	b_x	B_x	RRO_x	Non-selective $E(R_{POPx})$	P_{samp}	Effort_x	Selective $E(R_{POPx})$
1	0.5	1	1000	0	0	0	0	0	0	0
2	0.5	0.5	500	0	0	0	0	0	0	0
3	0.8	0.250	250	0.284	71	0.00028	0.68	0.03	7.3	0.1
4	0.8	0.200	200	0.568	114	0.00057	1.09	0.08	15.2	0.3
5	0.8	0.160	160	0.851	136	0.00085	1.31	0.18	29.2	0.7
6	0.8	0.128	128	1.135	145	0.00114	1.40	0.38	48.3	1.6
7	0.8	0.102	102	1.419	145	0.00142	1.40	0.62	63.7	2.6
8	0.8	0.082	82	1.703	139	0.00170	1.34	0.82	67.0	3.3
9	0.8	0.066	66	1.987	130	0.00199	1.25	0.92	60.6	3.5
10	0	0.052	52	2.270	119	0.00227	1.14	0.97	50.9	3.4
		ΣN_x	2540		*TRO*	1000	$\Sigma E(R_{POPx})$	9.61	ΣEffort_x	342.2	$\Sigma E(R_{POPx})$ 15.5
		ΣN_x(3+)	1040							

Table 5. Life table as in Table 3, except close-kin matches of interest are maternal half-sibling pairs (MHSPs). Random samples of 100 progeny are taken from two consecutive cohorts and all across-sample comparisons are made to identify MHSPs. Within each age class, E(R_{MHSPx}) = (number of comparisons)*(K_x)*(fraction of comparisons involving a parent of age x) = 100*100*K_x*N_x/ΣN_x. K_x is the probability of a MHSP involving a mother of age x at the time the first cohort was produced; K_x = RRO_x*RRO_{x+1}*s_x = the product of the relative reproductive outputs of the parent at ages x and x+1 and the probability of surviving from age x to x+1. Annual mortality reduces the expected number of MHSPs and, unless accounted for, will bias the naive estimate of female abundance.

Age(x)	s_x	l_x	N_x	b_x	B_x	RRO_x	E(R_{MHSPx})
1	0.5	1	1000	0	0	0	0
2	0.5	0.5	500	0	0	0	0
3	0.8	0.250	250	0.962	240	0.00096	1.85
4	0.8	0.200	200	0.962	192	0.00096	1.48
5	0.8	0.160	160	0.962	154	0.00096	1.18
6	0.8	0.128	128	0.962	123	0.00096	0.95
7	0.8	0.102	102	0.962	98	0.00096	0.76
8	0.8	0.082	82	0.962	79	0.00096	0.61
9	0.8	0.066	66	0.962	63	0.00096	0.48
10	0	0.052	52	0.962	50	0.00096	0.00
			ΣN_x 2540		*TRO* 1000	Σ E(R_{MHSPx})	7.31
			ΣN_x(3+) 1040				

Table 6. Summary of major factors that, unless explicitly accounted for, can influence performance of CKMR methods to estimate abundance.

Factor	POPs	HSPs
Ageing individuals	Only necessary if required to separate parents/offspring or account for changes in fecundity with age	Required to sort individuals into cohorts
Sampling of cohorts	Samples from single cohorts can be used for semelparous species. Sampling multiple cohorts is essential for most iteroparous species	Sampling multiple cohorts is essential because only cross-cohort comparisons provide information about census size
Age-specific fecundity	Affects precision; confounded with mortality and selectivity using POP data only	Affects precision and bias; can be estimated independently or from POP s+ ages
Adult mortality	Confounded with selectivity and fecundity using POP data only	Required to adjust probability a parent produces sibs in different years, but can be estimated from HSP data
Overdispersed variation in RS	Does not affect P(POP) but reduces precision	Reduces precision. For bias, see below
HSPs from the same cohort		Siblings from same cohort estimate N_b, not N
HSPs from different cohorts		
Residual RS uncorrelated across years		No bias in $\widehat{N}$
Persistent individual differences in RS		Increases P(HSP) and will bias $\widehat{N}$ unless accounted for
Skip breeding	Downward bias in $\widehat{N}$ if skip breeders not sampled	Complex effects on P(HSPs)
Non-random sampling concerns	POPs collected together more often than by chance; Probability of sampling an adult correlated with its RS	Siblings born in different years collected together more often than would occur by chance
Precision	CV($\widehat{N}$) = 0.15 requires about 50 POPs, which implies sample sizes on the order of $\sqrt{N}$ individuals	Similar considerations
Benefits of genomics-scale datasets	Scant: performance asymptotes at low numbers of genetic markers after POPS reliably identified	~10^3 SNPs generally will be required to reliably identify HSPs

Table 7. Summary of major factors that, unless explicitly accounted for, can influence performance of some genetic methods to estimate effective size.

Factor	Sibship method	LD method
Ageing individuals	Required to sort individuals into cohorts to estimate N_b or to estimate N_e from mixed-age samples	Required to sort individuals into cohorts to estimate N_b; not required to estimate N_e from mixed-age samples
Sampling of cohorts	A single-cohort sample provides a point estimate of N_b. Multiple such samples provide a temporal series of estimates	A single-cohort sample provides a point estimate of N_b. Multiple such samples provide a temporal series of estimates. Mixed-age samples can estimate N_e
Changes in fecundity or mortality with age; skewed sex ratio; overdispersed variance in RS	No bias; these are part of the signal of reduced N_e and N_b	No bias; these are part of the signal of reduced N_e and N_b
Skip breeding and persistent individual differences in RS	No bias to single-cohort samples estimating N_b	No bias to estimates of N_b or N_e, but this affects the ratio N_b/N_e and influences the bias adjustments to account for age structure
Non-random sampling issues	Estimate is downwardly biased if siblings are collected together more often than would occur by chance	Estimate is downwardly biased if relatives are collected together more often than would occur by chance
Precision	For large populations, sample sizes on the order of $\sqrt{N_e}$ individuals	Precision jointly determined by samples of individuals and loci
Benefits of genomics-scale datasets	~10^3 SNPs generally are required to reliably identify HSPs	Potentially large, but pseudo-replication of non-independent pairs of loci limits increases in precision
Changes in N and/or N_e	No effect on estimates based on single cohorts	Some bias toward recent N_e for 3-5 generations

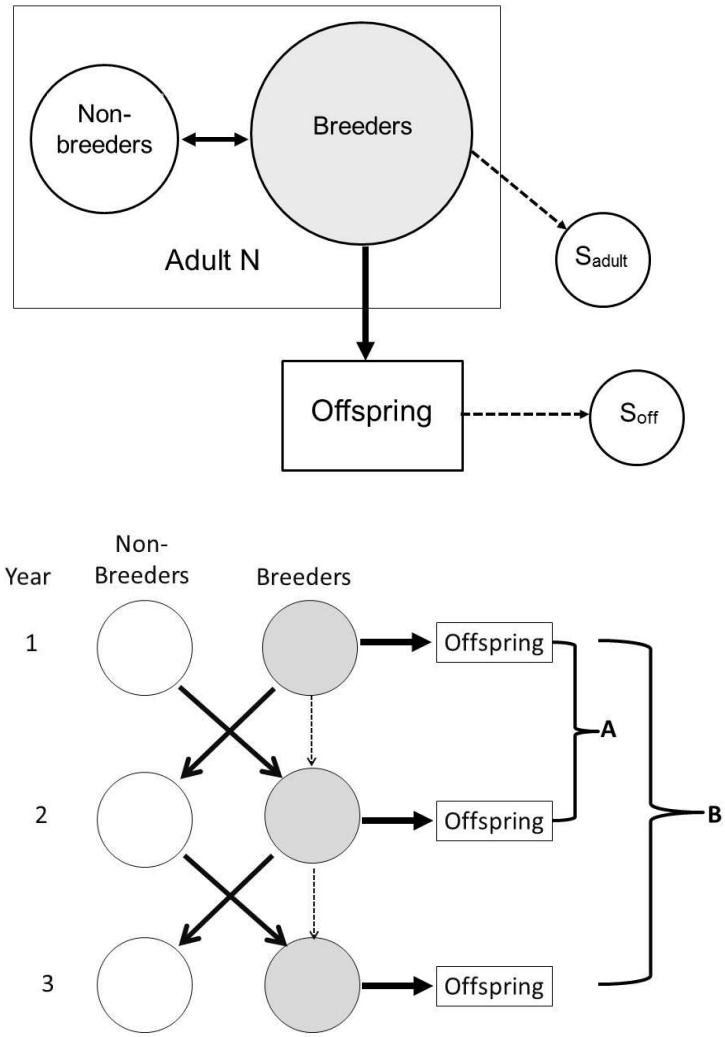

Figure 1. Schematic diagram showing possible effects of skip breeding on CKMR estimates. Top: If non-breeders are not sampled, POPs will provide an abundance estimate only for the breeding population. Bottom: A multi-year study for a population in which most adults alternate between being breeders and non-breeders; the thin dashed arrows indicate that a fraction of adults breed in consecutive years. Two years of POPs collections will sample the entire adult population. Few if any HSPs will be found for pairs of individuals separated in age by odd numbers of years (sampling scheme A), while the number of HSPs for comparisons of individuals an even number of years apart (sampling scheme B) will reflect the number of breeders per year more than the adult census size. Genetic methods that estimate N_b based on single-cohort samples also would produce an estimate related to the number of actual breeders each year, rather than the total number of adults.

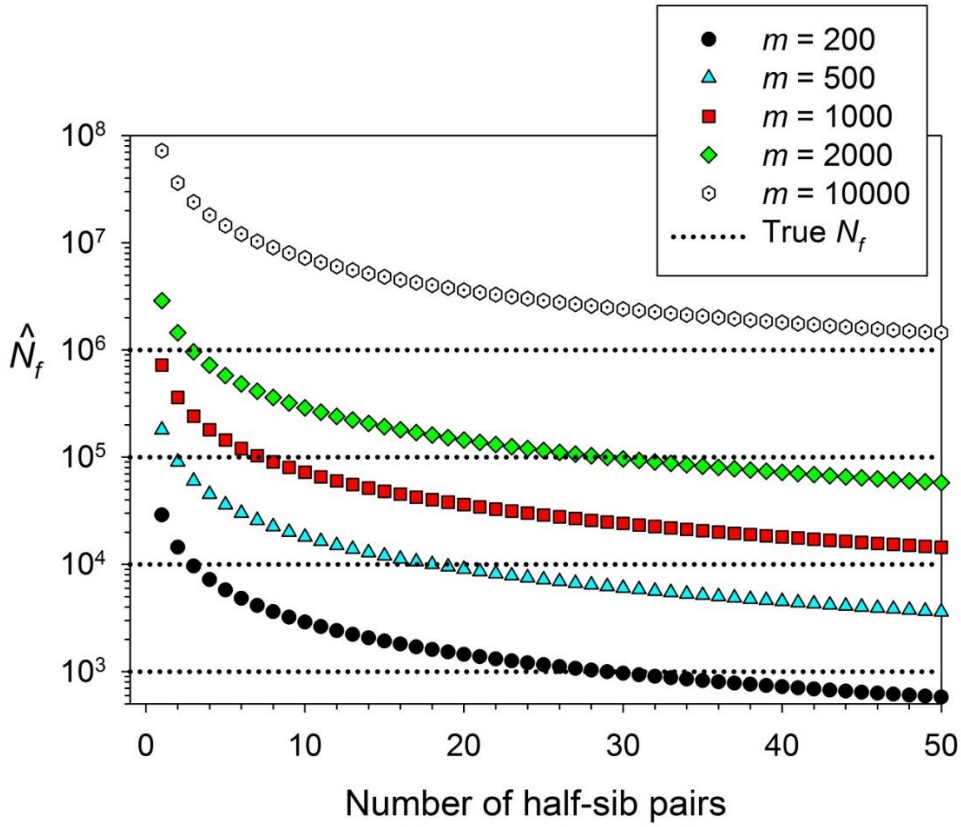

Figure 2. Granularity in estimating female census size using CKMR analyses based on maternal halfsiblings when true N_f is at least moderately large (indicated by dotted lines at 10^3, 10^4, 10^5, and 10^6). m is the total number of individuals sampled, assumed to be equally split among two consecutive cohorts. Annual adult survival is assumed to be 0.85, so there are $(0.85m/2)^2$ effective pairwise comparisons. Colored symbols represent possible estimates of N_f if 1-50 pairs of half siblings are found, given the indicated sampling effort and assuming all parents are equally likely to produce offspring. [$\widehat{N}_f$ is infinitely large if no siblings are identified in the sample.] The experimental-design objective is to identify a sampling regime that will provide a robust estimate of N_f, which occurs when the curves have flattened after ~40-50 HSPs have been found. For example, if true N_f were 10^4, m = 1000 would provide high precision. However, this level of sampling would not be adequate if true N_f were 10^5 or higher, because small changes in number of HSPs identified (through errors or stochastic effects) would cause large changes in $\widehat{N}_f$.

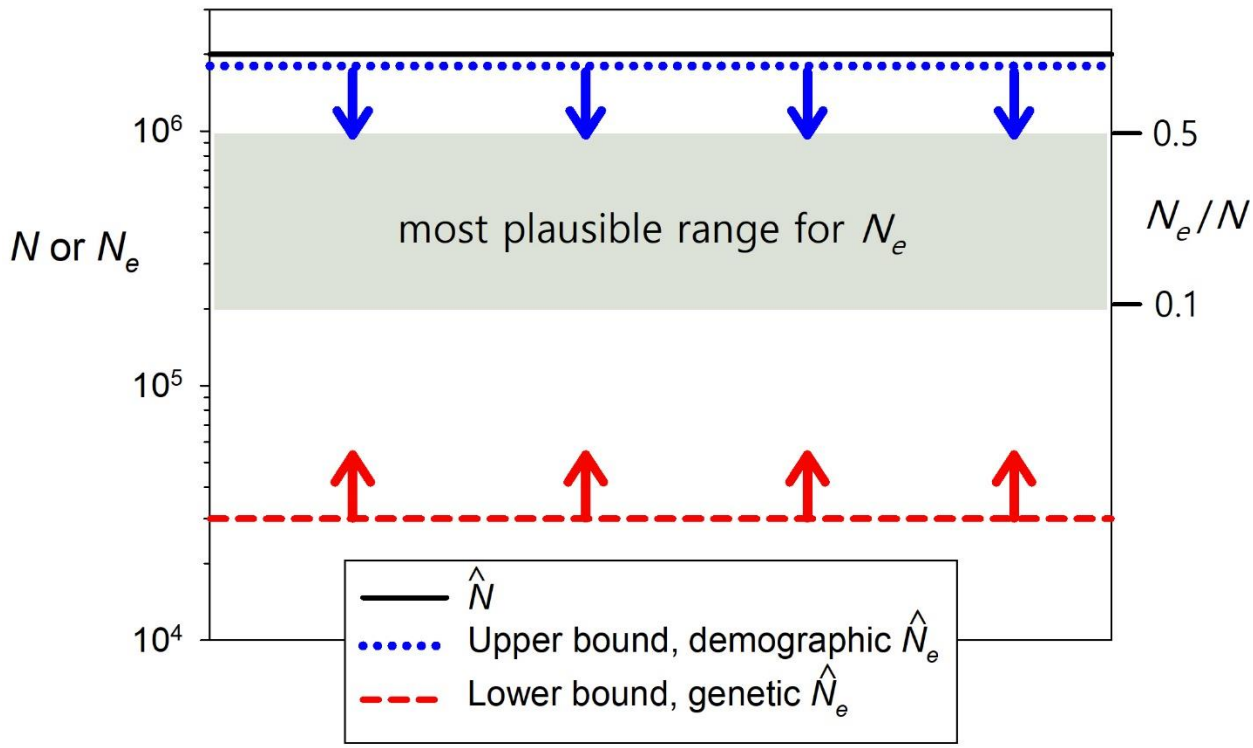

Figure 3. Using complementary properties of genetic and demographic estimators to bracket the plausible range for the ratio N_e/N in southern bluefin tuna. The solid black line is the CKMR estimate of adult abundance ($2x10^6$) from Bravington et al. (2016a); Waples et al. (2018a) used the same genetic and demographic data, respectively, to delimit a lower bound (dashed red line) and an upper bound (dotted blue line) to N_e. Genetic methods can provide robust lower bounds for N_e but, because the drift signal is so small, have difficulty distinguishing large from very large or infinite effective sizes. Conversely, life history constrains how large N_e can be but, without very detailed demographic information, cannot rule out very small N_e/N ratios. Collectively, and in conjunction with a robust estimate of N from CKMR, the two effective-size methods constrain the plausible range of N_e/N to about 0.1-0.5.