Methods in Ecology and Evolution

PROFESSOR DANIEL RUZZANTE (Orcid ID : 0000-0002-8536-8335)

Article type : Research Article

Validation of close-kin mark-recapture (CKMR) methods for estimating population abundance

Daniel E. Ruzzante[1], Gregory R. McCracken[1], Brage Førland[2], John MacMillan[3], Daniela Notte[1], Colin Buhariwalla[3], Joanna Mills Flemming[4], Hans Skaug[2].

[1]: 1355 Oxford St., Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4R2

[2]: Department of Mathematics, University of Bergen, Postboxs 7803, 5020 Bergen, Norway

[3]: Inland Fisheries Division, Nova Scotia Department of Fisheries and Aquaculture, Halifax, Nova Scotia, Canada B3J 2R5

[4]: Department of Mathematics and Statistics, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4R2

Corresponding author: Daniel Ruzzante: daniel.ruzzante@dal.ca

Running headline: Close-kin mark-recapture and population size

Abstract

(1) Knowing how many individuals there are in a population is a fundamental problem in the management and conservation of freshwater and marine fish. We compare abundance estimates (census size, N_c) in seven brook trout (*Salvelinus fontinalis*) populations using standard mark-recapture (MR) and the close-kin mark-recapture (CKMR) method. Our purpose is to validate CKMR as a method for estimating population size.

(2) CKMR is based on the principle that an individual's genotype can be considered a "recapture" of the genotypes of each of its parents. Assuming offspring and parents are sampled independently, the number of parent-offspring pairs (POPs) genetically identified in these samples can be used to estimate abundance. We genotyped (33 microsatellites) and aged ~2400 brook trout individuals collected over 5 consecutive years (2014-2018).

(3) We provide an alternative interpretation of CKMR in terms of the Lincoln-Petersen estimator in which the parents are considered as tagging the offspring rather than the offspring "recapturing" the parents.

(4) Despite various sources of uncertainty, we find close agreement between standard MR abundance estimates obtained through double-pass electrofishing and CKMR estimates, which require information on age-specific fecundity, and population- and age-specific survival rates. Population sizes ($\widehat{N}$) are estimated to range between 300 and 6000 adult individuals. Our study constitutes the first *in situ* validation of CKMR and establishes it as a useful method for estimating population size in aquatic systems where assumptions of random sampling and thorough mixing of individuals can be met.

Keywords: abundance estimation, brook trout, census size, close kin mark recapture, genetic tagging, Lincoln-Petersen, parent-offspring pairs, population size

Introduction

The estimation of abundance constitutes a fundamental problem in ecology and conservation biology, particularly in the management of exploited marine, anadromous or freshwater populations. In marine fisheries, abundance estimation has traditionally relied on the relationship between fishery catches and effort (catch per unit effort or CPUE) which can be subject to bias and uncertainty and is therefore often considered relatively unreliable and contentious. In the present study, we validate an alternative approach for estimating census size (N_c) or population abundance using genomics and close-kin mark-recapture (CKMR).

Introduced in Bravington, Skaug, and Anderson (2016a, but see also Skaug, 2001; and Rawding, Sharpe, & Blankenship, 2014), the CKMR method is based on the principle that an individual's genotype can be considered a "recapture" of the genotypes of each of its parents and analyses the number and pattern of parent-offspring pairs (POPs) in a mark-recapture (MR) framework. Assuming the sampling of offspring and parents to be independent of each other, the number of POPs genetically identified in samples from both groups can be used to estimate abundance. Further, we show that the CKMR estimator can be viewed as a Lincoln-Petersen type estimator, with the offspring being tagged by their parents.

By avoiding the need for CPUE data, CKMR has the potential to change the way marine harvested populations are monitored. It has recently been used to estimate population abundance of southern blue fin tuna (Bravington, Grewe, and Davies, 2016b) and white sharks (Hillary et al., 2018) as well as to estimate the ratio of effective to census size (or abundance) for southern blue fin tuna (Waples, Grewe, Bravington, Hillary, & Feutry, 2018). For both species, however, available abundance estimates are rather uncertain which makes validation of the CKMR method difficult with these species. Once validated, CKMR is applicable to populations (or species) of conservation concern as well as to those for which little information is available (i.e., those that are considered data limited or data deficient).

The objective of the present study is to validate CKMR using a set of seven independent brook trout (*Salvelinus fontinalis*) populations. For each population, we compare estimates of census size based on standard MR with those obtained with the CKMR method. Populations were sampled annually between 2014 and 2018, with individuals classified into age classes and assessed for polymorphism at 33(31) microsatellite DNA loci. Estimates of population abundance or census size using the CKMR method are obtained under the modeling assumption that while recruitment varies between years, the sampling is representative of age structure. We find that CKMR abundance estimates under these assumptions are statistically indistinguishable from those obtained by standard MR. We discuss sampling requirements and the need for life history information including age at maturity, age-specific fecundity and population and age-specific mortality rates.

Material and Methods

Study sites and sample collection: Brook trout (*S. fontinalis*) were collected from 4 coastal streams along the northwest shore of Nova Scotia in the summers of 2014-2018. The 4 streams are in independent watersheds that drain into the Bay of Fundy from near the top of the North Mountain (maximum relief 265 m; Fig. 1). Three of the streams contain waterfalls somewhere along the stream length that prevent the upstream movement of fish thus creating upstream landlocked populations and downstream populations that may receive immigrants from upstream. Collections therefore correspond to 7 distinct populations: Ross Creek Upstream (RCU), Ross Creek Downstream (RCD), Woodworth Upstream (WWU), Woodworth Downstream (WWD), Church Vault (CV), Saunders Brook Upstream (SBU) and Saunders Brook Downstream (SBD) (Table 1). Sampling was non-lethal. Fish were measured (fork length, FL) and fin clipped (adipose fin) before release. Fin clips were stored in 95% ethanol for subsequent DNA analysis. DNA analysis was conducted on all $n \approx 2400$

individuals at a total of 33 (31 for one population) microsatellite DNA markers chosen for their polymorphism, ease of scoring and absence of null or large alleles.

Life history characteristics: Brook trout breed in the fall, the fertilized eggs develop through the winter while buried in gravel, and hatching occurs the following spring. Age and sexual maturity information and their relationship with size (fork length) was taken from Ruzzante et al. (2016) where *n*=426 brook trout were used to develop age-length criteria and *n*=66 brook trout were used to develop sexual maturity criteria. These criteria were subsequently applied to the full number of genotyped fish in the present study to determine age and sexual maturity. Age was assessed by counting scale annuli, and in these streams brook trout live only up to 3+ years of age (Ruzzante et al. 2016). Electrofishing took place in July of each year at which time the populations consist of young-of-year (YOY) which are juveniles that are 2-4 months old, 1+ fish (14-16 months old), 2+ fish (26-28 months old) and 3+ fish (38-40 months old). Maturity in these brook trout populations is reached at age 1+ (50% of individuals are mature by age 1+) (Ruzzante et al. 2016). The sampling was conducted under fishing permit # 321158 issued to the Inland Fisheries Division of the Nova Scotia Department of Fisheries and Aquaculture.

Population size ($\widehat{N}_c$) estimation by standard mark-recapture: Population (census) size N_c was estimated each year by MR using the Lincoln-Petersen method [See Electronic Supplementary Material (ESM) for details] with each population sampled twice, two weeks apart in 2014, and the next day (or, on one occasion, the same day) from 2015 onwards. To estimate census population size, fish were captured, measured, fin-clipped and released on the first day of electrofishing. Electrofishing proceeded until >80 fish (2014, 2015, 2016) or > 160 fish (2017, 2018) had been captured, fin-clipped, and released. The length of the sampled section was determined from GPS coordinates. The following day (or, on one occasion, the same day), the same section of each stream was re-sampled, and the number of recaptures

determined. In 2014 only one section of streambed was electrofished while in the years following either the entire stream bed (RCD) or two or three sections separated by at least 500-750 m were electrofished. We used the recapture rate and the number of fish (1+ or older) caught in the sampled length of stream to estimate the density of adult fish (# fish per m stream length electrofished). Population census size was then estimated by multiplying density by the stream segment length, which is the length of stream over which there are no physical barriers to fish movement, this is a conservative estimate of N_c (Table S1). The total lengths of the streams were estimated using ARCGIS (1:50 000). Streams were walked to locate waterfalls and other significant barriers (e.g., impassable culverts) and GPS coordinates were used to position the barriers on the stream map. Electrofishing was conducted very thoroughly and included examining structures that could interfere with currents (e.g., pools under tree roots that stick out from the banks). Additionally, size differences between 1+ and 3+ individuals are not large enough to be affected differentially by the electrical fields generated by electrofishing. We can therefore assume that electrofishing selectivity did not differ among age classes 1+, 2+ and 3+.

Molecular protocol: Adipose fin tissue samples were digested using proteinase K (Bio Basic Inc., Markham, ON, Canada) for approximately 8 hours at 50 °C. Subsequently, DNA was extracted following a glassmilk protocol modified from Elphinstone, Hinten, Anderson, and Nock (2003) using a Robotic Perkin Elmer Multiprobe II Plus Liquid Handling System (Perkin Elmer, Waltham, MA, USA). A random subset of the extracts was then tested for DNA quality and quantity via visualization on 1% agarose gel stained with GelGreen (BioTium Fremont, CA, USA). We used the MEGASAT (Zhan et al., 2017) software and pipeline for the automated genotyping of 33 polymorphic microsatellites from sequencing data. Further details on the molecular protocol, and on the primers and loci used are available in ESM.

Within-sample analysis: Microchecker (v2.2.3) (van Oosterhout, Hutchinson, Wills, & Shipley, 2004) was used for detection of potential null alleles and large allele drop-out. Tests for Hardy-Weinberg proportions (HWE) and for linkage disequilibrium (LD) between pairs of loci were conducted in Arlequin 3.5 (Excoffier & Lischer 2010).

Population structure: These brook trout populations were shown to be distinguishable in Ruzzante et al. (2016). We therefore do not repeat a population structure analysis here.

Population size estimation by CKMR: CKMR is based on the identification of POPs between a sample of offspring and a sample of potential parents, all genotyped for the same suite of genetic markers. CKMR estimates of population size were obtained for the year 2015. In all cases sampling took place in the summer (July-August), shortly before the spawning season. For 2015 we used as offspring, the age 1+ individuals sampled in 2017 (i.e., individuals which in the summer of 2017 when they were sampled, were approximately 14-16 months old since hatching), and as potential parents, all individuals that were likely present during the 2015 spawning season. This pool thus included all individuals (ages 1+, 2+, 3+) sampled in 2015, those aged 1+ and 2+ sampled in 2014, those aged 2+ and 3+ sampled in 2016, and those aged 3+ sampled in 2017.

In its simplest form, the CKMR estimate of 2015 census size is (Bravington et al., 2016a)

$$\widehat{N}_{c(CKMR)} = \frac{2n_J n_A}{H}, \qquad (1)$$

where n_J and n_A are the number of offspring and adults genotyped at the suite of markers, and H is the number of POPs identified. The factor 2 in the numerator reflects the fact that each individual has two parents, otherwise the formula is analogous to a Lincoln-Petersen standard MR abundance estimate. This formula assumes that: i) adults are sampled after the offspring are born (or simultaneously); and ii) either fecundity does not depend on age, or adults that are 1+, 2+, 3+ in 2015 have the same probability of ending up in the adult sample

(during 2015-2017 sampling). Sufficient conditions for the latter part of ii) are that the sampling probability is the same for all age classes, and mortality does not depend on age.

In the current study assumption i) and the latter part of ii) (i.e., mortality does not depend on age) are violated, and hence we propose an alternative estimator. This new estimator can be used when any of fecundity (F_a, the average number of offspring per adult of age a), survival (S_a) and sampling probability depend on age a. We consider the situation where males (♂) and females (♀) have different age-specific fecundities. Using the notion of "relative reproductive output" (Bravington et al., 2016b, eq. 3.1), we get the following estimator for 2015 census size,

$$\widehat{N}_{c(CKMR)} = \frac{n_J}{H+1} \sum_{a=1+}^{3+} \left(\frac{F_a^{(♂)}}{\bar{F}^{(♂)}} + \frac{F_a^{(♀)}}{\bar{F}^{(♀)}} \right) \times \left(S_{a-1} \times n_{a-1,2014} + n_{a,2015} + n_{a+1,2016} + n_{a+2,2017} \right), \quad (2)$$

where $n_{a,y}$ is the number of adult individuals aged a sampled in year y, and $\bar{F}^{(♂)}$ and $\bar{F}^{(♀)}$ are the estimated mean fecundities across the 2015 populations. The use of $H+1$, rather than H, is a bias correction of Chapman type needed if the expected value of H is low. Age specific survival rates are relevant only for the fish collected before 2015, the relevant spawning season. This involves the 2014 collections only, since individuals collected in 2016 aged *a+1* and *a+2* and individuals collected in 2017 and aged *a+2* were alive in 2015 (Table S2). If male and female age-specific fecundities are the same, i.e. $F_a^{(♂)} = F_a^{(♀)}$, the following simplification of equation (2) is achieved:

$$\frac{F_a^{(♂)}}{\bar{F}^{(♂)}} + \frac{F_a^{(♀)}}{\bar{F}^{(♀)}} = \frac{2F_a}{\bar{F}} . \quad (3)$$

The average fecundity $\bar{F}$ is estimated assuming that sample sizes $n_{a,2015}$ are proportional to the corresponding cohort sizes in 2015, with the following formula

$$\bar{F} = \frac{\sum_{a=1}^{3} F_a \times n_{a,2015}}{\sum_{a=1}^{3} n_{a,2015}}. \quad (4)$$

Female fecundity values were taken from Halfyard, MacMillan, and Madden (2008; see Table S3). In the present study we estimated population abundance under two contrasting assumptions regarding male fecundity: in the first case male fecundity followed the same relationship with age (length) as female fecundity (i.e., equation (3) above), and we report these results here. In the second case, male fecundity was assumed to be invariant with size, i.e. $F_a^{(\sigma)}/\bar{F}^{(\sigma)} = 1$ for all ages a. Results under this somewhat unrealistic assumption are reported in ESM (Table S4). The CKMR approach as described above can be thought of as a general representation of the "transgenerational genetic mark recapture (tGMR)" method designed to estimate spawner abundance in semelparous chinook salmon (*Oncorhynchus tshawytscha*) populations (Rawding et al., 2014).

Lincoln-Petersen interpretation We next explain how the estimator given by equation (2) can be derived from a Lincoln-Petersen estimator of 1+ abundance. When the estimator in equation (1) was introduced in Bravington et al. (2016a) the point of view was that each sampled offspring tags both of its parents. However, the direction of tagging is chosen somewhat arbitrarily, and may be reversed, as described in detail in the Discussion. Fig. 2 illustrates the process in which the sampled adults (parents) tag, say, 10,000 individuals in the cohort born in 2015. Among these 10,000 individuals, a number $m = 100$, say, survive until 2017, and can serve as the tagged individuals in a standard Lincoln-Petersen estimator of 1+ population size in 2017:

$$\widehat{N}_{1+} = \frac{mn_J}{H}. \qquad (5)$$

Here, n_J and H are the same quantities as in equation (2), while

$$m = S_{Larvae} \cdot S_{0+} \cdot \sum_{a=1+}^{3+} F_a \times \left(S_{a-1} \times n_{a-1,2014} + n_{a,2015} + n_{a+1,2016} + n_{a+2,2017}\right)$$

is an estimator of the quantity m in Fig. 2. To simplify the argument, here we have assumed that male and female fecundities are the same (F_a). Because of the dependence on the unknown quantities S_{Larvae} and S_{0+}, neither m nor $\widehat{N}_{1+}$ can be calculated in the present study. However, both S_{Larvae} and S_{0+} cancel out in the process of converting (5) into an estimate of 2015 adult population size. To see this, let $\tilde{F}$ be the average number of offspring (per adult) born in 2015 that survive until 2017. This quantity is related to the average fecundity $\bar{F}$, which does not take juvenile mortality $S_{Larve} \cdot S_{0+}$ until 2017 into account, through $\tilde{F} = S_{Larve} \cdot S_{0+} \cdot \bar{F}$. Now, we can compactly express the relationship between equations (2) and (5): $\widehat{N}_{c(CKMR)} = 2\widehat{N}_{1+}/\tilde{F}$, where the factor 2 occurs because $\widehat{N}_{1+}/\tilde{F}$ is the number of male-female pairs that mated in 2015.

Identification of parent-offspring pairs (POPs): Estimating abundance via CKMR crucially depends on the accurate identification of POPs in the samples of genotyped offspring and potential parents. We used the software COLONY (version 2.0.6.4, Jones & Wang, 2010) to identify and count the number of POPs in our samples. In these analyses we assumed female monogamy and male polygamy, without inbreeding. Allele frequencies were updated during the run by taking family structure into account and the probability that a parent is included in the sample of potential parents (p) was set equal to the fraction of the habitat (streambed) electrofished for a given population. Doing so assumes that individuals have the capacity to disperse throughout the stream or portion of the stream corresponding to that population from

one year to the next. This assumption is consistent with the fact that no population structure is apparent within streams or stream sections (Ruzzante et al., 2016). For each population, COLONY was run five times, each with a different random seed. POP probabilities were summed up within each run and the final number of POPs set to the median sum across all five runs. This was necessary because of the low within population polymorphism exhibited by the 33(31) microsatellite markers. An alternative approach would be to consider only the POPs that appeared in at least three of the five runs. We present results with the first method; results were similar with the second method.

The term n_A in Eqns. (1) and (2) includes individuals sampled (non-lethally) between 2014 and 2017 and thus has the potential to include interannual recaptures. To eliminate such recaptures from n_A, we first identified pairs of individuals sharing the same multilocus genotypes using COLONY; we then assessed whether they could be the same individual based on their length and growth during the period that elapsed between sampling (1 or 2 years). The number of recaptures was then subtracted from the adult sample size to obtain n_A. The individual was assigned to the last year in which it was sampled (See details in ESM).

Results

Individuals collected between 2014 and 2017 (2018 for Church Vault, CV) were aged as a function of size and classified into cohorts for each population (Table 1). Age 1+ individuals collected in 2017 (as well as age 2+ individuals collected in 2018 [CV population] were bred in the fall of 2015 (and hatched in the spring of 2016; blue numbers in Table 1). Those that were present during the 2015 breeding season, including those of the appropriate age sampled in 2014, 2016 and 2017 were thus the potential parents (Table 2). Standard MR estimates of

abundance indicate that populations range in census size between $N_{c(MR)} \approx 400$ (RCD) and $N_{c(MR)} \approx 6000$ (CV, Table 2 and Figure 3).

Individuals in all population/year combinations were genotyped at 33 microsatellite loci (except those in CV which were genotyped at 31 loci). There were no significant deviations from Hardy-Weinberg Equilibrium for any of the population/year combinations nor was there consistent evidence for gametic phase disequilibrium (LD) between pairs of loci (See ESM for details). CKMR estimates of population abundance $N_{c(CKMR)}$ obtained under the assumptions of variable recruitment between years with the sampling being representative of age structure provided results that were indistinguishable from those obtained with standard MR ($\widehat{N}_{c(MR)}$) for 6 of the 7 populations. The CKMR estimate for the seventh population (SBU) was significantly lower than the MR estimate.

Whether male fecundity increases with size at the same rate as it does in females or remains invariant with size did not affect results in any significant way (Table S3). Confidence intervals for the $\widehat{N}_{c(CKMR)}$ were generally wider except for RCD, WWD and SBU where the sampling effort was sufficient for the detection of > 50 (RCD) or > 35 (WWD, SBU) POPs (Table S4). Regardless, to potentially achieve precision levels similar to those obtained for RCD (i.e., a CV≈0.15; Table S4), the sampling effort would have had to have been increased by approximately 33% in WWU and WWD, while in the remaining four populations it would have required doubling (100% increase).

Discussion

We estimated abundance for seven brook trout populations using standard MR as well as the CKMR method. CKMR estimates based on the assumptions that recruitment varied across years, yet sampling was representative of age structure ($\widehat{N}_{c(CKMR)}$), provided results statistically indistinguishable from the standard MR estimates. This provides evidence of the

effectiveness of CKMR as a powerful approach to estimate population abundance despite uncertainties in mortality, fecundity rates, ageing and fecundity-length relationships. We discuss these uncertainties in detail below.

CKMR requires information on age- and population-specific fecundity and mortality rates. Our census estimates were based on population-specific mortality rates, but these were assumed to be constant across years. The fact that the CKMR estimates ($\widehat{N}_{c(CKMR)}$) were similar to abundance estimates obtained by standard MR suggests that if mortality rates in these systems varied between the years 2014 and 2017, the variation was not large enough to create discordance in our population abundance estimates. Our estimates were also based on a female fecundity-length relationship for riverine brook trout obtained from the literature (Halfyard et al., 2008). The uncertainty in this relationship was trivial (results not shown) compared to the uncertainty introduced by the low number of POPs we detected in most populations.

Although the CKMR estimates ($\widehat{N}_{c(CKMR)}$) were largely concordant with standard MR ($\widehat{N}_{c(MR)}$), some of them had larger associated estimates of uncertainty. That is, coefficients of variation and corresponding confidence intervals were wider than those obtained by MR for at least five of the seven populations, a consequence of the relatively low number of POPs identified. In our study the POPs were ≥ 50 for one, ≥ 35 for 2, ≥10 for 3 and ≈8 for 1 population, respectively. Bravington et al. (2016a) required 13000 genotypes (adults and juveniles) to recover 45 POP in a southern blue fin tuna population estimated to contain 2,000,000 individuals with a coefficient of variation, CV≈0.15. Assuming the sample size required to obtain an estimate with a similar coefficient of variation is a square root function of the expected population size (Bravington et al., 2016a), the required sampling effort for an equivalent level of uncertainty in our brook trout systems should be doubled or nearly doubled for four populations (RCU, CV, SBU and SBD, Table S3) , increased by 33% for

two other populations (WWU, WWD) while it is sufficient for RCD (Table S3). The CKMR estimate for one population (SBU) was significantly lower than the MR estimate and we speculate this discrepancy may at least be partially attributable to the relatively low percentage of stream bed covered by the electrofishing in this population (0.09%, Table S4).

Other sources of uncertainty in our estimates included ageing. We used the age-length relationship described in Ruzzante et al. (2016) and the length-frequency distributions in the current data to assign ages to individuals collected from 2014 to 2017 (2018 for CV population). Errors in the age assignation can lead to bias in either direction in the age class representation among the parental genotypes and hence to unknown errors in the relative contribution of these age classes to the offspring generation. The close relationship we encountered in the present study between the MR and the CKMR estimates of population size suggest that if there were errors in the age assignation for some individuals, these were minor and did not adversely impact the estimation of average fecundity used in the estimation of population size in equation (2).

COLONY was run assuming female monogamy and male polygamy, a realistic assumption for these brook trout populations. We estimated $\widehat{N}_{c(CKMR)}$ under two alternative assumptions for the relationship of male fecundity with length. First, we assumed the fecundity-length relationship for males was the same as that for females (Fig 2) and in the second case we assumed male fecundity was invariant with length (Table S3). Results did not vary significantly even for the population for which we detected over 50 POPs (RCD).

The estimation of abundance using CKMR crucially depends on the ability to reliably identify POPs in the samples of juveniles and adults genotyped, which in turn is a function of the number of markers used, their polymorphism, and the reliability and ease of scoring (genotyping). The present study is based on 33 (31) moderately polymorphic markers as assessed in moderately large and widespread systems. The river systems examined in this

study are neither geographically widespread nor large, generally resulting in relatively low within population polymorphism. The number of missing markers among parent-offspring pairs (POPs) was low, <1% for parents and <2% for offspring (Table S3) and the proportion of mismatched markers between identified POP was 0.17% while that between parents and randomly chosen individuals not members of the POP was 38 times higher (6.5%) (Table S5). These results suggest the identified POP in our study can be considered real. Nevertheless, to overcome the potential uncertainties resulting from the low polymorphism in our markers we conducted five independent replicate COLONY runs of moderate length. We then summed up the POP probabilities within runs and used the median estimate across runs as our measure or POP (*H* in equation 2*).* An alternative approach of considering only the POP that appeared in at least 3 of the 5 COLONY runs resulted in estimates that were essentially indistinguishable from those obtained with the previous method (not shown).

Who is tagging who in CKMR?

Bravington et al. (2016a, b) took the viewpoint that offspring tag their parents, while here we have reversed the direction of tagging. This may appear arbitrary, but Skaug (2017) pointed out that the direction of tagging (called the offspring-centric versus parent-centric perspectives by him) can affect the complexity of the statistical derivations. Both perspectives were shown to give the same end result (estimator), in the same way as it is also possible to derive our eqn. (2) from the viewpoint that each offspring tags its two parents. The purpose of this section is to further contrast the two views.

When parents are tagged by their offspring, the number of "tags" in the population is an observed quantity. It is given as two times the number of genotyped offspring corrected for the number of siblings in the offspring cohort sample (e.g., for a half-sibling pair the common parent will be tagged twice but should only count as a single tagged adult). On the other

hand, when offspring are considered as the target of tagging, the actual number of tagged juveniles in the population will be unknown, as the number of offspring produced by a given set of adults will be unknown. In this case, the number of marks must be treated as a random quantity from a statistical point of view. The fact that some adults will have zero offspring, and hence will not contribute to the tagging process, does not cause problems in the subsequent statistical analysis.

We have used the term "Lincoln-Peterson" for our estimator. By this we mean a two stage sampling approach, with the second sample being a simple random sample from the offspring population (electrofishing of 1+ individuals in year *2017*). It is then possible to construct a conditional likelihood, given the first sample (1+ individuals tagged by their sampled parents). Although we have not emphasized this in our analysis and instead made reference to the familiar Lincoln-Peterson form, it can be seen as a fairly general principle that can be applied whenever the offspring constitute a random sample from the offspring population. Sampling variation associated with obtaining the first (adult) sample need not be accounted for. However, the resulting abundance estimate is for the 2015 cohort in 2017 ($\widehat{N}_{1+}$), which needs to be converted into an estimate of adult population size in 2015 ($i.e., \widehat{N}_{c(CKMR)} = 2\widehat{N}_{1+}/\tilde{F}$).

Conclusion: Our study validates the close-kin mark-recapture approach as a method for estimating abundance in populations where the assumptions of random sampling and thorough mixing of individuals can be met. This could include harvested marine populations and populations of conservation concern, and those for which limited other information is available. Depending on how the sampling is conducted, the method additionally requires some degree of understanding of how fecundity and mortality rates vary with age (length). Because of the dependence of the sample size requirements on the expected population size,

the method is likely not applicable to systems numbering in the tens of millions of individuals or larger.

Acknowledgements: We thank Quentin Kerr, Annie Simons, Connor Booker, Samantha Parmelee, Sarah Salisbury, David Malloy, Morgan Colp, Shreeram Sentivasan, and Hilary Brewis for assistance collecting samples across five years of fieldwork. We also thank Paul Conn and one anonymous reviewer for their insightful comments on an earlier version of this manuscript. Fieldwork was funded by an NSERC Discovery grant and by grants from the Nova Scotia Department of Fisheries and Aquaculture to DER. DN acknowledges partial support from an NSERC Undergraduate Student Research Award (USRA) and a Faye Sobey award. DER and JMF acknowledge support from the Ocean Frontier Institute at Dalhousie University.

Authors' contributions: DER conceived the idea to test and validate the CKMR approach with the brook trout data at hand. HS, BF and JMF provided the CKMR statistical framework. GRM, JMM, CB, DN and DER were involved in fieldwork collections. GRM and DN produced the molecular genetic data. DER conducted the analysis and led the writing of the MS with input from HS, BF and JMF. All authors contributed critically to the drafts and gave final approval for publication.

Data Accessibility Statement: Microsatellite genotypes have been submitted to DRYAD

DOI: https://doi.org/10.5061/dryad.md21ns4/1

Reference list

Bravington M.V., Grewe P.M., & Davies C.R. (2016a). Absolute abundance of southern bluefin tuna estimated by close-kin mark-recapture. Nature Communications, 10.1038/ncomms 13162

Bravington M.V., Skaug, H.J., & Anderson, E.C. (2016b). Close-Kin Mark-Recapture. Statistical Science, 31(2), 259-274. DOI: 10.1214/16-STS552

Elphinstone, M.S., Hinten, G.N., Anderson, M.J., & Nock, C.J. (2003). An inexpensive and high-throughput procedure to extract and purify total genomic DNA for population studies. Molecular Ecology Resources, 3(2), 317–320. doi:10.1046/j.1471-8286.2003.00397.x

Excoffier L, Lischer HEL. (2010). Arlequin suite v3.5: a new series of programs to perform population genetic analysis under Linux and Windows. Molecular Ecology Resources, 10, 564–567.

Halfyard E.A., MacMillan J.L., & Madden R.J. (2008). Fecundity and sexual maturity in select Nova Scotia trout populations. Unpublished report. Pictou, Nova Scotia: Inland Fisheries Division, Nova Scotia Department of Fisheries and Aquaculture.

Hillary R.M., Bravington M.V., Patterson T.A., Grewe P., Bradford, R., Feutry, P., ... Bruce, B.D. (2018). Genetic relatedness reveals total population size of white sharks in eastern Australia and New Zealand. Scientific Reports, 8, 2661 DOI: 10.1038/s41598-018-20593w

Jones O.R., & Wang J. (2010). COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources, 10 (3), 551-555

Rawding, D.J., Sharpe, C.S. & Blankenship, S.M. (2014). Genetic-based estimates of adult Chinook Salmon spawner abundance from carcass surveys and juvenile out-migrant traps. Transactions of the American Fisheries Society, 143(1), 55-67.

Ruzzante D.E., McCracken G., Parmelee S., Hill K., Corrigan A., MacMillan J., & Walde S.J. (2016). Effective number of breeders, effective population size and their relationship with census size in an iteroparous species, *Salvelinus fontinalis*. Proceedings of the Royal Society of London, **B,** 283, DOI: 10.1098/rspb.2015.2601.

Skaug H.J. (2001). Allele-Sharing Methods for Estimation of Population Size. Biometrics, **7**, 750-756

Skaug H.J. (2017) The parent–offspring probability when sampling age-structured populations. Theoretical population biology, **118**, 20-26.

van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., & Shipley P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4, 535–538.

Waples R.S., Grewe P.M., Bravington M.V., Hillary R.M., & Feutry P. (2018). Robust estimates of a high Ne/N ratio in a top marine predator, southern bluefin tuna. Science Advances, **4,** eaar7759

Zhan L., Paterson I.G., Fraser BA, Watson B., Bradbury I.R., Ravindran P.R., …. Bentzen P. (2017). MEGASAT: automated inference of microsatellite genotypes from sequence data. Molecular Ecology Resources, 17(2), 247-256.

Figure 1. Geographic location of the four streams (7 brook trout populations) sampled. All streams drain into the Bay of Fundy in Nova Scotia, Canada. Three of the streams have a waterfall (represented by a black bar) somewhere along the stream length that prevent the upstream movement of fish thus creating upstream landlocked populations and downstream populations that may receive immigrants from upstream. These are: Ross Creek Upstream (RCU), Ross Creek Downstream (RCD), Woodworth Upstream (WWU), Woodworth Downstream (WWD), Saunders Brook Upstream (SBU) and Saunders Brook Downstream (SBD). Church Vault (CV) has a waterfall near its outlet into the Bay of Fundy. Modified from Ruzzante et al. (2016).

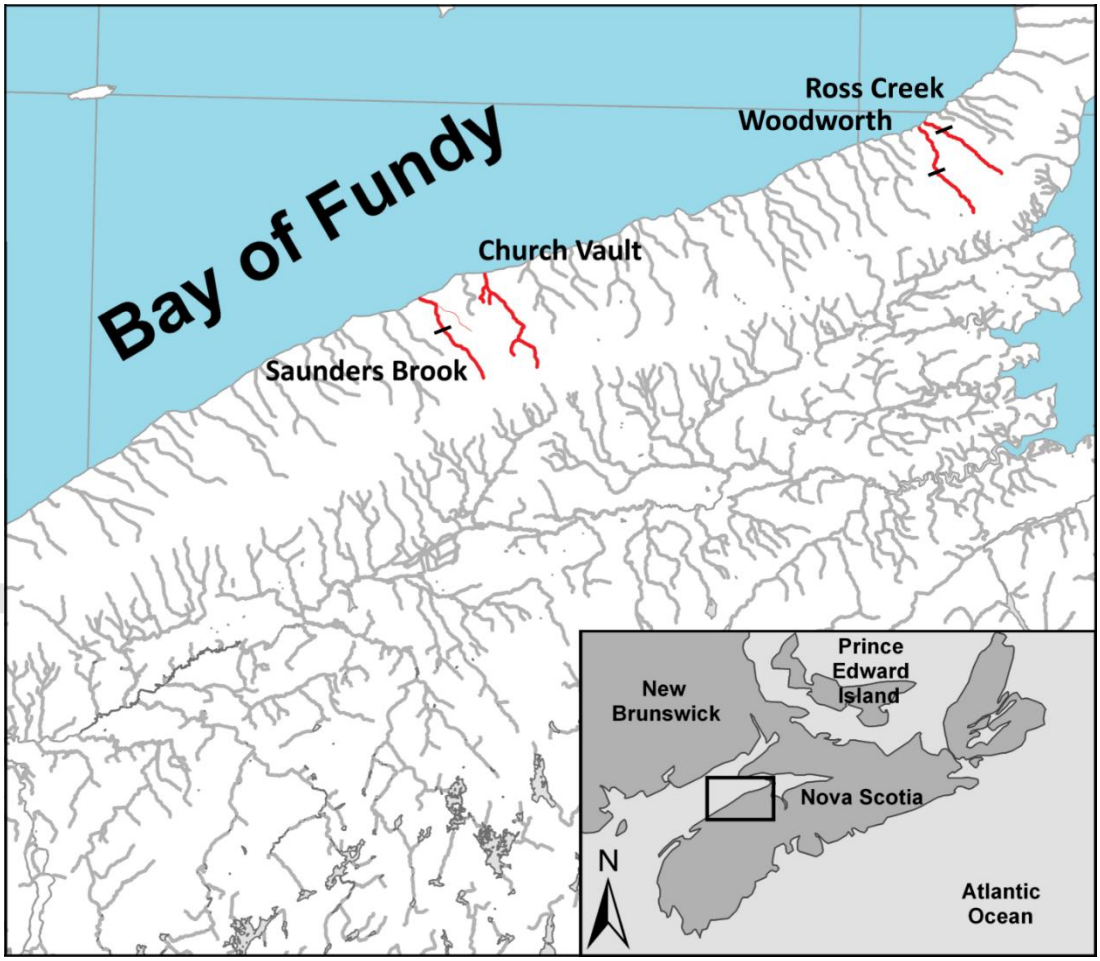

Figure 2 Illustration of how the sampled adults (lower panel) «tag» a part of the 2015 juvenile cohort (upper panel) through being their parents. Sample sizes for the RCU population are used, and colors distinguish the 4 different cohorts that are involved in the CKMR estimate. The dashed box indicates the reproduction event in which the juveniles were born. Adults sampled earlier (downwards pointing arrows) must be "mortality adjusted". For example, if we assume a 1-year mortality of 0.5, there were $0.5 \times 46 + 49 + 10 = 82$ (in expectation ●) individuals in the blue cohort that contributed in the genetic tagging of (a subset of) the 10,000 juveniles born in 2015. Quantities needed for the Lincoln Petersen estimator are m (number of "tagged" juveniles alive in 2017) and n_J (number of sampled individuals from the 2015 cohort).

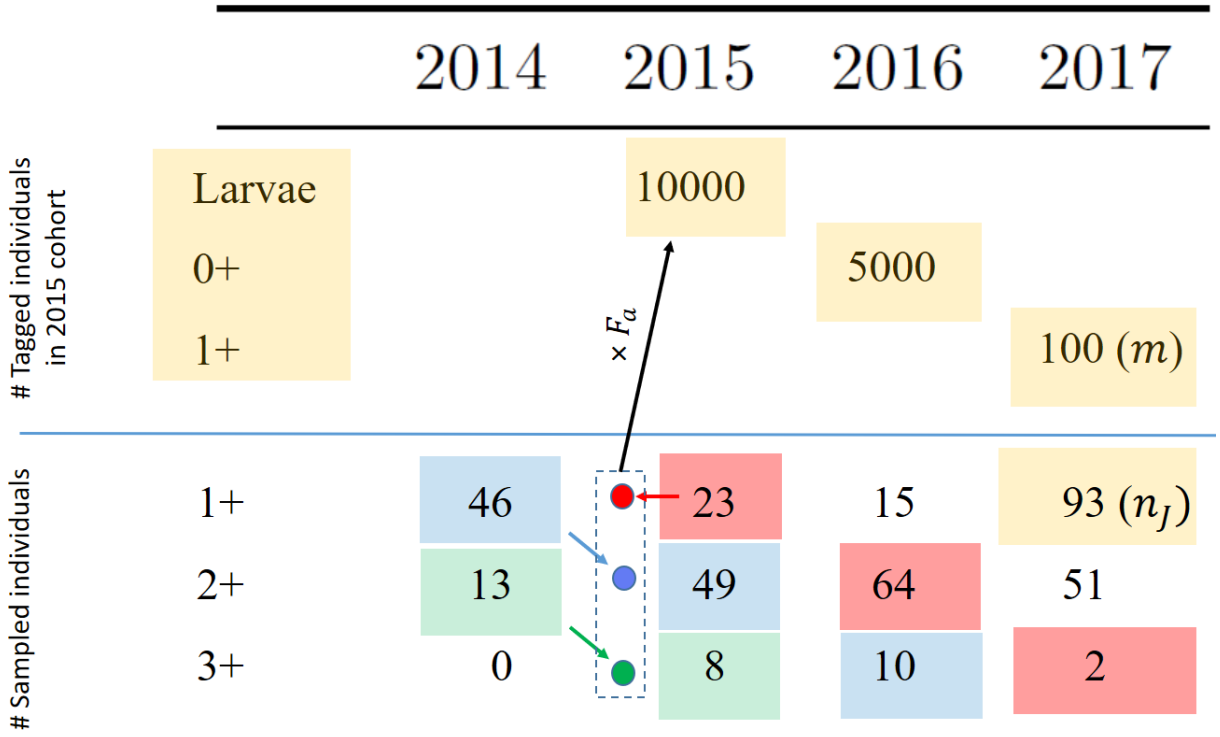

Figure 3. Abundance estimates ($\widehat{N}_c$) based on standard mark-recapture (MR,black) and close-kin mark-recapture (CKMR, red) for each of seven brook trout populations for the year 2015. Error ars are the 95% Confidence Intervals. Stream abbreviations as in legend to Fig. 1. See Table S4 for relevant summary statistics.

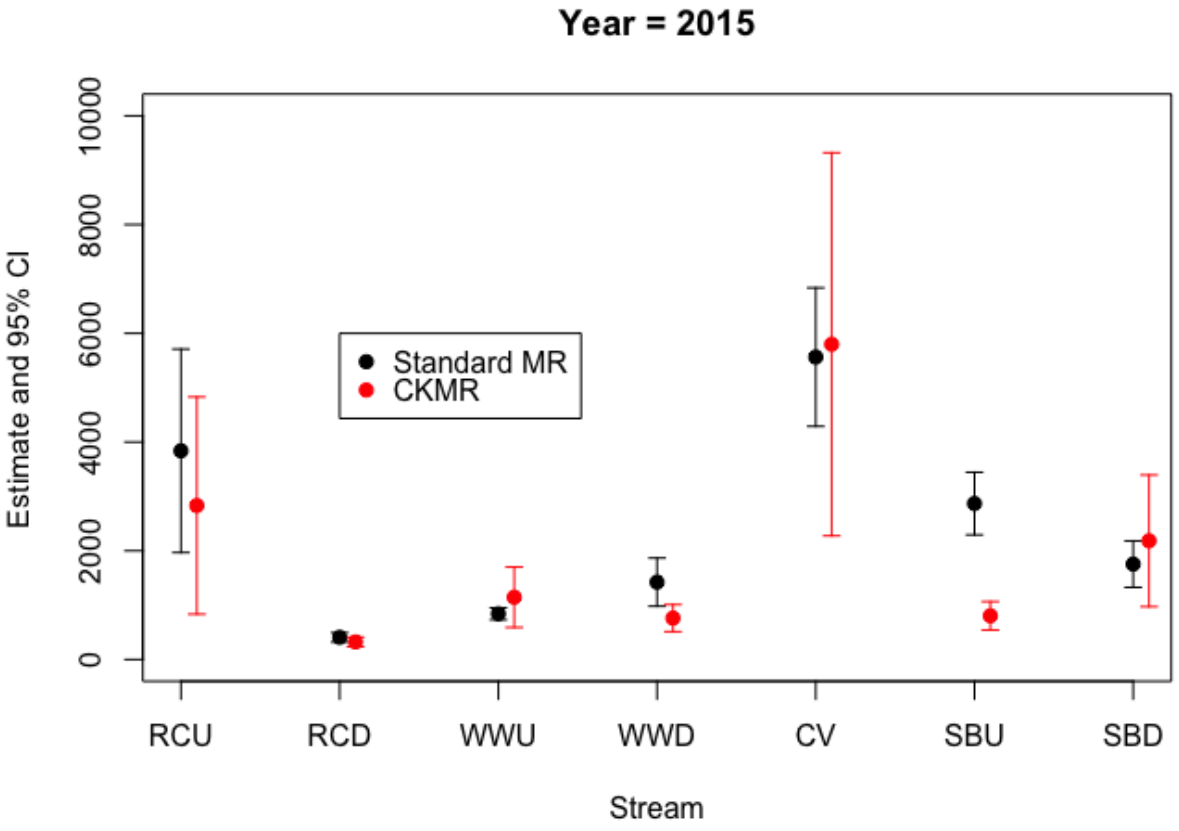

Table 1. Sample size by age class and year of collection. Numbers in red indicate individuals considered as potential parents of the individuals aged 1+ in 2017 (offspring, in blue). Negative within-bracket numbers indicate number of fish that were recaptured in subsequent years and were thus subtracted from the first sample (Details in Table S6). Brook trout eggs are fertilized in the fall and hatching takes place the following spring. Fish were sampled in the summer (July), thus 1+ individuals are individuals that are ~ 14-16 months old since hatching. Sx: Age specific survival rate

	Age class	***2014***	***2015***	***2016***	***2017***	***2018***	s_x
Ross Creek	**1+**	**46**	**23(-1)**	**15**	**93**		**0.42**
Upstream	**2+**	**13**	**49**	**64**	**51**		**0.11**
(RCU)	**3+**	**0**	**8**	**10**	**2**		**0**
	Total	**59**	**80**	**89**	**146**		
Ross Creek	**1+**	**52(-1)**	**7**	**6**	**86**		**0.42**
Downstream	**2+**	**20**	**33**	**60**	**11**		**0.06**
(RCD)	**3+**	**3**	**3**	**12**	**8**		**0**
	Total	**74**	**43**	**78**	**105**		
Woodworth	**1+**	**-**	**1**	**25**	**116**		**0.33**
Upstream	**2+**	**-**	**61**	**25(-1)**	**32**		**0.19**
(WWU)	**3+**	**-**	**10**	**9**	**5**		**0**
	Total		**73**	**61**	**153**		

Woodworth Downstream (WWD)	**1+**	**16(-1)**	**40**	**35**	**107**		**0.33**
	2+	**43**	**13**	**47**	**13**		**0.19**
	3+	**18**	**15**	**11**	**13**		**0**
	Total	**77**	**68**	**93**	**133**		
Church Vault (CV)	**1+**	**60**	**68**	**13**	**85(-4)**	**-**	**0.8**
	2+	**30**	**47**	**37**	**45**	**43**	**0.29**
	3+	**0**	**1**	**1**	**23**	**-**	**0**
	Total	**90**	**125**	**51**	**127**		
Saunders Brook Upstream (SBU)	**1+**	**17(-2)**	**49**	**42**	**97(-1)**		**0.9**
	2+	**49**	**34**	**33**	**45**		**0.18**
	3+	**0**	**3**	**4**	**5**		**0**
	Total	**72**	**86**	**79**	**147**		
Saunders Brook Downstream (SBD)	**1+**	**52**	**58**	**30**	**74**		**0.78**
	2+	**5**	**23**	**19**	**71**		**0.34**
	3+	**0**	**4**	**3**	**6**		**0**
	Total	**57**	**85**	**52**	**151**		

Table 2 Estimates of population abundance based on standard mark-recapture

Site	LENGTH (M)	2014	2015	2016	2017	2018	Median
RCU	3600	3924	3800	1971	5380	4243	3924
RCD	900	1369	408	310	321	516	408
WWU	2500	-	854	821	2065	1456	1155
WWD	2000	1370	1421	718	922	1310	1310
CV	5900	12154	5214	6783	5367	5957	5957
SBU	3900	5066	3022	3140	4063	9100	4063
SBD	1800	2682	1833	2331	2859	7244	2682