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Interannual variation in effective number of breeders and estimation of effective population size in long-lived iteroparous lake sturgeon (*Acipenser fulvescens*)

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Abstract

Quantifying interannual variation in effective adult breeding number (N_b) and relationships between N_b, effective population size (N_e), adult census size (N) and population demographic characteristics are important to predict genetic changes in populations of conservation concern. Such relationships are rarely available for long-lived iteroparous species like lake sturgeon (*Acipenser fulvescens*). We estimated annual N_b and generational N_e using genotypes from 12 microsatellite loci for lake sturgeon adults ($n = 796$) captured during ten spawning seasons and offspring ($n = 3925$) collected during larval dispersal in a closed population over 8 years. Inbreeding and variance N_b estimated using mean and variance in individual reproductive success derived from genetically identified parentage and using linkage disequilibrium (LD) were similar within and among years (interannual range of N_b across estimators: 41–205). Variance in reproductive success and unequal sex ratios reduced N_b relative to N on average 36.8% and 16.3%, respectively. Interannual variation in N_b/N ratios (0.27–0.86) resulted from stable N and low standardized variance in reproductive success due to high proportions of adults breeding and the species' polygamous mating system, despite a 40-fold difference in annual larval production across years (437–16 417). Results indicated environmental conditions and features of the species' reproductive ecology interact to affect demographic parameters and N_b/N. Estimates of N_e based on three single-sample estimators, including LD, approximate Bayesian computation and sibship assignment, were similar to annual estimates of N_b. Findings have important implications concerning applications of genetic monitoring in conservation planning for lake sturgeon and other species with similar life histories and mating systems.

Keywords: demographic parameters, effective number of breeders, effective population size, iteroparity, lake sturgeon, linkage disequilibrium

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Introduction

Effective population size (N_e) is the number of individuals in a theoretically 'ideal' population exhibiting the same magnitude of genetic drift and inbreeding as a population of size N (Wright 1931; Nunney 1993; Charlesworth 2009). N_e reflects the effects of evolutionary processes on population levels of genetic diversity and therefore has been an important conceptual focus

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in evolutionary studies and in conservation biology (Palstra & Ruzzante 2008; Charlesworth 2009; Waples 2010). Since Frankham's (1995) revealed that the ratio of effective to census population size (N_e/N) across wild species was smaller than previously perceived, more attention has been focused on investigations of N_e and causal factors affecting N_e in small natural populations (Mackay 2007; Palstra & Ruzzante 2008). Factors contributing substantially to reductions in N_e relative to N include fluctuations in population size over time, skewed sex ratios and nonrandom variation in lifetime reproductive success (Frankham 1995; Charlesworth 2009; Waples 2010). In addition, N_e is usually smaller than N in age-structured populations, or populations experiencing inbreeding, selection, migration (Charlesworth 2009), or are reproductively isolated in space or time (Hendry & Day 2005; Doctor & Quinn 2009). Ecological characteristics of a species including attributes of the mating system (Nunney 1991, 1993; Pearse & Anderson 2009), generation length (the average age at spawning, Waples 1990), lifespan, and levels of iteroparity (Waples 1990; Nunney 1996; Lippe *et al.* 2006; Serbezov *et al.* 2012a) also influence N_e and N_e/N.

The effective number of breeding adults (N_b) represents a measure of effective size for a single reproductive season. Factors that affect N_e also influence N_b but at different temporal scales (i.e. generations vs. single years, respectively; Waples 2002a). Moreover, N_b can vary among years within a generation, especially when interannual fluctuations in environmental conditions affect recruitment (see Myers 1998; for a review). In natural fish populations, large variability in year class recruitment is commonly observed (Winemiller & Rose 1992) and has unknown consequences to population levels of genetic diversity and N_e over time. For example, in some years, if few adults produce a large proportion of offspring, N_b/N could be low. Fluctuations in N_b across years also result in low N_e/N (Waples *et al.* 2010). Quantifying interannual variation in annual N_b and N_b/N over the course of a long-term study is thus important to quantify effects of interannual variation in recruitment on N_e/N.

The relationship between N_b and N_e is of importance for long-lived iteroparous species where estimates of N_e are rarely feasible (Waples 2010). For example, in the absence of empirical data, researchers have assumed relationships between annual N_b and N_e are comparable to semelparous species and developed supplementation programs with an annual target N_b to obtain generational goals for N_e (Tringali & Bert 1998; Kincaid 1999; Welsh *et al.* 2010). In semelparous species such as Pacific salmon (*Oncorhynchus spp.*), N_e can be approximated as the product of arithmetic mean N_b across years and generation length (g) (Hill 1979; Waples 1990, 2002a). For long-lived iteroparous species, however, the relationship between N_e and N_b remains an important question. N_b is difficult to extrapolate to N_e because breeders each year consist of a mixture of adults from multiple age classes, and often include representatives from several generations (Luikart *et al.* 2010; Waples 2010). Researchers have predicted that N_e could be somewhere between N_b and gN_b (Wang 2009; Luikart *et al.* 2010; Waples 2010), and several studies have shown that N_e could be close to N_b (e.g. Gautschi *et al.* 2003; Beebee 2009).

Lake sturgeon (*Acipenser fulvescens*) is an important species to study N_e, the relationship between N_e and N_b, and the ecological variables that affect effective sizes in long-lived iteroparous species. Aspects of the species life history probably influence relationships among effective sizes and census size (N) in ways not previously described in well studied salmonid species (Ardren & Kapuscinski 2003; Shrimpton & Heath 2003; Araki *et al.* 2007). Sturgeon is characterized by long generation intervals; age at sexual maturity can range from 12 to 22 years for males and from 14 to 33 years for females (Harkness & Dymond 1961). The increase in generation length is expected to increase the ratio of N_e/N (Felsenstein 1971; Hill 1972; Waples 2010). Lake sturgeon are long-lived (more than 50 years; (Birstein 1993) and iteroparous (Rochard *et al.* 1990; Auer 1999). Iteroparity can decrease variance in lifetime reproductive success, because differences in reproductive success among individuals are often not consistent across reproductive episodes (Nunney 1996; Turner *et al.* 2002; Lippe *et al.* 2006). Nonetheless, longevity and iteroparity may increase the possibility of intergenerational inbreeding and accrual of coancestry among offspring from different cohorts, especially in small populations, which can decrease N_e/N (Cervantes *et al.* 2011).

Promiscuous mating which is characteristic of aggregate spawning species, such as lake sturgeon (Duong *et al.* 2011a), should also decrease male and female variance in reproductive success (Sugg & Chesser 1994; Pearse & Anderson 2009), thereby buffering potential reductions in N_b/N. Lake sturgeon spawn in groups and broadcast gametes on to rocky substrate without postovulatory parental care, suggesting that variance in reproductive success during a single reproductive season can be high, resulting in low annual N_b/N ratios. Factors that affect lake sturgeon spawning, demographic parameters, and levels of promiscuity can vary across years (Duong 2010), resulting in interannual variation in N_b/N and ultimately per generation N_e/N.

Our first objective was to estimate annual inbreeding and variance N_b and test whether N_b and N_b/N covary across years with interannual variation in larval production, adult spawning number, sex ratios and variance in adult reproductive success. These relationships could reveal underlying genetic consequences of interannual variation in demographic parameters. For example, a negative association between annual N_{b} and larval production (i.e. smaller N_{b} in years with higher larval production) could reduce N_{e} (Waples *et al.* 2010), and potentially decrease population levels of genetic diversity. We also quantified proportional reductions in N_{b} associated with sex ratio skew and variance in adult reproductive success. Our second objective was to estimate contemporary N_{e} and examine relationships between N_{e} and annual N_{b}. Iteroparity and age structure of long-lived species complicate characterizations of associations between N_{b} and N_{e} (Waples 2010). Results from this study provide important empirical data and quantitative examination of relationships among inbreeding and variance effective breeding number and demographic characteristics of spawning adults and can be used to guide conservation supplementation programs when genetic data are not available.

Material and methods

Study site and sample collection

The study was conducted in the Black Lake watershed in Michigan, USA (Fig. 1). Lake sturgeon adults spawn within a 1.5-km section in the Upper Black River, the only spawning area and the largest tributary to Black Lake (3500 ha). The Black Lake population has been reproductively isolated from other Great Lakes population since 1903 (Baker & Borgeson 1999). The river is shallow and wadable allowing us to sample most spawning adults from late April through early June each year (Forsythe *et al.* 2012b).

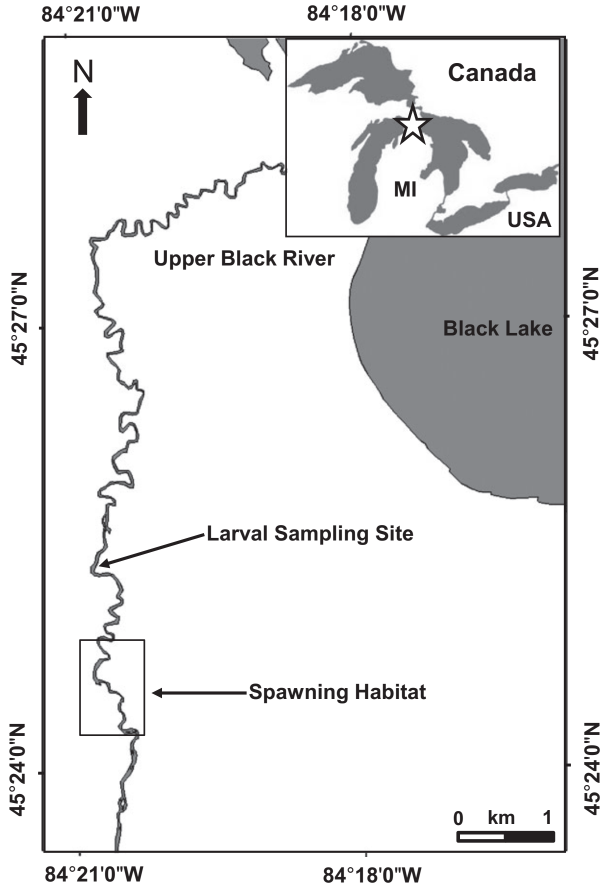

Fig. 1 Map of the study area including Black Lake and locations of adult spawning and collection of larval samples in the Upper Black River.

Spawning adults were sampled annually from 2001 to 2010 and larvae were sampled in each of 8 years (2001–2007 and 2010, Table 1, see also Duong *et al.*

Table 1 Sample sizes of adult and larval lake sturgeon captured and genotyped in 8 years (2001–2007 and 2010) and summary measures of adult reproductive success

	No. Captured adults			Number of larvae collected	% Larvae genotyped	% adults reproducing		Mean mate number		Male RS			Female RS		
Year	Male	Female	M/F			Male	Female	Male	Female	k	V_k	SDV	k	V_k	SDV
2001	71	44	1.61	1691	34	90.1	90.9	5.06	7.82	4.51	10.05	0.49	7.27	35.04	0.66
2002	71	33	2.24	1320	10	76.1	93.9	1.76	3.09	1.31	1.36	0.79	2.82	3.03	0.38
2003	80	40	2.00	16 417	3.0	80.0	87.8	3.86	7.49	2.95	11.79	1.36	5.90	32.96	0.95
2004	76	25	3.04	437	55	71.1	88.0	2.46	6.46	1.47	1.99	0.91	4.48	22.68	1.13
2005	106	47	2.26	7800	4.6	57.5	83.0	2.37	4.31	2.12	13.88	3.08	4.79	50.43	2.20
2006	162	63	2.57	5587	6.8	65.4	98.4	2.33	4.48	1.31	1.99	1.16	3.31	12.88	1.17
2007	143	63	2.27	1444	100	93.7	88.9	6.76	15.08	6.16	27.05	0.71	13.98	149.8	0.77
2010	169	55	3.07	2259	12	50.3	74.5	1.71	3.54	0.93	1.60	1.83	2.87	13.63	1.65

M/F, sex ratios of males to females; RS, reproductive success; k and V_k, mean and variance in RS; SDV, standardized variance in RS calculated for all adults captured in the same year. Percentage of adults reproducing is the percentage of captured adults that had at least one offspring assigned.

2011a,b). Adults were sampled using long-handled nets as we waded the river daily during the spawning season. Individuals were marked with 125-kHz passive integrated transponder (PIT) tags and a 1-cm^2 portion of the dorsal fin was removed for genetic analysis. Larval sampling started 10 days after the first observed spawning event and lasted for 25–40 days, until no larvae were captured for two consecutive nights. Larvae were sampled using the same protocols each year. We deployed five D-frame nets evenly spaced across the river (25 m; description in Smith & King 2005). Nets were checked hourly from 2100 to 0200 h during the period larvae dispersed downstream from the spawning site. Larvae captured represented ~15% of the total number of annual larvae produced that survived to this period following hatch (Smith & King 2005). Larvae were taken to a rearing facility and reared for 12–15 weeks. A portion of the caudal fin was taken from a random subset of individuals selected proportionally based on the number captured each night for genetic analysis (Table 1).

Genetic analysis

DNA was extracted from adult and larval samples using QIAGEN DNeasy(R) kits (QIAGEN Inc.). Samples were genotyped at 12 tetra-nucleotide microsatellite loci including Spl 120 (McQuown *et al.* 2000); AfuG 68B (McQuown *et al.* 2002). 2002); Aox 27 (King *et al.* 2001); AfuG 68, AfuG 9; AfuG 63, AfuG 74, AfuG 112, AfuG 56, AfuG 160, AfuG 195 and AfuG 204 (Welsh *et al.* 2003). Conditions for polymerase chain reaction (PCR) for these loci were as described in the primary references. All gels were scored independently by two experienced lab personnel. Ten per cent of all individuals were genotyped a second time at all loci to empirically estimate genotyping error. Scoring errors (percentage of alleles mis-scored) were experimentally estimated to be <1.0% over all years (see Duong *et al.* 2011a).

Data analysis

Testing assumptions on marker loci used in the study. Deviations of observed genotypic frequencies from Hardy–Weinberg (HW) equilibrium were tested using exact tests in GENEPOP 4.0 (Raymond & Rousset 1995; Rousset 2008). Of the 12 loci, one (AfuG 68) departed from HW equilibrium but was retained for analyses. FSTAT 2.9.3 (Goudet 2001) was used to test for genotypic disequilibrium among loci but no significant linkage was detected.

Estimating relative reproductive success of males and females. We define relative reproductive success as the number of genotyped larvae assigned to each adult. We conducted parentage analysis using two programs, the Parentage Allocation of Singles on Open Systems (PASOS) program, version 1.0 (Duchesne *et al.* 2005) and CERVUS version 3.0 (Kalinowski *et al.* 2007). Assignment of offspring to parent pairs was based on concordance of maternal and paternal assignment in PASOS (correctness probability 86.7–89.4%) and the most probably parental pair assigned in CERVUS at or above the 70% confidence level. Details of parentage assignment incorporating output from both programs are provided in Duong *et al.* (2011a,b).

Mean and variance in reproductive success among individuals were calculated for all adults captured in the same year and for only the subset of adults that produced at least one larva. These estimates of mean and variance in reproductive success were used to evaluate effects of ‘null parents’ (parents with no offspring assigned) on differences between variance (N_{bV}) and inbreeding (N_{bI}) effective breeding number (Table S1, Supporting information). We estimated standardized variance (variance/mean^2) in reproductive success (SDV) for males and females to compare the magnitude of variance (V_k) to the mean (k) of reproductive success among years (Nunney 1996).

Estimating effective number of breeders. We used three methods to estimate effective number of breeders: (i) linkage disequilibrium (LD); (ii) demographic data estimated using genetic determination of parentage; and (iii) a moment-based temporal method. When microsatellite data are used, LD can provide greater precision compared to the temporal (two-sample) method (Waples 2010). We used program LDNE (Waples & Do 2008) to estimate inbreeding effective number (N_{bD}) using multilocus genotype data of larval lake sturgeon. Results of estimates were reported at the allele exclusion criterion $P_{crit} = 0.02$ (see England *et al.* 2006; Waples 2006; Waples & Do 2008 Waples & Do 2010).

We estimated inbreeding and variance-effective breeding number (N_{bI} and N_{bV}, respectively) based on estimates of mean and variance in male and female reproductive success. First, estimates of the inbreeding effective numbers for each sex, for males (N_{bIm}), for example, were calculated as (Crow & Denniston 1988; equation 1; Caballero 1994, equation 22):

$$N_{bIm} = \frac{k_m N_m - 1}{k_m - 1 + V_m/k_m} \tag{1}$$

where N_m is the number of males captured, k_m is the mean number of offspring assigned to males (k_m), and V_m is the variance in the number of offspring for males. Inbreeding effective number of females (N_{bIf}) was calculated using the same equation. The inbreeding effective number of breeders was then computed as (Caballero 1994; equation 7 modified; Araki *et al.* 2007, equation 5):

$$N_{bI} = \frac{4N_{bIm}N_{bIf}}{N_{bIm} + N_{bIf}} \tag{2}$$

Estimates of the variance effective number of breeding males (N_{bVm}) and females (N_{bVf}) were calculated using equation 19 in Crow & Denniston 1988 (or equation 4 in Waples & Waples 2011). For males:

$$N_{bVm} = \frac{k_m(2N_m - 1)}{2(1 + V_m/k_m)} \tag{3}$$

Estimates for females were similarly estimated and the variance effective number of breeders was computed as in eqn 2.

We also employed a moment-based temporal method (Waples 1989) implemented in program NeEstimator 1.3 (Peel *et al.* 2004) using genotypes of adults and larvae each year to estimate variance N_{b} (here after N_{bT}) based on equation 9 in Waples (1989).

Associations between N_{b} derived using different estimators were assessed using Spearman correlation (ρ) because of small sample size (n = 8 years). Statistical comparisons between annual effective sizes derived based on different estimators were made using *t*-tests conducted using R (R-Development-Core-Team 2009).

Reductions in N_b associated with sex ratio skew and variance in reproductive success. Effects of a reduction factor (sex ratio skew or variance in individual reproductive success) on annual N_{b} were evaluated by calculating predicted N_{b} under an assumed ideal condition for each factor and computing a reduction rate as 1- N_{b}/N (Araki *et al.* 2007). The predicted N_{b} when sex ratios are balanced was calculated using eqn 2 where N_{bIf} and N_{bIm} were replaced by the number of females and males captured. The predicted N_{b} assuming a binomial distribution of variance in reproductive success was calculated using a scaled index of variability (V_k/k) where scaled V_k/k was computed to its expected value of $k = 2$ (using eqn 3) for a stable population (Crow & Morton 1955; Waples 2002b; Araki *et al.* 2007). To evaluate the effect of variance in reproductive success alone, sex ratio was ignored and the predicted N_{b} was estimated as the sum of N_{bf} and N_{bm} (see Table S2, Supporting information for detailed calculations; see Waples 2002b; Araki *et al.* 2007; for details).

Estimating contemporary N_e. We estimated N_{e} using three single-sample estimators including linkage disequilibrium (Hill 1981; Waples 1991), Approximate Bayesian Computation, ABC (Tallmon *et al.* 2008), and sibship assignment, SA (Wang 2009) using genotypes of adults captured during 10 years (2001–2010). The LD method was expected to provide accurate estimates of contemporary N_{e} for this population because lake sturgeon data are consistent with suggested requirements (Bartley *et al.* 1992; Schwartz *et al.* 1998; Lippe *et al.* 2006) including (i) small and isolated populations; (ii) sufficient sample sizes (>90 individuals) and numbers of microsatellite loci used (12); and (iii) samples consisted of a number of age classes that is approximately equal to generation length (Luikart *et al.* 2010; Waples & Do 2010; Robinson & Moyer 2012). Ages of adults captured during 10 years (n = 796) ranged from 13 to 59 years for males and from 26 to 60 years for females. Because annual sample sizes of adults were large (N = 101–225, Table 1) and contained the same range of age classes, samples pooled across years and single-year samples were used to estimate N_{e}.

We also used program ONeSAMP that uses an approximate Bayesian computation method to estimate N_{e} (Tallmon *et al.* 2008). We used three a priori ranges of N_{e} for all single-year data including 2–500 (based on the upper limit of LD-based N_{e} estimates); 2–2000 (based on the estimated census size of the population, approximately 1200; E. Baker unpublished data); and 2–10 000 (suggested by DA. Tallmon, personal communication). We also used a sibship assignment (SA) method implemented in program COLONY (Wang 2009) to estimate N_{e}. Estimates from program COLONY were based on the assumption of polygamous mating and no inbreeding.

Results

Population levels of genetic variability estimated from adult multilocus genotypes

Measures of genetic diversity suggest the 12 loci surveyed for adults and larvae provided sufficient discriminatory power to estimate parameters associated with N_{b} and N_{e} estimation. Levels of genetic diversity estimated for spawning adults were moderately high. The mean number of alleles per locus estimated for spawning adults ranged from 4.7 to 5.2 across years. Mean expected multilocus heterozygosity ranged from 0.586 to 0.591, and the combined (multilocus) probability of nonparental pair exclusion was 5.5×10^{-5}.

Annual population demographic data

Demographic parameters including adult sex ratios and mean and variance in male and female reproductive success were estimated each year (Table 1) to characterize relationships with N_{b}. Sex ratios were male-biased in all years, ranging from 1.61 to 3.01 males to females.

Parentage data revealed that males and females were highly promiscuous; males and females had many mates (annual means 1.76–6.76 mates for males and 3.09–15.08 mates for females; Table 1). Parentage analysis also revealed that high proportions of adults were assigned offspring during each of 8 years (ranges 50.3–94.7% for males and 74.5–98.4% for females). Analyses conducted with and without adults with no offspring assigned did not greatly affect estimates of mean and variance in reproductive success each year (Table S1, Supporting information). Standardized variance (SDV) in reproductive success (V_k/k^2) varied among years, 5.8-fold (range among years 0.38–2.20) and 6.2-fold (range 0.49–3.08) for females and males, respectively. Over the 8-year period, annual larval production varied approximately 40-fold across years (437–16 417), but was not significantly correlated with annual SDV ($\rho = 0.64$, $P = 0.10$ for males, and $\rho = 0.50$, $P = 0.21$ for females).

Annual estimates of N_b

Annual estimates of N_b were generally concordant across estimators (Table 2). Estimates of inbreeding effective number based on the LD method (N_{bD}) were more precise and closer to inbreeding N_{bI} and variance N_{bV} estimated based on demographic data than the temporal variance effective size estimator (N_{bT}). Annual estimates of N_{bD} ranged from 47 to 167 (95% CIs 39–425; Table 2). Demographic estimates of inbreeding N_{bI} (ranged 41–106) were lower than but not statistically different from N_{bD} and variance N_{bV} (t-tests ~ 1.2, d.f. = 14, $P \sim 0.2$) (Table 2). The harmonic mean (across years) inbreeding (N_{bI}), variance (N_{bV}), and LD-based (N_{bD}) effective breeding numbers were 66, 81 and 83, respectively. The temporal estimator (N_{bT}) yielded similar point estimates (harmonic mean across years 133) but higher confidence intervals in 5 of 8 years (2003–2005, 2007, and 2010) compared to other estimators N_{bD}, N_{bI}, and N_{bV} (all t-tests $P > 0.2$). In the other 3 years (2001, 2002 and 2006), point estimates of N_{bT} were high and the upper limit was infinite, confounding calculations of the ratio of N_{bT}/N. Interannual variation in N_{bI} was highly concordant with N_{bV} ($\rho = 0.86$, $P < 0.01$) and N_{bD} ($\rho = 0.72$, $P = 0.04$). The ratios of N_b/N from all estimators were similar across years, ranging from 0.27 to 0.86 (Table 2).

We used LD genetic estimates (N_{bD}) to test the associations of demographic parameters with interannual variation in N_b/N ratios. No significant correlations between annual N_{bD}/N and annual larval production ($\rho = -0.47$, $P = 0.24$), or SDV of males ($\rho = -0.52$, $P = 0.20$) or females ($\rho = -0.59$, $P = 0.13$) were found. Ratios of N_{bD}/N were higher in years when higher proportions of females produced larvae (females that had offspring assigned) ($\rho = 0.81$, $P = 0.02$) (Tables 1 and 2). However, the proportion of successful males was not associated with annual estimates of N_{bD}/N ($\rho = 0.38$, $P = 0.35$).

Reductions in N_b associated with sex ratio skew and variance in reproductive success

Reductions in annual N_b associated with sex ratio skew and variance in reproductive success were evaluated for each year. Reduction rates associated with unequal sex ratios each year ranged from 5.51 to 25.9% (mean ± SD = 16.3 ± 7.0%). Variance in reproductive success among adults resulted in comparatively higher reduction rates, averaging 36.8 ± 19.6% (Table 3), indicating that variance in reproductive success had a comparatively greater effect on annual N_b. For example, in 2005, high reduction rates due to variance in reproductive success (70.7%) resulted in a low observed and

Table 2 Comparisons of estimators of effective breeding number (95% CI) based on a temporal moment-based method, (variance effective breeding number; N_{bT}), demographic variables (variance N_{bV} and inbreeding N_{bI} effective breeding number) and linkage disequilibrium (inbreeding effective number; N_{bD}), and ratios of effective breeding numbers to the annual number of adults captured (a measure of adult census breeding size; N) in 8 years (2001–2007 and 2010)

Year	N	N_{bT}	N_{bI}	N_{bV}	N_{bD}	N_{bI}/N	N_{bV}/N	N_{bD}/N
2001	115	317 (94–∞)	76	140	63 (55–72)	0.66	1.22	0.55
2002	104	296 (92–∞)	87	89	167 (95–425)	0.83	0.86	1.61
2003	120	88 (43–206)	58	82	69 (57–83)	0.47	0.68	0.57
2004	101	75 (35–202)	43	52	77 (59–104)	0.43	0.52	0.76
2005	153	78 (40–165)	41	47	47 (39–57)	0.27	0.31	0.31
2006	225	1448 (203–∞)	104	113	147 (112–197)	0.47	0.50	0.65
2007	206	124 (66–247)	106	205	126 (105–150)	0.51	0.99	0.61
2010	224	132 (63–342)	76	140	88 (68–115)	0.34	0.33	0.39

Inbreeding N_{bI} based on eqns 1 and 2; Variance N_{bV} based on eqns 2 and 3 in this manuscript.

Table 3 Effects of sex ratio and variance in adult reproductive success on annual reductions in variance effective breeding number (N_{bV})

Year	Sex ratio		Variance in RS (scaled at $k = 2$)		Expected N_{bV}/N	Observed scaled N_{bV}/N
	Predicted N_{bV}	Reduction rate (%)	Predicted N_{bV}	Reduction rate (%)		
2001	109	5.51	84	27.0	0.69	0.65
2002	90	13.4	100	3.68	0.83	0.83
2003	107	11.1	62	48.6	0.46	0.47
2004	75	25.5	74	26.8	0.55	0.42
2005	130	14.9	45	70.7	0.25	0.27
2006	181	19.4	149	33.8	0.53	0.46
2007	175	15.1	129	37.5	0.53	0.51
2010	166	25.9	120	46.6	0.40	0.34

Predicted N_{b} for reduction factor of sex ratio was estimated assuming a balanced sex ratio. Predicted N_{b} for reduction factor of variance in reproductive success was estimated at conditions of $k = 2$, V_k = scaled variance V_k (calculated using eqn 3 in Waples 2002b), and balanced sex ratios. Expected N_{bV}/N was calculated by multiplying [1 − reduction rate] of sex ratio and variance in adult reproductive success. Observed scaled N_{bV}/N was estimated using eqn 3 (where $k = 2$, V_k = scaled variance V_k) and eqn 2 using observed sex ratios.

expected N_{b}/N (at an ideal $k = 2$ and its scaled variance V_k) (Tables 2 and 3, respectively). In years 2001 and 2004, the N_{b} reduction attributed to variance in reproductive success was comparable or lower than reduction rates attributed to skewed sex ratios. Sex ratio skew and variance in reproductive success together reduced N_{b} on average 53% relative to N (expected N_{b}/N; Table 3), which was similar to the observed mean N_{bI}/N across years (N_{bI}/N˜50%; Table 2).

Estimates of per-generation effective population size

Point estimates of N_{e} were similar among single-year samples and estimation methods LD, SA and ABC-500 (Table 4). For example, the LD-based N_{e} estimates ranged from 72 to 198 (harmonic mean 126). The harmonic mean of LD-N_{e} was close to the estimate (mean = 114; 95% CI: 95–136) based on all unique adults ($n = 796$) pooled from samples over 10 years. Among estimators, SA estimates were consistently lower with narrower confidence intervals than estimates based on LD and ABC-500 ($P < 0.001$ for all t-tests) (Table 4). LD and ABC-500 methods yielded comparable point estimates of N_{e} (t-test = 1.62, $P = 0.12$) but estimates were not consistent across years ($\rho = 0.29$; $P = 0.42$). Estimates from ABC-2000 and ABC-10000 yielded N_{e} estimates that varied and were generally larger than the other estimators among single-year samples (Table 4). The mean of ABC-based estimates for three a priori ranges (113, 287, and 426) increased with increasing prior

Table 4 Estimates of effective population size (95% CI) based on linkage disequilibrium (LD- N_{e}), sibship assignment (SA- N_{e}), and approximate Bayesian computation (ABC) methods with three a priori ranges of N_{e} based on annual adult genotypes from 10 years (2001–2010)

Year	LD- N_{e}	SA- N_{e}	ABC500- N_{e}	ABC2000- N_{e}	ABC10000- N_{e}
2001	110 (73–186)	51 (35–78)	101 (75–212)	305 (188–1093)	172 (95–660)
2002	122 (80–220)	55 (39–82)	109 (77–235)	77 (50–191)	975 (422–5868)
2003	154 (93–330)	53 (38–80)	73 (54–149)	48 (33–116)	137 (74–408)
2004	198 (108–640)	56 (39–83)	94 (73–174)	133 (91–394)	315 (161–1576)
2005	126 (83–220)	59 (42–87)	104 (78–214)	390 (229–1345)	397 (203–1980)
2006	129 (92–193)	88 (66–119)	94 (70–150)	616 (319–1731)	294 (161–985)
2007	119 (88–170)	77 (56–106)	159 (117–313)	192 (122–632)	292 (155–1038)
2008	159 (119–225)	84 (62–116)	117 (81–238)	167 (102–485)	1178 (470–7995)
2009	163 (112–268)	66 (48–95)	140 (102–255)	864 (434–3139)	397 (184–1580)
2010	72 (57–268)	67 (49–95)	139 (100–304)	81 (53–202)	106 (62–349)
Harmonic Mean	126	63	108	136	253

Three a priori ranges of ABC-based N_{e} include 2–500 (ABC500- N_{e}), 2–2000 (ABC2000- N_{e}), and 2–10 000 (ABC10000- N_{e}).

upper limits (t-test = 2.05, $P = 0.05$ for ABC-500 vs. ABC-2000; t-test = 2.74, $P = 0.01$ for ABC-500 vs. ABC-10000). However, this trend was not consistent among single-year samples as indicated by the lack of correlation among the estimates (ρ ˜ 0.2, P ˜ 0.5, $n = 10$), indicating estimates based on higher upper prior limits are probably upwardly biased. In addition, the 95% CIs of ABC-2000 and ABC-10000 estimates were unrealistically large, indicating imprecise estimation of N_e.

Mean estimates of N_e across years from LD, SA and ABC-500 were also close to annual N_b estimated based on annual larval collections (Table 2 and 4). Based on LD estimates, for example, the mean of N_b (99) was 23% smaller than N_e estimated for the entire sample of adults (LD-N_e = 126). The ratios of N_e to the annual number of spawning adults averaged 0.81, and the ratio of N_e to the total number of adults in the Black Lake population (N ˜1200 (E. Baker, unpublished data) was approximately 0.11.

Discussion

Our long-term study incorporating demographic data derived from parentage assignments and measures of linkage disequilibrium allowed quantification of annual N_b, intergenerational N_e, and the relationship between N_b and N_e for long-lived iteroparous lake sturgeon. Data allow predictions of future genetic changes within the population associated with interannual variation in adult census size (N), sex ratios, and larval production. Several important findings are highlighted. First, estimates of N_b derived using different estimators were similar and relatively stable over an 8-year period. Annual numbers of spawning adults were likewise similar, though larval production varied 40-fold across years, suggesting rates of mortality during early life stages (i.e. from eggs to the time of larval sampling) were probably random with respect to family. Second, variance in reproductive success and unequal sex ratios reduced annual N_b/N. However, their relative effects varied among years. Third, N_e estimated for the entire population was similar to the average N_b for larval cohorts produced each year, suggesting N_e for long-lived iteroparous species may not be approximated as the product of mean annual N_b and generation time, as has been suggested for semelparous species (Waples 1990).

N_b and aspects of the species' biology

Relative effects of demographic factors including variance in reproductive success and sex ratio skew on annual N_b can differ among species (Osborne *et al.* 2010). Most studies have shown that variance in family size is the primary factor reducing N_b/N (e.g. Waples 2002a; Araki *et al.* 2007; Serbezov *et al.* 2012a). We also found that reduction in N_b/N in the Black Lake lake sturgeon population due to variance in reproductive success was higher than reduction associated with skewed sex ratios (average 36.9% vs 16.3%, respectively). However, N_b/N reduction rates associated with both variables varied from year to year (Table 3), indicating environmental conditions and features of the species' reproductive ecology interact to affect demographic parameters and N_b/N. Annual estimates of N_b were correlated with the number of breeding adults (correlation between scaled N_{bV} and N: $\rho = 0.68$, $P = 0.06$). However, no significant correlations between N_b estimates (including N_{bD}, N_{bI}, and scaled N_{bV}) and sex ratio, larval production, and variance in relative reproductive success were observed. Importantly, although larval production varied greatly from year to year (40-fold), the number of spawning adults and standardized variance in reproductive success were relatively stable, as were estimates of N_b/N. These results imply that in years of comparatively poor larval production, adult reproductive success decreased proportionally across all spawning adults compared to years of high larval production.

Estimates of annual N_b/N for lake sturgeon (N_{bI}/N range 0.27–0.83 across years, Table 2) were not as low as predicted for a highly fecund and broadcast-spawning species (Bruch *et al.* 2006) where offspring experience high rates of mortality during early life stages (Hedgecock 1994; Hedrick 2005), which is common in lake sturgeon (Caroffino *et al.* 2010; Forsythe 2010). Most lake sturgeon adults captured each year contributed offspring (Table 1; Table S1, Supporting information), and standardized variance in reproductive success was comparable across years. Our estimates of annual N_b/N were within the range of N_b/N and N_e/N observed in other fish species that are characterized by different life histories and mating systems (Nunney 1996; Araki *et al.* 2007).

The reduction of N_b/N associated with skewed sex ratios in lake sturgeon was higher than has been described in salmonid species [2.3–5.4% for steelhead *Oncorhynchus mykiss* (Araki *et al.* 2007); 1–3% for brown trout (Serbezov *et al.* 2012a)]. Skewed sex ratios are expected in iteroparous species with different interspawning intervals between sexes. Sex ratios of lake sturgeon, for example, are commonly male-biased because males have shorter interspawning intervals (2.3 ± 0.08 years) than do females (3.7 ± 0.16 years; Forsythe *et al.* 2012a). Effects of sex ratios on N_b/N can also be buffered by polygamous mating (Table 1) which decreases variance in reproductive success, thereby moderating potential effects on N_b/N (Nunney 1996; Pearse & Anderson 2009).

Contemporary N_e and the relationship between N_e and N_b

Harmonic mean N_e (across years) estimated using three-one-sample estimators were comparable and thus are believed to provide a robust estimate of contemporary N_e. The N_e/N ratio estimated in this study (~ 0.11) was similar to the median value in different populations from other species (Frankham 1995; Palstra & Ruzzante 2008). The low N_e/N ratio can result in increased rates of loss of genetic diversity (Frankham 1995). However, for long-lived species, longevity and iteroparity could act as a buffer against negative effects of low N_e and N_e/N (DeHaan *et al.* 2006; Lippe *et al.* 2006).

The relationship between N_b and N_e for long-lived and iteroparous species is complicated (Waples 2010), and has rarely been investigated using single-sample methods (Waples & Do 2010). We found that N_e estimated from adults was comparable to annual N_b estimated from single larval cohorts. Studies in other taxa have shown similar results. In iteroparous natterjack toads (*Bufo calamita*), N_b was also documented to be close to N_e (Beebee 2009). In brown trout, another iteroparous fish, N_b was estimated to be approximately half of N_e (Serbezov *et al.* 2012a). Based on simulations, Robinson & Moyer (2012) demonstrated that N_b estimates were 60–72% of N_e for two long-lived iteroparous species (Atlantic sturgeon *Acipenser oxyrinchus* and three-ridge mussel *Amblema neislerii*; generation length approximately 14 years).

Similarities between N_b measured at early life stages and adult N_e could be expected if rates of mortality from the time of larval sampling to the adult stage are random with respect to family (Crow & Morton 1955; Waples 2002b). This expectation should hold for demographic estimators (Crow & Morton 1955; Waples 2002b) and LD and SA estimators (Wang 2009). Therefore, the observed estimates based on scaled N_{bV} (based on demographic estimators) and N_{bD} (LD method) may provide a robust estimate of N_b at adult stages under assumption of random family mortality. In another study conducted on the Black Lake population in a hatchery setting, N_b of larvae was comparable to N_b of juveniles 90 days posthatch (Crossman *et al.* 2011), the period when mortality is highest in wild lake sturgeon (Caroffino *et al.* 2010). Phillipsen *et al.* (2010) also found that there was no reduction of N_b estimated at the juvenile stage compared to egg stage for spotted frog (*Rana pretiosa*).

Alternatively, if offspring from different families experience different rates of mortality, N_b estimated at the adult stage would be smaller compared to larval N_b (Crow & Morton 1955; Waples 2002b). The factor that causes lower N_b/N at adult stage compared to early stage is the increase in variability index (V_k/k). A large V_k/k is expected when the mean number of progeny produced from a single spawning season (k) is large (Waples 2002b). In the Black Lake population, k could be <2 because the population size has been relative stable or slightly declining since a dam closed the population to outside immigration in 1903. Therefore, V_k/k could not differ greatly from the scaled V_k/k at the larval stage. Waples (2002b) showed that the N_b/N at adult stages of Pacific salmon were from 0 to 62% (mean 19%) smaller than N_b/N at larval stages. If the same reduction in N_b/N of 62% is applied to lake sturgeon, average N_b/N across 8 years (0.5) at the larval stage would be reduced to 0.19 at the adult stage, or N_b could range between 19 and 43 (based on the range in annual spawning adult number, 101–225, Table 2). In either two situations of random or nonrandom family survival after juvenile stage, the observed N_e (126) in our population was smaller than the product of mean annual N_b and generation length (20 years), as suggested for semelparous species with constant census sizes (Waples 1990).

Comparisons of N_b and N_e estimators

Violations in assumptions of effective size estimators due to species-specific life histories and genetic processes at population levels can affect accuracy and precision of effective size estimates (Beebee 2009; Saarinen *et al.* 2010; Barker 2011; Phillipsen *et al.* 2011; Serbezov *et al.* 2012b). Therefore, comparisons of different estimators provide greater understanding of contributions of demographic and behavioural factors to N_b and N_e (Waples & Do 2010) and provide a measure of estimator accuracy and precision (Beebee 2009). In this study, concordant estimates of variance N_b (unscaled N_{bV}) compared to inbreeding N_b (N_{bI}) indicate that a large proportion of the adult population each year contributed to offspring sampled at the larval stage. If survival among families after the time of larval sampling was random, scaled N_{bV} estimates (adjusting to expected N_b at $k = 2$) should be comparable to N_{bI} (Waples 2002b). The demographic-based estimates (N_{bI} and scaled N_{bV}) and single-sample genetic estimates of N_b (LD method; N_{bD}) were similar (t-test = 1.24, d.f. = 14, $P = 0.24$) and correlated ($\rho = 0.72$, $P = 0.04$). Both estimators were also correlated with annual spawning adult number, indicating robust estimates of N_b.

Our data show that in age-structured populations, single-year LD estimates provide unbiased estimates of population N_e when based on large sample sizes with a large number of age classes as predicted (Waples & Do 2010). We observed small variation in point estimates of

LD-N_e across 10 years, and the harmonic mean across 10 years was comparable to N_e estimates from the entire adult sample, supporting our prediction that spawning adults each year were a random subset of the whole population. In contrast, the SA estimator provided consistently lower point estimates and narrow CIs. The SA program can generate narrow CIs for N_e estimates in cases of small sample sizes compared to actual N_e and when a small number of loci characterized by low levels of variability are used (Wang 2009), which was not the case in our study. Adult sample sizes (range 101–225), the number of loci (12), and heterozygosity levels of markers (0.59) were high. For estimators derived using ONeSAMP program (ABC method), the point estimates and CIs were sensitive to a priori ranges used, indicating that the method is unreliable for long-lived iteroparous animals like lake sturgeon.

Study implications and further information needs

This study has important implications for the conservation of lake sturgeon and other species with similar life histories and mating systems. Comparisons of N_e and N_b suggest that for lake sturgeon and probably other long-lived, iteroparous species, N_e may not be approximated as the product of average annual N_b and generation length (g) as has been demonstrated for semelparous salmon ($N_e \sim gN_b$; Waples 1990, 2002a; Ardren & Kapuscinski 2003). Supplemental breeding programs would be ill advised to divide the targeted N_e into a series of (g) annual target effective numbers (N_b). For example, if a conservation goal of $N_e = 200$ is targeted and generation length (g) is 20 years, annual N_b would be 10 as suggested previously (Kincaid 1999; Welsh *et al.* 2010). Further, because N_b was sensitive to adult breeding number, maintaining a sufficient number of breeding adults each year will probably reduce effects of other factors (sex ratio skew and V_k) that we demonstrate will reduce N_b. Moreover, given that annual estimates of N_b/N were similar despite large fluctuations of larval production, random mortality among families is probably at early life stages, which we previously demonstrated based on a small sample from a single year (Duong *et al.* 2011b). Therefore, environmental variability, a key determinant of recruitment variability in lake sturgeon species, does not appear to disproportionally affect different subsets of spawning adults (e.g. as a function of size/age, location or timing of reproduction).

Our findings of relationships between annual N_b and population N_e leads to the question, can N_e in long-lived and iteroparous species be inferred based on estimates derived from samples collected during early life stages? Although temporal methods are available for age-structured populations (Jorde & Ryman 1995), frequently researchers are not able to accurately age individuals, and information on age-specific mortality is typically lacking. Further research focusing on relationships between census, annual breeding, and population effective sizes should be explored through simulations, given the paucity of data elucidating changes in N_b over sequential life stages in long-lived species with delayed sexual maturity. If relationships between N_b and N_e are robust, genetic monitoring (Schwartz *et al.* 2007) based on sampling of individuals at early and comparatively more accessible life stages may be an important conservation tool to predict demographic and genetic population trends for lake sturgeon and other species with similar life histories and mating systems.

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Data accessibility

Sampling date and microsatellite data of adult and larval lake sturgeon can be found at DRYAD entry doi: 10.5061/dryad.72242.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Comparisons of mean (k), variance (V_k), and standardized variance (SDV) in reproductive success, and inbreeding effective breeding number (N_{bI} and N'_{bI}) based on all adults captured and adults that had at least one offspring assigned (Adults reproducing).

Table S2 Reduction factors of sex ratio and variance in reproductive success (RS) among adult males and females on variance effective breeding number (N_{bV}).