Egg and Larval Collection Methods Affect Spawning Adult Numbers Inferred by Pedigree Analysis

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Running title: Egg and Larval Collection Methods Affect Pedigree Analysis

[A]*Abstract.*—Analytical methods that incorporate genetic data are increasingly used in monitoring and assessment programs for important rate functions of fish populations (e.g., recruitment). Because gear types vary in efficiencies and effective sampling areas, results from genetic based assessments likely differ based on the sampling gear used to collect genotyped individuals; consequently, management decisions may also be affected by sampling gear. In this study, genetic pedigree analysis conducted on egg and larval Lake Sturgeon collected from the St. Clair-Detroit river system using three gear types was used to estimate and evaluate gear-specific differences in the number of spawning adults that produced eggs and larvae sampled (N_s), effective number of breeding adults (N_b), and individual reproductive success. Combined across locations and sampling years, pooled estimates were 349 (N_s, point estimate) and 314 (N_b, 95% CI: 271-368). Mean reproductive success was 4.26 with a variance of 6.26 individuals per spawner. Mean (± SE) estimated numbers of unique parents per genotyped egg or larvae (i.e., adult detection rate) from 2015 samples were 1.104 ± 0.003 for vertically-stratified conical nets, 0.716 ± 0.002 for D-frame nets, and 0.709 ± 0.001 for egg mats. Using samples from 2016, adult detection rates were 0.725 ± 0.001 for D-frame nets and 0.629 ± 0.001 for egg mat collections. Coancestry results were negatively correlated with adult detection rate. Although, genetic pedigree analyses can improve understanding of recruitment in fish populations, this study demonstrates that estimates from genetic analyses can vary with targeted life stage (a biologically informative outcome) and sampling methodology. This study highlights the influence of sampling methods on interpretation of genetic pedigree analysis results when multiple gear types are used to collect individuals. Development of standardization approaches will facilitate spatial and temporal comparisons of genetic based assessment results.

[A]*Introduction.*—

In recent years, genetic data have been increasingly used to assess population recruitment (Jay et al. 2014; Tsehaye et al. 2016; Brenden et al. 2018). Accurate and precise understanding of recruitment in natural fish populations is fundamental to management because of its importance to a population's long-term sustainability (Houde 2008; Jay et al. 2014; Ludsin et al. 2014). Recruitment estimates can be obtained from samples collected using many different gear types, the selection of which is contingent on a species' life history (Casselman et al. 1990) and habitat-imposed constraints on gear sampling effectiveness (Cassleman et al. 1990; Erős et al. 2009). Efficiencies of different gear types vary (Cassleman et al. 1990), and sampling events typically involve varying levels of effort (Jackson and Harvey 1997). Consequently, analytical approaches have been proposed to standardize traditional fisheries data to allow comparisons among samples collected at different times, locations, sampling durations, and gear types (Maunder et al. 2004; Lee et al. 2010).

The sampling properties of different gears with respect to estimated genetic diversity of sampled individuals has not been previously evaluated, which may influence resulting measures of recruitment. Specimens from which genetic data are extracted are often obtained as part of standardized fisheries assessment or with the same suite of gears used during standardized assessments. As a result, genetic-based assessments of population recruitment may be improved if differences among gear types were quantified and new relative metrics were developed to account for differences in parameter estimates among collection methodologies; such metrics would facilitate spatial and temporal comparisons of recruitment estimates.

In this study we focus on the genetic assessment of population recruitment in Lake Sturgeon of the St. Clair-Detroit River system (SCDRS). Lake Sturgeon is a species of conservation concern that was once abundant throughout the Laurentian Great Lakes (Auer

1999). However, due in part to habitat modifications (Manny et al. 1988; Auer 1996; Bennion and Manny 2011; Hondorp et al. 2014), Lake Sturgeon populations have declined and not recovered despite implementation of more restrictive harvest regulations (Auer 1996) and improvements in water quality. Recent habitat remediation efforts in the SCDRS included the construction of spawning reefs with the intent of increasing spawning habitat availability and ultimately recruitment of lithophilic fishes (Hondorp et al. 2014, Manny et al. 2015; Roseman et al. 2011a).

Prior research conducted in the SCDRS immediately after construction of spawning reefs found that adult Lake Sturgeon successfully spawned over the reefs (Roseman et al. 2011a; Bouckaert et al. 2014; Manny et al. 2015; Prichard et al. 2017). Assessments also showed that eggs deposited at constructed reef sites survived to the larval drift stage (Roseman et al. 2011a; Bouckaert et al. 2014).

Despite evidence of successful spawning, questions nevertheless remained as to how many spawning adults contributed the eggs or larvae that were sampled (N_s), the effective number of breeders (N_b), and variance in individual reproductive success of Lake Sturgeon associated with the constructed reef sites. Genetic pedigree analysis is a useful method to characterize adult contributions to recruitment associated with remediation efforts (Christie et al. 2010; Sard et al. 2015). Information on how spawner abundance and reproductive success may affect cohort levels of genetic diversity are provided by estimates of N_b (i.e., it is the effective breeding population size for a spawning period). The effective number of breeders is usually smaller than the population-abundance because of sex-ratio skew (Frankham 1995; Charlesworth 2009; Waples 2010). High levels of variation in adult reproductive success also result in reduced N_b (Araki et al. 2007; Duong et al. 2013). Estimates of N_s, N_b, and variance in adult

reproductive success generated using sibship reconstruction methods based on genetic data further inform managers of the benefits of constructed spawning habitat for lithophilic spawning fishes. However, estimates based on traditional assessments as well as genetic data may be affected by the methodologies used to collect samples and the biology of the species of interest.

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The number of larvae collected in individual sampling events is often highly variable due to spatially and temporally heterogeneous distributions of individuals in the environment (Cyr et al. 1992; D'Amours et al. 2001). Because of the potential for passive and active dispersal during early (egg and larval) ontogenetic stages, measures of spawning success generated using genetic pedigree analysis may be influenced by specific sampling methodologies. In the case of Lake Sturgeon in the SCDRS, large numbers of egg or larvae collected from sampling conducted at a reef site does not necessarily indicate a large number of spawning adults. Lake Sturgeon are highly fecund (Bruch et al. 2006) and a single adult pair ostensibly could produce all eggs or larvae collected in a sample. For example, sampling gear such as small (38 × 24 × 0.5 cm) egg mats positioned directly on an constructed reef may sample offspring from many males, as females mate with multiple males (Bruch and Binkowski 2002). However, it is possible offspring were contributed by an individual female due to heterogeneous dispersal of eggs and dispersion of egg mats during spawning. Additionally, if eggs from individual females are not evenly distributed across a spawning site, positioning sampling gear such as D-frame nets directly downstream of constructed spawning reefs may collect larvae dispersing from small proportions of the reef and thus underestimate adult spawning number when collected larvae are used for genetic pedigree analysis. Gear types such as vertically-stratified conical nets positioned further away from constructed reefs may be more likely to sample larvae from proportionately more spawning adults because of the potential for larvae to mix in the water column during dispersal. Additionally, the sampling efficiency of each gear type in terms of the

number of eggs and larvae collected per sampling event can lead to potential differences in N_s and N_b estimated from genetic pedigree analysis.

The objective of this study was to examine the effects of early-life stage collection methods on estimates of N_s and N_b obtained using genetic pedigree analysis. We hypothesized that estimates of N_s, N_b, and measures of reproductive success would differ among sampling methods due to unequal sample sizes, per net or egg mat frequency of collection of eggs and larvae, and within sample relatedness of collected individuals.

[A]*Materials and Methods*

[B]Study Area.—

The SCDRS is a 145 km barrier-free connecting channel between Lake Huron and Lake Erie (Figure 1). The head of the SCDRS located near the city of Port Huron, MI is a known, natural spawning site for Lake Sturgeon (Manny and Kennedy 2002). Sites included in this study were located downstream of this natural spawning area. The 1.7 ha (~56.4 × 306.6 m) Harts Light Reef was located approximately 35 km downstream from the head of the St. Clair River, near East China, MI (Figure 1), and was constructed from 10-15 cm sorted limestone in 2014. Water depths are approximately 16 m with discharge of 1.35 m^3/s. Further downstream (28 km) in the St. Clair River was the 0.6 ha (~32.9 × 184.4 m) Pointe Aux Chenes Reef (Figure 1), constructed in 2014 from 10-15 cm sorted limestone, and water depths are approximately 15 m with discharge of 1.03 m^3/s. Finally, in the Detroit River, the 1.6 ha (~43.6 × 371.6 m) Grassy Island Reef (Figure 1) was constructed in 2015 from 10-15 cm sorted limestone. Water depths at Grassy Island Reef are approximately 12 m with discharge of 0.80 m^3/s.

[B]Egg and Larval Collection Methods.—Egg and larval stage Lake Sturgeon were collected using egg mats (egg stage: Roseman et al. 2011b), D-frame nets (larval stage: Roseman et al. 2011b), and vertically-stratified conical nets (larval stage: Bouckaert et al. 2014) at Harts Light and Pointe Aux Chenes and two additional spawning sites in the North and Middle Channel, St. Clair River in 2015 and 2016, and at Grassy Island Reef and two additional drift sites in the Trenton Channel, Detroit River in 2016 (Figure 1). Egg mats consisted of a furnace filter surrounding a 38 × 24 × 0.5 cm steel frame secured using 5 × 2.5 cm binder clips. Egg mats were deployed on constructed reefs in sets of three mats separated by one meter (Manny et al. 2010, Roseman et al. 2011b). Two sets of egg mats were deployed on each reef. One egg mat set was placed on the upstream portion of the reef and one egg mat set was placed on the downstream portion of the reef. Egg mats were retrieved weekly, and eggs were identified to species and enumerated. Each egg mat retrieved was treated as a separate sample. A total of 363 and 322 egg mats were retrieved across all study sites and checked for Lake Sturgeon eggs during the spawning period in 2015 and 2016, respectively. Numbers of Lake Sturgeon eggs collected on upstream egg mats compared to downstream egg mats on the reefs were similar for Hearts Light Reef and Grassy Island Reef. However, Lake Sturgeon eggs were collected only on the downstream egg mats at Grassy Island Reef (Craig et al. 2017). A subset of Lake Sturgeon eggs from each egg mat were selected at random and reared (Sutherland et al. 2014) until the yolk sac was absorbed to allow for DNA extraction.

D-frame nets were 76 cm at the base by 54 cm high made of 1600 µm mesh. D-frame nets were deployed as paired sets of two nets on the river bottom directly upstream and two nets directly downstream of each constructed reef. D-frame nets were fished at depths of approximately 14.5 m (Harts Light reef upstream), 17.3 m (Harts Light reef downstream), 15.7 m (Pointe Aux Chenes reef upstream), 16.2 m (Pointe Aux Chenes reef downstream), 9.8 m

(Grassy Island reef upstream), and 10.2 m (Grassy Island reef downstream). Nets were deployed at each reef from approximately 2000 hours to 0400 hours, during the peak larval drift period for Lake Sturgeon in the SCDRS identified by Bouckaert et al. (2014). In 2015 and 2016, 91 and 181 D-frame nets were deployed and retrieved in the SCDRS, respectively.

Vertically-stratified conical nets were deployed in paired sets with two groups of three nets each directly upstream and two groups of nets directly downstream of each constructed reef. A group consisted of three conical nets that were 0.15 m in diameter with 750 µm mesh. Nets were suspended on a buoy line in the water column; one net was set one meter below the surface, one net was set in the middle of the water column, and one net was set one meter off the river bottom (D' Amours et al. 2001, McCullough et al. 2015). Vertically-stratified conical nets were deployed at sunset and retrieved at sunrise. In total, 211 and 240 vertically stratified conical nets were deployed and retrieved in 2015 and 2016, respectively. Each net and night was treated as a separate sample.

[B]DNA Extraction and Amplification.—Caudal fin tissue was sampled from collected larvae and DNA was extracted using QIAGEN DNeasy® kits (QIAGEN, Valencia, CA, USA) according to the manufacturer's protocol. A nano-drop spectrophotometer was used to determine DNA concentration and DNA was diluted to a concentration of 20 ng/µl.

Polymerase chain reaction (PCR) was used to amplify DNA for each of 13 disomic loci: LS-68 (May et al. 1997), Afu68b (McQuown et al. 2002), Spl120 (McQuown et al. 2000), Aox27 (King et al. 2001), AfuG9, AfuG56, AfuG63, AfuG74, AfuG112, AfuG160, AfuG195, AfuG204 (Welsh et al. 2003) Atr113 (Rodzen and May 2002). Analyses also included 5 polysomic loci adapted from Jay et al. (2014), (Atr100, Atr114, Atr117, AciG35, and AciG110;

Rodzen and May 2002). Conditions for PCR for each of the 13 disomic loci were as described in Duong et al. (2013), and for the 5 polysomic loci as described in Jay et al. (2014).

Polymerase chain reaction was performed in 25-µl reactions with 100-ng of genomic DNA. Based on optimizations described in Scribner et al. (in review), reactions used 10x PCR buffer (0.1 M Tris-HCL, 15 mM $MgCl_2$, 0.5 M KCL, 0.1% gelatin, 0.1% NP-40, 0.1% Triton-X), $MgCl_2$, 2 mM each dNTP, 10 pmol of forward and reverse primer, and Denville Taq polymerase (Denville Scientific, Metuchen, NJ, USA. After multiplexing and dilution to concentrations optimized for analysis, PCR product was analyzed on an ABI3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) at the Michigan State University Research Technology Support Facility. All allele sizes were analyzed with MapMarkerTM size standards (BioVentures Inc., Murfreesboro, TN, USA), and included three Lake Sturgeon samples of known genotype, and a negative sample (no DNA). Results were visualized using GeneMarker (Softgenetics, State College, PA, USA). Alleles were scored independently and confirmed by two experienced laboratory personnel. Approximately 10% of all individuals were selected randomly and re-genotyped as a further quality control check, resulting in empirical scoring error rates of 0.5% and 1.8% in 2015 and 2016, respectively.

[B]Pedigree Analysis.—Allele scores (base pairs) were assigned using the method of Rodzen et al. (2004) and Wang and Scribner (2014). This method treats individual alleles as pseudo-disomic loci resulting in a presence (1), absence (2), or missing data (0) score for each individual and locus. Data were recorded as missing if an individual failed to amplify at a locus despite two separate amplification attempts. A total of 741 larvae collected in nets or reared from eggs collected on egg mats were analyzed using 164 alleles (pseudo-disomic loci) in 2015 and 2016; total sample size was 741 eggs and larvae. COLONY was used to assign larvae to FS and HS

groups and to infer N_s and N_b contributing to offspring sampled using a full-maximum likelihood approach (Wang 2004). COLONY parameters included polygamy for males and females, high likelihood precision, unique random number seeds for each run, and no prior sib-ship knowledge. All other COLONY parameters were run at default settings.

Accuracy in pedigree analysis is dependent on the number of loci analyzed and the amount of information provided by the markers (Wang and Scribner 2014). Wang and Scribner (2014) found that treating polysomic markers as pseudo-disomic loci allowed for accurate assessment of FS and HS relationships. Simulations from Hunter et al. (in review) demonstrate sufficient power to accurately assign larvae to FS and HS groups and infer N_s.

Pedigrees were generated in program COLONY consisting of each unique larval identity, a putative mother identity, and a putative father identity. Using only larval genotypes to generate the pedigree provides no information on the actual sex of the putative parents so further analysis considered only unique parent identities.

[B]Statistical Analysis.—To account for potential within sample variation and variation in results due to the order that larvae were included in the pedigree, each collection method and location-specific pedigree underwent bootstrap resampling (total # of iterations = 1,000). Resampling resulted in bootstrapped pedigrees for which the cumulative sums of unique parents per larvae genotyped were generated. A simple linear regression model (*e.q.* 1) was fit to each bootstrapped pedigree for each collection method

$$Sum_{par} = \beta_0 + \beta_1 N_{off} \qquad (e.q.1)$$

where, Sum_{par} is the cumulative sum of unique parents detected, β_0 is the intercept, β_1 is the model slope (i.e., the rate of detection of unique parents per larvae genotyped), and N_{off} is the number of larvae genotyped. The mean slope was calculated as the mean of all slopes estimated from 1,000 bootstrapped pedigrees. Regressing the cumulative sum of unique parent detected per larvae genotyped for each collection method yielded an estimate of the rate of detection of unique parents per larvae genotyped for a particular sampling method (i.e., a measure of gear sampling efficiency with respect to adult numbers represented in progeny sampled). These slopes were hypothesized to differ between collection methods due to within sample variation or due to the sequential order in which larvae, and subsequently the parents that contributed them, were included in the analysis. Differences in numbers of parents detected per larvae genotyped between gear types were examined for each bootstrap sample for each sampling gear using a varying-slope and varying-intercept linear model (*e.q.* 2)

$$Sum_{par_t} = \beta_{0_t} + \beta_{1_t} N_{off_t} + \varepsilon \qquad (e.q.2)$$

where Sum_{par_t} is the cumulative sum of unique parents detected by sampling gear *t*, β_{0_t} is the model intercept for sampling gear *t*, β_{1_t} is the model slope for sampling gear *t*, N_{off_t} is the number of larvae genotyped for sampling gear *t*. Error (ε)was assumed to be normally distributed with a mean of zero and a variance of σ^2. The varying-intercept and varying-slope, linear model was fit in R 3.4.3 (R Core Team 2017) using the *lm* function from the Stats package (R Core Team 2017). After fitting the linear model, we tested whether slopes for the sampling gears were significantly different (α=0.05) using the *linearHypothesis* function from the CAR package (Fox and Weisberg 2011). Testing the differences in slopes effectively tested for significant differences in the rate of detection of unique parents per larvae genotyped for the

sampling methods for each pair of bootstrapped pedigrees. Repeating this across all bootstrapped samples resulted in 1,000 significance test results as to whether model slopes differed between the sampling gears. To conclude there were significant differences in the rate of detection of unique parents per larvae genotyped for two sampling methods, the test for slope equality had to be rejected for more than 950 ($\alpha = 0.05$) of the bootstrap draws. Because of small sample sizes, captures from vertically-stratified conical nets in 2016 were not included in the slope comparisons.

In addition to rates of detection of unique parents per larvae genotyped, we also examined coancestry as a measure of relatedness among individuals sampled. Coancestry (θ) was calculated for each bootstrapped pedigree according to the methods described in Bartron et al. (2018). Individual coancestry values were calculated for each of 1,000 bootstrapped pedigrees as (*e.q.* 3),

$$\theta = \frac{n_{fs}(0.25) + n_{hs}(0.125) + n_u(0)}{n_t} \qquad (e.q.3)$$

where n_{fs} are the number of full-sibling dyads, n_{hs} are the number of half-sibling dyads, and n_u are number of unrelated dyads identified in each bootstrapped pedigree (Cockerham 1969; Crossman et al. 2011, Bartron et al. 2018). Mean coancestry was calculated as the sum of all individual coancestry values for 1,000 bootstrapped pedigrees divided by the number of bootstrapped iterations. Standard errors for mean coancestry, the number of unique parents included in bootstrapped pedigrees (N_{par}), and for the slope (rate of detection of unique parents genotyped were also calculated from the bootstrapped results.

Two-sided Student's *t*-tests based on the bootstrap results were used to test for significant differences between coancestry for each gear type by location and year. Pearson's correlation

tests were used to examine correlations between coancestry (θ), the number of parents included in a pedigree, and the rate of detection of unique parents per larvae genotyped.

[A]*Results*

[B]Sample Sizes Varied by Gear Type.—Collections of egg-stage and larval Lake Sturgeon were generally low for all gear types (Table 1). Egg mats had the highest mean catch at approximately 7 individuals per mat in 2015 and 6.5 individuals per mat in 2016. Catches in D-frame nets were substantially lower, at 3.8 and 4.3 individuals per net in 2015 and 2016 respectively. The mean number of individuals captured per conical net was far lower than egg mats or D-frame nets, with only 6.0% and 0.4% of the mean number of individuals captured per egg mat in 2015 and 2016, respectively. Comparison of non-zero catches showed even greater disparity among gear types. In 2015, the mean non-zero catch per D-frame net and conical net was only 6.3% and 2.4%, respectively, of the mean non-zero catch per egg mat. Similarly, in 2016 the mean non-zero catch was 13.9% and 1.2% of the mean non-zero catches per egg mat for D-frame net and conical net mean non-zero catches. However, averaged across years, over 90% of egg mats and conical net sets had a catch of zero (Table 1). In contrast, 57.3% of D-frame net sets collected one or more larval Lake Sturgeon when averaged across years. Gear types considered frequently collected zero individuals, with D-frame nets collecting individuals more often than egg mats and conical nets. However, when egg mats collected individuals they tended to collect more individuals than D-frame and conical nets.

[B]Number of Spawning Adults Contributing Eggs and Larvae (N_s).—Point estimates of N_s were sample size dependent and increased linearly with the number of larvae that were genotyped (Figure 2). To account for sample size dependence, and to compare N_s point estimates between

gear types and across locations, N_s values were divided by the total number of larvae genotyped in each sample and averaged across years. There was a 1.4% difference in average point estimates of N_s per larvae genotyped across all reefs and years between egg mats (0.556 N_s per larvae genotyped) and D-frames (0.564 N_s per larvae genotyped). However, vertically-stratified conical nets had a 68% and 66% larger estimate of N_s per larvae genotyped (0.936 N_s per larvae genotyped) compared to egg mats and D-frame nets, respectively. At Harts Light Reef, the average N_s per larvae genotyped was 77% and 39% larger for vertically-stratified conical nets (1.148 N_s per larvae genotyped) compared to egg mats, D-frame nets (0.649 and 0.829 N_s per larvae genotyped, respectively). Average estimates of N_s for egg mats (0.978 N_s per larvae genotyped) were 17% larger than that of D-frame nets (0.833 N_s per larvae genotyped), but vertically-stratified conical nets (1.35 N_s per larvae genotyped) were 62% and 38% larger than D-frame nets and egg mats, respectively, at Pointe Aux Chenes Reef. Finally, estimates of N_s at Grassy Island Reef in 2016 for D-frame nets were 40% larger than egg mats (0.500 and 0.702 N_s per larvae genotyped, respectively). Between-year variation in detection of unique parents per larvae genotyped for each of the sampling methods was generally low for individual reefs and when reefs were combined (Table 2).

[B]Effective Number of Breeders (N_b).—Estimates of N_b were also sample size dependent (Figure 2). When taking into account the effect of sample size, vertically-stratified conical nets consistently had greater N_b values estimated compared to egg mats and D-frame nets. Egg mats and D-frame nets had similar point estimates for N_b per larvae genotyped, but generally D-frame nets had larger N_b per larvae genotyped values than egg mats. However, 95% confidence intervals around N_b estimates for all gear types overlapped across all constructed reef sites for all years (Table 2).

[B]Rates of Detection of Unique Parents.—Mean slopes between years were similar within collection methods over all locations (Figure 3). Trends for differences in mean slope remained consistent between reefs and years. Vertically-stratified conical nets detected unique parents at a higher rate per larvae genotyped (range: 1.104 – 1.230), followed by D-frame nets (range: 0.716 – 1.019), and then by egg mats (range: 0.629 – 0.981) (Table 3). Significant differences in rates of unique parents detected per larvae genotyped were observed between vertically-stratified conical nets and D-frame nets (P=0.002) and egg mats (P=0.001) in 2015 pooled across all sites (Table 4). No significant difference was found between D-frame nets and egg mats (P=0.189) in 2015 across all reef sites. However, a significant difference in rates of detection of unique parents per larvae genotyped was observed between egg mats and D-frames (P=0.017) in 2016 for eggs and larvae pooled across all reef sites with D-frames detecting unique parents at a slightly higher rate. Trends in rates of detection of unique parents per larvae genotyped were relatively similar across locations and between years (Table 3). No significant differences were observed for the rate of detection of unique parents per larvae genotyped between any of the collection methods in 2015 (Table 4). Differences were not always significant but, for each location and year, vertically-stratified conical nets had the highest rate of detection followed by D-frame nets and finally egg mats (Table 3).

[B]Levels of Coancestry In Bootstrap Resampled Pedigrees.—Mean coancestry was lower for pedigrees generated using samples collected with vertically-stratified conical nets compared to pedigrees generated using samples from D-frame nets and egg mats in 2015 (Table 3). In 2016 pedigrees from samples collected using D-frame nets resulted in significantly lower coancestry than egg mats across all sites (P<0.001). However, this pattern was not always consistent in

2015 where higher coancestry was observed in D-frame collections using samples from all reefs and Harts Light Reef (Table 3). Student's *t*-tests indicated significant differences in levels of coancestry among all gear types ($P<0.001$) (Table 5). Significant negative correlations were observed between levels of coancestry (θ) and the number of parents included in a pedigree (Npar) and between levels of coancestry and the slope (rate of detection of unique parents per larvae genotyped in 2015 (Table 6) and 2016 (Table 7)) across all locations. There were significant ($P<0.001$) positive correlations between mean slope and the number of parents in 2015 (Table 6) and 2016 (Table 7) across all locations.

[A]*Discussion*.—

The study focus was to evaluate sampling gear effects on estimates of adult use of constructed spawning reefs as measured by N_s and N_b. Critical assessment of the effects of sampling methodology on the number of spawning adults contributing offspring for traditional assessment and genetic pedigree analysis will afford opportunity for statistical comparison and allow proper interpretation of results to inform management. Cyr et al. (1992) demonstrated that the number of replicate samples required for precise estimates of larval abundance using traditional sampling methods rises rapidly as the mean number of larvae captured per sample decreases. Pritt et al. (2014) similarly found evidence for differences in detection probabilities among species with differing early life history traits that impact site occupancy and abundance estimates. Similarly, precision in parameters estimated based on genetically reconstructed pedigrees may be improved by sampling larger numbers of individuals and more intensively spatially and temporally. In combination, findings here indicate that selection of sampling methods with high sampling efficiencies is important. Parameters estimated (N_s and N_b) via reconstructed pedigrees were sample size dependent, and each gear collected different numbers

of eggs and larvae per sampling event. Additionally, the relationship between sample size and accuracy of N_b estimates has been previously established (England et al. 2006; Ackerman et al. 2017). Ackerman et al. (2017) used COLONY to estimate N_b and suggest the desired sample size to produce accurate estimates should equal or exceed the true N_b for the sampled population. However, parameter estimates generated using genetic pedigree analysis are not just dependent on sample size, replicate sampling, and species early life history traits, but also different levels of relatedness among individuals collected with different gear types.

Sample coancestry was correlated with the rate at which unique parents were detected and generally differed between collections made using different gear types. Results may be due to the fact that eggs deposited by Lake Sturgeon are clustered by family group across a spawning site. Consequently, sample collection using egg mats may generate pedigrees with high coancestry and a relatively lower rate of detection of unique parents per larvae genotyped. The heterogeneous spatial distribution of fish larvae (Cyr et al. 1992; D'Amours et al. 2001) may also affect levels of relatedness within samples based on gear type and placement, and variance in detection probability for each gear type may vary based on early life history traits of the species (Pritt et al. 2014). For example, fewer family groups of larvae may have been collected by D-frame nets positioned close to the constructed spawning reefs. In contrast, vertically-stratified conical nets positioned further from the spawning site may have collected individuals after mixing in the water column as individuals disperse from a spawning site. Differences in measures of coancestry and rate of detection of unique parents among collections made using different gear types, supports the hypothesis that each gear type samples FS, HS, and unrelated eggs and larvae at different rates, likely due to spatial and temporal (ontogenetic) differences in degree of clustering of offspring within family groups at spawning sites.

Collection methodology influenced estimates of coancestry and detection rates of unique spawning adults, however each methodology can answer critical questions for assessment of constructed spawning habitat use. Egg mat collections allowed quantification of spawning effort following reef construction based on the enumeration of eggs deposited on the reef relative to sampling effort. Benthic D-frame nets allowed quantification of larvae that dispersed from constructed spawning reef sites, and vertically-stratified conical nets allowed insight into larval position in the water column during dispersal. When used with genetic pedigree analysis, egg mats on constructed spawning reefs provided the location at which unique parents contributed offspring, allowing estimates of N_s and N_b at a specific location and the point of origin to subsequently estimate larval dispersal (e.g., Derosier et al. 2007). However, differences in gear collection rates and between parameter estimates from methodologies presented in this study suggest further consideration of differences between parameter estimates generated using genetic pedigree analysis from different gear types to improve interpretation of results used to inform management decisions.

It is important to consider the differences among collection methodologies and inherent differences in gear sampling properties relative to sample size and the genetic makeup of the samples collected. However, this study does not offer direct insight into how to best fully account for these differences. Rather, we provide evidence for the importance of considering new methods to address differences inherent in sampling methodologies when the collected individuals are used with genetic pedigree analysis. One possibility to account for such differences may be to use samples collected with a single gear type, such as egg mats, that collected the most individuals per mat or net.

The importance of standardized field sampling and analytical techniques has long been recognized for traditional assessment and monitoring efforts (Bonnar and Hubert 2002; Hayes et

al. 2003; Maunder and Punt 2004). Bonar and Hubert (2002) called for standardization of sampling methods for assessment of inland fishes, citing benefits such as improved comparisons between sampling locations, and improved communication of results particularly for large systems where multiple agencies participate in sampling and management efforts. Likewise, Hayes et al. (2003) described the development of standardized sampling procedures for statewide monitoring and assessment to inform management of Michigan's aquatic resources, and the advantages that such an approach afford.

Increasingly, molecular methods are being combined with traditional sampling protocols to further inform management decisions (Schwartz et al. 2007). Although the emergence of this technology provides unique insights into the reproductive ecology of fishes, there also emerges a need to develop analytical methods to allow for standardization or adjustments in results obtained with sampling gear that differs in the coancestry of individuals captured. Future research may allow development of new analytical methods that account for variation in the rate of detection of unique parents per larvae genotyped between collection methodologies that will improve interpretation of parameter estimates such as N_s and N_b generated by genetic pedigree analysis.

Early life history sampling techniques combined with genetic pedigree analysis allow for quantification of adult numbers that are otherwise difficult to attain due to species ecology and effort required to sample large study systems. Sampling of eggs and larvae can be relatively non-invasive on the population level (e.g., species that are known to exhibit high natural early life mortality) and is of particular importance for threatened and endangered species such as Lake Sturgeon. In combination with genetic pedigree analysis, use of multiple gear types allows insight into a suite of critical ecological questions surrounding adult spawning numbers and recruitment. Better understanding of the effects of these collection methods on pedigree analysis

parameter estimates will improve management decisions by increasing the precision of estimated adult spawning population numbers and levels of recruitment. Results presented in this study demonstrate the importance of considering collection methodology and call for further pursuit of analytical methods that will account for variation in parameter estimates generated using genetic pedigree analysis given the observed differences in coancestry between samples obtained using varied collection methodologies.

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Figure Captions

Figure 1.—Map of the St. Clair-Detroit river system. Locations of constructed reef sites are highlighted, and net locations are indicated by triangles for D-frame nets, and circles for vertically-stratified conical nets. Egg mats were deployed on the constructed reefs.

Figure 2.—Plots of N_s and N_b by the number of larvae genotyped in the pedigree used to generate the estimate. N_s and N_b are positively correlated with sample size. Points are parameter estimates for individual reefs and all reefs combined in 2015 and 2016.

Figure 3.—Plot of the rate of detection of unique parents per additional larvae genotyped by each collection method in 2015 and 2016 for each of 1,000 bootstrapped gear and year specific pedigrees. Mean slope (solid line) represents the mean rate of detection of unique parents for each collection method each year. The cloud of lines surrounding the mean slope represents the cumulative number of unique parents detected per larvae genotyped for each of 1,000 pedigrees bootstrapped with replacement.

Table 1.—Trends in egg and larval Lake Sturgeon capture rates by year and gear type. Mean catch (± SD) is the mean for each gear type by year including egg mats or nets that collected zero eggs or larvae. Mean non-zero catch (mean ± standard deviation) is the mean for each gear type by year for only egg mats or nets that collected ≥ 1 egg or larvae. Percent zero catch is the percent of egg mats or nets that caught zero eggs or larvae by year.

	Egg mats		D-frame nets		Conical nets	
	2015	2016	2015	2016	2015	2016
Mean catch	7.02 ± 42.11	6.49 ± 37.80	3.75 ± 9.17	4.30 ± 13.38	0.42 ± 1.15	0.03 ± 0.16
Mean non-zero catch	110.74 ± 131.03	83.08 ± 111.18	6.96 ± 11.62	11.61 ± 20.05	2.62 ± 2.74	1.00 ± 0.00
Percent zero catch	93.66%	92.19%	46.15%	62.98%	83.89%	97.50%

Table 2.—Empirical results from genetic pedigree analysis for each gear in 2015 and 2016 using larvae pooled across all reefs and at each reef location. $N_{individuals}$ is the number of larvae genotyped, N_s is the estimated number of spawning adults, N_b is the effective number of breeders (bracketed N_b values are 95% confidence intervals around estimates of N_b).

		2015			2016	
Location	Parameter	Conical nets	D-frame nets	Egg mats	D-frame nets	Egg mats
All reefs	$N_{individuals}$	47	122	138	207	207
	N_s	44	71	83	122	102
	N_b	62 [40, 98]	76 [55, 109]	85 [63, 116]	115 [83, 151]	92 [68, 123]
Harts Light	$N_{individuals}$	27	21	112	95	105
	N_s	31	21	72	64	66
	N_b	50 [30, 98]	31 [18, 63]	73 [53, 105]	60 [42, 89]	50 [30, 98]
Pointe Aux Chenes	$N_{individuals}$	20	63	23	36	24
	N_s	27	43	22	36	24
	N_b	54 [30, 139]	46 [31, 72]	27 [15,55]	49 [31, 80]	32 [19, 59]
Grassy Island	$N_{individuals}$	NA	NA	NA	57	56
	N_s	NA	NA	NA	39	28
	N_b	NA	NA	NA	22 [13, 42]	43 [28, 68]

NA = data not available

Table 3.—Mean (± 1 SE) for the number of spawning adults (N_s) included in each simulation, the slope (rate of detection of unique parents per larvae genotyped), and coancestry (θ) for 1,000 pedigrees bootstrapped with replacement in 2015 and 2016 for each gear type and reef site.

		2015			2016	
Location	Parameter	Conical nets	D-frame nets	Egg mats	D-frame nets	Egg mats
All reefs	N_s	49 ± 0.115	87 ± 0.128	98 ± 0.135	149 ± 0.142	129 ± 0.142
	Slope	1.104 ± 0.003	0.716 ± 0.002	0.709 ± 0.001	0.725 ± 0.001	0.629 ± 0.001
	θ	0.008 ± 0.0002	0.019 ± 0.0002	0.016 ± 0.0001	0.010 ± 0.0000	0.015 ± 0.0001
Harts Light	N_s	28 ± 0.091	20 ± 0.080	86 ± 0.124	88 ± 0.138	89 ± 0.134
	Slope	1.164 ± 0.005	1.019 ± 0.005	0.765 ± 0.001	0.937 ± 0.002	0.852 ± 0.002
	θ	0.008 ± 0.0004	0.029 ± 0.0002	0.016 ± 0.0002	0.010 ± 0.0001	0.013 ± 0.0002
Pointe Aux Chenes	N_s	23 ± 0.089	60 ± 0.119	20 ± 0.078	41 ± 0.107	23 ± 0.090
	Slope	1.230 ± 0.006	0.966 ± 0.003	0.904 ± 0.005	1.167 ± 0.004	0.981 ± 0.005
	θ	0.002 ± 0.0003	0.014 ± 0.0002	0.056 ± 0.0016	0.006 ± 0.0002	0.036 ± 0.0011
Grassy Island	N_s	NA	NA	NA	54 ± 0.113	30 ± 0.079
	Slope	NA	NA	NA	0.955 ± 0.003	0.547 ± 0.002
	θ	NA	NA	NA	0.015 ± 0.0003	0.072 ± 0.0010

NA = data not available.

Table 4.—*P*-values for tests for significant differences in the rate of detection of unique parents per larvae genotyped between gear types across and at all reefs in 2015 and 2016.

Location	2015			2016
	Conical nets vs. D-frame nets	Conical nets vs. egg mats	D-frame nets vs. egg mats	D-frame nets vs. egg mats
All reefs	0.002*	0.001*	0.189	0.017*
Harts Light	0.462	0.034*	0.231	0.114
Pointe Aux Chenes	0.437	0.246	0.225	0.241
Grassy Island	NA	NA	NA	0.002*

* Represents significant differences in the rate of detection of unique parents between gear types (α=0.05). NA = data not available.

Table 5.—*P*-values calculated using a student's t-test comparing coancestry for 1,000 pedigrees bootstrapped with replacement between gear types across and at all reefs in 2015 and 2016.

	2015			2016
Location	Conical nets vs. D-frame nets	Conical nets vs. egg mats	D-frame nets vs. egg mats	D-frame nets vs. egg mats
All reefs	<0.001*	<0.001*	<0.001*	<0.001*
Harts Light	<0.001*	<0.001*	<0.001*	<0.001*
Pointe Aux Chenes	<0.001*	<0.001*	<0.001*	<0.001*
Grassy Island	NA	NA	NA	<0.001*

* Represents significant differences in the rate of detection of unique parents between gear types (α=0.05). NA = data not available.

Table 6.—Pearson's correlation tests resulted in significant negative correlations between coancestry (θ) and the number of spawning adults detected in the pedigree (N_s), significant negative correlations between coancestry and slope (rate of detection of unique parents per larvae genotyped), and a significant positive correlation between slope and the number of parents detected in the pedigree in 2015.

Location		Conicals θ/N_s	Conicals slope/N_s	Conicals θ/slope	D-frames θ/N_s	D-frames slope/N_s	D-frames θ/slope	Egg mats θ/N_s	Egg mats slope/N_s	Egg mats θ/slope
All reefs	t	-10.53	42.41	-7.45	-15.68	33.28	-11.20	-14.65	34.23	-9.75
	P-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	correlation	-0.32	0.80	-0.23	-0.45	0.73	-0.33	-0.42	0.74	-0.30
Harts Light	t	-8.32	43.72	-6.82	-13.10	41.50	-9.65	-13.95	35.17	-9.03
	P-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	correlation	-0.26	0.81	-0.21	-0.38	0.80	-0.29	-0.40	0.74	-0.28
Pointe Aux Chenes	t	-5.03	45.07	-4.98	-15.34	40.70	-10.76	-15.56	40.01	-10.14
	P-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	correlation	-0.16	0.82	-0.16	-0.44	0.79	-0.32	-0.44	0.79	-0.31

* Represents significant differences in the rate of detection of unique parents between gear types (α=0.05).

Table 7.—Pearson's correlation tests between coancestry (θ) and the number of spawning adults (N_s) detected in the pedigree in 2016.

Location		D-frames θ/N_s	D-frames slope/N_s	D-frames θ/slope	Egg mats θ/N_s	Egg mats slope/N_s	Egg mats θ/slope
All reefs	t	-12.24	30.17	-8.09	-12.24	30.17	-8.09
	P-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	correlation	-0.36	0.69	-0.25	-0.36	0.69	-0.25
Harts Light	t	-15.05	39.19	-12.16	-13.09	34.72	-9.09
	P-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	correlation	-0.43	0.78	-0.36	-0.38	0.74	-0.28
Pointe Aux Chenes	t	-7.14	40.08	-5.48	-16.81	43.27	-12.76
	P-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	correlation	-0.22	0.79	-0.17	-0.47	0.81	-0.38
Grassy Island	t	-14.77	36.96	-11.91	-15.02	28.71	-10.51
	P-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	correlation	-0.42	0.76	-0.35	-0.43	0.67	-0.32

* Represents significant differences in the rate of detection of unique parents between gear types (α=0.05).

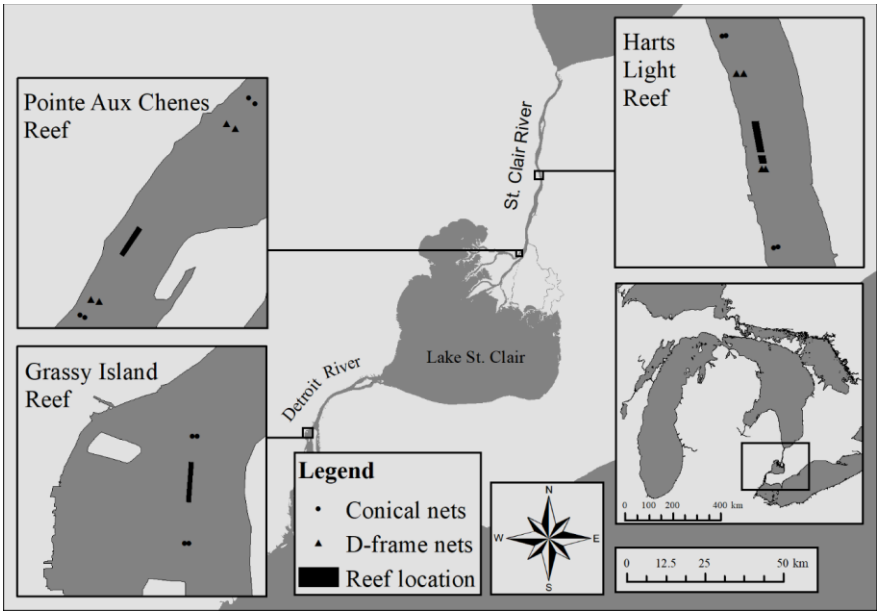
Pointe Aux Chenes Reef
Harts Light Reef
St. Clair River
Grassy Island Reef
Detroit River
Lake St. Clair
Legend
Conical nets
D-frame nets
Reef location
N
W
E
S
0 100 200 400 km
0 12.5 25 50 km

Figure 1.

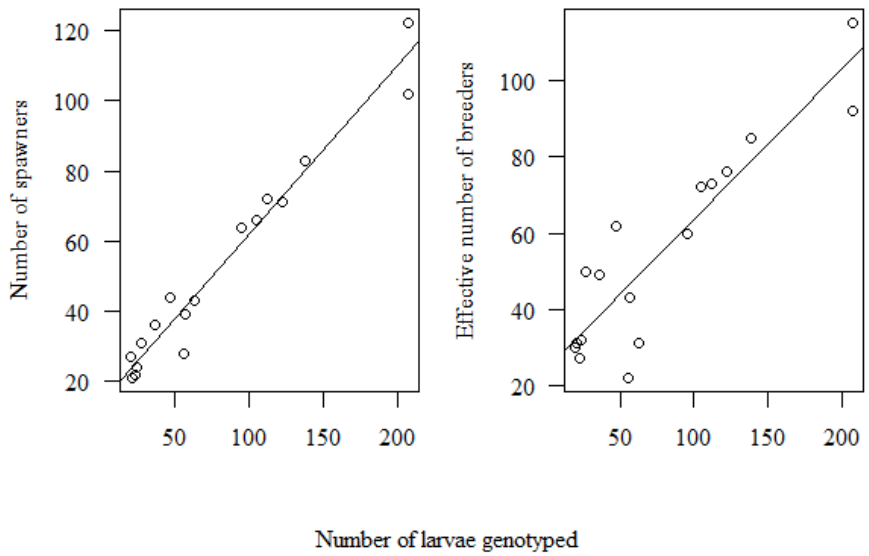
Number of spawners
120
100
80
60
40
20
50
100
150
200
Effective number of breeders
100
80
60
40
20
50
100
150
200
Number of larvae genotyped

Figure 2.

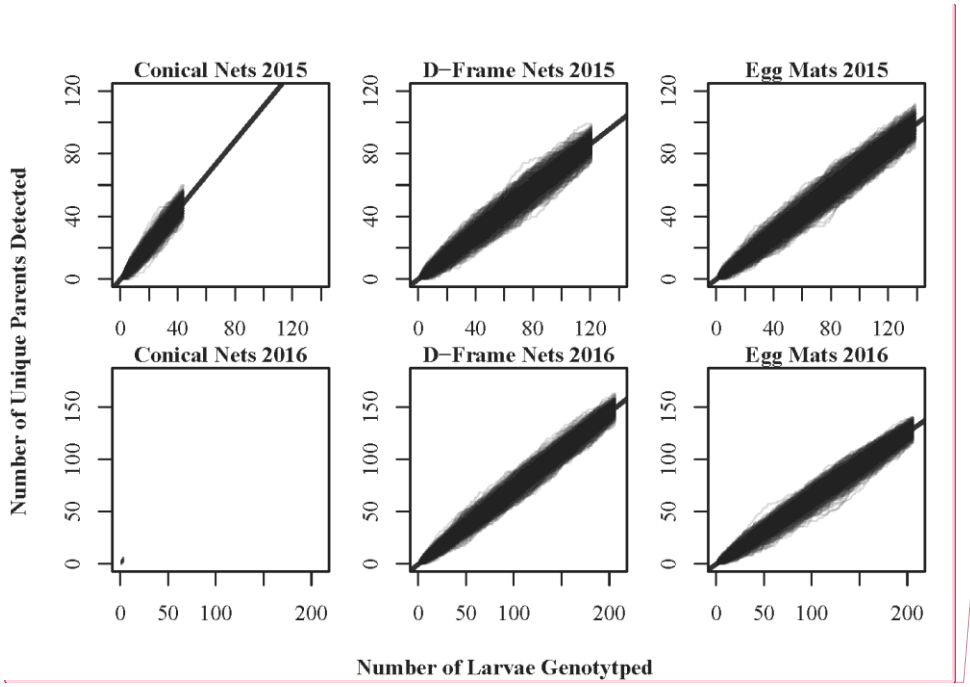

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Figure 3.