ASSESSING REPRODUCTIVE SUCCESS OF LAKE STURGEON (*ACIPENSER FULVESCENS*) ASSOCIATED WITH NATURAL AND CONSTRUCTED SPAWNING REEFS IN A LARGE RIVER SYSTEM USING PEDIGREE ANALYSIS

By

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ABSTRACT

ASSESSING REPRODUCTIVE SUCCESS OF LAKE STURGEON (*ACIPENSER FULVESCENS*) ASSOCIATED WITH NATURAL AND CONSTRUCTED SPAWNING REEFS IN A LARGE RIVER SYSTEM USING PEDIGREE ANALYSIS

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Habitat modification including barriers to migration, poor water quality, and modification of benthic habitat has contributed to the decline of lake sturgeon (*Acipenser fulvescens*) abundances in the Great Lakes. Lack of habitat was identified as a limiting factor for lake sturgeon recovery in the St. Clair-Detroit River System (SCDRS). To increase habitat availability in the SCDRS with the intent of increasing lake sturgeon populations, seven spawning reefs were constructed. Using 741 eggs and larvae collected during traditional assessments, genetic pedigree analysis was used to further quantify spawning habitat use. In 2015 and 2016, 339-349 spawners were estimated to have contributed offspring across all sites. The effective number of breeders was estimated at 295-314 spawners, with mean (4.26-4.37 larvae) and variance (6.26-7.20) in individual reproductive success across all reefs and in 2015 and 2016. Evidence of adults spawning at multiple reefs within and between rivers was revealed by shared sib-ship of offspring collected at multiple locations. Comparison between gear types revealed that differences in the way individuals are collected can affect estimates generated from genetic pedigree analysis. Finally, species richness estimators were combined with genetic pedigree analysis to estimate the total number of spawners contributing offspring at constructed reefs (11-92 spawners per reef per year). Detailed information regarding lake sturgeon spawning behavior associated with spawning habitat construction in the SCDRS informs future assessment and management action for conservation of lake sturgeon throughout their range.

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THESIS INTRODUCTION

Conservation of threatened and endangered species receives a great deal of attention and resources. Critical post-project assessment of conservation efforts is often difficult because of species' ecology and habitats occupied. Assessment of spawning success for lake sturgeon (*Acipencer fulvescens*) is particularly difficult given the species is long lived, spawns intermittently, is iteroparous, and is suspected to spawn over a range of disparate sites. Traditional abundance assessment methods such as mark-recapture (Thomas and Haas 2002; Lallaman et al. 2008; Pledger et al. 2013) and catch-per-unit-effort can provide estimates of the number of adults (N) or relative abundance of offspring produced (Caroffino et al. 2011). However, in large river systems, low recapture rates and small sample sizes result in considerable uncertainty in parameter estimation (Wirgin et al. 1997; Thomas and Haas 2002). Additionally, traditional methods are unable to provide accurate estimates of the number of individuals contributing offspring at spawning sites or between years. Genetic Pedigree analysis provides an alternative method for estimation of the number of spawning adults and relatedness of offspring (Pemberton 2008; Jay et al. 2014; Duong et al. 2013). Genetic data can provide critical insight into the spatial structure and temporal spawning distribution of lake sturgeon based on spatial distributions of larvae of the same or different parentage and levels of relatedness among offspring. Additionally, population genetic structure of adults between spawning locations and over time can be inferred.

We are interested in understanding lake sturgeon reproductive success associated with the use of natural and constructed spawning sites in the St. Clair-Detroit River System. Lake sturgeon is an important cultural, ecological and economic species (Hay-Chmielewski and

Whelan 1997; Roseman et al. 2011). Once abundant in areas surrounding the Laurentian Great Lakes and Mississippi River basin, lake sturgeon populations have declined to about one percent of their historic abundance (Auer 1996; Hay-Chimelewski and Whelan 1997). Throughout the majority of their native range, lake sturgeon are listed as threatened or endangered, and have been extirpated from many areas (Hay-Chimelewski and Whelen 1997).

Currently, individual spawning behavior for lake sturgeon specific to the SCDRS has not been well described. In the Great Lakes Basin, lake sturgeon are long lived, intermittent spawners that return with high probability to natal streams (Burch and Binkowski 2002; DeHaan et al. 2006; Forsythe et al. 2011). Male lake sturgeon mature at 12-22 years (Burch and Binkowski 2002; Hay-Chimelewski and Whelen 1997) and spawn every 2.3 ± 0.08 years in the Black River, though some males have been found to spawn in consecutive years (Forsythe et al. 2012). Females mature at a range of 14-33 years (Burch and Binkowski 2002; Hay-Chimelewski and Whelen 1997), and typically spawn at 3.7 ± 0.16 year intervals in the Black River, (Forsythe et al. 2012). A high degree of variability in spawning periodicity has been found with males spawning every 1-7 years and females 2-7 years in the Black River (Forsythe et al. 2012). Females participate in repeated spawning events with 2 to 8 males over a period of 2 to 4 days at a given spawning site in the Winnebago system (Bruch and Binkowski 2002). Spawning sites are selected for a set of physical parameters such as 30-50mm diameter rock substrate providing at least 20mm of interstitial space, minimum water velocities, and temperature (Auer 1996; Dumont et al. 2011; Roseman et al. 2011). Mature adults migrate to spawning sites when water temperatures are 8.8-19.1˚C and peak spawn occurs when temperatures are 11.5-16.0˚C (Bruch and Binkowski 2002).

Lake sturgeon are highly fecund with females producing 49,000-667,000 eggs in a spawning year (Peterson et al. 2007) though this varies with female size. Eggs are deposited on rocky substrate. Duong et al. (2011a) showed that time from fertilization to dispersal varies due to maternal effects, spawning date, and temperature. In the Black River, time to dispersal ranged from 4-36 days and was inversely related to temperature (Duong et al. 2011a). Post-hatch larvae remain in substrate interstitial spaces until their yolk sac is absorbed (Duong et al. 2011b; Auer and Baker, 2002). Once the yolk sac is absorbed, larvae enter the water column and drift nocturnally (Auer and Baker, 2002). Despite high fecundity and low adult natural mortality, lake sturgeon experience low rates of recruitment at early life history stages. Populations are particularly affected by anthropogenically-induced adult mortality and habitat modification (Hay-Chmielewski and Whelan 1997).

Beginning in the early 1900s large-scale habitat modifications such as the dredging of shipping canals, construction of dams, and hardening of riparian habitat were common throughout the Great Lakes region (Bennion and Manny 2011). From 1874 to 1966, 96.5 kilometers of commercial shipping channels were dredged, and disposal of dredge spoils covered 4,050 hectares of river bottom destroying historic lithophilic spawning habitat in the SCDRS (Bennion and Manny 2011). Additionally, lake sturgeon throughout the Great Lakes were over harvested as a result of commercial bycatch, intentional harvest (mainly for caviar), and recreational fishing (Peterson et al. 2007). Despite restrictions on recreational harvest and bans on commercial fishing, the lack of suitable spawning habitat is recognized as a significant barrier to lake sturgeon recovery in the SCDRS (Roseman et al. 2011).

The 1987 Great Lakes Water Quality Agreement recognized a number of impairments in the SCDRS, including the loss of fish and wildlife habitat resulting from anthropogenic

modifications. This led to the SCDRS being recognized as an area of concern by the United States and Canada. Large scale collaborative efforts to address the loss of fish and wildlife habitat have been ongoing throughout the system. These efforts include removal of contaminated sediments, shoreline restoration, and construction of spawning reefs. Between 2004 and 2016 seven spawning reefs were constructed throughout the SCDRS ranging in size from 0.2 acres to 4.0 acres (Manny et al. 2015; Briggs et al. 2016). Reefs constructed of mixed rock, coal cinders, and or limestone, were designed to create additional spawning habitat, and specifically targeted threatened and endangered lithophilic spawners such as lake sturgeon (Manny et al. 2010; Roseman et al. 2011). Critical assessment of restoration efforts can ensure effective management of threatened and endangered species such as lake sturgeon by evaluating if project goals are met and providing information to guide future management decisions.

Accurate assessment of the number of adults contributing offspring, effective number of breeders, individual patterns and variation in reproductive behavior and success, and larval dispersal in large non-wadable rivers is difficult. Population assessment of adult lake sturgeon in the SCDRS has traditionally been performed using setlines, trawling, and mark-recapture of adult and juvenile fish (e.g., Thomas and Haas 2002). Catch per unit effort and abundance data using lake sturgeon eggs and larvae have also been used to assess use of artificial reefs in the SCDRS (Bouckaert 2014). However, these methods are limited by a number of factors. The SCDRS is an open system where emigration and immigration are possible, tags may be lost due to harvest or physical loss, and low recapture rates may impede robust estimates of the number of adult sturgeon in the system (Thomas and Haas 2002). Additionally, delayed maturity and low early-life recruitment levels limit the potential for immediate observation of restoration efforts. Direct estimates of spawning success based on relative abundance of dispersing larvae is

difficult in large rivers. For highly fecund fish such as lake sturgeon, larval abundance provides neither an estimate of spawning effort in a season nor a reliable quantitative estimate of the number of adults contributing offspring (Chiotti et al. 2008; Jay et al. 2014).

Rehabilitation goals for lake sturgeon have been established and it has been suggested that an empirical estimate of the effective population size (N_e) is called for. N_e varies as a function of unequal sex ratios, variance in individual reproductive success, and variance in the population size over time (Waples 1990). The effective population size is the standard measure of the loss of gene diversity in a population, change in allele frequency, and occurrence of inbreeding (Allendorf et al. 2013). N_e is influenced by a number of factors including population size over time, sex ratios, and lifetime reproductive success (Frankham 1995; Charlesworth 2009; Waples 2010). Waples (1990) showed that there are difficulties in predicting the decline in heterozygosity for iteroparous species such as lake sturgeon due to overlapping year classes and fragmentation into spatially or temporally discrete spawning groups. N_s is the total number of adult spawners contributing offspring.

N_b is the effective breeding number and is a measure of N_e within a single spawning season. Since N_e is difficult to determine for long lived iteroparous species (Hill 1972; Waples 2010), N_b is a more clearly obtained measure of the effective number of breeders contributing offspring at natural and artificial spawning sites in a single spawning season. Estimates of N_b are important for assessment of constructed spawning habitat as variation in the number of adults contributing offspring has direct effects on the genetic diversity of the population and is linked to lake sturgeon spawning success. Genetic pedigree analysis allows for accurate estimates of the number of adults contributing offspring (N_s), the number of breeders (N_b) and variation in

reproductive success annually and between locations (Pemberton 2008; Duong et al. 2013, Jay et al. 2014).

Estimates from genetic pedigree analysis can be affected by differences in the spatial and temporal manner different gear types collect individuals for genotyping. Crossman et al. (2011) found that levels of coancestry varied between collection of dispersing larvae and naturally produced eggs. It is possible for the gear type used to collect lake sturgeon egg and larval samples to effect estimates from genetic pedigree analysis. Comparing estimates from genetic pedigree analysis between sample collection methods can provide insight into potential gear biases that may impact estimates of N_s and N_b using pedigree analysis.

Finally, combining community ecological theory and pedigree analysis provides a novel method for estimating the total number of spawners contributing offspring at a location based on rarefaction techniques. Estimates of the number of spawners contributing offspring using genetic pedigree analysis are sample size dependent and managers and stakeholders are interested in knowing the total number of adults contributing offspring at a location. Species accumulation techniques have long been used to provide reliable estimates of the total number of species at a sample location, based on rates of detection of unique individuals (Walther and Moore 2005; Gotelli and Colwell 2011). The basis of the theory is that when sampling begins species in high abundance will be detected at a rapid rate. However, as sampling continues only rare species remain and rates of detection of new species will asymptote (Ugland et al. 2003). This asymptote represents the most likely total number of species at a sampling location. Adapting this technique to pedigree analysis by substituting parents for species and larvae genotyped for sampling events allows estimation of the total number of spawners contributing offspring at a sampling location.

The objectives of this study are to: (1.) describe use of constructed spawning habitat by lake sturgeon in the SCDRS by estimating N_s, N_b, N_b/N_s ratios, and mean and variance in reproductive success; (2.) describe spatial and temporal patterns in individual spawning behavior of lake sturgeon in the SCDRS; (3.) describe patterns of post-hatch larval dispersal associated with constructed spawning habitat; (4.) quantify the effects of sample collection method on estimates of N_s, N_b, and N_b/N_s ratios from genetic pedigree analysis; and (5.) use the combination of community ecological theory and genetic pedigree analysis to estimate the total number of spawners contributing offspring.

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CHAPTER 1: USING PEDIGREE ANALYSIS TO DISCRIBE THE USE OF CONSTRUCTED SPAWNING REEFS BY LAKE STURGEON (*ACIPENSER FULVESCENS*) IN THE ST. CLAIR-DETROIT RIVER SYSTEM

ABSTRACT

Habitat modifications have widely removed spawning habitat required by lithophilic spawners like lake sturgeon (*Acipenser fulvescens*) including in the St. Clair-Detroit River system (SCDRS). To address loss of habitat and to facilitate increases in lake sturgeon abundance, 7 artificial spawning reefs were introduced to the SCDRS since 2004. Assessment through collection and enumeration of eggs and larvae provided evidence of spawning by adult lake sturgeon and survival of eggs to larval drift at constructed reef sites. However, the number of spawners contributing offspring, spawning location and spawning periodicity, and the extent of larval dispersal during drift remained undescribed in the SCDRS. Pedigree analysis was used to assign larvae (N=741) collected in 2015 and 2016 from three artificial reefs to full- and half-sibling groups and estimate the number of breeding adults. The number of spawners contributing offspring (N_s) and effective number of breeders at a reef or in a spawning season (N_b) were estimated. Across all reefs, estimates of N_s ranged from 156-227 spawners in 2015 and 2016, respectively. Overall estimates of N_b ranged from 145 in 2015 to 199 in 2016. Mean and variance in individual reproductive success averaged across all locations in 2015 and 2016 was 3.68 offspring per spawner and 4.86, respectively. Evidence of full- and half-sibling larvae collected at multiple reefs reveal lake sturgeon spawning at multiple locations within and between years. Detection of larvae in downstream D-frame nets suggest larvae may be dispersing great distances through the SCDRS. Incorporating genetic data from traditional sampling methods informs management of threatened populations and directs future habitat remediation efforts in large river systems.

INTRODUCTION

Conservation efforts for threatened and endangered species are often hindered by species ecology, habitats occupied, or the rarity of the species in the study area. One example of this is lake sturgeon (*Acipencer fulvescens*), which was once abundant in the Great Lakes region and Mississippi River basin (Thomas and Haas 2002). However, overharvest and habitat modification have resulted the decline of lake sturgeon populations (Auer 1996; Roseman et al. 2011a). Currently, lake sturgeon populations are at less than 1% of historic abundance (Auer 1996; Hay-Chimelewski and Whelan 1997) or have been extirpated.

The St. Clair-Detroit River System (SCDRS) is believed to contain a large remnant populations of lake sturgeon within the Great Lakes (Thomas and Haas, 2002). The SCDRS lake sturgeon population is as a potential source for stocking efforts within its current genetic stocking unit which extends from the western basin of Lake Erie around the Michigan shoreline to Southern Lake Michigan (Welsh et al. 2010). As such, remediation efforts aimed at expanding current lake sturgeon population numbers in the SCDRS are of critical importance for population maintenance in this and other systems throughout GSU-1.

Assessment work throughout the SCDRS has identified lack of spawning habitat as a limiting factor for conservation of lithophilic spawning fishes including lake sturgeon (Hondorp et al. 2014; Manny et al. 2015). In response to this conservation need, 7 artificial reefs have been constructed in the SCDRS since 2004 to restore lost spawning habitat for lithophilic spawners (Manny et al. 2015). Following reef construction, immediate assessment of the reefs was performed to quantify use by spawning lake sturgeon. Lake sturgeon eggs were detected on the reefs following construction, where few or no eggs were detected prior to construction,

indicating that lake sturgeon immediately located and utilized the artificial reefs for spawning (Roseman et al. 2011a; Manny et al. 2015; Prichard et al. 2017; Fischer et al. 2018). Additional assessment during the larval drift period demonstrated that lake sturgeon eggs deposited on the reefs were successfully recruited to the larval drift stage (Roseman et al. 2011a; Bouckaert 2014).

Successful spawning demonstrated by recruitment to the larval stage is one measure of success for the constructed spawning reefs (McLean et al. 2014). However, if spawners are spatially distributed over a number of smaller spawning sites there is potential for depensatory effects due to a reduction in individual reproductive success possibly due to density dependent reductions in fertilization rates which have been observed in other broadcast spawners (Levitan et al. 1995, Levitan 2004). Additionally, concern exists regarding the fate of larvae following hatch and drift. Little is known about the dispersal of lake sturgeon larvae in this large river system or habitat required for recruitment to the juvenile stage (Boase et al. 2014). Questions remain regarding (1) how many spawners contribute offspring at a reef, (2) if adults spawn in more than one location in a single year, (3) and how larvae disperse through the system.

Genetic techniques can provide answers to critical management questions. Through the use of pedigree analysis, the number of spawners (N_s), effective number of breeders (N_b), and mean and variance in individual reproductive success of adults contributing offspring at a location, in a year, and across years by genotyping larvae and inferring the likely number of parents contributing those offspring was estimated. Estimates of N_s, N_b, and mean and variance in reproductive success further quantify use of constructed reefs by spawning lake sturgeon and are critical for post-construction assessment. N_s is an estimate of the total number of spawners detected in a sample of progeny (eggs or larvae) and provide an index for comparison between

reefs and over time assuming equal sampling and catchability between sites and years. N_b is the effective number of breeders defined as the size of a breeding population within a single spawning season in which the rate of change in allelic diversity is equal to the rate of change by genetic drift alone (Allendorf et al. 2013). N_b is typically lower than N_s due to skewed sex ratios and variance in individual reproductive success (Waples 1990, Allendorf et al. 2013). N_b/N_s ratios provide insight into the risk of population level losses of genetic diversity. When N_b/N_s ratios are low risk increases due to a large proportion of offspring being contributed by a relatively small number of spawners. Quantifying use of artificial reefs by spawning lake sturgeon and quantifying variation in reproductive success is critical to inform management and help guide future remediation efforts.

The objectives of this study were to (1) estimate the number of adults contributing offspring at artificial reefs (N_s), the effective number of breeders (N_b), and mean and variance in individual reproductive success, (2) determine if adults are spawning at multiple locations within a spawning season, (3) and describe patterns in larval dispersal from artificial reef sites. Estimating N_s, N_b, and N_b/N_s ratios reveals the number of spawners contributing offspring at artificial reefs and insight into potential population level genetic consequences for lake sturgeon in the SCDRS. Additionally, adult spawning frequency and location indicates levels of potential gene flow between reef sites and across the riverscape. High levels of interconnectivity between locations may suggest that multiple spawning sites are capable of functioning as a single large spawning habitat incorporating potential benefits associated with a spawning portfolio effect (Schindler et al. 2010, Dufour et al. 2015). Additionally, it is important to know how far larvae disperse, at what rate they are retained in the river, and what possible habitat features are required to ensure that larvae recruited to drift reach suitable nursery habitat to ensure the highest

possible survival. In the SCDRS, it is possible that larvae may disperse great distances from their spawning location. Inferred sib-ship (full- and half-sibling relationships) of larvae dispersing from artificial reefs and captured in downstream D-frame and vertically stratified conical nets can describe larval dispersal through the system. Larvae collected at a downstream location that are full- or half-sibs with larvae collected at an upstream location suggest that the possible extent of larval dispersal is at least as large as the distance between full- and half-sib detections assuming additional spawning is not occurring between sampling sites. Describing patterns of larval dispersal allows identification of habitat use and requirements for early life stages that may inform future conservation decisions.

METHODS

Study Area

The St. Clair-Detroit River System is a 145-kilometer waterway flowing from Lake Huron into Lake Erie that consists of the St. Clair River, Lake St. Clair, and the Detroit River (Figure 1). The system has been heavily modified by dredging of shipping channels, draining of wetlands, and hardening of riparian areas to accommodate anthropogenic uses. As a result, the SCDRS was declared an area of concern (AOC) due in part to the loss of fish and wildlife habitat. Despite impairments, the SCDRS is believed to house a large remnant populations of lake sturgeon (Thomas and Haas 2002). Once thought to contain numerous lake sturgeon spawning sites, the construction of shipping channels in the SCDRS has resulted in the removal of 46.2 million cubic meters of substrate and disposal of those dredge spoils has resulted in the burial of approximately 4000 hectares of remaining benthic habitat (Bennion and Manny 2011). Subsequently, the number of known spawning sites was reduced from 15 to only 3 (Goodyear et al. 1982; Manny and Kennedy 2002; Nichols et al. 2003). Extensive efforts were undertaken to detect areas where natural spawning occurred for lake sturgeon in the SCDRS resulting in lack of spawning habitat being identified as a factor limiting lake sturgeon spawning success (Manny et al. 2015). The largest naturally occurring spawning location is thought to be at the head of the St. Clair River under the Blue Water Bridge, Port Huron, MI (Manny and Kennedy 2002) with additional spawning sites detected in the North Channel of the St. Clair River (Thomas and Haas 1999; Manny and Kennedy 2002) and near Zug Island in the Detroit River (Manny and Kennedy 2002; Caswell et al. 2004).

To increase suitable spawning habitat for lithophilic spawners such as lake sturgeon, 7 artificial reefs were constructed throughout the SCDRS (Fisher et al. 2018). To determine the extent of use of artificial reefs by spawning lake sturgeon 3 reefs including, Harts Light Reef (2015-2016), Pointe Aux Chenes Reef (2015-2016), and Grassy Island Reef (2016) were assessed. Harts Light Reef is the northern most artificial reef site in the St. Clair River (Figure 2). Constructed in 2014 from 8-15 cm diameter fractured limestone, this 3.8-acre reef had depths exceeding 16m and water velocities at the time of drift were up to 1.4 m^3/s. Downstream from Harts Light Reef was Pointe Aux Chenes Reef (Figure 3). Also constructed in 2014 from 8-15 cm diameter crushed limestone, this 1.5-acre reef was up to 15m deep with water velocities at the time of drift up to 1.2 m^3/s. Further downstream from Pointe Aux Chenes in the St. Clair River were the North and Middle Channel control sites which were sampled in 2015 and 2016 to assess spawning and larval drift in alternate locations. In 2015, Grassy Island Reef was constructed in the Detroit River (Figure 4). Grassy Island is 4.0-acres, constructed of 8-15 cm diameter crushed limestone, was up to12m deep, with water velocities at the time of drift up to 0.8 m^3/s.

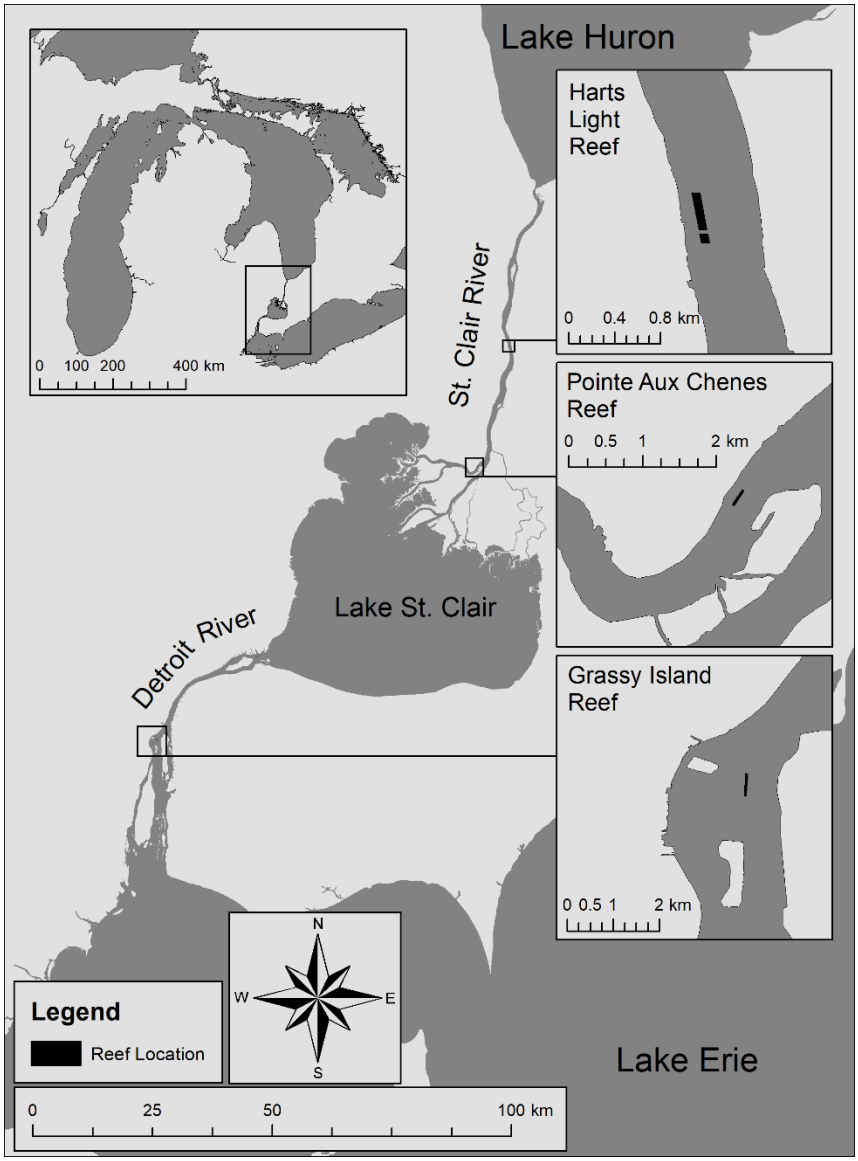

Figure 1. Overview of the St. Clair-Detroit River system. Study site location is shown in the upper left inset. Locations of each reef are highlighted and pullouts on the right show reef locations.

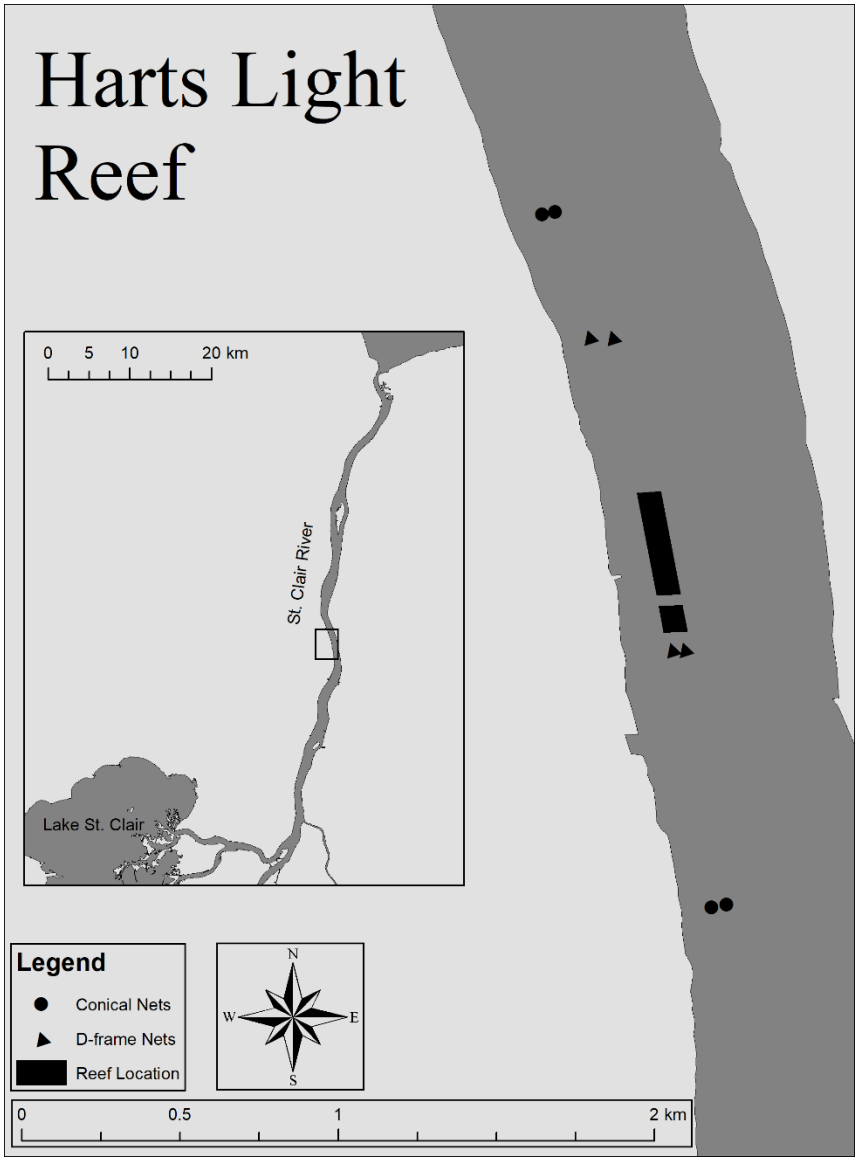

Figure 2. Overview of Harts Light Reef. The site location in the St. Clair River is shown in the inset map on the left. Reef location is represented by black rectangles. Conical net sampling locations are represented by black circles and D-frame net sampling locations are represented by black triangles.

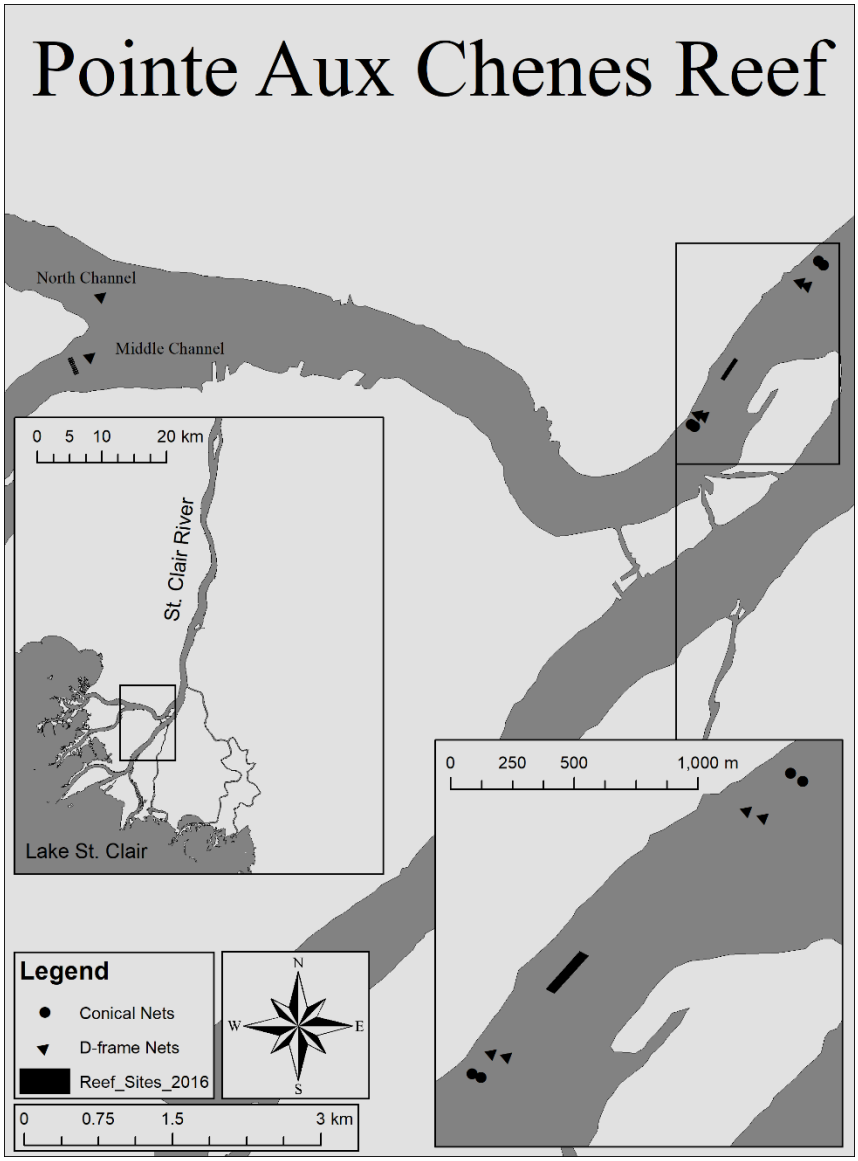

Figure 3. Overview of Pointe Aux Chenes Reef in the St. Clair River. Reef location is shown in the inset map on the left. The reef location is represented by a rectangle. Conical net sampling locations are represented by circles, and D-frame net sampling locations are represented by triangles. D-frame sites in the North and Middle channels were located upstream of known spawning sites to examine patterns of larval dispersal from Harts Light Reef and Pointe Aux Chenes Reef. Egg mats were sampled on spawning sites in the North and Middle Channels to examine alternate spawning locations.

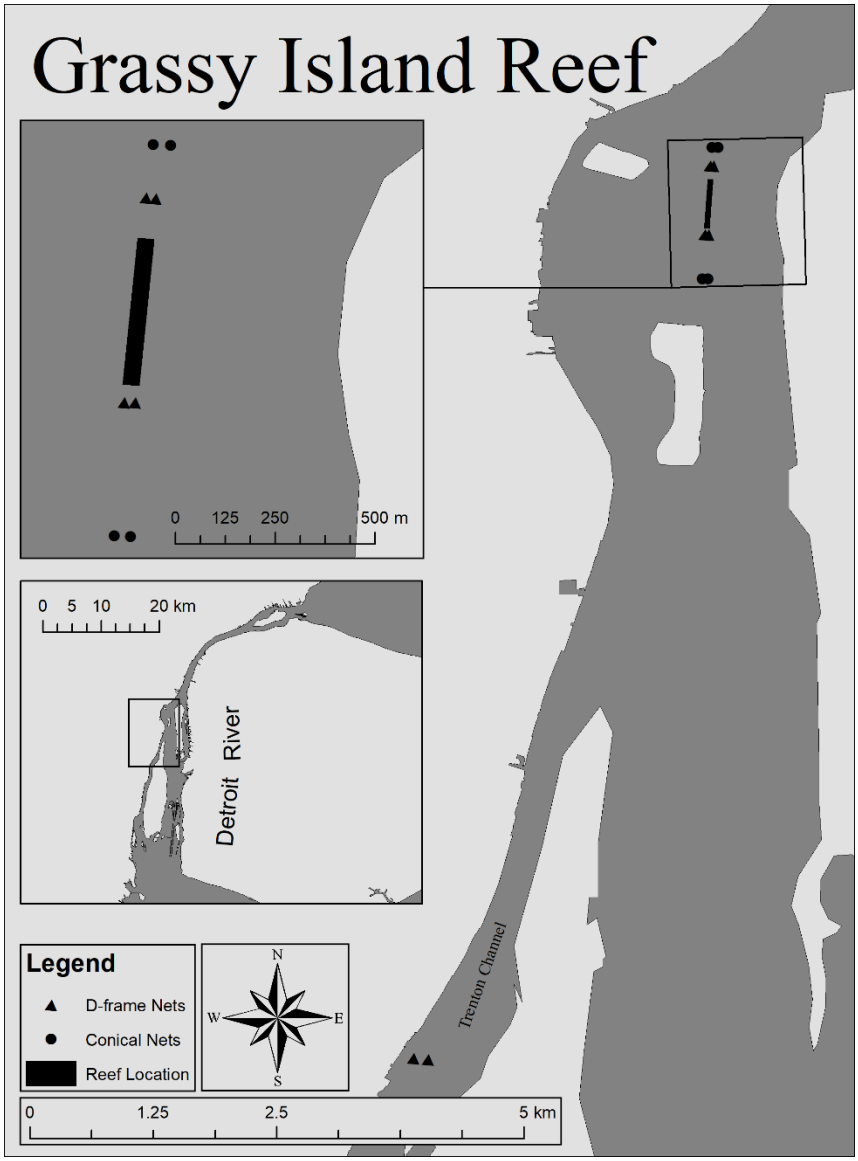

Figure 4. Overview of Grassy Island Reef in the Detroit River. Reef location is shown in the inset map on the bottom-left. The reef location is represented by a rectangle. Conical net sampling locations are represented by circles, and D-frame net sampling locations are represented by triangles. D-frame nets were deployed in the Trenton Channel to examine patterns of larval dispersal in the Detroit River. Egg mats and vertically stratified conical nets were not deployed in the Trenton Channel.

Sample Collection

All samples were collected by U.S. Geological Survey and U.S. Fish and Wildlife Service in 2015 and 2016 in the St. Clair River and in 2016 in the Detroit River. All sampling and handling of fish during research were carried out according to guidelines for the care and use of fishes by the American Fisheries Society (http://fisheries.org/docs/wp/Guidelines-for-UseofFishes.pdf).

Egg mats were used to collect lake sturgeon eggs on the artificial reefs. Egg mats were 38-24-0.5-cm metal frames wrapped in natural-fiber furnace filter material secured with 5-2.5-cm binder clips and were deployed in a set of 3 mats separated by approximately 1 meter of line (Manny et al. 2010, Roseman et al. 2011b). Egg mats were deployed and retrieved weekly, checked for eggs, and immediately redeployed. Lake sturgeon eggs were collected from 28 May through 9 June 2015 in the St. Clair River and 11 May through 31 May 2016 in the St. Clair and Detroit Rivers. Prior to sampling for larval lake sturgeon drift, egg mat gangs were removed from the reef area to safely deploy D-frame and vertically stratified conical nets. As a result, egg mats were likely not sampled on the reefs for the full duration of the spawning period. Eggs were reared by U.S. Geological Survey personnel (Sutherland et al. 2014) until yolk sacs were absorbed and larvae were preserved in 95% ethanol for genetic analysis.

Larvae were collected from the three constructed spawning reefs and three alternate spawning sites located downstream from artificial reefs in the St. Clair and Detroit rivers during post hatch nocturnal drift. Alternate spawning sites were located in the North (Maslinka Reef - historic spawning site) and Middle Channels (Middle Channel Reef - constructed spawning site) of the St. Clair River, and the Trenton Channel (no known spawning) of the Detroit River. Two 76-cm base by 54-cm high, 1600-µm mesh benthic D-frame nets were set above and below each reef site to compare catch rates of larvae drifting off the reef with that of larvae drifting from

upstream (Auer and Baker 2002, Roseman et al. 2011b). Larvae collected above the reef were assumed to be dispersing from an upstream spawning location, while larvae collected below a reef were likely dispersing from a combination of reef and upstream locations allowing for the determination of the relative contribution of larvae drifting from a reef to the overall abundance of larvae sampled. One additional D-frame net was set at each of two alternate drift sites in each river to detect larvae dispersing in areas separated hydrologically or by distance from artificial reefs. Alternate sites were located in the North Channel and Middle Channel of the St. Clair River in 2015 and 2016, and in the Trenton Channel of the Detroit River in 2016 (Figures 3 & 4). There is a historic coal cinder reef in the North Channel and another artificial reef in the Middle Channel, but D-frames were sampled upstream of these reefs with the intention of collecting larvae drifting from upstream locations such as Harts Light Reef and Pointe Aux Chenes Reef. The alternate drift sites in the Detroit River in 2016 were not in the area of any known spawning locations constructed or natural, but were instead placed in an area believed to be largely separated hydrologically from the recently constructed Grassy Island Reef. In 2015 and 2016, nets were deployed at approximately 2200hrs and retrieved after 0400hrs to sample the peak drift period in the SCDRS determined by Bouckaert et al. (2014). D-frame sampling began at each reef following egg collection, allowing for a brief incubation period (5-13 days after egg detection), and ceased when larvae were no longer detected in samples. Harts Light Reef and Pointe Aux Chenes Reef were sampled twice per week on alternate nights from 2 June through 22 July 2015 and 8 June through 7 July 2016 when weather conditions permitted. North Channel and Middle Channel alternate drift sites were sampled concordant with Pointe Aux Chenes Reef sampling events in 2015 and 2016. Grassy Island Reef and Trenton Channel sites were sampled

from 24 May through 13 June 2016. All net contents were euthanized and preserved in 95% ethanol in the field for processing at the lab.

To determine the vertical distribution of larvae throughout the water column, vertically stratified conical nets were used to collect dispersing lake sturgeon larvae at Harts Light and Pointe Aux Chenes Reef in the St. Clair River in 2015 and Grassy Island Reef in the Detroit River in 2016. Two sets of conical nets were deployed upstream and downstream of the reefs. Each net set consisted of three 0.15-meter diameter 750-µm mesh nets fished 1 meter below the surface, mid water column, and 1 meter off the bottom. Nets were deployed and retrieved in concert with D-frame sampling at each site. All contents of the nets were preserved in 95% ethanol in the field for processing at the lab.

All lake sturgeon larvae were identified and removed from preserved D-frame and conical net samples at the lab by U.S. Geological Survey personnel. Each larvae was photographed and measured for total length using digital imaging analysis software (Image Pro Plus 7.0, Media Cybernetics, Inc., Rockville, MD). Tissue samples were obtained from individual larvae by removing caudal tissue posterior to the vent. Tissue was preserved in 95% ethanol for DNA extraction. Samples were cataloged in a database containing a unique larvae identification number, collection information, and measurement data.

Genetic Analyses

DNA extraction followed manufacturer's protocol using the QIAGEN DNeasy® kits (QIAGEN Inc.). A NanoDrop ND-100 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) was used to quantify DNA. Prior to amplification using polymerase chain reaction (PCR), samples were diluted to 20ng/µl with DNA suspended in sterile water.

To ensure sufficient power for pedigree analysis, DNA was amplified across 18 microsatellite loci, 13 disomic and 5 polysomic. The power to assign sib-ship and subsequently infer the number of parents likely contributing offspring is positively correlated with the number of loci analyzed and the allelic variation among those loci (Ryman et al. 2006; Wang and Scribner 2014) and the use of additional loci has the potential to provide marked increases in the power of assignment. Genotyping was performed by combining 13 standardized disomic markers including: 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), with 5 polysomic markers including: Atr100, Atr114, Atr117, AciG35, and AciG110 (Rodzen and May (2002) that have been adapted from previous use with white sturgeon (*Acipenser transmontanus*) (Jay et al. 2014) to provide sufficient power for the accurate assignment offspring to full- and half-sib groups and allow for the accurate estimation of the number of parents contributing offspring to a sample using pedigree analysis.

PCR was conducted in 25µl reactions with 5 µl of 20 ng/µl DNA suspended in sterile water. Reactions were performed using 10x PCR Buffer (1M Tris-HCl, 1M MgCl2, 1M KCl, 10% gelatin, 10% NP-40, 10% Triton-X), $MgCl_2$ as called for based on optimizations (Scribner et al. in review), 2mM of each dNTP, 10 pmol forward and reverse primer and 0.5 µl Taq polymerase. PCR products were then multiplexed and diluted to standardized concentrations optimal for analysis at the MSU Research Technology Support Facility on an ABI 3730xl DNA analyzer. Allele sizes were determined through the use of size standards obtained from (MapMarkerTM, and BioVentures Inc.) as well as the incorporation of 3 samples of lake sturgeon DNA with known genotypes with the analysis of each loci. Results were visualized

using GeneMapper (Softgenetics, State College, PA) and all genotypes were determined through scoring performed by 2 experienced lab personnel to ensure accuracy. Loci that were not identically scored were reanalyzed or were eliminated from further analysis. Approximately 10% of individuals were genotyped blindly for all loci as a further means of quality control.

Scores for alleles were treated as pseudo-disomic loci in the method of Rodzen et al. (2004) and Wang and Scribner (2014). Using this method, Wang and Scribner (2014) found that 10 loci had sufficient power to infer 90% of dyadic relationships. A total of 205 possible alleles were analyzed across 18 microsatellite loci in 2015 and 2016 for lake sturgeon in the St. Clair and Detroit Rivers, resulting in the creation of a vector with a length of 205 for each of 741 individuals. Resulting vectors were filled using a presence or absence score of 1 and 2 respectively, with the addition of 0 representing missing data for the case where an individual failed to amplify at a given locus despite two separate amplification attempts. Individuals that failed to amplify at >9 loci were automatically excluded from further analysis. Vectors for individual genotypes were verified by first generating the series of vectors for each locus for each individual through the use of an automated function created in R (R Core Team 2017) and then hand coding the same vectors for each locus for a subset of those same individuals to ensure genotypes were generated accurately.

Genotypes were analyzed using Program COLONY (Wang 2004) to assign full- and half-sibling groups and infer the most likely number of parents contributing offspring in our samples. Analysis with Program COLONY was performed in two replicate runs with different random number seeds (Jay et al. 2014). Program COLONY uses a full-maximum likelihood approach to assign sib-ship and infer parentage meaning that it considers a group of likelihoods instead of multiple pairwise comparisons thus allowing for inferences to be made beyond the full-sib level

(Wang 2004). This approach is sensitive to genotyping errors, and mutation rates. Mutation rates have been shown to be as high as $1.4x10^{2}$ for microsatellite loci (Talbot et al. 1995; Wang 2004). Measures were taken to minimize the possibility of errors due to amplification, scoring, typing, and data entry. However, Program COLONY does account for some level of error described as Class 1: allelic dropout, and Class 2: mutation, genotyping, contamination, and data entry (Wang 2004). Error rates were determined by comparing genotypes from the original data set to individuals re-amplified in a 10% error check. Mean allelic error rates were calculated at 0.5% and 1.8% in 2015 and 2016 respectively by dividing the number of allelic differences between the original genotypes and the 10% error check genotypes by the total number of alleles. Final analysis assumed a slightly higher error rate than observed; 2% for allelic dropout and 0.1% for all other error.

Power Analysis

To demonstrate the power to assign larvae to full- and half-sib groups and to infer the number of parents contributing the offspring in our samples we simulated pedigrees consisting of a known number of male and female parents and offspring with known genotypes. The simulated ratios of males to females represent random selections from a uniform distribution of sex ratios ranging from 3.15 observed in 16 years of data on a spawning lake sturgeon population from the Black River, Michigan (Scribner, unpublished data). A matrix was constructed consisting of unique males in the rows, and unique females as the columns. The matrix was filled with the simulated number of offspring produced by each female-male pair which was selected from a Poisson distribution with a rate parameter of 4.0 approximating the observed mean and variance in individual reproductive success from SCDRS empirical data. A total of

500 unique breeding matrices with 10-75 parents with simulated genotypes and subsequent offspring with simulated genotypes were constructed based on population allele frequencies observed in 2015 and 2016, SCDRS empirical data. Program COLONY was used to infer full- and half-sibs and parentage from the simulated genotypes of larvae in the 500 breeding matrices. Simulated full- and half-sibling relationships and parentage were then compared to the inferred relationships from Program COLONY to demonstrate power.

RESULTS

Quantifying Spawning Success

A total of 741 larvae were genotyped, 307 in 2015 and 434 in 2016. Concordance between replicate COLONY analyses was high for all estimates within years (Table 1-4). Confidence intervals for estimates of N_b did not include zero (Table 1-4). Estimates of N_s were 156, 220, and 344 in 2015, 2016 and 2015-16 combined respectively, when averaged across two replicate COLONY runs (Table 1). Averaged estimates of N_b were 146, 195, and 304 in 2015, 2016 and 2015-16 combined. Estimates of N_s and N_b varied across years and at individual reef sites, but were dependent on sample size (Tables 1-4). As more larvae were genotyped, more spawners were detected and the effective breeding size increased. Mean and variance in reproductive success were also sample size dependent with noticeably lower estimates where small sample sizes were analyzed. Mean reproductive success was estimated at 3.68 larvae per spawner, averaged across all years, with a variation in individual reproductive success estimated at 4.86. Estimates of N_b/N_s ratios in the SCDRS are high for both years and at each reef site within years ranging from 0.96 to 1.07 across all reefs and years and from 0.83 to 1.17 at individual reef sites across years.

Table 1. Estimates of the number of spawners (N_s), effective number of breeders (N_b), N_b/N_s ratio, mean and variance in individual reproductive success using larvae pooled across all reef sites for 2015, 2016 and 2015-2016 combined.

Estimates Across All Reefs								
2015 (N=307)			2016 (N=434)			2015 &2016 (N=741)		
	Run 1	Run 2		Run 1	Run 2		Run 1	Run 2
N_s	158	154	N_s	213	227	N_s	349	339
N_b	148	145	N_b	191	199	N_b	314	295
CI95(L)	120	116	CI95(L)	157	164	CI95(L)	271	250
CI95(U)	186	185	CI95(U)	233	247	CI95(U)	368	351
N_b/N_s	0.94	0.94	N_b/N_s	0.90	0.88	N_b/N_s	0.90	0.87
Mean Rs	3.99	4.11	Mean Rs	4.08	3.82	Mean Rs	4.26	4.37
Variance Rs	5.27	7.13	Variance Rs	6.00	5.83	Variance Rs	6.26	7.20

Table 2. Estimates of the number of spawners (N_s), effective number of breeders (N_b), N_b/N_s ratio, mean and variance in individual reproductive success using larvae collected at Harts Light Reef in 2015, 2016.

Harts Light Reef					
2015 (N=160)			2016 (N=200)		
	Run 1	Run 2		Run 1	Run 2
N_s	94	96	N_s	121	119
N_b	101	101	N_b	116	122
CI95(L)	77	77	CI95(L)	90	95
CI95(U)	135	136	CI95(U)	150	157
N_b/N_s	1.07	1.05	N_b/N_s	0.96	1.03
Mean Rs	3.40	3.33	Mean Rs	3.31	3.36
Variance Rs	2.59	2.79	Variance Rs	3.75	3.10

Table 3. Estimates of the number of spawners (N_s), effective number of breeders (N_b), N_b/N_s ratio, mean and variance in individual reproductive success using larvae collected at Pointe Aux Chenes Reef in 2015, 2016.

Pointe Aux Chenes Reef					
2015 (N=106)			2016 (N=60)		
	Run 1	Run 2		Run 1	Run 2
N_s	68	67	N_s	46	46
N_b	67	67	N_b	53	54
CI95(L)	49	48	CI95(L)	36	37
CI95(U)	98	95	CI95(U)	79	83
N_b/N_s	0.99	1.00	N_b/N_s	1.15	1.17
Mean Rs	3.12	3.16	Mean Rs	2.61	2.61
Variance Rs	3.18	3.11	Variance Rs	1.67	1.49

Table 4. Estimates of the number of spawners (N_s), effective number of breeders (N_b), N_b/N_s ratio, mean and variance in individual reproductive success using larvae collected at Grassy Island Reef in 2016.

Grassy Island Reef		
2016 (N=117)		
	Run 1	Run 2
N_s	57	58
N_b	48	48
CI95(L)	33	34
CI95(U)	72	71
N_b/N_s	0.84	0.83
Mean Rs	4.11	4.03
Variance Rs	7.13	7.51

Individual Spawning Site Selection

Detection of full- and half-sibling larvae reared from eggs collected on egg mats provides evidence of lake sturgeon spawning at multiple locations in the same year (Table 5). Additionally, genetic pedigree analysis allows for estimates of the number of unique parents contributing offspring between reefs and years in the SCDRS (Table 5).

Averaged across replicate COLONY runs, 2% and 80% of lake sturgeon larvae reared from eggs collected on egg mats at Pointe Aux Chenes in 2015 were full-sibs and half-sibs, respectively with larvae reared from eggs collected on egg mats at Harts Light Reef in 2015. For larvae reared from eggs collected on egg mats at the North and Middle Channel sites in 2015 no full-sibs were detected, but 13% were half-sibs with larvae reared from eggs collected on egg mats at Harts Light Reef. Finally, in 2015, no full sibs were detected and 7% of larvae reared from eggs collected on egg mats were half-sibs with larvae reared from eggs collected on egg mats at Pointe Aux Chenes. Capture of half-sibling larvae reared from eggs collected on egg mats at multiple sites reveal adults spawning at multiple locations within the same spawning year in the St. Clair River in 2015. The incidence half-sibling larvae at multiple reef sites is evidence of at least one parent spawning at multiple locations. Results of genetic pedigree analysis reveal that the number of parents contributing offspring at multiple reef locations ranged from 1-19 parents in 2015.

Further evidence of adults spawning in multiple locations was revealed in 2016. Of larvae reared from eggs collected on egg mats at Pointe Aux Chenes, 38% were full-sibs and 92% were half-sibs with larvae reared from eggs collected at Harts Light reef. Larvae genotyped from eggs collected on egg mats at the North and Middle Channel Sites were full- and half-sib with larvae reared from eggs collected on egg mats at Harts Light Reef (23% and 89%

respectively). No full-sibling relationships were detected between the North and Middle Channel Reefs and Pointe Aux Chenes Reef in 2016. However, 16% of larvae reared from eggs collected on egg mats at the North and Middle Channel Reef were half sibs with larvae reared from eggs collected on egg mats at Pointe Aux Chenes Reef. The number of parents contributing offspring at multiple reefs sites ranged from 1-26 in the St. Clair River in 2016. Collection of full- and half-sibling larvae reared from eggs collected on egg mats in 2016 at multiple reef sites provides additional evidence of adults contributing offspring at multiple reef sites within the St. Clair River in a single spawning period.

With the addition of sampling on Grassy Island Reef in the Detroit River, the 2016 pedigree analysis revealed evidence of lake sturgeon spawning in multiple rivers in a single spawning season. From larvae reared using eggs collected on egg mats at Grassy Island Reef 55%, 2% and 4% were half-sibs with larvae reared from eggs collected on egg mats at Harts Light Reef, Pointe Aux Chenes Reef, and the North and Middle Channel Reefs, respectively. The number of parents contributing offspring between rivers in 2016 ranged from 1-17 spawners.

Evidence for Spawning in Consecutive Years

To determine if adults were spawning in consecutive years, COLONY was run using all larvae from 2015 and 2016 across all sites. Analysis revealed 10 and 18 full-sibs and 403 and 397 half-sibs in replicate colony runs. However, 3 half sibling pairs were concordant between replicate COLONY runs with random number seeds. Detection of half-sibling larvae between years provides evidence of a limited number adults spawning in consecutive years. With 741 larvae included in the analysis some of these sibship assignments may be reflective of false assignment. However, with concordance between runs and assignment probabilities ranging

from 37.6-93.6%, data provide evidence of adults spawning in consecutive years. Also, pedigree analysis using larvae reared from eggs collected on egg mats in 2015 and 2016 revealed evidence of adults spawning consecutively at the same and on different reefs in the St. Clair River and between the St. Clair and Detroit rivers.

Table 5. Number of parents contributing offspring reared from eggs collected on egg mats at a downstream site that were also detected spawning at the upstream site; and the number of larvae reared from eggs collected on egg mats at a downstream site that were full- and half-sibs with larvae reared from eggs collected on egg mats at an upstream site. Results provide evidence of parents contributing offspring at multiple reefs in the St. Clair River in 2015 and at multiple reefs in both rivers in 2016.

Harts Light & Pointe Aux Chenes Reefs

	2015		2016	
	Egg Mats		Egg Mats	
	Run 1	Run 2	Run 1	Run 2
Parents	19	19	26	23
Full Sibs	0	1	9	9
Half Sibs	21	16	22	22

Pointe Aux Chenes & North/Middle Channel Reefs

	2015		2016	
	Egg Mats		Egg Mats	
	Run 1	Run 2	Run 1	Run 2
Parents	1	3	2	2
Full Sibs	0	0	0	0
Half Sibs	1	2	5	2

North/Middle Channel & Grassy Island Reefs

	2015		2016	
	Egg Mats		Egg Mats	
	Run 1	Run 2	Run 1	Run 2
Parents	NA	NA	1	1
Full Sibs	NA	NA	0	0
Half Sibs	NA	NA	2	2

Harts Light & North/Middle Channel Reefs

	2015		2016	
	Egg Mats		Egg Mats	
	Run 1	Run 2	Run 1	Run 2
Parents	3	4	18	14
Full Sibs	0	0	2	8
Half Sibs	3	3	21	18

Pointe Aux Chenes & Grassy Island Reefs

	2015		2016	
	Egg Mats		Egg Mats	
	Run 1	Run 2	Run 1	Run 2
Parents	NA	NA	2	0
Full Sibs	NA	NA	0	0
Half Sibs	NA	NA	2	0

Harts Light & Grassy Island Reefs

	2015		2016	
	Egg Mats		Egg Mats	
	Run 1	Run 2	Run 1	Run 2
Parents	NA	NA	14	17
Full Sibs	NA	NA	1	0
Half Sibs	NA	NA	29	33

Patterns of Larval Dispersal

Patterns of larval dispersal using larvae reared from eggs collected on egg mats at upstream locations, and larvae captured in downstream D-frame and vertically stratified conical nets suggests that larvae may be dispersing large distances through the SCDRS. In 2015, larvae were collected in downstream D-frame and vertically stratified conical nets at Pointe Aux Chenes and the North and Middle Channel control sites that were full- and half-sibs with larvae reared from eggs collected on egg mats at the upstream Harts Light Reef (Table 6). Also in 2015, larvae were collected in downstream D-frame nets at the North and Middle Channel control sites that were half-sibs with larvae were reared from eggs collected on egg mats at Pointe Aux Chenes Reef. In 2016, larvae were collected in downstream D-frame and vertically stratified conical nets at Pointe Aux Chenes that were full-sibs with larvae reared from eggs collected on egg mats at Harts Light Reef. Also in 2016, larvae were collected in downstream D-frame and vertically stratified conical nets at Pointe Aux Chenes, the North and Middle Channel control sites, and Grassy Island Reef that were half-sibs with larvae reared from eggs collected on egg mats at Harts Light Reef. In both years, larvae captured in downstream D-frame nets at the North and Middle Channel control sites that were half- sibs with larvae reared from eggs collected on egg mats at Pointe Aux Chenes Reef. In 2016 larvae were collected in downstream D-frame and vertically stratified conical nets at Grassy Island Reef that were full- and half-sibs with larvae reared from eggs collected on egg mats at Pointe Aux Chenes Reef. Finally, in 2016 larvae were collected in downstream D-frame and vertically stratified conical nets that were half-sibs with larvae reared from eggs collected on egg mats at the North and Middle channel control sites.

Table 6. Number of parents contributing offspring collected in D-frame and vertically stratified conical nets at a downstream site that were also detected spawning at the upstream site; and the number of larvae collected in D-frame and vertically stratified conical nets at a downstream site that were full- and half-sibs with larvae reared from eggs collected on egg mats at an upstream site. Detection of larvae that are full- and half-sibs with larvae that were reared from eggs collected on egg mats at upstream sites may indicate larval dispersal from the upstream location.

Harts Light Egg Mats & Pointe Aux Chenes D-frames And Conicals

	2015		2016	
	Drift		Drift	
	Run 1	Run 2	Run 1	Run 2
Parents	70	76	41	37
Full Sibs	6	1	1	2
Half Sibs	75	79	31	29

Pointe Aux Chenes Egg Mats & North/Middle Channel D-Frames

	2015		2016	
	Drift		Drift	
	Run 1	Run 2	Run 1	Run 2
Parents	7	9	3	0
Full Sibs	1	0	0	0
Half Sibs	9	10	2	0

North/Middle Channel Egg Mats & Grassy Island D-frame And Conicals

	2015		2016	
	Drift		Drift	
	Run 1	Run 2	Run 1	Run 2
Parents	NA	NA	11	10
Full Sibs	NA	NA	0	0
Half Sibs	NA	NA	14	15

Harts Light Egg Mats & North/Middle Channel D-frames

	2015		2016	
	Drift		Drift	
	Run 1	Run 2	Run 1	Run 2
Parents	33	28	15	15
Full Sibs	3	1	0	0
Half Sibs	29	28	12	12

Pointe Aux Chenes Egg Mats & Grassy Island D-frame And Conicals

	2015		2016	
	Drift		Drift	
	Run 1	Run 2	Run 1	Run 2
Parents	NA	NA	13	10
Full Sibs	NA	NA	2	1
Half Sibs	NA	NA	20	15

Harts Light Egg Mats & Grassy Island Reef D-frame And Conicals

	2015		2016	
	Drift		Drift	
	Run 1	Run 2	Run 1	Run 2
Parents	NA	NA	42	36
Full Sibs	NA	NA	0	0
Half Sibs	NA	NA	48	43

Power of Assignment

At small sample sizes estimates of the number of parents and mate pairs was not consistently accurate, however, with greater than 15 parents representing the progeny in the pedigree, the ability to infer the correct number of parents and mate pairs was high (Figure 5). When adult numbers represented in the pedigree are low, sib-ship inferences are also not consistently accurate. However, with greater than 25 parents in the pedigree the ability to correctly infer full- and half-sib relationships was high (Figure 6). It is notable that given the power of the markers and analysis, regardless of sample size, unrelated offspring are inferred as unrelated reliably.

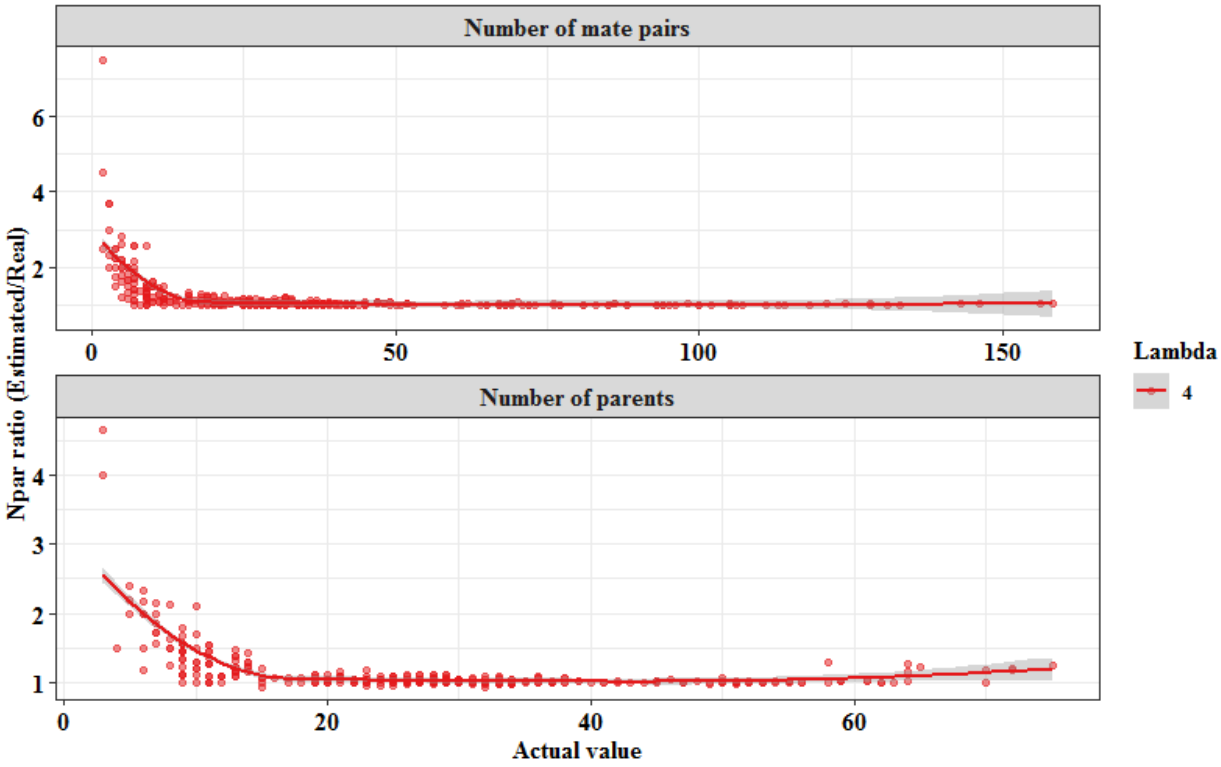

Figure 5. Ratio of true to estimated number of parents from COLONY simulations. A correct estimate of the number of parents in a sample results in a 1 on the y-axis. The red line is a fitted loess line with 95% confidence intervals represented by the grey bar.

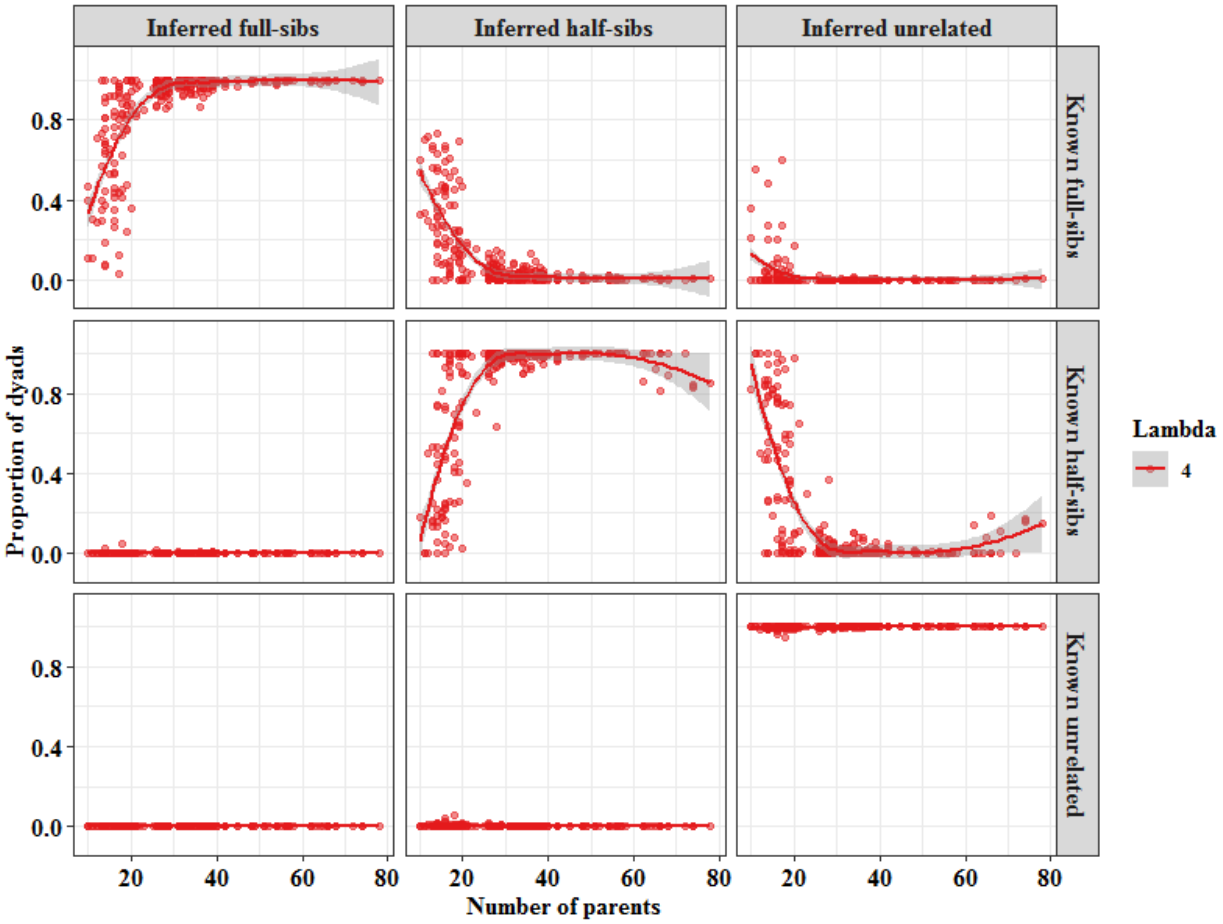

Figure 6. Matrix showing the ratio of true to inferred full-sibs, half-sibs, and unrelated offspring for the number of parents observed in a sample. Individual plots show the ratio of inferred (x-axis) to known (y-axis) full-sibs, half-sibs, and unrelated offspring. The red line is a fitted loess line with 95% confidence intervals represented by the grey bar. The number of parents observed for each simulation is plotted on the x-axis for each individual plot. As simulations include more observed parents the ability to correctly infer sib-ship increases. Unrelated individuals are almost always correctly inferred as unrelated.

DISCUSSION

Concern existed regarding the potential for depensatory effects caused by artificial reefs spreading out a finite number of spawners over a large area (Levitan et al. 1995, Levitan 2004). However, results of genetic pedigree analysis indicate that many spawners are using artificial reefs in the same year. N_b/N_s ratios represent the ratio of the number of spawners detected to the effective breeding size, and the N_b/N_s ratios approach and even exceed 1 for all reefs in 2015 and 2016. Duong et al. (2013) observed N_b/N_s ratios ranging from 0.27-0.86 for lake sturgeon in the Black River, MI. N_b estimates for the SCDRS in 2015 and 2015 are high in comparison. High N_b/N_s ratios are reflective of the near Poisson distribution of the observed mean and variance in individual reproductive success. Low N_b/N_s ratios reflect increased risk for accelerated losses of population level genetic diversity. Additionally, high N_b/N_s ratios provide evidence against the hypothesis that artificial spawning reefs are likely to accelerate loss of population level genetic diversity due to reduced numbers of spawners participating in spawning events at artificial reef sites. However, all estimates based on genetic pedigree analysis were sample size dependent.

Genetic pedigree analysis using larvae reared from eggs collected on egg mats provides a positive spawning location for inferred parents contributing full- and half- sibs. Detection of full- and half-sibling larvae reared from eggs collected on egg mats at multiple locations provides evidence that many spawning lake sturgeon are utilizing multiple artificial reefs in a single year in the SCDRS. Additionally, detection of full-sibling larvae reared from eggs collected on egg mats at multiple reef sites in the St. Clair River is evidence that male and female lake sturgeon are participating in multiple spawning events at multiple locations in the same year. Finally, detection of half-siblings between larvae reared from eggs collected on egg mats

from the Detroit River and larvae reared from eggs collected on egg mats in the St. Clair River reveal lake sturgeon are spawning in both rivers in a single year. This provides evidence of potential for gene flow between the two rivers. Telemetry studies have suggested that there is some movement by adult lake sturgeon between the St. Clair and Detroit rivers (Kessel et al. 2018), and this study confirms some adults are moving between rivers and are spawning in both.

Additionally, genetic pedigree analysis of larvae reared from eggs collected on egg mats suggests that individual spawning efforts are being dispersed across the riverscape. Spawning at multiple locations may contribute to a more resilient spawning portfolio (Schindler et al. 2010; Dufour et al. 2015) for lake sturgeon in the SCDRS that may buffer the effects of site specific mortality events occurring at individual locations across the riverscape in a single year. Because the same spawners are utilizing multiple reefs, the portfolio effect may not only buffer overall mortality, but act as a buffer against potential losses of genetic diversity within generations due to high site-specific mortality events. Determining levels of connectivity and gene flow between reefs will help inform future determination of artificial reef placement. Additional research is warranted to quantify movement of spawning lake sturgeon between rivers and levels of gene flow per generation across reefs and the entirety of this large 160km system.

When designing habitat remediation projects with limited resources, there is often a compromise between size and numbers of remediation sites to be constructed. Larger sites may attract more spawners and reduce concerns regarding depensatory effects. Diamond (1975) laid out a series of principals for management of habitat patches including prioritization of large protected areas and connectivity in terrestrial systems. The same principals established by Diamond (1975) regarding size and connectivity may apply to spawning habitat restoration in large, barrier-free rivers. However, a single large site may reduce population resiliency by

negating the potential benefits of a diverse spawning portfolio. Additionally, hydrology and anthropogenic factors such as commercial shipping channels may not allow for a single large-scale design. Levels of connectivity between smaller habitats may overcome these limitations. With spawners utilizing multiple locations within the same year, the multi-reef design may in fact be functioning as a single-large spawning habitat. Evidence of adults spawning at multiple locations within years provides compelling evidence for spawning habitat connectivity within the St. Clair River in 2015 and limited connectivity between rivers in 2016. Additional monitoring of use of artificial reefs by spawning lake sturgeon would allow quantification of levels of connectivity between reefs by relative distance and reef size.

Detection of sibling larvae between years provides evidence of adults spawning in consecutive years. Smith and Baker (2005) indicate around 14% of male lake sturgeon spawn in consecutive years in the Black River, MI. However, in an 8-year study, Forsythe et al. (2012) did not find evidence of females spawning in consecutive years in the Black River, MI. Additional years of data are needed in the SCDRS to adequately describe spawning periodicity of lake sturgeon between sexes, even though detection of multiple half-sibling larvae between years supports observations of at least male lake sturgeon spawning in sequential years.

Detection of full- and half-sibling larvae in downstream D-frame and vertically stratified conical nets demonstrate commonly observed trends of nocturnal downstream dispersal of lake sturgeon larvae from spawning site locations. Collection of larvae in drift nets that are full- or half-siblings with larvae reared from eggs collected on egg mats at upstream locations suggest that larvae in the SCDRS may be moving distances >40 km in the St. Clair River in 2015 and 2016 and >120 km between the St. Clair and Detroit rivers in 2016. The Detroit and St. Clair rivers have water velocities up to 1.35 m^3/s (observed at the time of larval dispersal in 2015), and

dispersal >40 km within rivers seems possible. However, while no hard barrier to dispersal exists between rivers, Lake St. Clair may act as a hydrological barrier to dispersal of larvae between the rivers. While it may seem unlikely that larvae are dispersing >120 km from Harts Light Reef in the St. Clair River to Grassy Island Reef in the Detroit River there is evidence in the literature for transport of deep water sculpin (*Myoxocephalus thompsonii*) and burbot (*Lota lota*) (Roseman et al. 1998; Lantry et al. 2007; McCullough et al. 2015). Additionally, while full- and half-sibling relationships between larvae reared from eggs on upstream egg mats and larvae captured in downstream D-frame nets suggests patterns of larval dispersal, this pattern is confounded by the fact that adults are observed spawning in multiple locations. Instead of larval dispersal alone, full- and half-sibling relationships may be representative of some combination of larval dispersal and adults spawning in multiple locations.

Further understanding of patterns of larval lake sturgeon dispersal in large barrier free river systems is critical when identifying larval and nursery habitat critical for conservation, and future work incorporating pedigree analysis and a spatially specific sampling design may allow for more detailed description of larval dispersal patterns. However, evidence of adults spawning in multiple locations would limit such a sampling design to river reaches where no additional spawning occurred between the spawning site and the furthest downstream collection location. Direct release of reared larvae that have been tagged by emersion in alizarin red as described in Schludermann et al. (2012) may provide an alternate method of examining larval dispersal timing and distance. However, this technique may have limited application in the SCDRS due to low probability of detection of tagged larvae given limitations in sampling due to the scale of the system and shipping traffic.

Results of this study further illustrate potential benefits of habitat remediation through the construction of spawning habit for lake sturgeon. Genetic pedigree analysis provided evidence that large numbers of lake sturgeon utilized constructed spawning habitat and that adults spawned at multiple locations within a single spawning season. Large numbers of spawners at individual constructed spawning sites reduces concerns for potential depensatory effects or loss of genetic diversity related to concerns regarding reduced spawner density. Additionally, adults spawning at multiple constructed spawning locations illustrates the potential for multiple spawning locations to contribute to the overall spawning portfolio for lake sturgeon. Examination of the effects of reef size on the number of spawners associated with a site, and connectivity based on distance between reefs would further inform future spawning habitat construction for lithophilic spawners such as lake sturgeon. Despite strong evidence of the immediate benefits of constructed spawning habitat little is known about the long term performance of these structures and population level responses of the fishes that use them (Fischer et al. 2018). Continued monitoring would describe use of constructed spawning habitat by lithophilic spawners as reefs over the course of reef maturation. However, results presented here provide support for the potential of constructed habitat to address spawning habitat limitations for threatened and endangered fishes. Further analysis of constructed spawning habitat in the SCDRS including reef size, connectivity, and describing patterns of larval dispersal has the potential to inform future remediation efforts aimed at addressing spawning habitat limitations for a host of threatened and endangered fish species.

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CHAPTER 2: ASSESSING POTENTIAL BIAS IN SAMPLE COLLECTION METHODS FOR USE WITH GENETIC PEDIGREE ANALYSIS TO ENSURE ACCURATE ASSESSMENT OF LAKE STURGEON (*ACIPENCER* FULVESCENS) SPAWNING

ABSTRACT

Dredging of commercial shipping channels in the St. Clair-Detroit River System (SCDRS) destroyed spawning habitats for lithophilic fishes including lake sturgeon (*Acipenser fulvescens*). As part of a large scale collaborative remediation effort to address the loss of habitat and increase levels of recruitment, lithophilic spawning habitat was constructed. Recently genetic pedigree analysis was performed to estimate the number of spawners (N_s), effective number of breeders (N_b), and mean and variance in individual reproductive success at three constructed reefs in the SCDRS. Genetic pedigree analysis resulted in high estimates of N_s (44-122), N_b (62-115), and low mean (2.14-4.06) and variance in reproductive success (0.80-5.48) across all sites. Additionally, there was concern around the effects sampling method on estimates based on genetic pedigree analysis due to spatial and temporal differences in how different methods collect eggs and larvae. Rates of detection of unique parents per larvae genotyped ranged from 0.63-1.38 parents/larvae across all sites, 0.77-1.20 parents/larvae at Harts Light Reef, 0.96-1.26 parents/larvae at Pointe Aux Chenes Reef, and 0.57-1.36 parents/larvae at Grassy Island Reef. Results of this analysis provide evidence that sample size and collection method affected estimates of N_s, N_b, and N_b/N_s ratios. Also, collection method effected the proportion of full- and half-sibling and unrelated larvae in each sample and resulted in significant differences in the rates of detection of unique parents per larvae genotyped. Quantifying the effects sampling methods have on the results of genetic pedigree analysis describes potential impacts of gear type on estimates of N_s, N_b, and N_b/N_s ratios to inform future assessment of conservation efforts for threatened and endangered fishes.

INTRODUCTION

Anthropogenic modifications to natural systems impede conservation efforts for threatened and endangered fishes. Lake sturgeon (*Acipenser fulvescens*) is a species of conservation concern that was once abundant in the Great Lakes (Auer 1999). However, due in part to habitat modifications (Manny et al 1988; Auer 1996; Bennion and Manny 2011) lake sturgeon populations have declined and have not recovered despite restructured harvest regulations (Auer 1996) and greatly improved water quality.

Large scale habitat modification due to the construction of commercial shipping channels destroyed critical spawning habitat for benthic spawners (Manny et al 1988; Bennion and Manny 2011) in the St. Clair-Detroit River System (SCDRS). Historic accounts identified fifteen naturally occurring spawning sites (Goodyear et al. 1982). However, habitat modifications including the construction of shipping channels destroyed large amounts of available benthic spawning habitat in the SCDRS (Bennion and Manny, 2011). Only three known natural spawning locations remained active (Manny and Kennedy 2002). As a result, lack of suitable lithophilic spawning habitat was identified as a limiting factor for conservation of fishes such as lake sturgeon and other lithophilic spawners in the SCDRS (Hondorp et al. 2014; Manny et al. 2015). Recent habitat remediation efforts in the SCDRS included the construction of spawning reef habitat (Roseman et al. 2011a; Bouckaert et al 2014) with the intent of mitigating lost lithophilic spawning habitat to increase recruitment (Fischer 2018).

Benthic egg mats, benthic D-frame nets, and vertically stratified conical nets were used in 2015 and 2016 to assess the numbers of lake sturgeon eggs and larvae associated with three constructed spawning reefs. Initial assessment provided compelling evidence that immediately

following construction, lake sturgeon used artificial reefs for spawning in the SCDRS (Roseman et al. 2011a; Bouckaert et al. 2014; Manny et al. 2015; Prichard et al. 2017; Fischer et al. 2018). Also, eggs that were spawned at artificial reef sites survived to the larval drift stage (Roseman et al. 2011a; Bouckaert et al. 2014).

Despite preliminary measures of success based on presence and numerical abundance of eggs and larvae, question remained about how many adults (N_s) were contributing the eggs or offspring sampled, the effective number of breeders (N_b), and variance in individual reproductive success of lake sturgeon associated with artificial reef sites. High egg and larval counts do not necessarily indicate large N_s. Lake sturgeon are highly fecund (Bruch et al. 2006) and a single pair could in principal populate whole samples from each gear type. In the assessment of artificial reefs effective population size (N_e) becomes important as N_e provides information on how artificial reefs may affect population levels of genetic diversity. N_e is considered the population size at which the rate of loss of allelic diversity is equal to that of genetic drift (Wright 1931; Allendorf et al. 2013). N_e is usually smaller than the population abundance because reductions in N_e are caused by changes in population size over time, skewness in sex ratios, and variation in individual reproductive success (Frankham 1995; Charlesworth 2009; Waples 2010; Duong et al. 2013). Similar to N_e, the effective number of breeding adults (N_b) is the effective population size for a spawning period, and in lake sturgeon is also influenced by the number of spawners, and mean and variance in reproductive success within a single spawning season (Duong et al. 2013). High levels of variation in individual reproductive success caused by few individuals contributing a high proportion of offspring in a spawning season can lead to low estimates for N_b, and low N_b/N_s ratios (Duong et al 2013). Low N_b/N_s ratios indicate the increased potential for losses of population level genetic diversity. Quantifying N_b and N_b/N_s

allows comparison of the effects of within season variation in recruitment on population levels of genetic diversity.

In Hunter (2018, Chapter 1) genetic pedigree analysis provided estimates of (N_s), (N_b), mean and variance in individual reproductive success, and provided evidence of lake sturgeon adults spawning in multiple locations in the SCDRS within a single spawning season. However, the number of spawners detected by genetic pedigree analysis was dependent on sample size (Hunter 2018, Chapter 1). There was also concern that potential differences inherent in the way certain gear types sample eggs and larvae associated with artificial reefs could affect interpretation of estimates based on genetic pedigree analysis results. Particularly, concern existed surrounding the way each gear type sample full-sibling (FS) and half-sibling (HS) larvae, and how spatial distribution of eggs, larvae, and sampling gear may affect estimates of N_s, N_b, N_b/N_s ratios, and mean and variance in reproductive success.

Lake sturgeon are lithophilic broadcast spawners, and female lake sturgeon have been described as participating in multiple spawning bouts whereby small proportions of eggs are released into sperm from multiple males (Bruch and Binkowski 2002). Eggs from individual females disperse widely at a spawning site (LaHaye et al. 1992; Auer and Baker 2002; Bruch and Binkowski 2002; Peterson et al. 2007). However, eggs from a single female are unlikely to be evenly distributed across a spawning site (Caroffino et al 2010, Finley et al. in press). Concern existed surrounding the likelihood that certain collection methods may disproportionately collect larvae contributed by relatively few females. For example, small (38 x 24 x 0.5 cm) egg mats positioned directly on an artificial reef may sample offspring from many males, but it is possible offspring were contributed by only a single female simply due to patchy patterns in dispersal of eggs and dispersion of egg mats during spawning. Additionally, if eggs from single females are

not evenly distributed across a site, positioning of relatively few D-frame nets directly downstream of artificial spawning reefs may also collect larvae drifting from only small proportions of the reef and thus disproportionately sample FS and HS larvae. Vertically stratified conical nets were positioned further away from artificial reef sites and may have sampled larvae contributed from proportionately more spawning adults because of the potential for larvae to have been mixed in the water column during drift. However, each gear type collects eggs and larvae in different numbers per sampling event. Accuracy of FS and HS assignment and the number of parents that contributed offspring has been shown to be sample size dependent (Hunter 2018, Chapter 1). The relative per-net or per-egg mat sampling efficiency of each gear type may affect the ability to estimate N_s, N_b, N_b/N_s, and mean and variance in reproductive success using genetic pedigree analysis.

To assess if adult spawning numbers estimated using pedigree analysis are comparable between gear types, analysis of how sampling method influences estimates is called for. Quantifying differences in the number of unique parents that were detected per larvae genotyped within samples from each gear type provided critical insight into differences in how egg mats, D-frames and vertically stratified conical nets collect eggs and larvae for use with pedigree analysis. The rate of detection of unique parents per larvae genotyped provides insight into the number of FS, HS, and unrelated larvae in a sample. The objective of this analysis was to examine the potential for systematic trends in unequal per-net or egg mat sample size and unequal rates of sampling of FS, HS, and unrelated offspring between collection methods to influence estimates of N_s, N_b, N_b/N_s ratios, and mean and variance in reproductive success based on genetic pedigree analysis. Results will inform future sampling designs for assessment of spawning habitat use by spawning lake sturgeon.

MATERIALS AND METHODS

Study Area

The St. Clair-Detroit River System is 145-km barrier-free connecting channel extending from Lake Huron to Lake Erie (Figure 7). The St. Clair and Detroit Rivers are heavily used by commercial shipping vessels, recreational boaters, and supports a popular recreational fishery. This extremely large river system has been heavily modified and degraded to accommodate human use resulting in a designation as a Great Lakes, Area of Concern (AOC) due to the loss of fish and wildlife habitat. Additionally, the extreme size and heavy use of the system by commercial shipping traffic are highly restrictive to certain methods of sampling.

Fed by waters from Lake Huron, the SCDRS is relatively stable in temperature and water velocity (Manny et al. 1988; Fischer et al. 2018). The head of the system is a known natural spawning site (Manny and Kennedy 2002) located near the city of Port Huron, MI. Constructed from 10-15cm sorted limestone in 2014, the 3.8-acre Harts Light Reef is located in the St. Clair River near East China, MI. Water depths can exceed 16m with water velocities of 1.35 m^3/s. Further downstream (28-km) in the St. Clair River is the 1.5-acre Pointe Aux Chenes Reef, also constructed in 2014 from 10-15cm sorted limestone, water depths exceed 15m with water velocities of 1.03 m^3/s. In the north channel of the St. Clair River, downstream from Pointe Aux Chenes Reef (8-km) is Maslinka reef, which is considered a historic spawning site. This 0.3-acre reef is constructed of coal cinders deposited by steam ships in the late 1800s (Fischer et al. 2018). Middle Channel reef (1.0-acre) also located downstream of Pointe Aux Chenes (8-km), is an artificial reef constructed in 2012. Finally, in the Detroit River, the 4.0-acre Grassy Island

Reef was constructed from 10-15cm sorted limestone in 2015. Water depths at Grassy Island Reef exceed 12m with water velocities of 0.80 m^3/s.

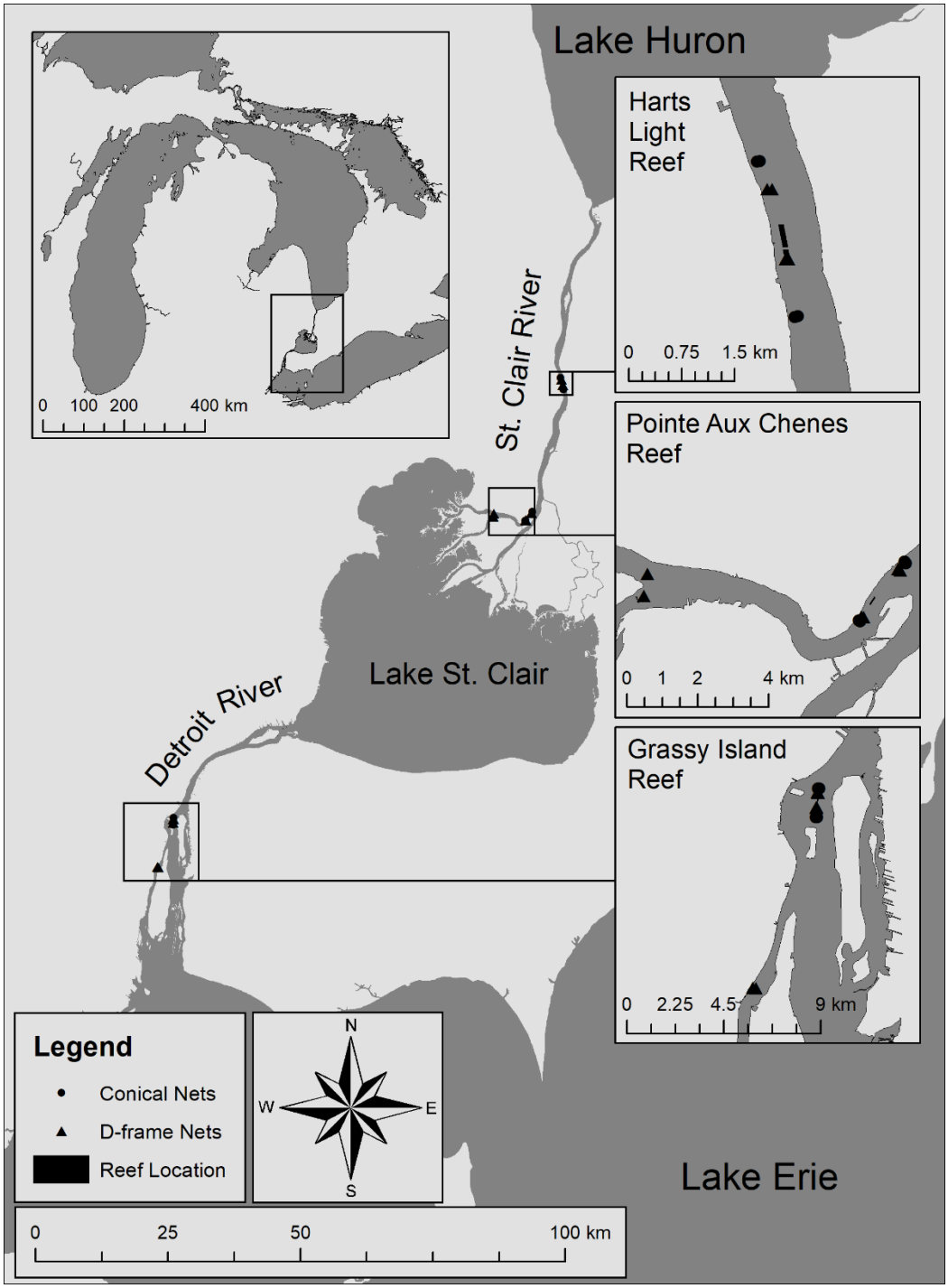

Figure 7. Map of the St. Clair-Detroit River system. Locations of constructed reef sites are highlighted, and net locations are indicated by triangles for D-frame nets, and circles for vertically stratified conical nets. Egg mats were deployed on the constructed reefs.

Egg and Larval Collection Methods

Samples 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 alternate spawning sites in the North and Middle Channel, St. Clair River in 2015 and 2016 (Figure 7), and at Grassy Island Reef and two alternate drift sites in the Trenton Channel, Detroit River in 2016 (Figure 7). Egg mats consisted of a furnace filter wrapped around a 38 x 24 x 0.5 cm steel frame secured using 5 x 2.5 cm binder clips, and were deployed on the artificial reefs in sets of 3 mats separated by 1 meter of line (Manny et al. 2010, Roseman et al. 2011b). Egg mats were retrieved weekly, weather permitting, and eggs were identified and enumerated. 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. Reared larvae were preserved in 95% ethanol for genotyping. Sampling and handling of fish was conducted according to the American Fisheries Society guidelines for the care and use of fishes (http://fisheries.org/docs/wp/Guidelines-for-UseofFishes.pdf).

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 directly upstream and two nets directly downstream of the artificial reefs. Nets were deployed at each reef from approximately 2000 hours to 0400 hours, during the peak larval lake sturgeon drift period in the SCDRS identified by Bouckaert et al. (2014). Samples were euthanized and preserved in 95 percent ethanol and were returned to the lab for processing where larvae were identified, enumerated, and preserved in 95% ethanol for genotyping.

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 artificial reefs. A

group consisted of 3 conical nets that were 0.15m in diameter and are made with 750 µm mesh. Nets were suspended on a buoy line in the water column; one net 1 meter below the surface, one net in the middle of the water column, and 1 net 1 meter off the river bottom (D' Amours et al. 2001, McCullough et al. 2015). Vertically stratified conical nets were deployed at sun set and retrieved at sun-up. Each net was treated as a separate sample, preserved in 95 percent ethanol, and returned to the lab for identification, enumeration, and preservation in 95% ethanol for genotyping.

DNA Extraction and Amplification

Tissue was extracted from larvae by dorso-ventrally bisecting the post-vent area. DNA was extracted using QIAGEN DNeasy® kits (QIAGEN Inc.) according to the manufacturer's protocol. DNA concentration was determined using a nano-drop spectrophotometer and was diluted to a concentration of 20 ng/µl.

DNA was amplified using polymerase chain reaction (PCR) across 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). PCR conditions for 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). Scribner et al. (in review) shows that the combination of 13 disomic and 5 polysomic markers had sufficient power to accurately assign larvae to FS and HS groups and in large river systems.

PCR was performed in 25-µl reactions with 5 µl of 20 ng/µl genomic DNA. Based on optimizations described in Scribner et al. (in review), reactions used 10x PCR buffer (1M Tris-HCL, 1M MgCl2, 1M KCL, 10% gelatin, 10% NP-40, 10% Triton-X), MgCl2, 2mM each dNTP, 10pmol of forward and reverse primer, and 0.5 µl Taq polymerase. After multiplexing and dilution to concentrations optimized for analysis, PCR product was then analyzed on an ABI 3730xl DNA analyzer at the Michigan State University Research Technology Support Facility. Results were visualized using GeneMapper (Softgenetics, State College, PA). All allele sizes were analyzed with size standards from (MapMarkerTM and BioVentures Inc.), three lake sturgeon samples with known genotypes, and a negative sample with no DNA. Alleles were scored independently and confirmed by a second experienced scorer, and approximately 10% of individuals were reanalyzed as a further quality control check resulting in empirical error rates of 0.5% and 1.8% in 2015 and 2016, respectively.

Pedigree Analysis

Allele scores 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), and missing data (0) score for each locus. Data was missing only if an individual failed to amplify at a locus despite 2 separate amplification attempts. Analysis were performed using 164 alleles (pseudo-disomic loci) in 2015 and 2016 for 741 eggs and larvae. Program COLONY (Wang 2004) was used to assign larvae to FS and HS groups and to infer the most likely number of parents (N_s) and effective number of breeders (N_b) contributing to offspring sampled using a full-maximum likelihood approach. 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. Parentage, FS, and HS relationships are listed here in order of increasing difficulty of assignment (Wang and Scribner 2014). Simulations from Hunter (2018, Chapter 1) demonstrated sufficient power to accurately assign larvae to FS and HS groups and infer N_s.

Pedigrees were generated in program COLONY (Wang 2004) consisting of each unique larval id, a putative mother id, and a putative father id (Table S1). 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 ID's. Plotting the cumulative sum of unique parent detected per larvae genotyped for each collection method reveals the differences in the rate of detection of unique parents per larvae genotyped between collection methods (Figures S1-S4). The slope of a linear regression model fit to the data for each gear type would represent the rate of detection of unique parents per larvae genotyped for each gear type. However, the slope may 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, are included in the analysis. To account for potential variation, each collection method and location specific pedigree was bootstrapped (R=1000) with replacement (Table S2). This resulted in 1000 bootstrapped pedigrees for which the cumulative sum of unique parents per larvae genotyped were generated (Table S3). A linear regression model was fit to each bootstrapped pedigree for

each collection method (Eq. 1) where Sum_{par} is equal to the cumulative sum of unique parents detected, β_0 is the intercept, β_1 is the slope, and N_{off} is the number of larvae genotyped. The mean slope was calculated as the mean of all slopes generated across 1000 bootstrapped pedigrees for each collection method.

$$Sum_{par} = \beta_0 + \beta_1 N_{off} \quad \text{Eq. 1}$$

Statistical Analysis

Significant differences between the rate of detection of unique parents per larvae genotyped were examined using F-tests between gear types using a linear regression model (Eq. 2) where Sum_{par} is the cumulative sum of unique parents detected, β_0 is the intercept, β_1 is the slope, N_{off} is the number of larvae genotyped, and Gear is the collection method. Linear hypothesis testing was performed using R 3.4.3 (R Core Team 2017) using the CAR package (Fox and Weisberg 2011) Linear Model function to construct the linear model. The STATS package (R Core Team 2017) Linear Hypothesis function was used to test for significant differences in the rate of detection of unique parents per larvae genotyped between bootstrap iterations using an F-test. Tests between the rate of detection of unique parents per larvae genotyped between each gear type resulted in 1000 p-values for tests between each gear type (e.g., vertically stratified conical nets vs. egg mats). For each of the 1000 comparisons between each gear type significant differences were scored as 1 (significantly different) or 0 (not significantly different). P-values for significant differences (α=0.05) in the rate of detection of unique parents between gear each gear type was determined as the percentage of pairwise tests that resulted in a significant difference (e.g., 950 out of 1000 tests were significant, p=0.05)

$$Sum_{par} = \beta_0 + \beta_1 N_{off} + \beta_1 Gear + \beta_1 N_{off} \times Gear$$ Eq. 2

RESULTS

Sample Size by Gear Type

Estimates of N_b and N_s were inherently sample size dependent (Hunter 2018, Chapter 1), and collection methods that sampled with a higher intensity would allow for better estimates with less sampling effort and cost. Frequency histograms of egg and larval catch numbers showed that all gear types most often collect few eggs or larvae when successful (Figure 8). However, egg mats collected hundreds of individuals when compared to D-frames and vertically stratified conical nets. D-frames had smaller numbers of individuals collected (<100 larvae) than egg mats (<500 eggs), and vertically stratified conical nets collected very few (<12 larvae) per sample when individuals are collected.

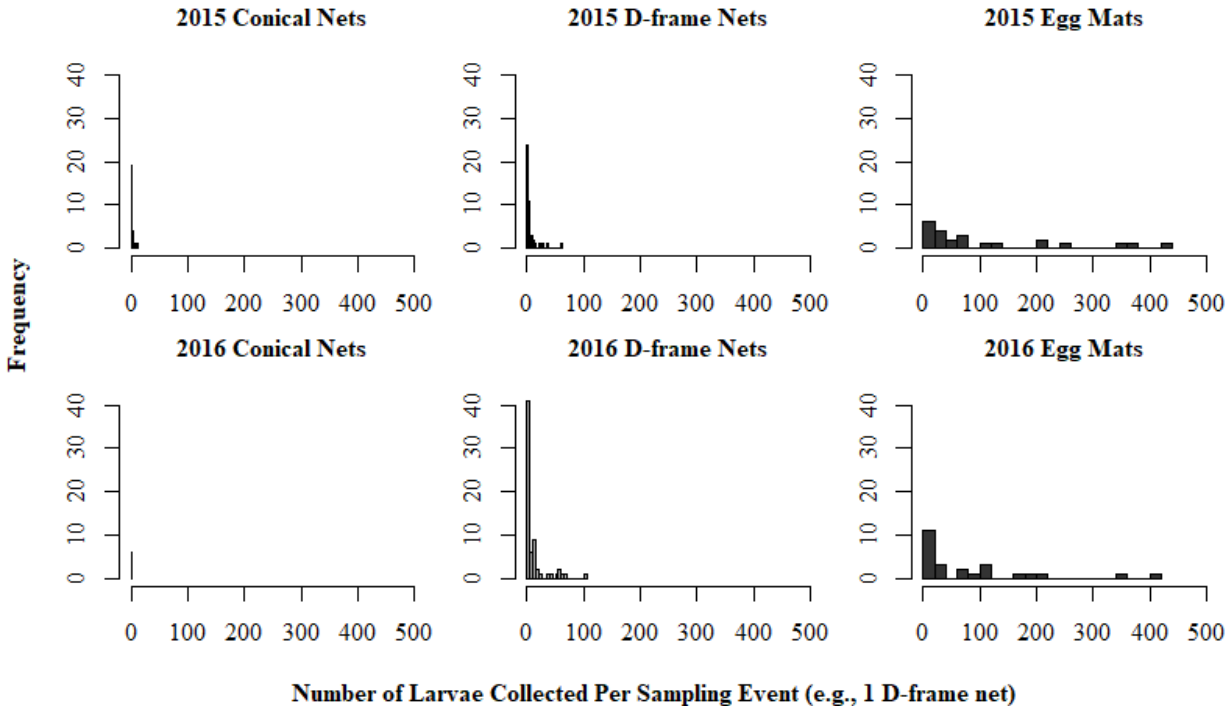

Figure 8. Histograms representing the frequency distribution for the numbers of individuals captured per sampling event that successfully captured individuals by each gear type in 2015 and 2016 in the SCDRS. A sampling event is defined as a single event where a net or egg mat was deployed and retrieved.

Number of Spawners Contributing Eggs and Larvae (N_s)

Estimates of N_s ranged from 44-122 for all reefs, 21-74 at Harts Light Reef, 22-43 at Pointe Aux Chenes Reef, and 28-41 at Grassy Island Reef (Tables 7-10). As expected, when more larvae were genotyped more unique spawners (N_s) were detected (Figure 9). To account for sample size dependence and compare N_s estimates between gear types and across locations, N_s estimates are divided here by the number of larvae genotyped in each sample and averaged across years and replicate COLONY runs. Across all reefs and years, average estimates of N_s per larvae genotyped were similar 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 higher estimates of N_s per larvae genotyped (0.936 N_s per larvae genotyped) compared to egg mats and D-frames. At Harts Light Reef, the average N_s per larvae genotyped was (0.649, 0.829, and 1.148 N_s per larvae genotyped) for egg mats, D-frame nets, and vertically stratified conical nets, respectively. Average estimates of N_s for egg mats were slightly higher for egg mats (0.978 N_s per larvae genotyped) compared to D-frame (0.833 N_s per larvae genotyped), but vertically stratified conical nets were still higher at (1.35 N_s per larvae genotyped) at Pointe Aux Chenes Reef. Finally, estimates of N_s at Grassy Island Reef in 2016 were (0.500 and 0.702 N_s per larvae genotyped) for egg mats and D-frame nets, respectively. The rates of detection of unique parents per larvae genotyped indicate that estimates for N_s are similar across reefs and years between D-frames and egg mats but are higher for samples collected with vertically stratified conical nets.

Effective Number of Breeders (N_b)

The effective number of breeders (N_b) ranged from 51-115 across all reefs, 30-76 at Harts Light Reef, 27-54 at Pointe Aux Chenes Reef, and 22-46 at Grassy Island Reef for all years (Tables 7-10). Like estimates of N_s, estimates of N_b was sample size dependent (Figure 9), and comparisons between gear types are made here by averaging N_b estimates divided by the number of larvae genotyped across years. Estimated average N_b per larvae genotyped was 0.542, 0.552, and 1.202 for egg mats, D-frame nets, and vertically stratified conical nets, respectively, across all sites. At Harts Light Reef N_b per larvae genotyped was higher for D-frames (1.042) compared to egg mats (0.683), but vertically stratified conical nets were comparatively higher (1.852). Pointe Aux Chenes shows similar patterns but with slightly higher estimates for N_b per larvae genotyped for egg mats (1.254) compared to D-frames (1.053). Vertically stratified conical nets showed an even higher estimate for N_b per larvae genotyped (2.70) compared to egg mats and D-frames. Finally, at Grassy Island Reef estimates of N_b per larvae genotyped were higher for D-frame nets (0.781) compared to egg mats (0.393). When taking into account the effect of sample size on estimates of N_b, data show that vertically stratified conical nets consistently have elevated estimates compared to egg mats and D-frame nets. Egg mats have similar estimates for N_b per larvae genotyped, but more often estimates from D-frame nets are higher.

Ratio of Effective Breeding Size to the Number of Spawners (N_b/N_s)

The ratio of effective breeding size to the number of spawners detected (N_b/N_s) is an important indicator of the potential consequences artificial reefs have on population level genetic diversity of lake sturgeon in the SCDRS. Sample size dependence is also evident for N_b/N_s

ratios (Figure 9). However, unlike N_s and N_b, the relationship between sample size and N_b/N_s ratios is negative and non-linear (Figure 9). In 2015 and 2016, N_b/N_s ratios were high in the SCDRS, ranged from 0.90-1.41 and were concordant between years for each gear type. N_b/N_s ratios presented here were averaged across years for comparison between gear types across locations. Across all reefs and years, N_b/N_s ratios were (0.970, 0.978, and 1.285) for egg mats, D-frame nets, and vertically stratified conical nets, respectively. Similarly, estimates of N_b/N_s ratios were higher for vertically stratified conical nets (1.610) than for egg mats (1.050) or D-frame nets (1.201) at Harts Light Reef. Unlike all other locations estimates for N_b/N_s ratios were higher for egg mats (1.280) compared to D-frames (1.235), but were still considerably higher for vertically stratified conical nets (2.000) at Pointe Aux Chenes Reef. Finally, at Grassy Island Reef estimates of N_b/N_s ratios were higher for D-frame nets (1.110) compared to egg mats (0.790). Consistent with estimates of N_s and N_b, estimates of N_b/N_s ratios per larvae genotyped are higher at each location for vertically stratified conical nets compared to estimates from egg mats and D-frame nets.

Rates of Detection of Unique Parents

Results of 1000 bootstrapped iterations of collection method and year specific pedigrees allowed comparison of rates of detection of unique parents per larvae genotyped by each gear type represented by the mean slope (Figures 10-13). Only 4 larvae were genotyped for vertically stratified conical nets in 2016, and due to small sample size, no comparisons with conical nets were made in 2016 with D-frames and egg mats. Mean slopes between years are generally concordant among collection method with a slightly lower slope in 2016 for egg mats over all locations (Figure 10). 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 followed by D-frame nets and then by egg mats (Figures 11-13). However, at Grassy Island Reef in 2016, the mean slope for D-frame nets (0.962) was considerably higher than for egg mats (0.545) (Figure 13).

Differences in the Rate of Detection of Unique Parents

Significant differences in rates of detection of unique parents detected per larvae genotyped were observed between vertically stratified conical nets and D-frame nets ($p=0.002$) and egg mats ($p=0.002$) in 2015, across all sites (Table 11). No significant difference was observed between D-frame nets and egg mats ($p=0.193$) 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 ($p=0.018$) in 2016 across all reef sites with D-frames detecting unique parents at a slightly higher rate.

In 2015, a significant difference in the rate of detection of unique parents detected per larvae genotyped was observed between conical nets and egg mats ($p=0.037$) but not for D-frame nets and conical nets ($p=0.490$) or D-frames and egg mats ($p=0.244$) at Harts Light Reef. No significant difference was observed between the rates of detection of unique parents per larvae genotyped for D-frames nets and egg mats in 2016 at Harts Light Reef ($p=0.244$). At Pointe Aux Chenes Reef, no significant difference was observed for the rate of detection of unique parents per larvae genotyped for any of the collections methods in 2015 or between D-frames and egg mats in 2016. Finally, at Grassy Island in 2016 the rate of detection of unique parents per larvae genotyped was significantly higher for D-frame nets compared to egg mats ($p<0.001$).

DISCUSSION

Population estimates based on sampling for rare species such as lake sturgeon and for early life stages is often fraught with difficulty (Wirgin et al. 1997; Thomas and Haas 2002; Caroffino et al. 2010) due to the ecology of the species and difficulty of detection. Additionally, sampling difficulties are compounded in large, non-wadable rivers. Assessment related to early life history of fishes is often aimed at describing the impacts of human activity on a sensitive life stage (Cyr et al. 1992). In the SCDRS, traditional assessment methods using benthic egg mats, benthic D-frame nets, and vertically stratified conical nets demonstrated use of constructed reefs by spawning lake sturgeon in the spawning season immediately following reef construction (Roseman 2011a; Bouckaert et al 2014). Additionally, assessment of artificial reefs provided evidence of successful survival of lake sturgeon eggs to the larval drift stage through the enumeration of collected lake sturgeon eggs and larvae (Roseman 2011a; Bouckaert et al 2014). Coupling traditional assessment methods with genetic pedigree analysis provided additional means for estimating the number of adults contributing offspring on artificial reefs in the SCDRS (Hunter 2018, Chapter 1). Quantification of N_s, N_b, and the mean and variance in individual reproductive success revealed high numbers of spawners participating in spawning events at artificial reefs and within the SCDRS across years (Hunter 2018, Chapter 1). N_b/N_s ratios were consistently high between years and at individual reefs (Hunter 2018, Chapter 1).

In this analysis the effect of egg and larval collection methods on estimates of N_s, N_b, mean and variance in reproductive success, and N_b/N_s ratios were examined. Cyr et al. (1992) demonstrated that the sample size required for precise estimates of larval abundance using traditional sampling methods rises rapidly as the mean number of larvae captured per sample

decreases. Examining frequency and numbers of larvae collected by each gear type shows differences in the number of larvae collected per sample between collection methods (Figure 8). Egg mats collect far more individuals than D-frame nets, which collect more individuals than vertically stratified conical nets. Additionally, precision in pedigree analysis is reduced at small sample sizes (Hunter 2018, Chapter 1) further emphasizing the importance of collection efficiency. Concern also existed regarding the potential of sampling method to influence estimates due to the difference in spatial and temporal scale at which different collection methods operate. Spatially heterogeneous distributions of larvae in the environment is known to cause high variation in replicate samples using traditional collection methods (Cyr et al. 1992). Similarly, egg deposition by individual lake sturgeon is believed to be inherently patchy (Caroffino et al. 2010, Finley et al. in press). Patchiness in egg and larval distribution may influence genetic pedigree analysis results through the unequal collection of FS and HS larvae between collection methods. Increased patchiness has the potential to increase levels of co-ancestry within a sample which will cause a direct decline in the accumulation rate of unique parents detected. Critical assessment of the effects of sampling methodology on population estimates for traditional assessment and genetic pedigree analysis can ensure high degrees of precision and accurate interpretation of results.

Estimates of N_s and N_b were sample size dependent, and each collection method collected different numbers of eggs and larvae when successful. Egg mats tended to result in the largest sample sizes, followed by D-frame nets. Conical nets tended to capture low numbers relative to other gear. Sample size also influenced estimated mean and variance in reproductive success of lake sturgeon spawning at artificial reef sites using pedigree analysis. When sample sizes of genotyped larvae were small (e.g., <30), variance in reproductive success was considerably

lower on average regardless of the collection method or year being considered. FS tend to be falsely assigned as HS and to a lesser degree unrelated, and HS tend to be assigned as unrelated at low sample sizes (Hunter 2018, Chapter 1). As a result, low samples sizes may not allow accurate construction of sib-groups and may mask true variance in reproductive success resulting in elevated estimates of N_b/N_s. Alternatively, by random chance, low sample sizes may not include enough FS and HS larvae to be fully representative of the actual mean and variance in reproductive success of lake sturgeon at an artificial reef by chance alone. With low sample size, reduced estimates of variance in reproductive success caused elevated estimates of N_b per larvae genotyped and subsequently N_b/N_s ratios compared to estimates made with sample sizes of (>30) genotyped larvae. However, when adequate sample sizes are used for pedigree analysis N_b/N_s estimates were concordant between years for each collection method and were similar for egg mats and D-frames. Using larvae collected in vertically stratified conical nets resulted in elevated estimates for N_b/N_s compared to D-frames and egg mats. This may be due to the way each collection method samples larvae or was positioned relative to the artificial reefs. Conical nets were positioned a greater distance from artificial reefs and were sampling larvae higher in the water column. It is possible that larvae collected in conical nets are more admixed than eggs and larvae that are being sampled using D-frames and egg mats that are positioned closer to and on the reef respectively.

Differences in the way collection methods include early ontogenetic stages can affect the results of genetic pedigree analysis. Though limited samples were available, vertically stratified conical nets resulted in higher N_b/N_s ratios compared to D-frame nets and egg mats. The difference between the rates at which collection methods detect unique parents per larvae genotyped showed a consistent trend across all reefs and at individual reefs. However,

differences were not always found to be significant. This may be representative of actual differences in the way the collection methods sample at each reef, or reduced sample sizes in individual reef analysis. With small sample sizes it is possible that by random chance, patterns in the way collection methods sample become less apparent. Larger sample sizes and additional years of sampling are likely required to sufficiently quantify differences in the way collection methods sample at individual reefs. However, given the largest sample sizes examined (all reefs combined), results of genetic pedigree analysis and rates of detection of unique parents per larvae genotyped were comparable between D-frame and egg mat collections.

Results of this analysis provide evidence for differences in estimates due to sample size and the way in which each collection method collects eggs and larvae. N_b is reduced as mean and variance in individual reproductive success increase because a higher proportion of offspring are being contributed by a smaller proportion of adult spawners (Frankham 1995; Charlesworth 2009; Waples 2010). Reduced estimates of N_b and subsequently N_b/N_s ratios suggest population level genetic consequences (Palstra and Ruzzante 2008; Charlesworth et al. 2009; Waples et al. 2010). Low N_b/N_s ratios indicate a population that is at risk for more rapid losses of population level genetic diversity. Genetic pedigree analysis resulted in overall high N_b/N_s ratios at artificial reefs in the SCDRS (Hunter 2018, Chapter 1). Differences in frequency and number of egg and larval collections as well as the rate of detection of unique parents suggests that collection method may affect how estimates of N_s, N_b, and N_b/N_s ratios are interpreted.

Each collection method plays a crucial role in assessing the use of artificial reefs by spawning lake sturgeon in the SCDRS. Egg mats allow quantification of spawning effort prior to and following reef construction. Benthic D-frame nets allow quantification of larvae collected as they disperse from reef sites, and vertically stratified conical nets allow insight into larval

position in the water column during larval dispersal from spawning sites. When used in combination with genetic pedigree analysis, egg mats provide a precise location at which an egg was spawned and thus the location at which parents participated in a spawning event. Vertically stratified conical nets and D-frame nets can allow examination of patterns of larval drift by comparing capture location of FS and half sibling larvae dispersing from spawning sites. However, D-frame nets and conical nets sample larvae drifting in the water column. Since the spatial and temporal extent of larval drift in the SCDRS has not been successfully described, there is no way to know with certainty the origin of larvae captured in either net type.

Regardless, more information is needed to fully quantify how each collection method may affect interpretation of estimates of N_s, N_b, and N_b/N_s when coupled with genetic pedigree analysis. Over all reef sites and years, data suggest that egg mats and D-frames nets produce similar estimates of N_s, N_b, N_b/N_s ratios, and mean and variance in reproductive success. Additionally, averaged over all reef sites and all sampling years, egg mats and D-frame nets detect unique parents per larvae genotyped at similar rates. Additional research would provide insight into the spatial and temporal variability surrounding estimates of N_s, N_b, N_b/N_s ratios, and mean and variance in reproductive success produced using larvae from each of these collection methods.

Results of this analysis demonstrate the importance of considering differences in typical sample sizes collected by each collection method and the potential influence of the patchy spatial distribution of eggs and larvae for future assessment of the use of artificial reefs by lake sturgeon when coupled with genetic pedigree analysis. The number of larvae genotype is highly important when estimating N_s, N_b, N_b/N_s ratios, and mean and variance in reproductive success. Additionally, it is important to consider that each gear type demonstrated consistent patterns in

the rate of detection of unique parents per larvae genotyped. Disproportionate levels of co-ancestry within samples will result in different rates of detection of unique parents per larvae genotyped. High levels of co-ancestry in samples would reduce the rate of detection of unique parents, and may affect estimates of variation in individual reproductive success that are used to estimate N_b. Results of this analysis suggest that collection method is an important consideration when obtaining samples for use with genetic pedigree analysis.

Table 7. Results from genetic pedigree analysis for each collection method in 2015 and 2016 using larvae pooled across all reefs.

Estimates Across All Reefs												
Egg Mats					**D-frame Nets**				**Conical Nets**			
2015 (N=138)			2016 (N=207)		2015 (N=122)		2016 (N=207)		2015 (N=47)		2016 (N=4)	
	Run1	Run2		Run1 Run2		Run1 Run2		Run1 Run2		Run1 Run2		Run1 Run2
N_s	83	85	N_s	102 106	N_s	71 64	N_s	122 116	N_s	44 44	N_s	NA NA
N_b	85	89	N_b	92 96	N_b	76 60	N_b	115 111	N_b	62 51	N_b	NA NA
CI95(L)	63	66	CI95(L)	68 72	CI95(L)	55 43	CI95(L)	89 84	CI95(L)	40 32	CI95(L)	NA NA
CI95(U)	116	120	CI95(U)	123 128	CI95(U)	109 88	CI95(U)	151 144	CI95(U)	98 80	CI95(U)	NA NA
N_b/N_s	1.02	1.05	N_b/N_s	0.90 0.91	N_b/N_s	1.07 0.94	N_b/N_s	0.94 0.96	N_b/N_s	1.41 1.16	N_b/N_s	NA NA
Mean Rs	3.33	3.27	Mean Rs	4.06 3.91	Mean Rs	3.44 3.44	Mean Rs	3.39 3.57	Mean Rs	2.14 2.47	Mean Rs	NA NA
Var Rs	2.98	2.70	Var Rs	5.82 5.48	Var Rs	2.56 4.79	Var Rs	4.09 4.09	Var Rs	0.77 0.80	Var Rs	NA NA

Table 8. Results from genetic pedigree analysis for offspring collected at Harts Light Reef in 2015 and 2016 by collection method.

Harts Light Reef																		
Egg Mats						**D-frame Nets**						**Conical Nets**						
2015 (N=112)			2016 (N=105)			2015 (N=21)			2016 (N=95)			2015 (N=27)			2016 (N=0)			
	Run1	Run2		Run1	Run2		Run1	Run2		Run1	Run2		Run1	Run2		Run1	Run2	
N_s	72	74	N_s	66	70	N_s	21	21	N_s	64	61	N_s	31	31	N_s	NA	NA	
N_b	73	76	N_b	72	75	N_b	31	30	N_b	60	60	N_b	50	50	N_b	NA	NA	
CI95(L)	53	57	CI95(L)	51	54	CI95(L)	18	16	CI95(L)	42	42	CI95(L)	30	30	CI95(L)	NA	NA	
CI95(U)	105	106	CI95(U)	102	105	CI95(U)	63	59	CI95(U)	89	87	CI95(U)	98	91	CI95(U)	NA	NA	
N_b/N_s	1.01	1.03	N_b/N_s	1.09	1.07	N_b/N_s	1.48	1.43	N_b/N_s	0.94	0.98	N_b/N_s	1.61	1.61	N_b/N_s	NA	NA	
Mean Rs	3.11	3.03	Mean Rs	3.18	3.00	Mean Rs	2.00	2.00	Mean Rs	2.97	3.11	Mean Rs	1.74	1.74	Mean Rs	NA	NA	
Var Rs	2.92	2.77	Var Rs	2.30	2.38	Var Rs	0.60	0.70	Var Rs	3.49	3.24	Var Rs	0.53	0.53	Var Rs	NA	NA	

Table 9. Results from genetic pedigree analysis for offspring collected at Pointe Aux Chenes Reef in 2015 and 2016 by collection method.

Pointe Aux Chenes Reef																		
Egg Mats						**D-frame Nets**						**Conical Nets**						
2015 (N=23)			2016 (N=24)			2015 (N=63)			2016 (N=36)			2015 (N=20)			2016 (N=0)		
	Run1	Run2		Run1	Run2		Run1	Run2		Run1	Run2		Run1	Run2		Run1	Run2
N_s	22	22	N_s	24	24	N_s	43	41	N_s	36	36	N_s	27	27	N_s	NA	NA
N_b	27	27	N_b	32	32	N_b	46	46	N_b	49	50	N_b	54	54	N_b	NA	NA
CI95(L)	15	16	CI95(L)	19	19	CI95(L)	31	32	CI95(L)	31	31	CI95(L)	30	29	CI95(L)	NA	NA
CI95(U)	55	50	CI95(U)	59	61	CI95(U)	72	72	CI95(U)	80	83	CI95(U)	139	139	CI95(U)	NA	NA
N_b/N_s	1.23	1.23	N_b/N_s	1.33	1.33	N_b/N_s	1.07	1.12	N_b/N_s	1.36	1.39	N_b/N_s	2.00	2.00	N_b/N_s	NA	NA
Mean Rs	2.09	2.09	Mean Rs	2.00	2.00	Mean Rs	2.93	3.07	Mean Rs	2.00	2.00	Mean Rs	1.48	1.48	Mean Rs	NA	NA
Var Rs	1.13	1.13	Var Rs	0.96	0.96	Var Rs	2.21	1.87	Var Rs	0.86	0.80	Var Rs	0.34	0.34	Var Rs	NA	NA

Table 10. Results from genetic pedigree analysis for offspring collected at Grassy Island Reef in 2016 by collection method.

Grassy Island Reef								
Egg Mats			**D-frame Nets**			**Conical Nets**		
2016 (N=56)			2016 (N=57)			2016 (N=4)		
	Run1	Run2		Run1	Run2		Run1	Run2
N_s	28	28	N_s	39	41	N_s	NA	NA
N_b	22	22	N_b	43	46	N_b	NA	NA
CI95(L)	13	13	CI95(L)	28	30	CI95(L)	NA	NA
CI95(U)	42	42	CI95(U)	68	74	CI95(U)	NA	NA
N_b/N_s	0.79	0.79	N_b/N_s	1.10	1.12	N_b/N_s	NA	NA
Mean Rs	4.00	4.00	Mean Rs	2.92	2.78	Mean Rs	NA	NA
Var Rs	8.00	8.00	Var Rs	2.13	1.88	Var Rs	NA	NA

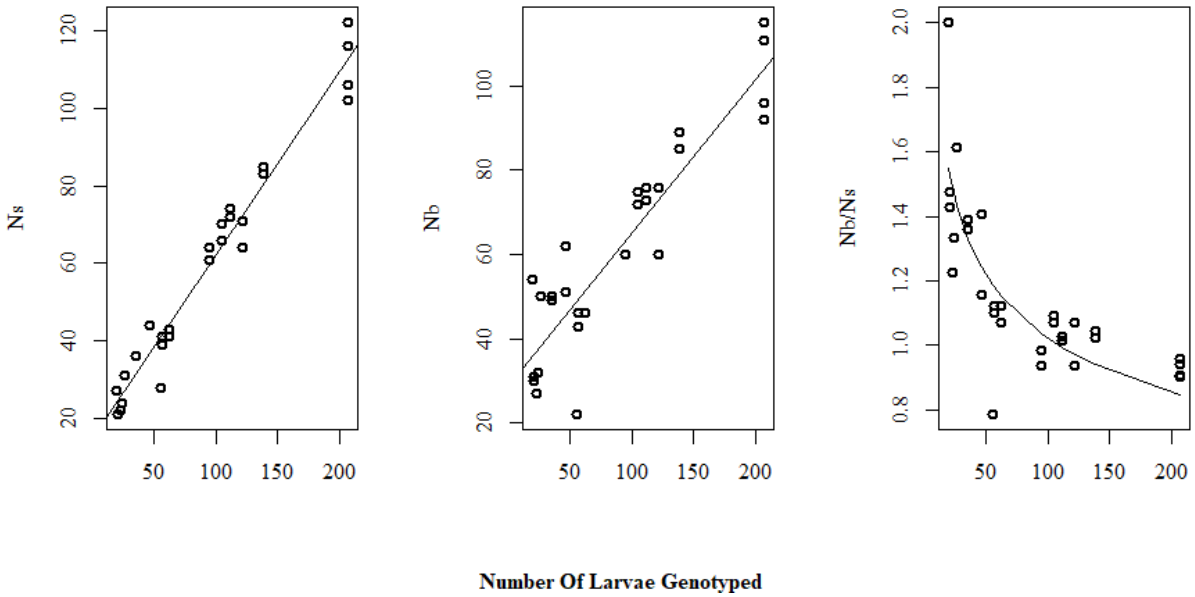

Figure 9. Plots of N_s, N_b, and N_b/N_s ratios by the number of larvae genotyped in the pedigree used to generate the estimate. N_s and N_b are positively correlated with sample size while N_b/N_s ratios are negatively correlated with sample size.

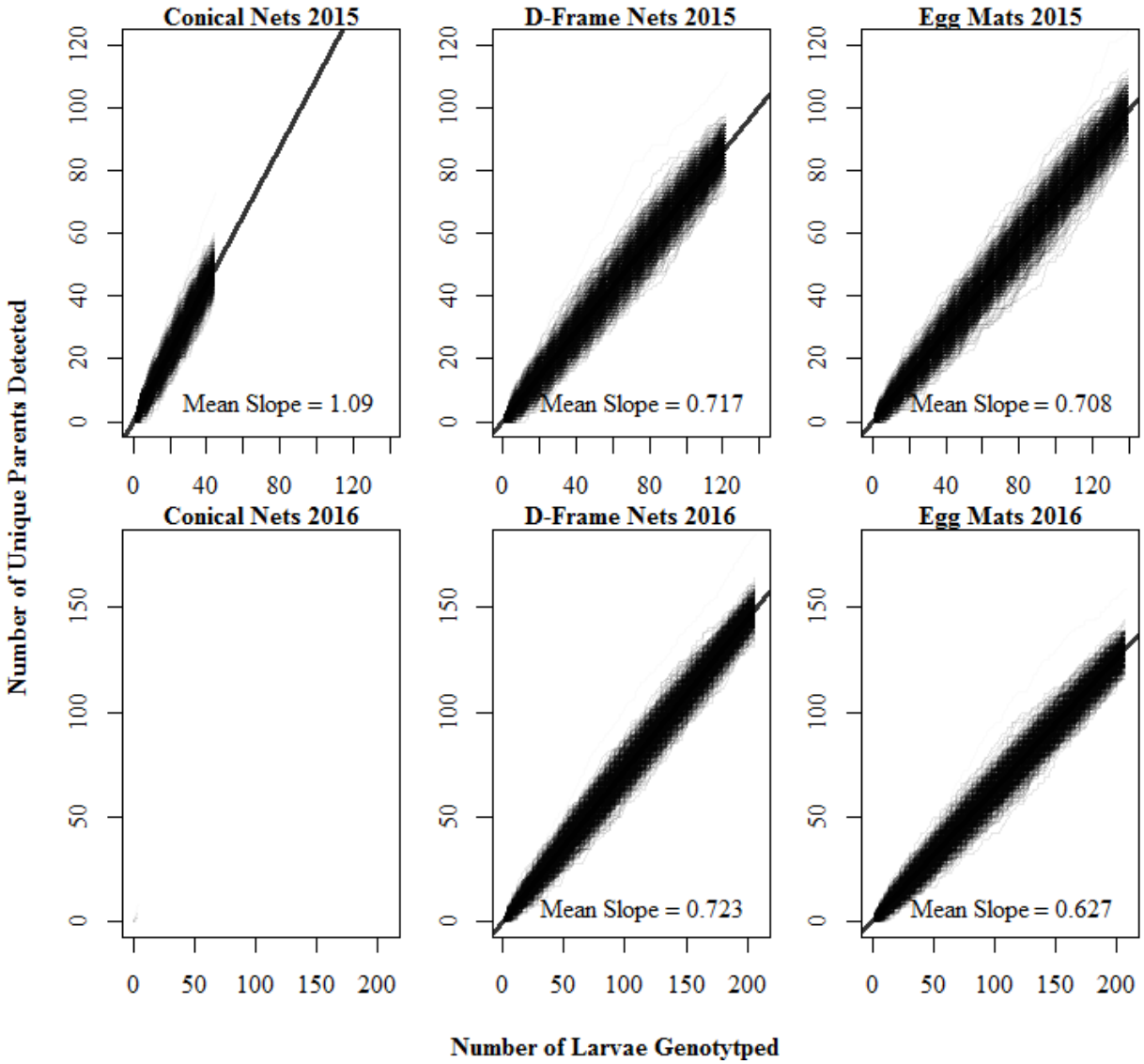

Figure 10. Plot of the rate of detection of unique parents per additional larvae genotyped by each collection method in 2015 and 2016 for each of 1000 bootstrapped gear and year specific pedigrees. Mean slope (solid line) represents the mean rate of detection of unique parents for each collection method each year. High degrees of concordance are observed across years for each collection method.

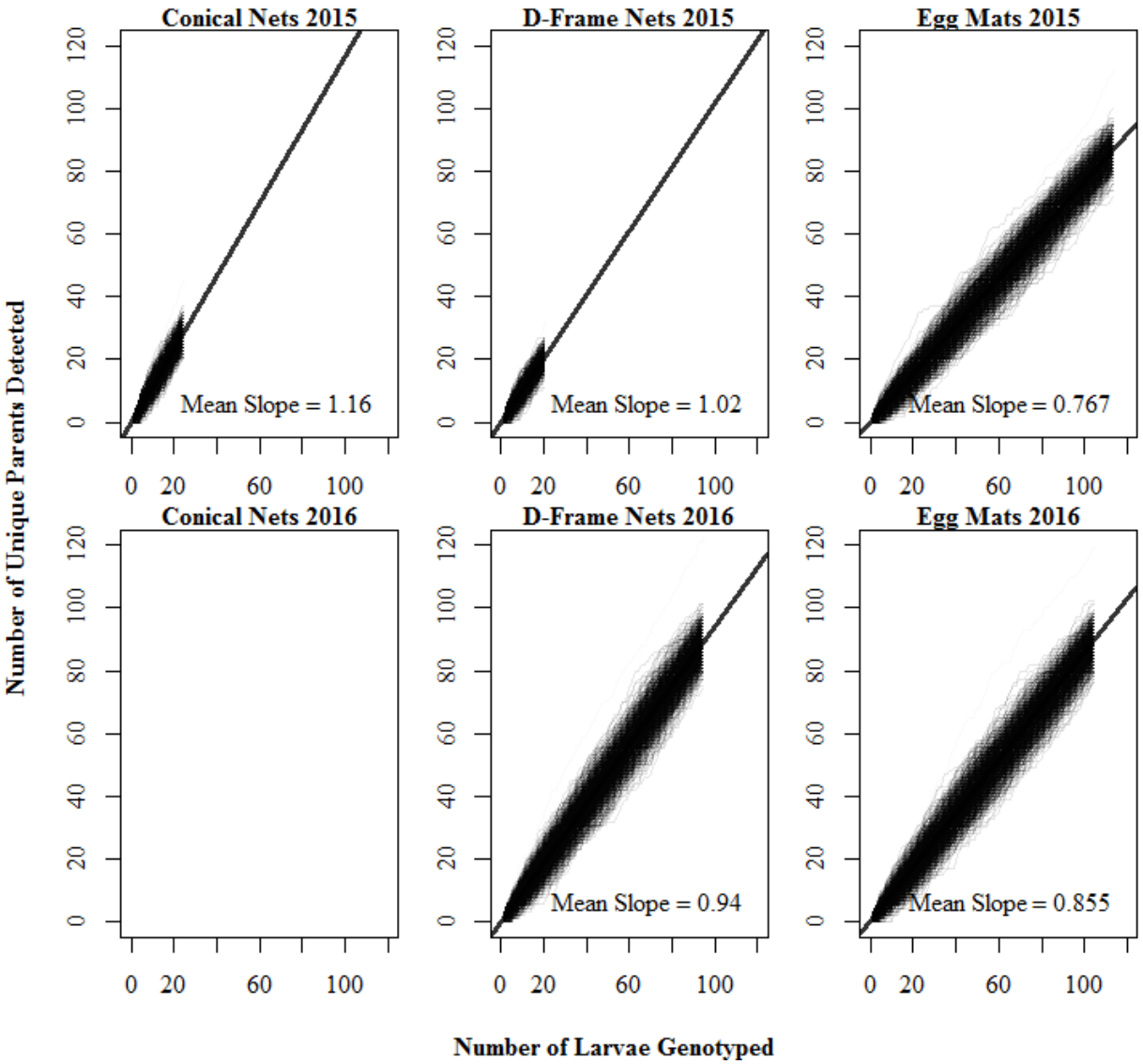

Figure 11. Plot of the rate of detection of unique parents detected per additional larvae genotyped for 1000 bootstrapped pedigrees for eggs and larvae collected at Harts Light Reef by collection method in 2015 and 2016. Mean slope (solid line) represents the mean rate of detection of unique parents for each collection method each year. No vertically stratified conical nets were sampled at Harts Light Reef in 2016.

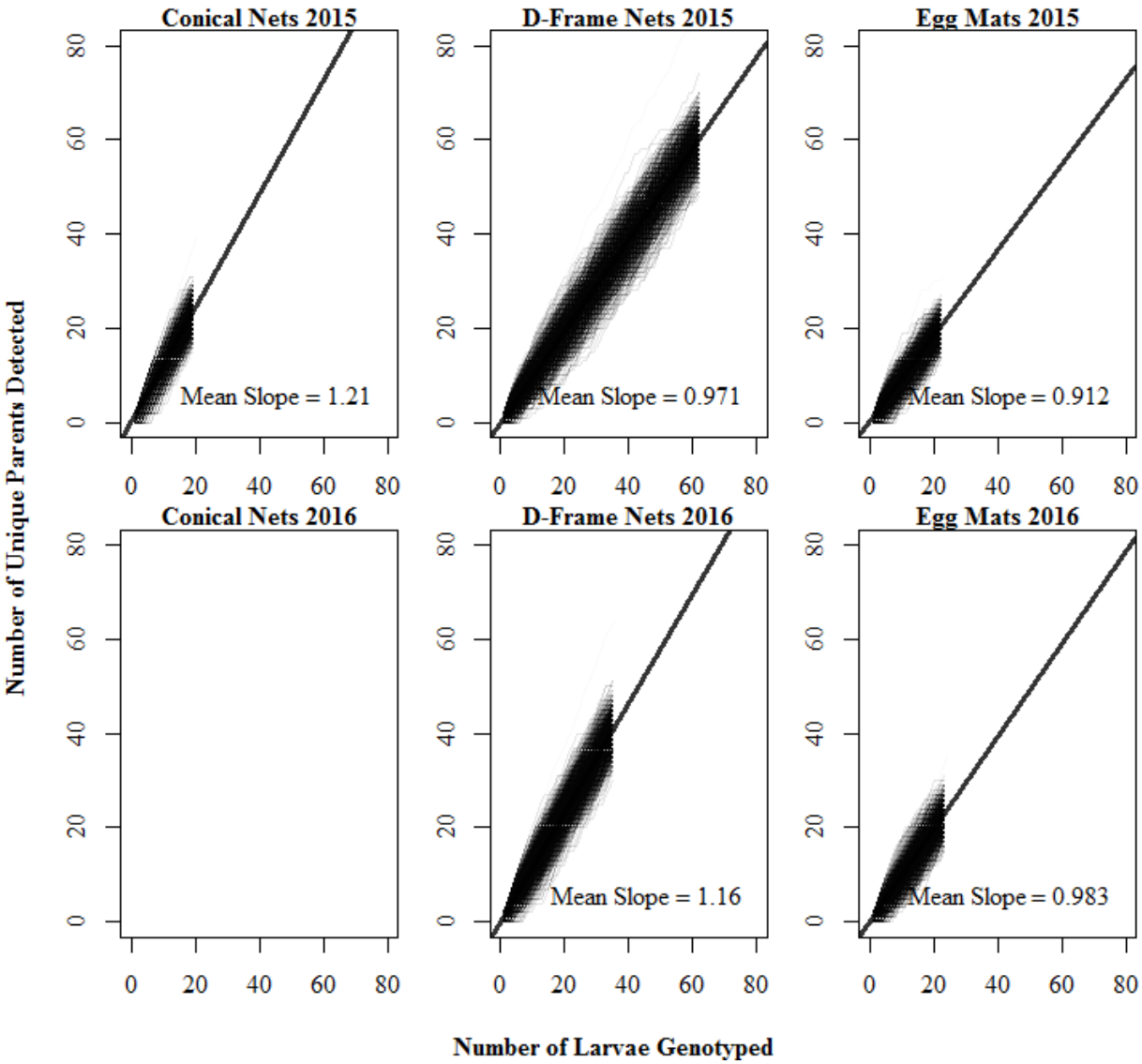

Figure 12. Plot of the rate of detection of unique parents detected per larvae genotyped for 1000 bootstrapped pedigrees for eggs and larvae collected at Pointe Aux Chenes Reef by collection method in 2015 and 2016. Mean slope (solid line) represents the mean rate of detection of unique parents for each collection method each year. No vertically stratified conical nets were sampled at Harts Light Reef in 2016.

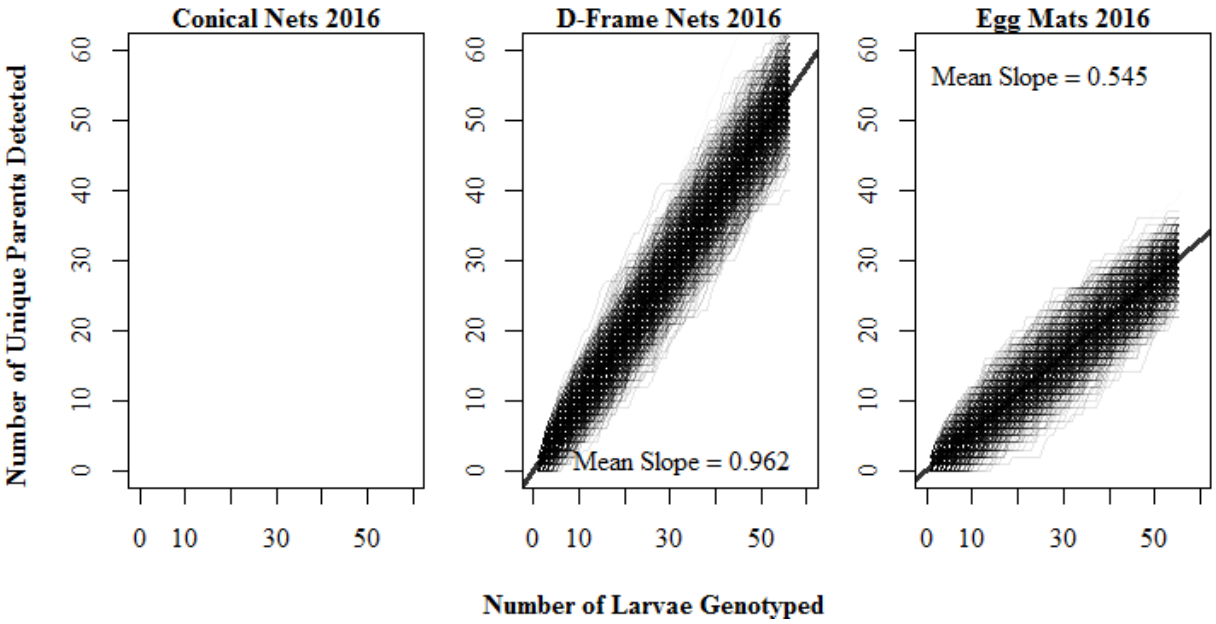

Figure 13. Plot of the rate of detection of unique parents detected per larvae genotyped for 1000 bootstrapped pedigrees for eggs and larvae collected at Grassy Island Reef by collection method in 2016. Mean slope (solid line) represents the mean rate of detection of unique parents for each collection method each year.

Table 11: 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.

	2015			2016
Location	**Conical vs Egg Mat**	**Conical Vs D-frame**	**D-frame Vs Egg Mat**	**D-frame Vs Egg Mat**
All Reefs	0.002*	0.002*	0.193	0.018*
Harts Light	0.037*	0.490	0.244	0.244
Pointe Aux Chenes	0.259	0.257	0.436	0.261
Grassy Island	NA	NA	NA	<0.001*

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

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CHAPTER 3: COMBINING COMMUNITY ECOLOGICAL THEORY AND MOLECULAR DATA TO ESTIMATE THE NUMBER OF SPAWNING ADULTS CONTRIBUTING OFFSPRING AT CONSTRUCTED SPAWNING REEFS

ABSTRACT

Assessment of constructed spawning habitat for lake sturgeon in the St. Clair-Detroit River System (SCDRS) was performed using enumeration of eggs and larvae collected with traditional methods and with genetic pedigree analysis. Egg and larval collections provided evidence of immediate use of recently constructed reefs in the SCDRS by spawning lake sturgeon (*Acipenser fulvescens*). Additionally, genetic pedigree analysis estimated the number of spawners contributing offspring to samples using lake sturgeon eggs and larvae collected at artificial reef sites. However, estimates from genetic pedigree analysis were strongly dependent on sample sizes for each site and collection method. Reliable estimates of the total number of spawners contributing offspring at an artificial reef site would inform future management efforts. The combination of genetic pedigree analysis and non-parametric species accumulation estimators provide a reliable method to extrapolate total spawner abundance from egg and larval fish samples. Simulations were used to compare the Chao and jackknife species accumulation estimation methods for use with pedigreed samples. Simulation results provided estimates of bias, accuracy, and precision for each method. Chao and jackknife were used to estimate total spawner abundance on artificial reef sites in the SCDRS in 2015 and 2016. From pedigrees constructed with between 23-138 larvae reared from eggs collected on egg mats, total spawner estimates at artificial reefs were estimated from 13-92 spawners over a two-week period for all reefs and years. Width of the 95% confidence intervals around estimates was tight averaging ±10% of the point estimate. Combining methods from community ecological and genetic techniques provide a novel means to overcoming difficulties of estimating spawner abundance.

INTRODUCTION

Due to expansive habitat modifications, limited spawning habitat availability has been identified as an important factor limiting lake sturgeon (*Acipenser fulvescens*) recruitment in the St. Clair-Detroit River System (SCDRS). Since 2004, seven artificial spawning reefs have been constructed to mitigate loss of spawning habitat for lithophilic spawners such as lake sturgeon (*Acipenser fulvescens*). To quantify the degree of restoration program success, levels of use of constructed spawning reefs by lake sturgeon are needed in the broad spatial context of the entire SCDRS. Specifically, researchers need methods to quantify the number of spawners contributing offspring (N_s) in spawning events at a reef.

Lake sturgeon populations have declined to less than 1% of their historic abundance (Hay-Chimelewski and Whelan 1997). Despite reduced fishing pressure (Auer 1996) and improved water quality in the SCDRS, lake sturgeon populations have not recovered. The ability of a fish stock to recover after severe population reductions is dependent on the population growth rate when the population is at low density (Liermann and Hillborn 1997). Prior to artificial reef construction only three known spawning sites remained in the SCDRS (Manny and Kennedy 2002). Given a finite number of spawners, dispersing individual spawning efforts over new, small sites, as opposed to two, historic spawning sites, could result in depensatory effects due to reduced spawner densities resulting in overall reduced probabilities of fertilization. In other broadcast spawners, low densities at spawning events has been attributed to reduced probabilities of fertilization due to sperm limitation (Levitan 1995) and other depensatory effects. Therefore, concern exists surrounding the construction of artificial reefs on the overall spawning success of lake sturgeon throughout the entire system.

Ensuring artificial reefs were utilized by lake sturgeon for spawning was the first measure of success for the reef program. Roseman et al. (2011a) provided compelling evidence that, following construction, lake sturgeon immediately used the reefs for spawning, and that eggs that were deposited on the reefs survived to the larval stage. However, lake sturgeon are highly fecund, producing 49,000-667,000 eggs per female (Peterson et al. 2007) with fecundity increasing with female body weight (Bruch et al. 2006, Peterson et al. 2007). It is possible that offspring from a single female could populate entire samples collected on egg mats, D-frame nets, and vertically stratified conical nets previously used to assess spawning at artificial reef sites in the SCDRS (Roseman et al. 2011a; Bouckaert et al. 2014). Due to high individual fecundity, numbers of eggs and larvae collected are not sufficient to estimate the number of spawners contributing offspring at artificial reefs. Even if large numbers of offspring are produced at artificial reefs, reducing the number of possible mates for polygamous specices like lake sturgeon can have population level genetic consequences. The effective number of breeders (N_b) is a measure of and ideal population size in a single spawning season where the rate of loss of population level genetic diversity is equal to genetic drift (Allendorf et al. 2013). Low ratios of N_b/N_s indicate the risk of accelerated rates of loss of population level genetic diversity.

The number of SCDRS spawners (N_s), mean and variance in individual reproductive success, and the effective number of breeders (N_b) that produced genotyped offspring were estimated using pedigree analysis (Hunter 2018, Chapter 1). Estimates of N_s, N_b, and N_b/N_s ratios were high in all years and at reef sites. All estimates were sample size dependent (Hunter 2018, Chapter 1 & 2) and only provide information on the number of parents detected. Managers and stakeholders would also like to have accurate estimates of the total number of spawners contributing offspring at an artificial reef.

Traditional mark-recapture methods allow for population size estimates, but capture and recapture rates for lake sturgeon are often low and confidence intervals around estimates are often wide (Wigrin et al. 1997; Thomas and Haas 2002). An additional limitation is that it is difficult to say an individual captured at a reef using traditional methods was indeed spawning at that reef. Determinations can be made regarding sex and stage for adults (Chiotti et al. 2016) that are captured, but the exact location at which they spawned is still largely unverified. However, larvae reared from eggs collected on egg mats used for pedigree analysis provided locations where adult lake sturgeon spawned (Hunter 2018, Chapter 1). Genetic pedigree analysis can allow for estimates of the number of spawners contributing offspring to a sample. However, estimates from genetic pedigree analysis were found to be sample size dependent (Hunter 2018, Chapter 2), and there is interest in estimating the total number of spawners contributing offspring at a location.

Community ecological theory has produced and evaluated estimators capable of generating point estimates and confidence around the number of unique species in a community (Walther and Moore 2005, Gotelli and Colwell 2011). The theory behind species accumulation estimators is that initially many new species are detected as new areas are sampled, and the species accumulation curve rises steeply. However, with greater sampling effort rarer species remain to be detected and the rate of accumulation of new species per sample asymptotes (Ugland et al. 2003). This asymptote is easily detected for species that were easily identified, or where a complete census of species can be obtained (Colwell and Coddington 1994). However, in cases where total enumeration was not possible, researchers must extrapolate to estimate the number of species in a community (Ugland et al. 2003). Many methods have been developed by which species accumulation data can be extrapolated to provide estimates of true species

accumulation (e.g., Chao 1987, Smith and van Belle 1984, Walther and Moore 2005). Common approaches to estimate species accumulation include rarefaction, parametric estimators, and non-parametric estimators (Hughes et al. 2001). In a review of species accumulation estimator performance, Walther and Moore (2005) found the non-parametric estimators (Chao2, Jack2, Jack1) performed best considering overall bias and accuracy.

Combining genetic pedigree analysis with established methods in community ecological theory would provide an estimate of the total number of spawners contributing offspring in a year at an artificial reef. The objectives of this study were to (1) demonstrate ability to estimate the total number of spawners contributing offspring at a spawning site using community ecological theory and simulated pedigrees and (2) apply the adapted methodology to estimate the most likely total number of spawners contributing offspring at artificial reef sites in the SCDRS.

METHODS

Simulating Known and Sampled Pedigrees

To simulate natural spawning behavior and offspring produced by lake sturgeon populations, simulations were constructed that incorporated assumptions based on literature and observations surrounding lake sturgeon reproductive success. First, the number of parents in the simulated population was randomly selected from a uniform distribution with a minimum of 20 and a maximum of 500. These values were chosen to represent the range of the number of spawners (N_s) detected in the SCDRS at individual reefs and within years. Simulated sex ratios were selected randomly from a uniform distribution of sex ratios ranging from 1.56-3.17 males to females as observed over a 16-year period for spawning lake sturgeon in the Black River, Michigan (Scribner unpublished data 2001-2017). The number of male and female parents was selected with a ratio that was closest to the randomly selected simulation sex ratio. If there was more than one possible combination of male and female parents that equaled the simulation sex ratio, one combination was randomly selected. If no combination of male and female parents exactly matched the simulation sex ratio, the closest combination was selected and the resultant sex ratio for that combination was calculated and used for the remainder of that simulation. The number of expected mates per mate pair was chosen from a probability distribution ranging from 2-8 mates (Peterson et al. 2007) with decreasing probabilities of mating with higher numbers of mates (Figure S5). The number of fertilized eggs per mate pair was assumed to be 2000-6000 viable fertilized eggs.

A breeding matrix was simulated that consisted of male parents as the number of rows and female parents as the number of columns. First, the number of males and female mates with

was determined based on the probability distribution for the number of mates (Figure S5). Males from the simulated population are randomly selected as possible mates for females. If the number of times a male was selected reaches the maximum number of mates, that male was eliminated as a possible mate for additional females. If males remain that have not mated, those males were assigned as mates to females that have not exceeded the maximum number of mates. The probability of mating between the unmated males and females was also based on the probability distribution for numbers of mates. The result was a matrix of simulated successful mate pairs (Table S4). The matrix was populated with the number of offspring produced from each parent pair, selected randomly from the assumed minimum and maximum number of fertilized eggs per mate pair (Table S5). A full simulated pedigree was constructed from the simulated breeding matrix consisting of all simulated larvae produced and the simulated mate pairs that contributed them.

To simulate the sampling of offspring using egg mats, a random number of offspring were drawn from a uniform distribution with a minimum of 20 and a maximum of 1000. Sub-sampling the simulated full population resulted in a subset simulated sample pedigree where the number of male and female spawners is not fully representative of the total number of spawners (Figure S6). The selected number of larvae were randomly drawn from the full simulated breeding matrix resulting in a mean and variance in individual reproductive success with a Poisson distribution ($\hat{\lambda}$=4) that approximates the observed mean and variance in the SCDRS in 2015-2015 (Figure S7). Larvae were the result of multiple simulated mate parings for both male and female lake sturgeon. Distributions of the number of simulated mates for male and female lake sturgeon are shown in Figure S4. Selected larvae and their contributing parents, were assembled in a simulated sample pedigree consisting of the unique larval identification number,

unique adult female identification number, and unique adult male identification number. The result was one simulated full spawning population pedigree and a corresponding simulated sample pedigree from a total of 1000 simulations.

The specpool function, package vegan (Oksanen et al. 2018) was used to generate estimates and standard error for the total number of parents contributing offspring using simulated sample pedigrees using the methods, Chao 1, Chao 2, jackknife 1, jackknife 2. Point estimates and 95% confidence intervals from simulated sample pedigrees were compared to the known numbers of adult spawners from the simulated full pedigrees.

The Chao Estimator

Chao (1984) developed a method of estimating the species accumulation by placing emphasis on rare species. Rare species provide greater information about the un-sampled species than common species do, so the method disproportionally weights singletons (species detected only once) and doubletons (species detected only twice) (Chao 1987). The Chao (1987) methods were modified by substituting the actual and observed number of species in a community with the actual and observed number of unique spawners, and the number of samples or quadrats with the number of offspring genotyped. The Chao method assumes continuous sampling, no error in parental assignment, and equal probability of collecting larvae from all spawners. Additionally, Chao assumes that the spawners contributing disproportionately fewer offspring contributed the most information about undetected spawners in the population. The Chao estimator used in this analysis is presented below (Eq. 3) where S_{pred} is the true number of spawners, S_{obs} is the number of spawners observed, a1 is the number of singletons or spawners occurring in pedigrees only once, and a2 is the number of doubletons or spawners occurring in pedigrees only twice.

$$S_{pred} = S_{0bs} + \frac{a1^2}{(2*a2)}$$ Eq. 3. (Chao estimator (Oksanen et al. 2018))

The Jackknife Estimator

Developed in 1949 and improved in 1958 by Quenoille and Tukey respectively, the jackknife estimation technique also weights rare species (Heltse and Forrester, 1983). The order of the jackknife estimate refers to the inclusion of singletons (1st order) (Gotelli and Colwell 2011). Similar to the Chao estimator, singletons refer to spawners that are only detected once. Bias in estimates from jackknife are reduced by sub setting the data and making estimates based on reduced sample sizes (Gotelli and Colwell 2011). For example, estimates for a 1st order jackknife are obtained using *n* samples where one sample is randomly eliminated from each of the *n* samples. Jackknife has been widely applied as a technique for estimating asymptotic species accumulation (Burnham and Overton 1978; Boulinier et al. 1998; Hellmann and Fowler 1999). In the jackknife estimator (Eq. 4), S_{pred} is the true number of spawners, S_{obs} is the number of spawners observed, a1 is the number of singletons, and *n* is the number of sampling sites.

$$S_{pred} = S_{obs} + a1 \times \frac{(n-1)}{n}$$ Eq. 4. (1st order jackknife (Oksanen et al. 2018))

Collection of Lake Sturgeon Eggs

The SCDRS (Figure 18) is a large, non-wadable international waterway connecting Lake Huron to Lake Erie. Three artificial reef sites were assessed in 2015 and 2016 to quantify the extent of use by spawning lake sturgeon. Harts Light Reef is the northern most artificial reef in the SCDRS and is 3.8-acres of 10-5cm sorted limestone. Harts Light Reef was assessed in 2015 and 2016 following construction in 2014. Also assessed in 2015 and 2016, Pointe Aux Chenes

Reef is 1.8-acres of 10-15cm sorted limestone. In 2015, Grassy Island Reef was constructed in the Detroit River. Grassy Island Reef is 4.0-acres of 10-15cm sorted limestone. High water velocities (0.80-1.35m^3/s), depth (12-16m), and heavy occurrence of commercial shipping and recreational boating traffic make these areas extremely difficult to sample. Additional sampling using benthic D-frame and vertically stratified conical nets required removal of egg mats in the areas of the reef. As a result, egg mats were not sampled for the duration of the spawning period. In 2015, egg mats were sampled from 28 May – 9 June at Harts Light and Pointe Aux Chenes Reefs. In 2016, egg mats were sampled from 11 May – 25 May at Grassy Island, and from 30 May – 13 Jun at Harts Light and Pointe Aux Chenes Reefs.

Larvae reared from eggs collected on egg mats were chosen for analysis because collection of eggs provides a precise location at which parents contributed offspring. Benthic egg mats were deployed on three artificial reefs to collect lake sturgeon eggs in 2015 and 2016 as described in Hunter 2018, Chapter 1. Egg mats were constructed from a 38 x 24 x 0.5 cm steel frame that was covered with a furnace filter secured by 2.5 cm binder clips (Manny et al. 2010; Roseman et al. 2011b). Egg mats were deployed in sets of three mats as described in Roseman et al. (2011b), and were collected weekly. Eggs were identified and enumerated and egg mats were returned to their original sampling location. Subsamples of lake sturgeon eggs were returned to the lab for rearing (Sutherland et al. 2014). Lake sturgeon larvae were reared until the yolk sac was absorbed to ensure adequate tissue for DNA extraction. Larvae were euthanized and preserved in 95% ethanol. Handling of fish was performed according to the American Fisheries Society guidelines for care and use (http://fisheries.org/docs/wp/Guidelines-for-UseofFishes.pdf).

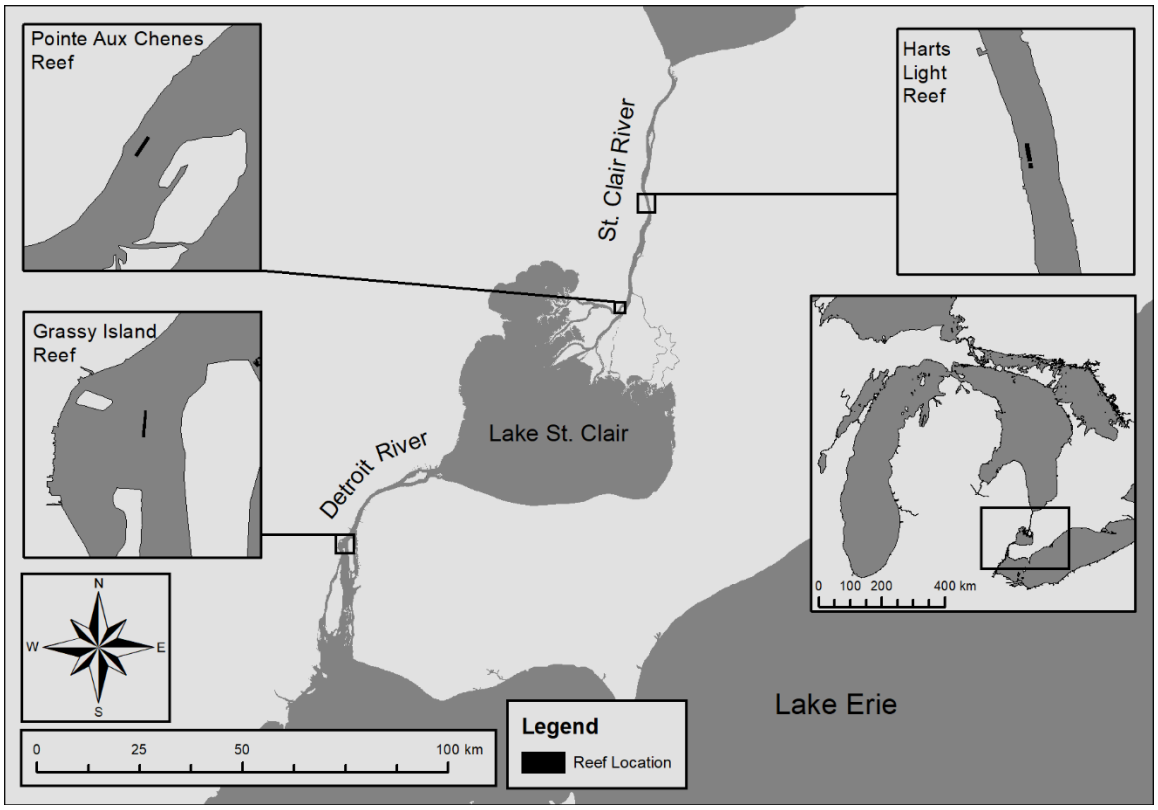

Figure 14. Map of three constructed spawning reef locations in the St. Clair-Detroit River System. Location of constructed reefs is highlighted in pullouts and reefs are indicated by black bars.

Extraction and Amplification of DNA

Post vent tissue was removed from larvae and DNA was extracted according to manufactures protocols using QIAGEN DNeasy® kits (QIAGEN Inc.). DNA was suspended in sterile water at a standard concentration (20 ng/µl) determined using a nano-drop spectrophotometer.

Amplification of DNA was accomplished using polymerase chain reaction (PCR). DNA was amplified across 13 optimized 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) and 5 polysomic loci: Atr100, Atr114, Atr117, AciG35, and AciG110 (Rodzen and May (2002). All PCR conditions are described in Duong et al. 2013 and Jay et al. 2014 for disomic and polysomic alleles respectively.

Reactions were performed using 25µl reactions containing: 5µl of 2µl DNA, 10x PCR buffer (1M Tris-HCL, 1M MgCl2, 1M KCL, 10% gelatin, 10% NP-40, 10% Triton-X), MgCl2, 2mM dNPT 10pmol primer (forward and reverse), and 0.5µl Taq polymerase. Amplified DNA was diluted and multiplexed for analysis on an ABI 3730xl DNA analyzer (Michigan State University Research Technology Support Facility). Allele sizes were determined through the use of commercial size standards (MapMarkerTM and BioVentures Inc.). Alleles scores were independently determined and confirmed by two scorers using GeneMapper (Softgenetics, StateCollege, PA). Quality assurance and control included three lake sturgeon with known genotypes, a DNA negative sample, and approximately 10% of all samples were reanalyzed across all 18 loci to determine genotyping error rates. Observed error rates in 2015 and 2016 were 0.5% and 1.8%, respectively

Genetic Pedigree Analysis

Genetic pedigrees were developed from genotypes of lake sturgeon larvae reared from eggs collected on egg mats at artificial spawning reefs in the SCDRS using program COLONY (Wang 2004). Rozden et al. (2004) and Wang and Scribner (2014) describe methods of treating mendelian allele scores as pseudo-disomic loci where alleles are score as present (1), absent (2), and missing data (0). Loci were considered missing data if despite 2 attempts, they failed to amplify. Genotyping from 741 total larvae yielded 164 pseudo-disomic loci. Hunter (2018, Chapter 1) demonstrated the ability of 205 microsatellite markers treated as pseudo-disomic loci to infer the number of parents and mate pairs that contributed offspring to a sample. Parameters in program COLONY were default except for, polygamy (males and females), high likelihood precision, and no prior sib-ship knowledge. Pedigrees consisted of unique larval ID's and unique parent ID's identical to simulated population and simulated sample pedigrees described earlier.

Estimates generated here assume that genotypes and subsequent pedigrees are estimated without error. When parent and offspring genotypes are used, this is likely violated to some extent due to rates of allelic dropout (the failure of individual alleles to amplify), mutation, genotyping error, contamination, and data management (Wang 2004). Program COLONY does allow incorporation of error in analysis (Wang 2004). Pedigrees from empirical data were subsequently constructed in Program COLONY using an error rate of 2% for allelic dropout and 0.1% for all other error. Estimates of the total number of spawners contributing offspring at a reef in the SCDRS in 2015 and 2016 were constructed using specpool, package vegan (Oksanen et al. 2018) in the same manner as estimates for simulated sample pedigrees.

RESULTS

Simulation Based Estimates – Bias, Accuracy, and Precision

Results of five of 1000 simulations estimating the total number of male and female lake sturgeon contributing offspring using simulated full population and simulated sample pedigrees are presented in Table 11. Low proportions of offspring were sampled in each simulation (<0.01%). Simulated observed numbers of parents was lower than the simulated true numbers of parents. Of the true number of parents, 20-97% were observed in simulated sample pedigrees. Probabilities the simulated true number of male and female lake sturgeon were outside the calculated 95% confidence intervals for estimates based on simulated sample pedigrees were examined (Figure 19). At low sample sizes, 95% confidence intervals for Chao estimates contain the simulated true number of male and female lake sturgeon more often than estimates made using Jackknife 1. However, as sample sizes increase, estimates made using Jackknife 1 outperform Chao estimates (Figure 19). Bias for each estimator was also calculated as the percentage of simulations where the simulated true number of spawners was above, within, or below the confidence intervals. Both estimators were negatively biased. The simulated true number of spawners was above 45.2% of the time and within 95% confidence intervals 54.8% of the time using Chao. Using Jackknife 1 simulated true numbers of spawners was above the confidence intervals 35.3% and within the 95% confidence intervals 64.7% of the time. To examine precision, deviation of simulated true numbers of male and female lake sturgeon contributing offspring outside 95% confidence intervals for estimates made with Chao and Jackknife 1 are presented in Figure 20. Deviations outside 95% confidence intervals are higher for estimates made with Jackknife 1 compared to Chao at low sample sizes. However, at sample

sizes above 200 larvae, Jackknife 1 quickly began to outperform estimates made using Chao (Figure 20). Accuracy was also examined by comparing the absolute difference between estimates using Chao and Jackknife 1 with the true simulated number of spawners. Over 1000 simulations Chao outperformed Jackknife 1 with an average deviation of 15.6 spawners. Jackknife deviated on average by 22.8 spawners. Width of the confidence intervals around estimates for 1000 simulations is presented in Figure 21. At low sample sizes, width of the 95% confidence intervals for estimates made using Chao are comparatively larger than those made with Jackknife 1. For both methods width of the 95% confidence intervals decreases as a function of sample size (Figure 21).

Table 12. Example of estimates of the total number of parents contributing offspring for five simulations generated using Chao and jackknife-1 estimators. The observed number of parents (Obs. N) is lower than the true number of parents (True N). True simulated numbers of offspring (True Noff) and simulated sample numbers of offspring (Sample Noff) are presented for each simulation. The number of males and females are estimated separately in each simulation. Variance in individual reproductive success and total number of spawners between sexes means that estimates for males and females are generated with different precision.

Estimates Of The Total Number Of Parents Contributing Offspring								
True N	Obs. N	Chao	Chao SE	Jack 1	Jack 1 SE	True Noff	Sample Noff	Sex
162	145	159.29	6.67	177.92	5.73	4446735	410	mom
292	207	274.41	18.27	299.77	9.62	4446735	410	dad
103	100	103.33	2.91	109.98	3.15	4672386	413	mom
318	219	296.82	20.05	321.75	10.12	4672386	413	dad
47	16	27.52	9.29	26.48	3.16	1489059	21	mom
99	20	191.90	189.59	38.10	4.15	1489059	21	dad
109	90	97.78	4.55	111.91	4.67	3169688	249	mom
207	140	222.02	24.87	216.69	8.74	3169688	249	dad
42	41	41.50	1.03	42.99	1.41	1448137	219	mom
97	86	108.03	10.70	114.87	5.36	1448137	219	dad

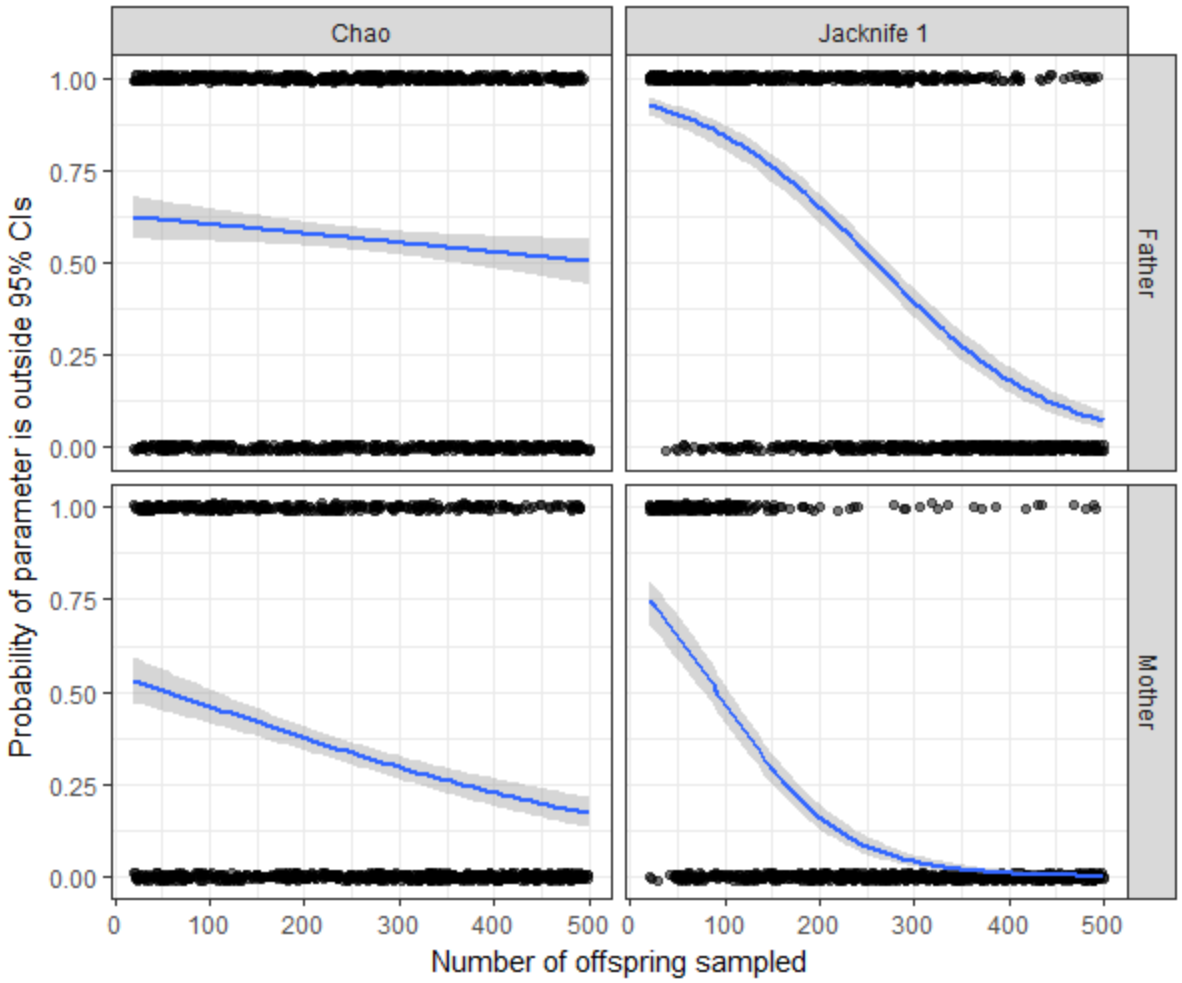

Figure 15. Plots of discordance between simulated true numbers of male and female lake sturgeon contributing offspring and estimates made using Chao and jackknife 1. Discordance is determined as the probability that the true number is outside the estimated 95% confidence intervals for each method. Points represent estimates from individual simulations, the blue line is a smoothed binomial function fitted to the points, and the grey bar represents the 95% confidence interval for the fitted line.

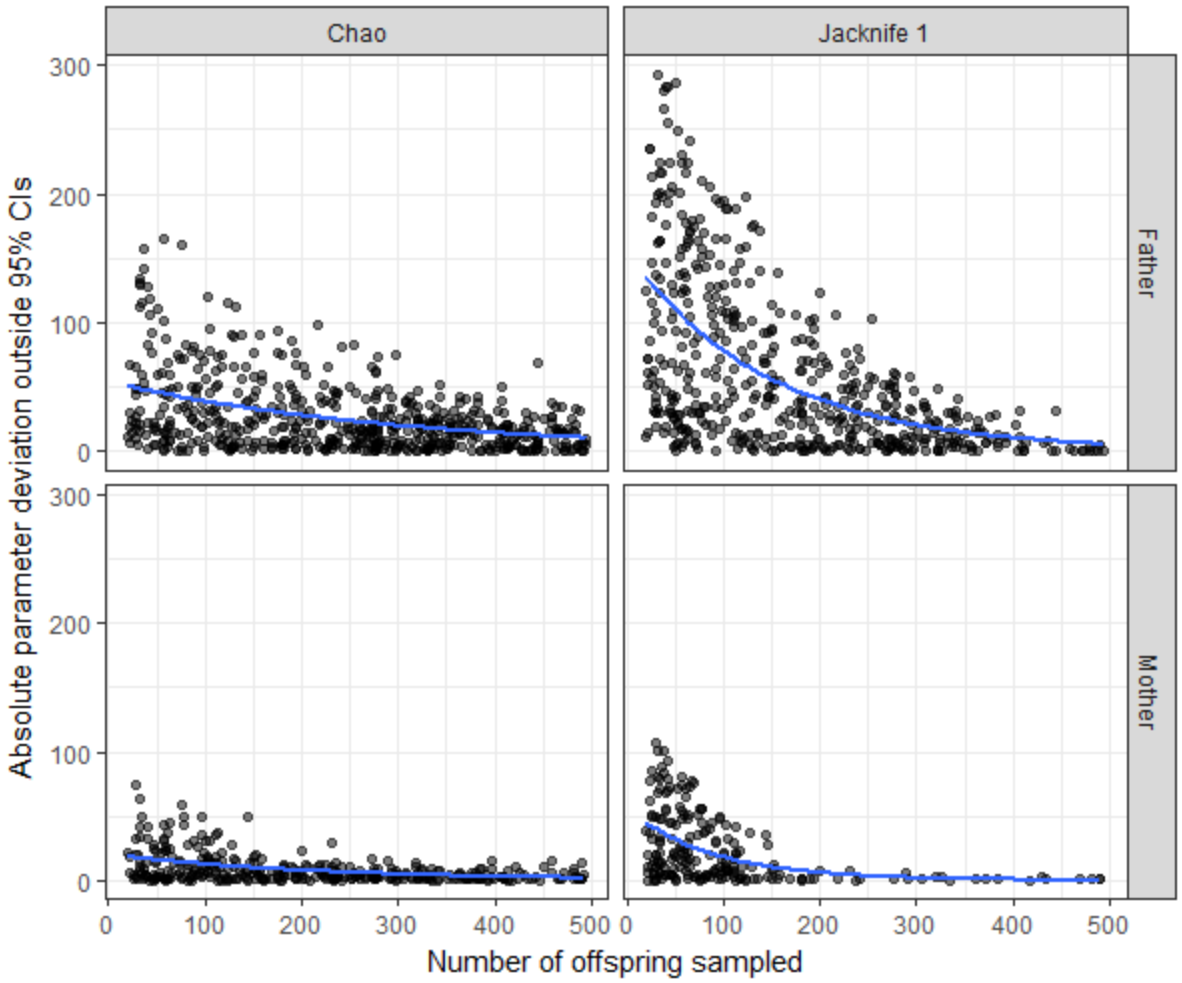

Figure 16. Deviation outside the 95% confidence intervals for estimates generated using Chao and jackknife 1 for 1000 simulations. Deviation refers to the difference between the simulated true number of male and female lake sturgeon contributing offspring and the upper or lower bound of the 95% confidence intervals surrounding estimates. Points represent individual simulations, and the blue line is a smoothed line fitted to the points.

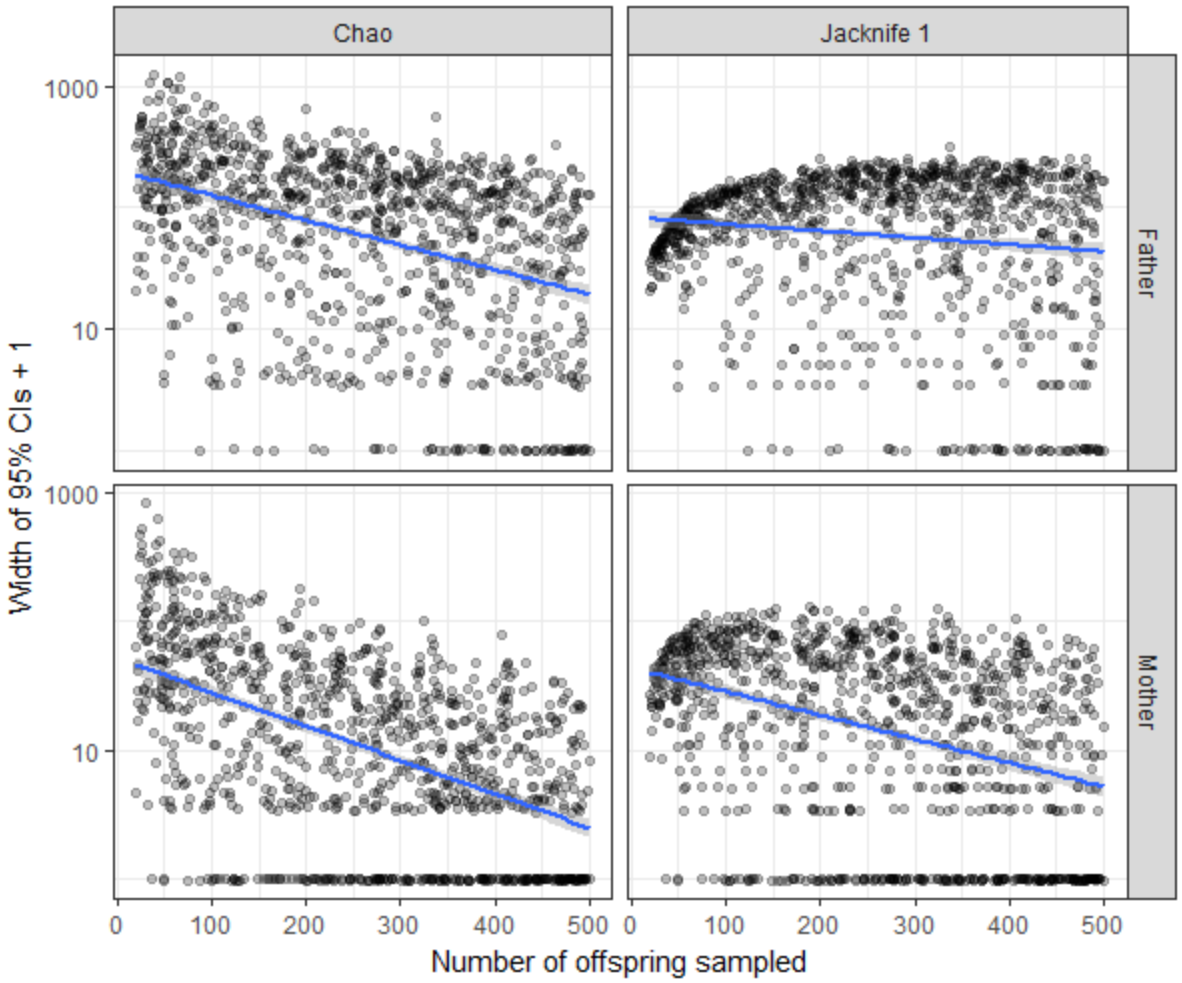

Figure 17. Plots of the width of the confidence intervals surrounding estimates of the total number of spawners contributing offspring made using Chao and jackknife. Individual points represent the width of the confidence intervals for individual simulations, the blue line is a linear regression fitted to the points, and the grey bar represents the 95% confidence interval around the regression line. Both estimates show width as a decreasing function of sample size.

Empirical Estimates of the Total Number of Spawners Contributing Offspring at Constructed Spawning Reefs

Estimates of the total number of spawners contributing offspring on three artificial reefs in 2016 and 2016 were made using genetic pedigrees generated of larvae reared from eggs collected on egg mats at each reef. Pedigrees generated from only larval genotypes of lake sturgeon do not provide definitive sex of the parents that contributed each larvae. Instead unique putative parent identification numbers are assigned. As a result, analysis of the number of parents using empirical data will consider only unique parent identification numbers for an estimate of total number of spawners pooled across sexes. Observed numbers of parents and estimated total number of parents increases as sample size increases (Table 12). Estimates of total numbers of parents are higher for each reef each year using Jackknife 1 compared to Chao. However, differences in estimates of total numbers of parents contributing offspring between methods vary by only 1-7 parents across years and reefs. From 138 larvae sampled in 2015, 83 unique parents were observed from genetic pedigree analysis at Harts Light Reef. Estimates for the total number of parents contributing offspring at Harts Light Reef in 2015 were 85 and 92 parents using Chao and Jackknife 1 respectively. Estimates at Pointe Aux Chenes ranged from 11-37 parents for Chao and 13-32 for Jackknife1 in 2015 and 2016 respectively. Grassy Island Reef was only sampled in 2016 and estimates for Chao and Jackknife were similar, 30 and 33, respectively. All estimates reach an asymptote and confidence intervals converge as sample size increases (Figures 22-24).

Table 13. Table of estimates of the total number of parents contributing offspring at constructed spawning reef sites in 2015 and 2016. The observed number of parents in the sample (Obs. N), number of offspring sampled (Sample Noff), and estimates and standard errors from Chao and Jack 1 are presented.

	Empirical Estimates Of Total Number Of Parents Contributing Offspring								
	Year	Obs. N	Chao	Upper 95%	Lower 95%	Jack 1	Upper 95%	Lower 95%	Sample Noff
Harts Light	2015	83	84.9	85.9	81.7	91.9	97.8	86.1	138
	2016	66	69.1	70.0	63.6	74.9	80.7	69.1	105
Pointe Aux Chenes	2015	10	11.4	12.9	9.3	12.9	16.1	9.6	23
	2016	24	26.8	27.7	21.9	31.7	37.6	25.7	24
Grassy Island	2015	NA	NA	NA	NA	NA	NA	NA	NA
	2016	28	30.1	31.2	26.7	32.9	37.2	28.6	56

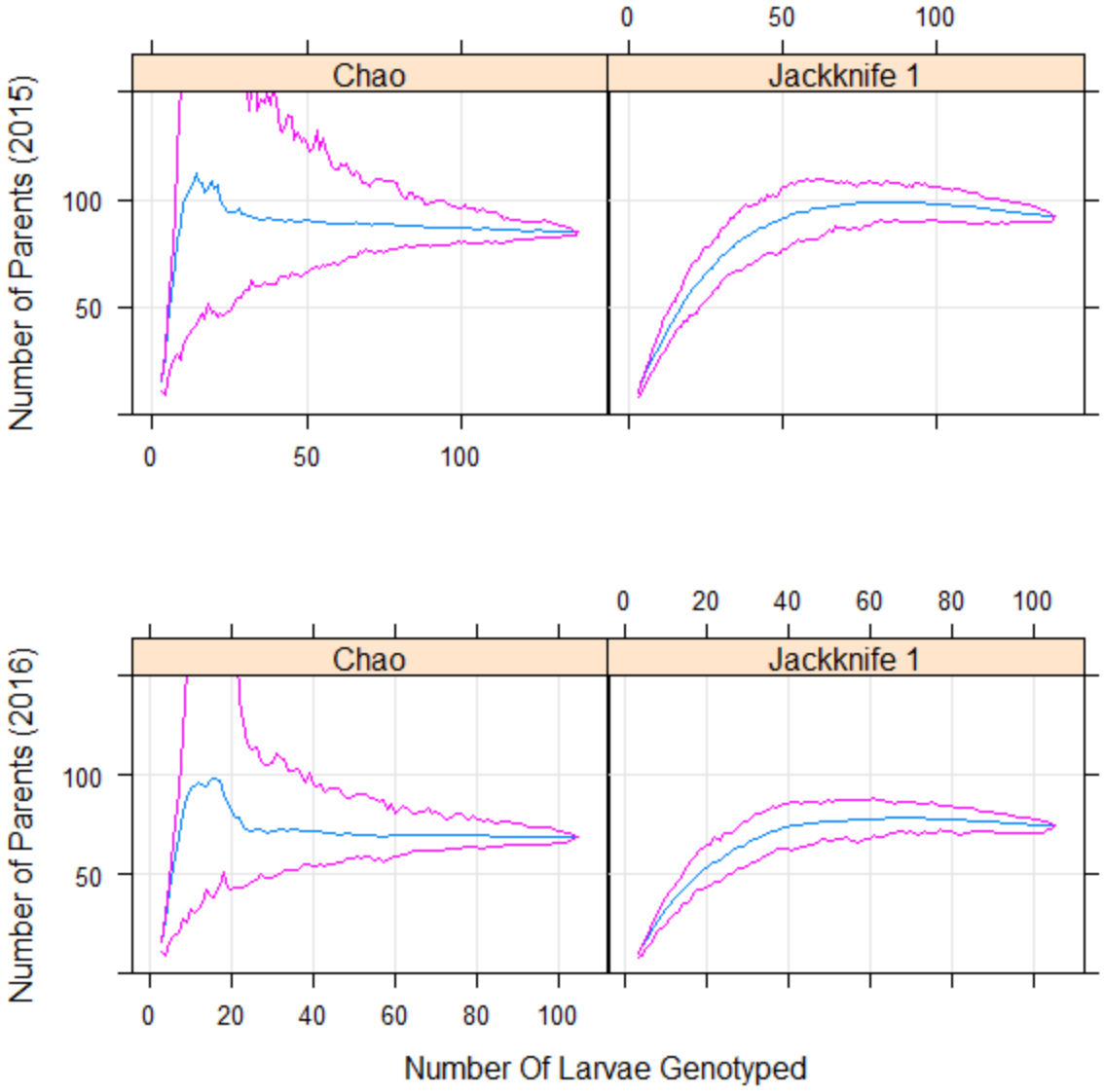

Figure 18. Plot of parent accumulation curves in blue with 95% confidence intervals in pink for Harts Light Reef in 2015 and 2016.

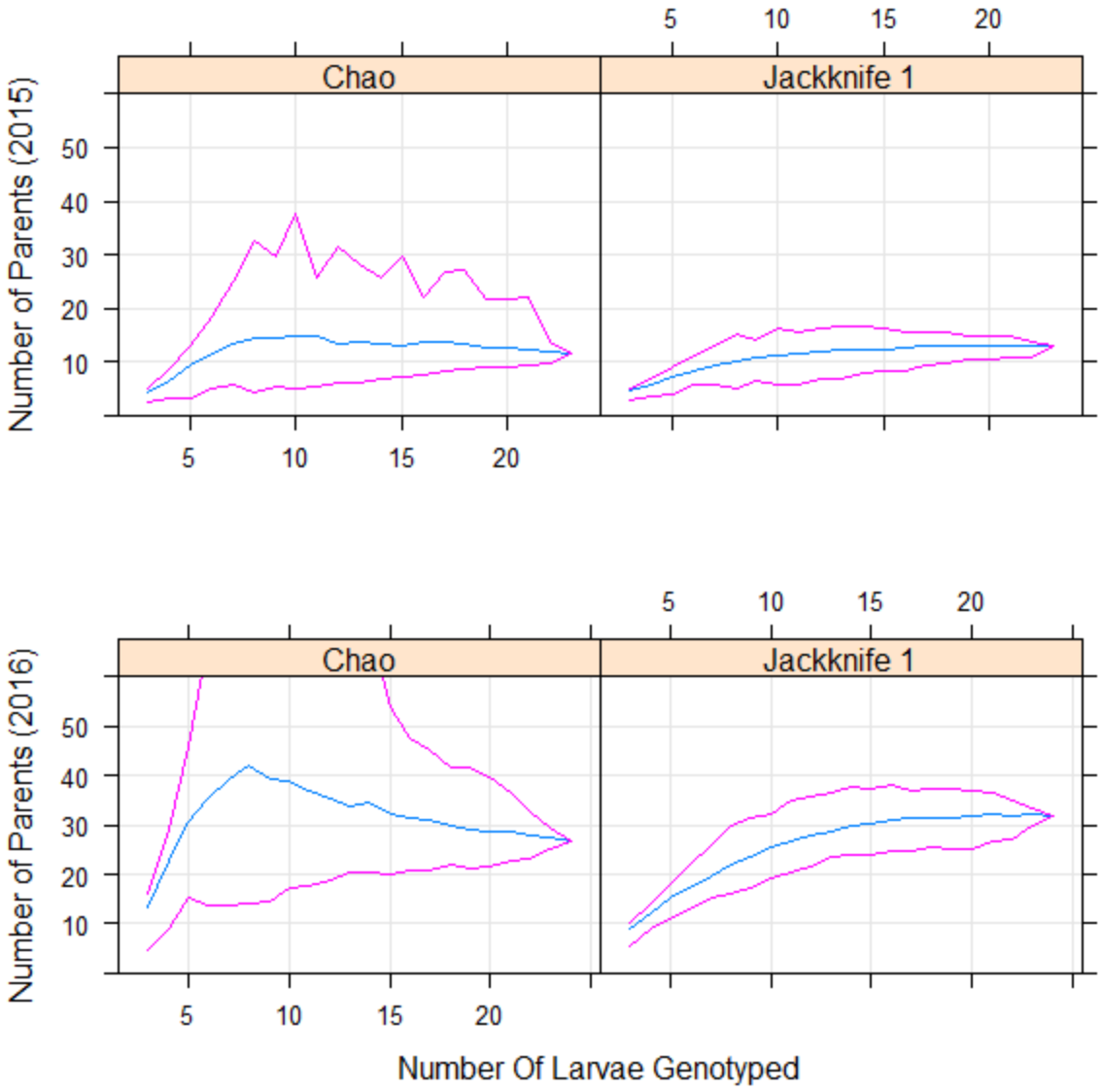

Figure 19. Plot of unique parent accumulation curves generated by Chao and jackknife 1 estimates for the total number of spawners at Pointe Aux Chenes Reef in 2015 and 2016. Blue lines represent estimated total number of spawners and pink lines represent 95% confidence intervals.

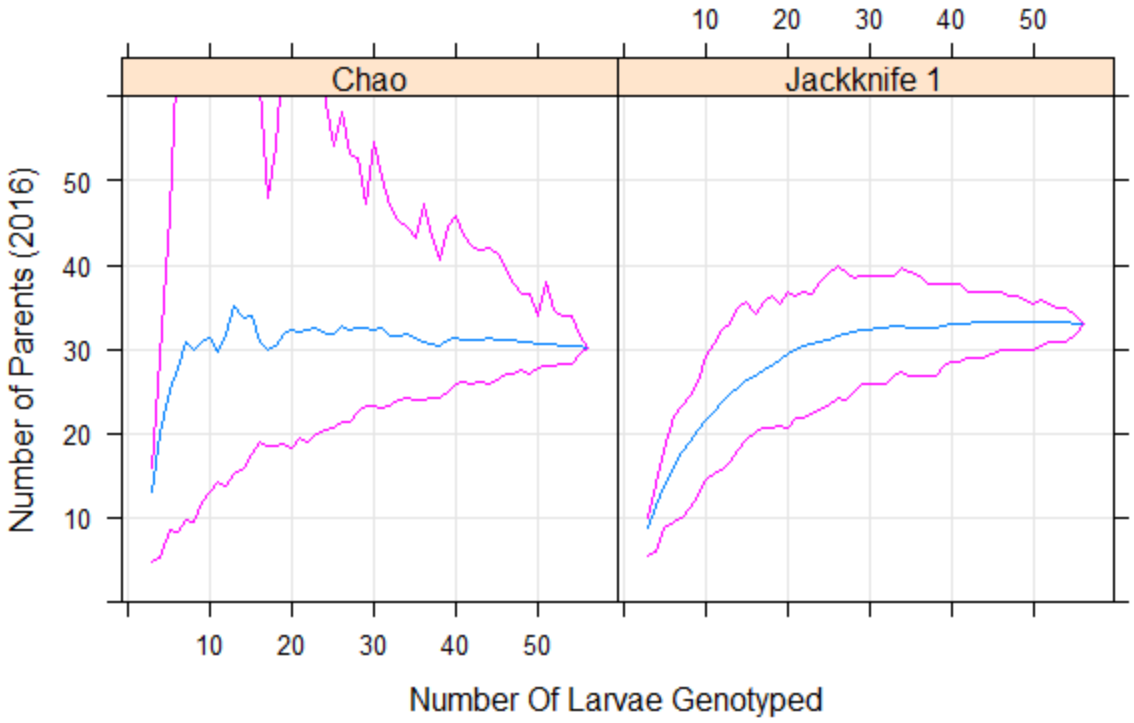

Figure 20. Plot of unique parent accumulation curves at Grassy Island Reef in 2015 and 2016. Estimates were generated using Chao and Jackknife 1. Estimated total number of parents is represented by the blue line and 95% confidence intervals around the estimate in pink.

DISCUSSION

Critical assessment of habitat remediation efforts aimed at increasing populations of threatened species like lake sturgeon is necessary to inform and direct future management action. Traditional assessment methods can provide estimates of the numbers of lake sturgeon eggs and larvae associated with restoration areas relative to un-restored habitat. However, lake sturgeon are a highly fecund species (Bruch et al. 2006; Caroffino et al. 2010) and larval abundances are unable to provide reliable inferences into the number of parents spawning at artificial reef sites. Genetic pedigree anlysis in concordance with traditional assessment methods provided estimates of N_s, N_b, and mean and variance in individual reproductive success (Hunter 2018, Chapter 1). However, all estimates were found to be a function of sample size (Hunter 2018, Chapters 1 &2). Managers and stakeholders could benefit from accurate methods to estimate the total number of spawners contributing offspring at an artificial reef site.

Comparison of the two methods revealed differences in bias, accuracy, and precision associated with estimates. Hellmann and Fowler (1999) list a series of criteria for selection of estimators and sample size that included examining estimator bias, precision, and accuracy. Analysis of simulated full pedigrees and simulated sampled pedigrees demonstrated that the application of species accumulation estimators like Chao and Jackknife 1 can provide reliable point estimates and confidence intervals around the true number of spawners. Additionally, estimates varied in the width of estimated 95% confidence intervals and degree of error present as a function of sample size. Both estimators were negatively biased, and across 1000 simulations the total number of spawners was never below the 95% confidence intervals. When examining precision across 1000 simulations, Jackknife 1 outperformed Chao. Estimates were

more often within the 95% confidence intervals for Jackknife, and the confidence intervals were often more conservative for Jackknife when compared to Chao. However, the accuracy of the Chao estimator averaged across 1000 simulations was superior compared to Jackknife 1.

Simulations provide compelling evidence that reliable estimates of the total number of spawners can be made using genetic pedigree analysis and community ecological theory. However, simulations consist of perfectly constructed pedigrees free of genotyping and assignment error. In reality, genotyping errors and mutation rates can have significant impacts on the accuracy of assignment in pedigree analysis (Wang 2004). When used with empirical data it is critical that typing error be minimized during amplification and accounted for in analysis as described in Wang (2004). Such measures ensure additional uncertainty added by genotyping error is minimized regardless of the estimator used.

Estimates based on empirical data analyzed here from the SCDRS in 2015 and 2016 are higher than the observed number of spawners in the samples. However, difference between the estimated and observed number of spawners were small. Unique parent accumulation curves generated for each reef and year by each method asymptote and confidence intervals begin to converge. Sample sizes for each estimate are small (23-138), and may not provide enough information for reliable estimates to be generated by either method. Hellmann and Fowler (1999) showed the effect of sample size on bias, accuracy, and precision for the Jackknife 1 estimator. Bias and accuracy are optimized for Jackknife 1 when greater than 37-44% of the total number of species are observed in the original sample, and estimates were downwardly bias for small sample sizes (Hellmann and Fowler 1999). When used with pedigree analysis, this means that >40% of the total number of spawners should be detected through sampling to minimize bias. It is unlikely that the 10 – 83 spawners observed in pedigrees from the SCDRS

are reflective of 40% of the total spawners contributing offspring at artificial reefs. As a result, it is likely that estimates are biased low. However, egg mats were only sampled at each location for about a two-week period, likely missing a portion of spawning activity given lake sturgeon spawning can last (19-43) days and may result in more than one peak in spawning activity (Forsythe et al. 2011). As a result, estimates may be interpreted as the total number of spawners contributing offspring at each artificial reef in a two-week period at the beginning of the spawning season. Results of simulations suggest reliable estimates of the total number of spawners at each reef could be generated using the described methods. To ensure reliable estimates are generated, sample sizes (number of larvae genotyped) at each location may be increased by sampling egg mats for the duration of the spawning period.

The combination of traditional and genetic methods with community ecological theory described here can allow estimation of the numbers of spawners contributing offspring at a location by collection of eggs and larvae. Such estimates were previously unattainable due to the difficulty of detection of the species and the limitations of working in a large, non-wadable river system. The ability to estimate the total number of spawners contributing offspring by combining species richness estimators and genetic pedigree analysis provides a novel method for the critical analysis of habitat remediation efforts for lake sturgeon throughout their range.

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LITERATURE CITED

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