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Influences of multilocus heterozygosity on size during early life

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Abstract

Genetic diversity has been hypothesized to promote fitness of individuals and populations, but few studies have examined how genetic diversity varies with ontogeny. We examined patterns in population and individual genetic diversity and the effect of genetic diversity on individual fitness among life stages (adults and juveniles) and populations of captive yellow perch (*Perca flavescens*) stocked into two ponds and allowed to spawn naturally. Significant genetic structure developed between adults and offspring in a single generation, even as heterozygosity and allelic richness remained relatively constant. Heterozygosity had no effect on adult growth or survival, but was significantly and consistently positively related to offspring length throughout the first year of life in one pond but not the other. The largest individuals in the pond exhibiting this positive relationship were more outbred than averaged size individuals and also more closely related to one another than they were to average-sized individuals, suggesting potential heritability of body size or spawn timing effects. These results indicate that the influence of heterozygosity may be mediated through an interaction, likely viability selection, between ontogeny and environment that is most important during early life. In addition, populations may experience significant genetic change within a single generation in captive environments, even when allowed to reproduce naturally. Accounting for the dynamic influences of genetic diversity on early life fitness could lead to improved understanding of recruitment and population dynamics in both wild and captive populations.

KEYWORDS

aquaculture, fish, heterozygosity–fitness correlation, microsatellite, *Perca flavescens*, relatedness

1 | INTRODUCTION

Heterozygosity has been generally linked to individual fitness and population persistence (i.e., heterozygosity–fitness correlations; HFCs) across a wide range of taxa (Chapman, Nakagawa, Coltman, Slate, & Sheldon, 2009). The benefits of heterozygosity, however, may not be consistent throughout the life of an individual. Relatively few studies have examined ontogenetic variation in HFCs within cohorts, but the few that have tend to find stronger HFCs during some life stages than others (Cohas, Bonenfant, Kempenaers, & Allainé, 2009; Pujolar, Maes, Vancoillie, & Volckaert, 2006). Collectively, such studies suggest that natural selection may most strongly act on individuals during specific periods of life, changing the frequency of heterozygotes at later life stages. For example, viability selection may result in the differential survival of juveniles by culling more homozygous individuals in favor of heterozygotes (Clegg & Allard, 1973). Despite numerous examinations of HFCs, ontogenetic variation in heterozygosity and HFCs has received relatively little attention, even though identifying the periods

of life most strongly influenced by genetic diversity and selection could improve our understanding of limits on individual fitness and population dynamics (Chapman et al., 2009).

These dynamics have been especially ignored in fish, even as genetic diversity appears to be an important contributor to variation in fish performance at both the individual and population levels (Allendorf, Berry, & Ryman, 2014). The first year of life in most fishes is characterized by extremely high mortality rates and represents the focus of most studies seeking to elucidate mechanisms controlling variance in fish population abundance and recruitment (Pepin & Myers, 1991). Early life mortality is also often highly size selective, with viability selection disproportionately removing smaller individuals and conferring higher fitness to larger individuals in the same cohort. During early life stages, larger individuals are able to forage more effectively for a wider size range of prey items while also exhibiting increased maneuverability, predator avoidance, and starvation resistance (Graeb, Dettmers, Wahl, & Caceres, 2004; Miller, Crowder, Rice, & Marschall, 1988). Later in life, many temperate species also face highly size-selective mortality overwinter, where large individuals with superior energy stores and lower mass-specific metabolic rates are better equipped to survive cold, resource-poor winter environments compared with smaller individuals (Post & Evans, 1989a). Depending on the environment, size-selective mortality during either the larval stage or later juvenile and overwintering stages may be most important to recruitment (Pepin & Myers, 1991; Pritt, Roseman, & O'Brien, 2014). Should heterozygosity differentially influence individual growth and survival during different stanzas of early life, elucidating its relative importance to individual fitness could provide more thorough and accurate estimates of recruitment dynamics as a function of viability selection.

An extreme example of the "bigger is better" hypothesis is the development of skewed or bimodal size distributions during the early life of fishes. In several species, individuals in a single cohort exhibit dramatically different growth patterns, resulting in some individuals becoming twice or three times as long, and up to an order of magnitude heavier, than others (DeAngelis & Coutant, 1982). Again, these large individuals may also be the fittest, able to consume a wide range of prey types, avoid predation, and better withstand overwinter conditions. Bimodal size distributions among juveniles in the same cohort have been hypothesized to arise from differences in initial egg or larval sizes, asynchronous hatching dates among clutches of siblings, or disproportionate mortality rates among size classes (DeAngelis & Coutant, 1982; Miller & Storck, 1984). If hatching dates or initial egg sizes were influenced by heritable factors, it might be expected that the largest individuals should be more closely related to one another (e.g., siblings) than to the overall population or that they exhibit similarly rapid growth or mortality rates. Moreover, individuals sharing similar beneficial alleles (either by identity or by descent), or harboring higher levels of genetic diversity, could experience much more rapid growth than less diverse individuals (Mitton, 1997), thereby contributing to highly skewed or bimodal size distributions within cohorts of offspring. Conversely, disparate growth among individuals could originate through small random differences in size that are exacerbated as larger individuals access and potentially deplete larger food items, rendering them unavailable to smaller members of the cohort (DeAngelis & Coutant, 1982), in which case larger individuals would not be expected to be more related to one another. These questions have largely been left unaddressed in both wild and cultured populations, leaving the mechanisms driving these disparate growth patterns poorly understood.

Most studies of heterozygosity effects in fishes have been limited to artificially spawned and raised salmonids (Danzmann, Ferguson, & Allendorf, 1988) and generally ignored potential ontogenetic variation in HFCs (DiBattista, Feldheim, Gruber, & Hendry, 2008; Borrell et al., 2011; but see Lieutenant-Gosselin & Bernatchez, 2006). Rapid changes in genetic structure in aquaculture populations and the contribution of heterozygosity to the disproportionately rapid growth of some individuals are also poorly understood, even though these dynamics may have important implications for best culture practices and management (Christie, Marine, French, & Blouin, 2012; Christie, Marine, French, Waples, & Blouin, 2012). We used an experiment in seminatural ponds to examine ontogenetic variation in the relative importance of genetic diversity to growth and survival of yellow perch (*Perca flavescens*) during juvenile and adult life stages. We specifically addressed three questions. First, do genetic diversity and genetic structure change from adults to offspring over a single generation? Second, is heterozygosity correlated to growth and survival of adult (age 2–3) or juvenile (age 0–1) yellow perch? Third, what is the relationship among different size classes of offspring to one another and to other individuals in the same cohort? These questions have important implications for our understanding of viability selection in the context of fish productivity for wild and aquaculture populations.

2 | METHODS

2.1 | Study species

Yellow perch are an economically and ecologically important species present throughout North America and are also commonly raised in aquaculture for both food production and to stock water bodies to provide recreational fishing opportunities (Kestemont, Dabrowski, & Summerfelt, 2015). Aquaculture production of yellow perch through small-scale facilities using the same or similar broodstock over several years may result in reduced genetic diversity and fitness of these populations, although few studies have examined the genetic consequences of aquaculture practices for yellow perch (Kestemont et al., 2015). Yellow perch are demersal spawners that do not exhibit any parental care or alternative life histories. They spawn in early spring at water temperatures of 7–11°C, and spawning in small lakes generally occurs over a 1- to 3-week period (Feiner & Höök, 2015). In the wild, yellow perch recruitment can be highly variable, with occasional recruitment failures interspersed with years of high recruitment success. Depending on environmental conditions, annual recruitment success may be sensitive to environmental quality in very early life (during the larval and young-of-year stages) or during the first winter (transition to yearlings) (Feiner & Höök, 2015).

2.2 | Pond experiment

The yellow perch used in this study originated from a Minnesota, USA, strain produced in an aquaculture facility at 10,000 Lakes Aquaculture, Inc., Osakis, MN, USA. These fish are progeny from an onsite broodstock population used for small-scale yellow perch production for pond and lake stocking. Initially, 278 age 2 yellow perch between 150 and 200 mm total length (TL) were transported to the Saline Fisheries Research Station in Saline, MI, USA. In October 2012, these fish were stocked (139 per pond) into two replicate 0.5 acre ponds that were empty of other fish. Each pond was a closed system and no migration of fish could occur between ponds. Both ponds contained natural vegetation and coarse woody debris and supported abundant zooplankton and macroinvertebrate food sources. To improve adult growth and promote maturation, each pond was additionally stocked with fathead minnows (*Pimephales promelas*) for forage. At the time of stocking, a fin clip was removed from the left pectoral fin of each adult yellow perch for future genetic analysis. All fin clips were stored in 95% ethanol, which was changed within 48 hr of sampling to avoid alcohol dilution during tissue dehydration. To enable unique identification, all fish were individually tagged by injecting a unique passive integrated transponder (PIT) tag (Oregon RFID, Portland, OR) into the right dorsal muscle just below the anterior end of the dorsal fin. Each pond (Pond 16 and Pond 17) was stocked with a random sample of adult yellow perch so there was no difference in initial fish size between ponds (see Results). The sex of individual fish was unable to be determined at the time of stocking, but assuming a 1:1 sex ratio, roughly 70 fish of each sex were stocked into each pond. The perch were allowed to overwinter in the ponds and spawn naturally in spring 2013.

Yellow perch offspring (hereafter collectively referred to as offspring) were sampled at three dates representing three life stages over time (young-of-year [YOY], juveniles, and yearlings; see below). The initial sampling of YOY occurred 11–13 August 2013 using seines and minnow traps. Seining sites were distributed throughout each pond to avoid biased collection of certain family groups, as previous research in the closely related Eurasian perch (*P. fluviatilis*) has demonstrated the potential for assortative shoaling behavior based on kin groups (Behrmann-Godel, Gerlach, & Eckmann, 2006). Pond 16 was divided into quadrants and a single seine haul using a 10-m seine was performed in each quadrant. Due to woody structure that could not be adequately seined, Pond 17 was divided into thirds and one seine haul was performed in each third. Collected fish were euthanized and stored in 95% ethanol and later measured (to 1 mm TL), weighed (to 0.01 g), and a pectoral fin clip removed in the laboratory, with all fin clips stored in 95% ethanol until analysis. From 28 to 31 October 2013, both ponds were completely drained and all adults and offspring (hereafter referred to as juveniles) were collected. Random samples of 240 juveniles per pond (roughly 8% and 16% of the total populations of Pond 16 and 17, respectively) were weighed, measured, and fin-clipped. All fin clips were stored in lysis buffer (100 mmol/L Tris-HCl pH 8.0, 100 mmol/L EDTA, 10 mmol/L NaCl, 2% SDS) for genetic analyses. All juveniles were counted and replaced in their respective ponds after tissue sampling. All adults were identified (via PIT tag), weighed, measured, and moved into a separate pond (Pond 5). Finally, as overwinter mortality may be a significant source of size-dependent mortality in yellow perch (Post & Evans, 1989a), ponds 16 and 17 were seined 23–24 April 2014 using a single seine haul with a 50-m seine that stretched to cover the entire width of each pond, and at least 200 randomly chosen offspring per pond (hereafter referred to as yearlings) were weighed, measured, and fin-clipped following the same protocol used the previous October (Figure 1).

2.3 | Genetic analyses

Adult DNA was extracted from fin clips following a standard protocol using proteinase-K digestion, extraction with phenol chloroform isoamylalcohol (PCI) and chloroform isoamylalcohol (CI), and precipitation of DNA with ice-cold 100% ethanol (Sambrook & Russell, 2001). DNA from offspring samples was extracted following a standard protocol using proteinase-K digestion followed by extraction using 5 mol/L KCl solution and precipitation with ice-cold 70% isopropanol (Sambrook & Russell, 2001). All stock DNA was resuspended in Tris–low EDTA (TLE) buffer following extraction. Working aliquots were diluted in ddH_20 to a concentration of ~20 ng/μl DNA following estimation of stock DNA concentration via NanoDrop (Thermo Fisher Scientific Inc., Wilmington, DE).

All individuals were genotyped at 11 microsatellite loci. Mpf4, Mpf7 (Grzybowski et al., 2010), Pfla2, Pfla6, Pfla9 (Leclerc, Wirth, & Bernatchez, 2000), and YP13, YP17, and YP109 (Li, Wang, Givens, Czesny, & Brown, 2007) were developed in yellow perch, whereas Svi4, Svi17, and Svi33 (Borer, Miller, & Kapuscinski, 1999) were developed in walleye (*Sander vitreus*). Ten loci were amplified across three separate multiplexes at specific annealing temperatures (Mpf4+ Svi33 at 48°C; Mpf7+ Pflal2+ PflaL6+ YP13 at 55°C; and Svi4+ Pflal9+ YP17+ YP109 at 56°C), whereas locus Svi17 was amplified separately at 53°C. Each PCR consisted of 1.25 mmol/L Tris-HCl, 6.25 mmol/L KCl, 6.25 mg/L BSA, 2.0 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 0.2 to 0.4 μmol/L of each forward primer and reverse primer (depending on the microsatellite and multiplex; see Table 1), 40 ng of DNA, and 1 unit of NEB *Taq* polymerase in a 20 μl reaction. The thermal cycles for Mpf4+Svi33 and Svi17 were an initial cycle of 94°C for 2 min, followed by 25 cycles of 94°C for 30 s, the annealing temperature for 1 min, and 72°C for 2 min, followed by a final extension step of 72°C for 45 min to promote full extension of large alleles. The thermal cycles for the two multiplexes Mpf7+ PflaL2+ PflaL6+ YP13 and Svi4+ PflaL9+ YP17+ YP109 contained an initial cycle at 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, the annealing temperature for 1 min, and 72°C for 2 min, which was followed by extension at 72°C for 10 min. All loci were amplified using fluorescently labeled forward primers, and individual genotypes at each locus were scored using an ABI 3130XL and GeneMapper 4.1 (Applied Biosystems, Foster City, CA). Initial scoring was automated using specified loci size ranges and allele size bins, and scoring was visually checked for assignment accuracy. All individuals successfully genotyped at all 11 loci were retained for analysis.

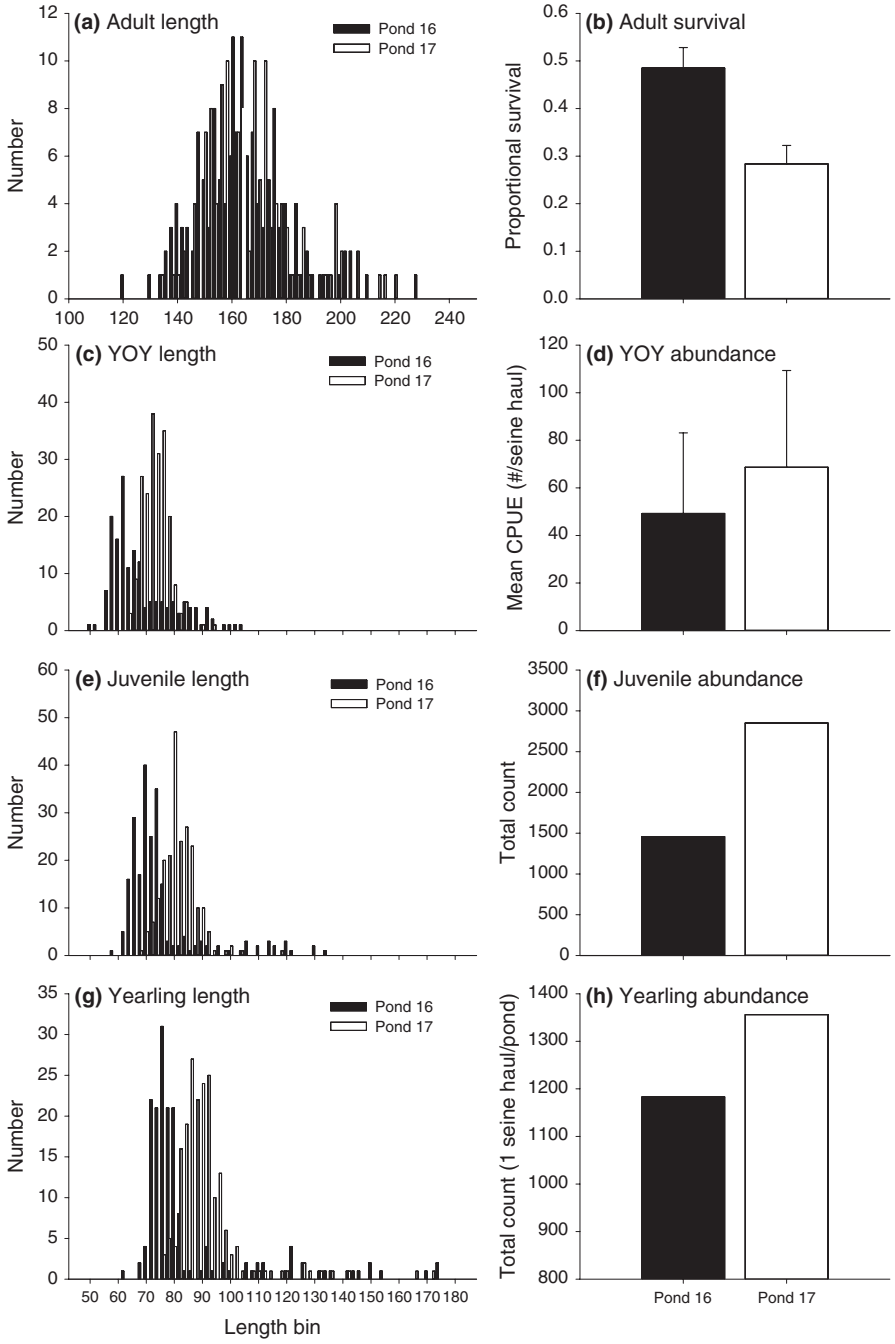

FIGURE 1 Length–frequency histograms (2-mm-length bins; left panels) of original adults (note different x-axis scale; a), young-of-year (YOY; c), juvenile (e), and yearling (g) yellow perch in Pond 16 (black) and Pond 17 (white). Adult proportional survival (panel b; error bars are one standard error), and the abundances of YOY (d), juvenile (f; error bars are one standard deviation), and yearling (h) yellow perch in either pond are shown in right panels

2.4 | Data analysis

All genotypes were checked for scoring errors due to large allelic dropout and stutter using Micro-Checker v. 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). In each pond, adult genotypes were tested for the presence of null alleles using GenePop v. 4.3 (Rousset, 2008). Observed and expected heterozygosity and deviations from Hardy–Weinberg equilibrium were tested at each locus within each pond with Fisher's exact tests using R package "diveRsity" (Keenan, McGinnity, Cross, Crozier, & Prodöhl, 2013). To assess the ability of our loci to represent genomewide heterozygosity, both the g^2 statistic (David, Pujol, Viard, Castella, & Goudet, 2007) and heterozygosity–heterozygosity correlations among loci (R package "inbreedR"; Stoffel, Esser, Hoffman, & Kardos, 2016) were used to determine pairwise covariance of heterozygosity across all markers that exhibited a low frequency of null alleles in adults (see Results). We also estimated levels of gametic-phase disequilibrium among loci using R packages "haplo.stats" (Sinnwell & Schaid, 2016) and "gap" (Zhao, 2007, 2015) and determined mean r^2 (i.e., mean squared pairwise correlation coefficient across all loci pairs) for adults in each pond. To evaluate levels of inbreeding in the adult stock of each pond, F_{IS} values were estimated and bootstrapped 1000 times using "diveRsity." For each pond and sample date (adults in October 2012 and October 2013; offspring in August 2013, October 2013, and April 2014), allelic

TABLE 1 Forward and reverse sequences, accession and reference information, final PCR concentrations, and total number and size range of alleles observed across all genotyped individuals for the eleven loci used to genotype all yellow perch in the study

Locus	Sequence (5′–3′)	Accession	Conc. (μmol/L)	Num. alleles	Size range	Reference
mpf4	F: AATGTCGCAGCTTCACTATC R: CAGGTGGTAGTATTGCCAA	EU153818	0.3	10	181–231	Grzybowski et al. (2010)
svi33	F: CAGGACTGCTGTGTATAGACTTG R: GATATAGCTTTCTGCTGGGGTC	G36967	0.4	18	125–195	Borer et al. (1999)
mpf7	F: CCAGCAGTCATTACTCCAAGC R: GCCTTGATCCTCCACTTCATT	EU153821	0.3	9	155–181	Grzybowski et al. (2010)
pfla2	F: GTAAAGGAGAAAGCCTTAAC R: TAGCATGACTGGCAAATG	AF211827	0.3	6	198–216	Leclerc et al. (2000)
pfla6	F: GCATACATATAAGTAGAGCC R: CAGGGTCTTCACTATACTGG	AF211831	0.4	7	161–173	Leclerc et al. (2000)
yp13	F: GGCACCCAAACTACCACT R: CAGTCGGGCGTCATCATCAAACAAGCCCCATACA	DQ826683	0.2	5	228–246	Li et al. (2007)
pfla9	F: GTTAGTGTGAAAGAAGCATCTGC R: TGGGAAATGTGGTCAGCGGC	AF211834	0.2	2	231–233	Leclerc et al. (2000)
svi4	F: ACAAATGCGGGCTGCTGTTC R: GATCGCGGCACAGATGTATTG	G36961	0.4	7	151–165	Borer et al. (1999)
yp109	F: CAGTCGGGCGTCATCATCCAGAGGTTGGCAAGACT R: CATTGTTCCGTGTTGCTTCA	DQ826718	0.4	11	152–188	Li et al. (2007)
yp17	F: CAGTCGGGCGTCATCACAGCGTTTCCACAGTATTGACC R: GGGTTTTACACTGTTGATGGGAT	DQ826686	0.2	3	227–233	Li et al. (2007)
svi17	F: GCGCACTCTCGCATAGGCCCTG R: CGTTAAAGTCCTTGGAAACC	G36963	0.3	5	157–223	Borer et al. (1999)

richness (rarefied to 134 individuals, the smallest sample from a single pond and date) was calculated using R package "PopGenReport" (Adamack & Gruber, 2014).

2.5 | Population genetic structure, heterozygosity, and allelic richness

We used ANOVA with post hoc Tukey's tests to determine whether mean allelic richness (measured as the mean rarefied richness across loci) or mean individual heterozygosity differed between life stages (adult to offspring and among offspring stages) in each pond. We used untransformed heterozygosity data, whereas allelic richness was natural-log-transformed to meet assumptions of normality. To examine differences in genetic structure between ponds and among life stages, genetic distances were determined through calculation of pairwise F_{ST} values in "diveRsity," where each F_{ST} was bootstrapped 1,000 times and estimates were deemed significant when the respective F_{ST} 95% confidence interval did not overlap zero. To determine variance in reproductive success among adults and test for parental effects on offspring traits, we attempted parentage analysis by exclusion and probability, but we were unable to perform reliable parentage analysis on the majority of sampled offspring due to relatively low genetic diversity in our stock population (mean exclusion probability = 0.45). Instead, to elucidate potential variance in reproductive success leading to changes in allelic frequencies due to drift, we also estimated the effective population size (N_e) of each pond at each offspring life stage using the temporal method outlined by Jorde and Ryman (2007) in program N_e Estimator v. 2.01 (Do et al., 2014) using a minimum allele frequency of 0.05 and estimating uncertainty with jackknifed 95% confidence intervals.

2.6 | Heterozygosity effects on size and survival

To avoid complications with independence of samples, we focused on changes in adult growth and mortality (identified by lack of recapture when the ponds were drained in October 2013) during the time when adults were separated into the two replicate ponds from October 2012 to October 2013. To examine whether there were differences in heterozygosity, size, or growth between ponds during this time, we used two-sample *t* tests with pond identity as the independent variable and adult length, absolute growth in length (i.e., change in mm TL between 2012 and 2013), and average individual heterozygosity as the response variables. To determine whether proportional survival differed between ponds, a chi-squared test was used to test for differences in the proportion of adults recaptured in 2013 between ponds. To evaluate whether size or heterozygosity was important to annual

survival in either pond, we used a logistic mixed model with survival (1 = survived, 0 = mortality) as the dependent variable, heterozygosity and length at stocking as additive fixed independent variables, pond as a random effect, and random slopes for the effect of heterozygosity nested within pond. Finally, to assess the importance of heterozygosity to individual length and growth of adults, we used a linear mixed model with a fixed effect of heterozygosity, random effect of pond, and random slopes of heterozygosity nested within pond as explanatory variables and either stocking length, length after 1 year, or growth (i.e., change in length) as response variables.

At each offspring life stage (YOY, juvenile, and yearling), we used Mann–Whitney U-tests to examine differences in mean offspring length between ponds because length distributions became increasingly skewed in each pond resulting in non-normally distributed error (Figure 1). To examine the effect of heterozygosity on fish size among ponds and life stages, we used a linear mixed model with a mean effect of heterozygosity, random intercepts for pond and stage to account for between-pond and between-stage variance in length, and random slopes for heterozygosity nested within each pond and life stage to assess variance in HFCs between ponds and stages. For these analyses, length was natural-log-transformed to approximate a normal distribution.

2.7 | Relationships between offspring size classes

We observed increasingly long rightward tails and bimodality in the length–frequency distributions of offspring in both ponds over time (Figure 1). To more closely examine whether these largest individuals (i.e., the tail of each distribution) were genetically different from the rest of the offspring (or more related to one another), we divided the offspring at each stage and pond into "large" and "average" size categories. We first identified the 2-mm-length bin between the main and secondary modes in each length distribution with the fewest observations (often, zero) and assigned all individuals larger than this bin as "large" offspring and all individuals smaller than this size bin as "average" individuals. Then, we tested for differences in mean individual heterozygosity between large and average individuals across life stages using repeated-measures ANOVA with life stage as a random effect and size class as a factor. We also estimated bootstrapped F_{IS} for each size class, stage, and pond to determine whether large individuals were more or less inbred. Pairwise F_{ST} between large and average-sized individuals in each pond and life stage were bootstrapped using the methods described above to determine whether there was significant genetic structure distinguishing size classes. Finally, we used the "relat" function in R (© 2009, K. Csillery) to determine mean levels of pairwise genetic relatedness among large individuals, between large and average-sized individuals, and among average individuals by calculating r_{xy} between each pair of individuals per pond and life stage, where positive r_{xy} indicates that the pair of individuals are more related than expected and negative r_{xy} indicates they are less related than expected given a random draw of individuals (Queller & Goodnight, 1989). We tested for differences in mean r_{xy} among large, between large and average, and among average individuals using a repeated-measures ANOVA with life stage as a random effect, the comparison as a factor, and r_{xy} as the dependent variable. All statistical analyses were carried out in program R version 3.2.1 (R Core Team 2014).

3 | RESULTS

3.1 | Population abundance, size, and mortality

There were no significant differences in adult total length between ponds at initial stocking ($t_{266} = -1.37$, $p = .17$; Figure 1a) or between survivors after 1 year ($t_{51.6} = -0.88$, $p = .38$). Similarly, there was no difference in absolute growth in length over that time period between ponds ($t_{46.4} = 0.14$, $p = .89$). However, there was a significant difference in survival rate of adults between ponds ($\chi^2_1 = 10.66$, $p = .001$), with more adults surviving in Pond 16 (48%) than Pond 17 (28%) (Figure 1b).

Offspring abundance was always higher in Pond 17 than Pond 16 during the experiment. A total of 207 YOY were sampled from Pond 17 (mean ± *SE*: 68.7 ± 16.9 fish/seine haul) and 197 from Pond 16 (mean ± *SE*: 49.3 ± 23.5 fish/seine haul) in August 2013 (Figure 1d). In October when ponds were drained, a total of 2851 juveniles were collected from Pond 17 and 1458 from Pond 16 (Figure 1f). Finally, a total of 1356 yearlings were collected in Pond 17 and 1183 from Pond 16 using single seine hauls in April 2014 (Figure 1h). In addition to being more abundant, yellow perch offspring were significantly longer on average in Pond 17 than Pond 16 at each of the YOY (Mann–Whitney *U*-test: $W = 9535$, $p < .001$), juvenile ($W = 8939$, $p < .001$), and yearling ($W = 6854$, $p < .001$) life stages (Figure 1c,e,g).

3.2 | Genetic analyses

Five loci demonstrated null allele frequencies greater than 0.25 and two of these (pflal2 and pflal9) were not in Hardy–Weinberg equilibrium in either pond (Table 2). Because a high frequency of null alleles can potentially bias estimates of relatedness and underestimate heterozygosity, these five markers were excluded from further statistical analyses. However, analyses both including and excluding these markers resulted in similar results (data not shown) so the choice of markers did not appear to qualitatively change the results of the study. Among loci with negligible null allele frequencies, there was no heterozygosity disequilibrium across loci (mean $g^2 = -0.003$, 95% confidence interval: −0.011–0.005) and low heterozygosity–heterozygosity correlations (HHCs) between loci (mean HHC = −0.015, 95% confidence interval: −0.089–0.072). There were also low levels of gametic-phase disequilibrium between loci in the adult stock of both ponds (Pond 16 mean $r^2 = .09 \pm 0.06$ *SD*; Pond 17 mean $r^2 = .08 \pm 0.03$ *SD*).

3.3 | Allelic richness, heterozygosity, and genetic structure

Mean allelic richness decreased from adults to offspring by about 2.5 alleles per locus in both ponds. There were significant differences

TABLE 2 Summary statistics for the original adult stock of yellow perch in each pond, including observed heterozygosity (H_O), expected heterozygosity (H_E), *p*-value from exact test for Hardy–Weinberg equilibrium (HWE), and bootstrapped estimates of inbreeding coefficient (F_{IS}; 95% confidence intervals in parentheses below each estimate) and null allele frequency (Null freq.; 95% confidence intervals in parentheses) for each microsatellite locus and across all loci (Overall). Only loci with a mean null allele frequency <0.25 were used in statistical analyses

	Pond 16					Pond 17				
Locus	H_O	H_E	HWE	F_{IS}	Null freq.	H_O	H_E	HWE	F_{IS}	Null freq.
mpf4	0.71	0.76	0.67	0.07 (−0.02, 0.17)	0.07 (0.02, 0.12)	0.74	0.74	0.99	0.00 (−0.09, 0.09)	0.02 (0, 0.07)
svi33	0.78	0.74	0.10	−0.05 (−0.13, 0.05)	0 (–, –)	0.72	0.74	0.05	0.03 (−0.06, 0.12)	0.01 (–, –)
mpf7	0.52	0.54	0.99	0.03 (−0.11, 0.17)	0.01 (0, 0.07)	0.6	0.51	0.07	−0.18 (−0.33, −0.03)	0 (–, –)
pflal2	0.98	0.73	<0.01	−0.34 (−0.39, −0.28)	0.27 (0.23, 0.31)	0.97	0.72	<0.01	−0.34 (−0.40, −0.28)	0.32 (0.28, 0.36)
pflal6	0.8	0.72	0.03	−0.11 (−0.20, −0.02)	0 (–, –)	0.58	0.53	0.29	−0.11 (−0.23, 0.03)	0 (–, –)
yp13	0.5	0.5	0.71	0.00 (−0.15, 0.14)	0.33 (0.26, 0.41)	0.47	0.52	0.49	0.10 (−0.04, 0.25)	0.38 (0.3, 0.45)
pflal9	0.39	0.48	0.03	0.19 (0.02, 0.36)	0.46 (0.38, 0.53)	0.13	0.48	<0.01	0.73 (0.61, 0.85)	0.74 (0.68, 0.79)
svi4	0.38	0.35	0.33	−0.08 (−0.15, −0.01)	0 (–, –)	0.59	0.47	0.13	−0.26 (−0.33, −0.18)	0 (–, –)
yp109	0.87	0.81	0.67	−0.07 (−0.13, 0.00)	0.27 (0.21, 0.33)	0.8	0.79	0.72	−0.02 (−0.09, 0.06)	0.36 (0.29, 0.42)
yp17	0.6	0.57	0.29	−0.06 (−0.20, 0.07)	0.39 (0.32, 0.46)	0.47	0.57	0.11	0.17 (0.03, 0.30)	0.52 (0.45, 0.58)
svi17	0.19	0.19	0.01	0.00 (−0.10, 0.17)	0.03 (–, –)	0.2	0.2	0.68	−0.01 (−0.09, 0.08)	0 (–, –)
Overall	0.61	0.58	<0.01	−0.05 (−0.08, −0.02)	–	0.57	0.57	<0.01	0.00 (−0.04, 0.03)	–

among life stages in Pond 16 ($F_{3,20}$ = 5.169, p = .01), where adult allelic richness was significantly higher than all offspring stages (Tukey's tests, p < .05), but there were no differences among offspring stages (p > .95). Allelic richness slightly differed in Pond 17 ($F_{3,20}$ = 3.247, p = .04), although post hoc tests revealed only marginal differences between adult and offspring stages (p > .05; Figure 2a). Mean individual heterozygosity significantly differed among stages in Pond 16 ($F_{3,685}$ = 33.70, p < .001), with significant differences among all life stages (p < .05), although with no discernable pattern (Figure 2b). Mean heterozygosity also differed among life stages in Pond 17 ($F_{3,750}$ = 3.90, p = .01), where YOY heterozygosity was higher than either adult or juvenile heterozygosity (p < .05). Corresponding to the depletion in allelic richness observed in offspring stages, the estimated N_e of offspring stages in both ponds was very low (range of mean N_e = 2.9–7.4 across ponds and offspring life stages) considering the potential parent pool of 139 adults originally stocked into each pond (Figure 2c). F_{ST} indicated significant genetic differentiation among all life stages within and between ponds (Table 3). The lone exception was nonsignificant differentiation between YOY and yearling offspring in Pond 16 (mean F_{ST} < 0.001).

3.4 | Heterozygosity effects on adult growth and mortality

Pond 16 exhibited significantly higher adult heterozygosity at the time of stocking (t_{266} = 2.39, p = .02), but adult heterozygosity did not differ between ponds when considering survivors of the first year (t_{101} = 0.69, p = .49). Heterozygosity had no effect on adult length at the time of stocking (β = −1.92 ± 6.01 *SE*) and this effect did not vary between ponds (random slopes σ^2 = 14.17, χ^2_3 = 0.15, p = .99). Heterozygosity also had no effect on adult length among survivors after 1 year (β = −8.11 ± 18.97 *SE*; random slopes σ = 438.5, χ^2_3 = 0.29, p = .96), or on growth in length over the year (β = −2.31 ± 7.817 *SE*; random slopes σ^2 < 0.01, χ^2_3 = 0.09, p = .99). Initial size was positively related to survival among adults (β = 0.02 ± 0.01 *SE*, p = .018), whereas the effect of heterozygosity did not differ between ponds and had no overall effect on survival (β = −0.98 ± 0.88 *SE*; random slopes σ^2 = 0.62, χ^2_2 = 1.30, p = .52).

3.5 | Heterozygosity effects on offspring size

Heterozygosity was significantly related to offspring length (β = 0.09 ± 0.10 *SE*; chi-squared test for effect of heterozygosity: χ^2_5 = 30.30, p < .0001). Importantly, these effects varied between ponds (random slopes between ponds σ^2 = 0.017, χ^2_4 = 11.90, p = .018) but not among stages within ponds (random slopes among stages within ponds σ^2 = 0.004, χ^2_1 = 0.36, p = .55), with strong positive effects of heterozygosity across life stages in Pond 16 (mean β = 0.18) and virtually no effect across life stages in Pond 17 (mean β = −0.001; Figure 3) explaining about 27% of the variance in offspring length. Despite positive size–heterozygosity relationships, there was only a marginal difference in heterozygosity between large and average-sized yellow perch offspring in Pond 16 (repeated-measures ANOVA: $F_{1,2}$ = 12.02, p = .07), and no difference in heterozygosity between large and average-sized offspring in Pond 17 ($F_{1,2}$ = 1.15, p = .40; Figure 4a).

3.6 | Genetic structure between size classes

Should increased offspring size result as a product of the timing of spawning, hatch, or other parental effects, it would be expected that large individuals would be genetically dissimilar and less related to average-sized individuals in the same cohort. Bootstrapped pairwise F_{ST} indicated significant differences between large and average-sized

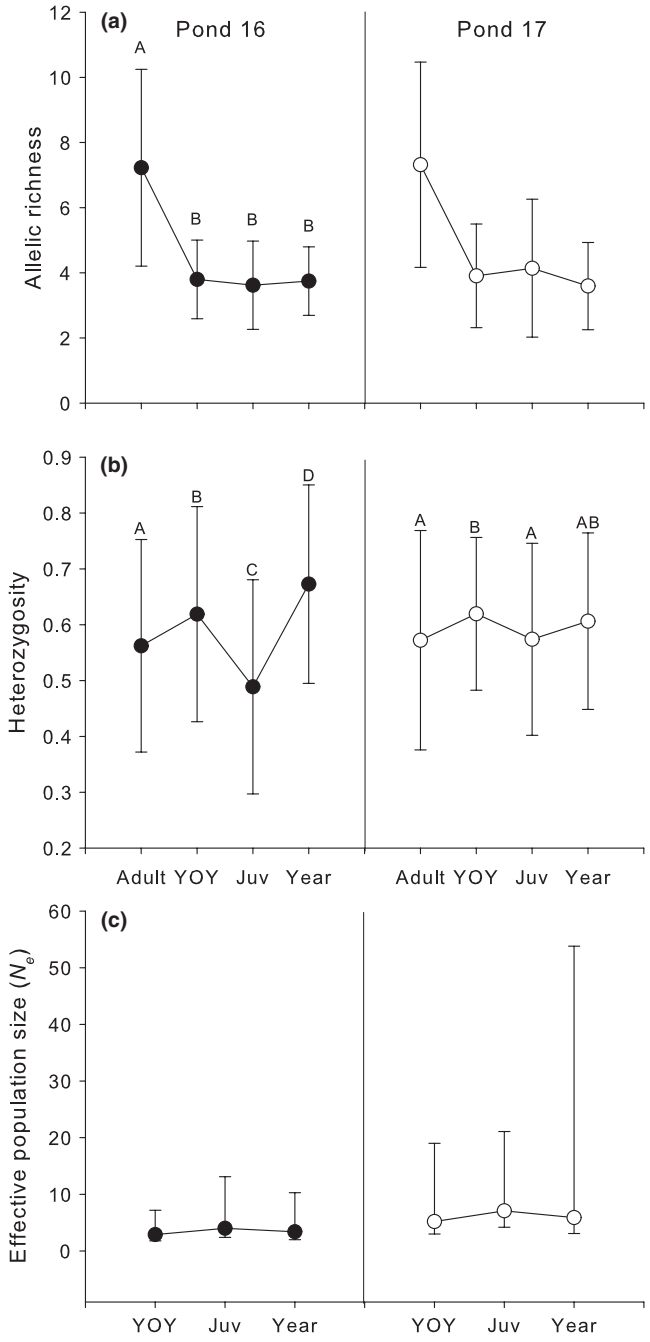

FIGURE 2 Estimates of mean allelic richness (a), mean individual heterozygosity (b), and effective population size (c) estimated from initially stocked adult yellow perch and their offspring collected at the young-of-year (YOY), juvenile (Juv), and yearling (Year) stages in Pond 16 (black; left plots) and Pond 17 (white; right plots). Error bars in a and b represent one standard deviation, and in c represent jackknifed 95% confidence intervals. There were significant differences in heterozygosity and allelic richness in Pond 16, but only heterozygosity differed among life stages in Pond 17. Effective population size was a fraction of the 139 potential adult parents stocked into each pond

offspring at each life stage in Pond 16 (no 95% confidence intervals overlapped zero). In contrast, pairwise F_{ST} values between large and average-sized offspring overlapped zero at each life stage in Pond 17 (Figure 4b). Both large and average-sized individuals at all life stages in both ponds were significantly more outbred than expected ($F_{IS} < 0$), with the exception of juveniles in Pond 16, where F_{IS} was not different from zero. In Pond 16, large offspring were significantly more outbred than their average-sized counterparts as YOY and yearlings (Figure 4c). In Pond 17, large offspring only exhibited significantly more negative F_{IS} values as YOY, but the size classes did not differ in F_{IS} as juveniles or yearlings.

Average pairwise relatedness marginally differed when comparing relatedness among large offspring, between large and average offspring, and among average offspring (repeated-measures ANOVA: $F_{2,4} = 5.99$, $p = .06$) in Pond 16. Pairwise comparisons indicated that large offspring were significantly more related to one another than they were to average-sized offspring (post hoc Tukey's test, $p < .001$) and significantly less related to average-sized offspring than average-sized fish were to one another ($p < .008$) (Figure 4d). An opposing trend was detected in Pond 17: marginal overall differences in relatedness ($F_{2,4} = 6.87$, $p = .05$) were driven by average-sized individuals being significantly more related to one another than to large fish, or than large fish were among themselves ($p < .02$).

4 | DISCUSSION

High genetic diversity has been hypothesized to promote fitness of both individuals and populations (e.g., Mitton, 1997). However, few studies have examined variability in the correlation between microsatellite heterozygosity and fitness driven by differences in ontogeny or environmental conditions. In addition, it is unclear how rapidly population-level genetic diversity can change in a single generation under natural reproductive conditions due to processes such as genetic drift and high variance in reproductive success. We observed significant genetic structure arising between offspring and their parents and a loss of allelic richness within a single generation. In addition, we observed considerable variation in the relationship between length and heterozygosity among different life stages and between ponds. The fittest (i.e., largest) individuals in one pond were also more related to one another and less inbred than average-sized individuals in the same cohort. Collectively, this information sheds new light onto the importance and variability of genetic diversity during early life in fish, a period of high selective mortality and a focus of biologists seeking to understand viability selection, variation in recruitment, and population productivity (Pepin, 2015).

Adaptation to captivity is a growing concern among aquaculturists and conservationists (Frankham, 2008). In fish, fitness may be reduced in individuals that have spent even a single generation in captivity, reducing their ability to adequately supplement wild populations and potentially decreasing the overall fitness of populations in which they are stocked through outbreeding depression (Christie, Marine, French, & Blouin, 2012). Our results suggest that gene frequencies may significantly change within a single generation in captive environments, even when relatively large adult populations of fish are allowed to spawn naturally. Offspring genotypes were significantly different from their

TABLE 3 Sample sizes (*N*) and pairwise F_{ST} statistics (below diagonal) and associated bootstrapped 95% confidence intervals (above diagonal) calculated between yellow perch samples from each life stage (adults, young-of-year [YOY], juveniles, and yearlings) and pond. Only Pond 16 YOY and yearlings were not significantly different

			Pond 16				Pond 17			
		N	Adult	YOY	Juvenile	Yearling	Adult	YOY	Juvenile	Yearling
Pond 16	Adult	134		(0.07, 0.10)	(0.05, 0.08)	(0.06, 0.09)	(0.02, 0.04)	(0.08, 0.11)	(0.06, 0.09)	(0.07, 0.10)
	YOY	164	0.084		(0.03, 0.04)	(0.00, 0.01)	(0.03, 0.06)	(0.07, 0.11)	(0.05, 0.09)	(0.04, 0.07)
	Juvenile	223	0.061	0.034		(0.03, 0.04)	(0.03, 0.06)	(0.11, 0.15)	(0.09, 0.12)	(0.09, 0.11)
	Yearling	168	0.073	<0.0001	0.034		(0.02, 0.05)	(0.07, 0.10)	(0.05, 0.08)	(0.04, 0.06)
Pond 17	Adult	134	0.027	0.047	0.045	0.037		(0.04, 0.06)	(0.02, 0.05)	(0.03, 0.05)
	YOY	205	0.091	0.087	0.131	0.083	0.048		(0.00, 0.02)	(0.01, 0.03)
	Juvenile	219	0.076	0.068	0.101	0.066	0.035	0.009		(0.00, 0.01)
	Yearling	196	0.088	0.050	0.100	0.050	0.041	0.016	0.007	

parents at all life stages in both ponds. Moreover, mean allelic richness decreased by 2–3 alleles per locus from adults to offspring and effective population size estimates were a fraction of the size of the potential adult stock (Figure 2), suggesting strong selection and/or drift altered allele frequencies and richness between adults and offspring and potentially between offspring life stages, whereas heterozygosity remained largely constant. High among-individual variance in reproductive success might be expected in highly fecund animals, especially in species such as yellow perch which display high among-female variation in egg production and egg size (Jansen, 1996; Lauer, Shroyer, Kilpatrick, McComish, & Allen, 2005), and such variance likely plays a role in the significant genetic differentiation we observed (Planes & Lenfant, 2002). Due to limitations of our study (e.g., number and variability of loci surveyed and lack of genomic context), it is unclear as to whether selection or drift was the main cause of the changes we observed. Nevertheless, our results support the notion that rapid genetic change can occur in a single generation in aquaculture or other captive propagation programs, and should be taken into account by managers seeking to develop stocking programs to repatriate or supplement wild populations.

Whereas population-level heterozygosity generally did not differ among life stages, we did find strong contrasts in the relationships between length and heterozygosity among life stages and between ponds. Heterozygosity had no association with length, growth, or survival of age 2–3 adult yellow perch in either pond, but we observed significant positive relationships between heterozygosity and length at all offspring life stages in Pond 16 (the pond exhibiting relatively higher adult survival and lower offspring abundances). These observations are consistent with the idea that selection is strongest during early life and fades as individuals reach adulthood. For example, American eels (*Anguilla anguilla*) exhibited positive HFCs for growth and survival at 12 months of age, but not at 22 months (Pujolar et al., 2006), whereas survival in alpine marmots (*Marmota marmota*) was positively associated with heterozygosity as juveniles but not as adults (Cohas et al., 2009). These studies suggested ontogenetic shifts in HFCs were the result of selection removing homozygous individuals (i.e., heterozygote advantage). We observed no clear trend toward more or less genetic diversity over time among offspring stages in either pond, which would seem to rule out this hypothesis. It is possible that observed HFCs arose as the result of fitness associations with specific loci genotyped in this study—however, lacking genomic data for yellow perch and the locations of these markers on the yellow perch genome, our ability to investigate this scenario is limited. Rather, we hypothesize that HFCs are strongest in young (small) fish and subsequently decline as size becomes less important to survival as fish age (i.e., viability selection is relaxed). Fish exhibit patterns of compensatory growth in small individuals when given access to improved resources (Ali, Nicieza, & Wootton, 2003), and a similar mechanism may have diluted HFCs in adults in our study. However, as recruitment in fish is often determined during the first 1–2 years of life and survival is often size selective during this time (Pepin & Myers, 1991), heterozygosity may play a role in determining individual recruitment success in many fishes. Examining the relationship between heterozygosity and recruitment variability could provide useful insights into the recruitment dynamics of many species, especially fish stocks that have suffered reduced genetic diversity and recruitment failures after being overfished (Allendorf et al., 2014).

Environmental effects on HFCs are not commonly assessed but have been noted in multiple taxa. Usually, populations experiencing more challenging environments exhibit stronger HFCs (Chapman et al., 2009; Markert et al., 2010). Heterozygous individuals appear to be more metabolically efficient, enabling them to withstand stressful environments better than homozygous counterparts while there is little difference in fitness in relatively benign environments (Danzmann et al., 1988). In this study, we detected significant positive correlations between size and heterozygosity in one study pond (Pond 16) over all offspring life stages, but found no such correlations in the other pond (Pond 17). Although the study ponds used in this experiment were designed as replicates, there was significant variation in adult survival and offspring abundance and growth between them. Adult survival was nearly twice as high in Pond 16, while offspring experienced significantly reduced growth and lower abundance when compared to Pond 17. Adults stocked into each pond exhibited no initial differences in size, and a similar number of parents appeared to have successfully

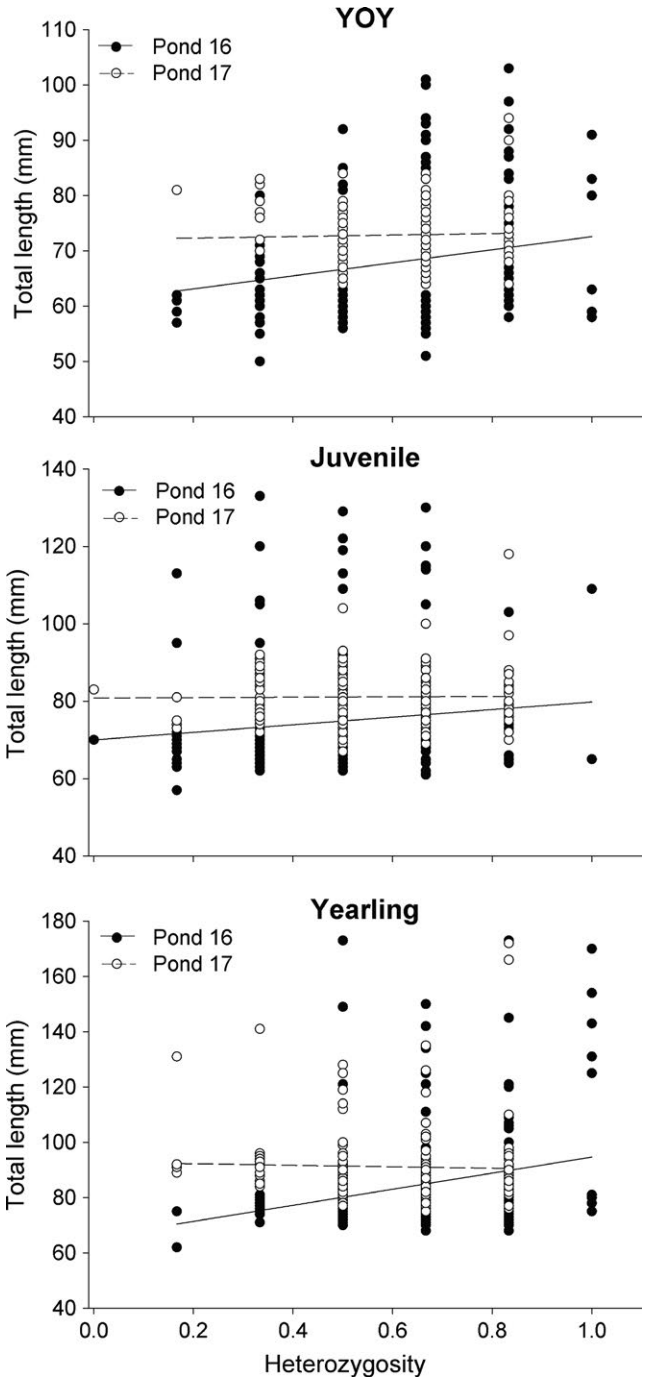

FIGURE 3 Relationships between total length (mm) and individual heterozygosity for yellow perch offspring collected at the young-of-year (YOY), juvenile, and yearling life stages from Pond 16 (black points, solid line) and Pond 17 (white points, dashed line). Lines represent estimated regression line. Heterozygosity was positively related to length across stages in Pond 16, but not Pond 17

reproduced in both ponds based on estimates of effective population size; thus, it is unlikely that one population started in poorer condition and subsequently suffered. Slower growth coinciding with lower abundance of offspring suggests Pond 16 comprised a relatively harsher environment for young yellow perch in this study. Although we did not quantify habitat characteristics in each pond, there was very little aquatic vegetation in Pond 16, whereas such vegetation was abundant in Pond 17, suggesting invertebrate resources for offspring may have been reduced in Pond 16 (Zimmer, Hanson, & Butler, 2000). In addition, adult yellow perch are known cannibals (Post & Evans, 1989b)—the higher survival of adult yellow perch in Pond 16, in tandem with little aquatic vegetation to serve as refuges, may have resulted in more intense predation pressure on offspring and reduced foraging activity, leading to reduced growth and survival. Thus, the stronger HFCs observed in Pond 16 may have resulted from the relatively poorer environment experienced by offspring in this pond, aligning with observations in birds, fish, and amphibians (see review by Chapman et al., 2009). We should note that other environmental factors, including differential warming rates or hatching dates, could also have contributed to the apparent differences in reproductive success and offspring fitness between ponds. Further experimentation to understand how the interaction between heterozygosity and environmental quality might influence subsequent individual fitness throughout ontogeny could identify the relative importance of each factor in influencing population resilience and recruitment in response to population fluctuations and environmental stress.

Skewed length distributions and the presence of rapidly growing individuals within cohorts appears to be a common phenomenon in fish populations (DeAngelis & Coutant, 1982). As these rapidly growing individuals may also represent the most fit individuals in the cohort, understanding the mechanisms driving such size disparities could inform a wide range of complex issues in fisheries management, from recruitment dynamics to aquaculture practices. Previous studies have suggested such differential patterns in growth may arise from differences in initial hatching size or date, or some individuals taking advantage of spatiotemporally patchy resources (Miller & Storck, 1984). Our data provide some support for the former hypothesis, as large individuals in one pond were genetically different, significantly more related to one another, and significantly less inbred than average-sized individuals in Pond 16. Potentially, these individuals represent full- or half-siblings that were spawned at the same time relatively early in the year, as might be expected if reproductive success was nonrandom among spawning females. Because large individuals in Pond 16 also tended to be significantly less inbred than other individuals, their parents appear to have been more genetically dissimilar than average parents, which may have improved their fitness (Kempenaers, 2007). Future studies examining the development of size bimodality within cohorts should consider parentage analysis (unable to be performed here) of naturally spawned offspring as a method to test these hypotheses under conditions for natural reproduction, something many studies have ignored (e.g., Venturelli et al., 2009). Moreover, we should note that large individuals in Pond 17 exhibited few of these patterns and that large and average-sized offspring exhibited only marginal differences in heterozygosity in one pond, despite the presence of generally positive length–heterozygosity correlations, similar effective population sizes, and no size differences between the adult stocks of either pond. This may not be surprising, as the correlations we observed were generally weak ($r \leq .2$), a common phenomenon in HFCs (Chapman et al., 2009), and sample sizes of large individuals were

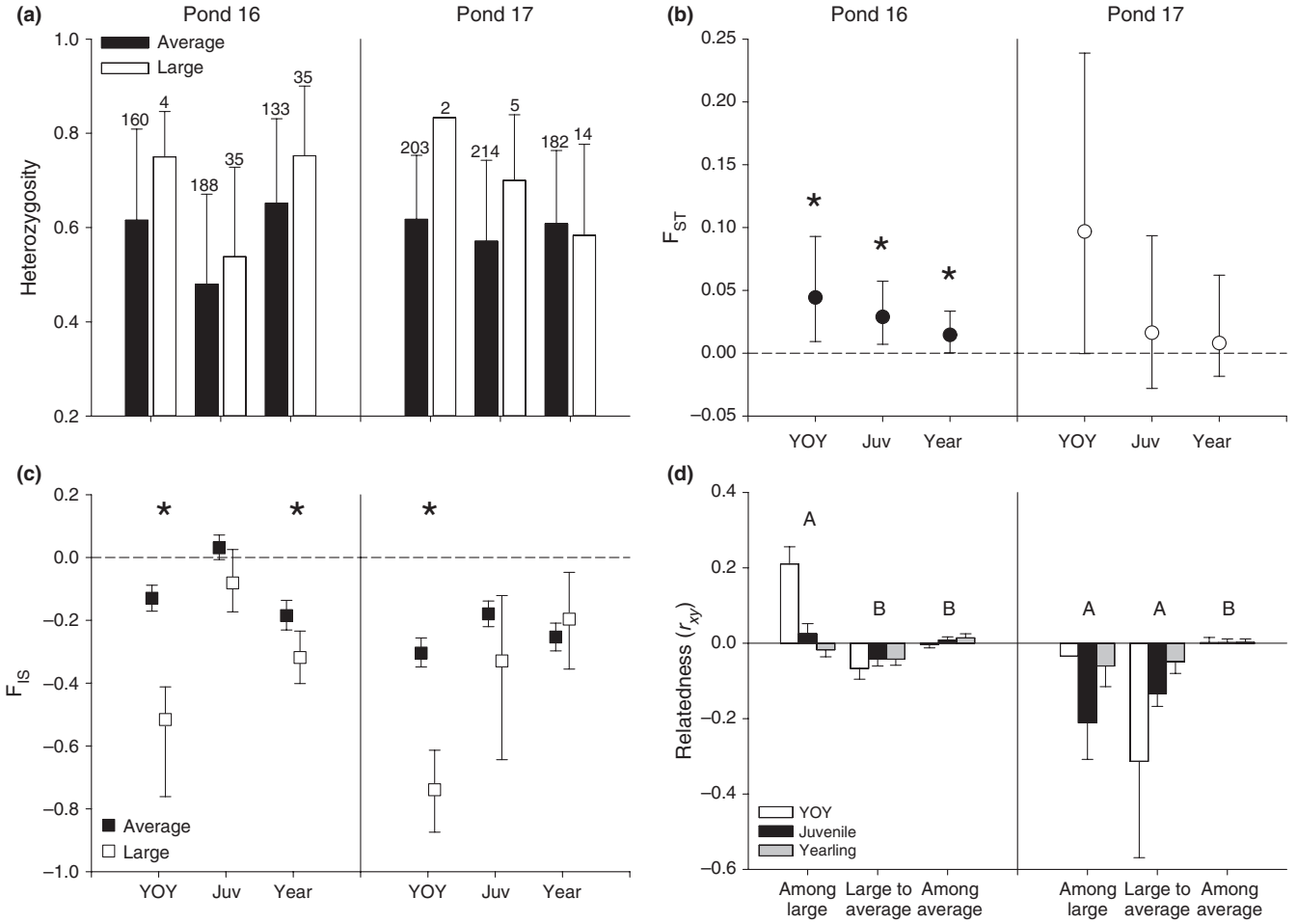

FIGURE 4 Comparisons of mean heterozygosity (a), mean pairwise F_{ST} values (b), inbreeding coefficients (F_{IS}; c), and mean pairwise relatedness (d) of average-sized and large yellow perch offspring collected at the young-of-year (YOY), juvenile (Juv), and yearling (Year) life stages from ponds 16 and 17. Error bars represent standard deviation of heterozygosity, bootstrapped 95% confidence intervals for F_{ST} and F_{IS}, and standard error of relatedness. Sample sizes for the number in each size class per pond and life stage are reported above mean heterozygosity bars. Asterisks in panels b and c and letters in panel d denote significant differences determined either via nonoverlapping 95% CIs or repeated-measures ANOVA. Large individuals exhibited significant genetic differentiation and were significantly more outbred and significantly more related to one another than to average-sized individuals in Pond 16, but not Pond 17

fairly low for most life stages, weakening our statistical power to detect such differences. Therefore, outbreeding, hatching date, and size at hatch may be important factors promoting large size during the first year of life, but may not be required depending on other environmental conditions.

There remains substantial debate in the literature on the value and accuracy of HFCs using relatively small numbers of molecular markers, as they may not represent genomewide genetic diversity (DeWoody & DeWoody, 2005; also note the low g^2 and HHC values observed in this study), and in many species, including yellow perch, little genomic information exists to examine whether these markers exist in or near functional genes. In addition, small numbers of loci may bias the measurement of genetic differentiation and relatedness within and among populations. Therefore, some caution should be taken in interpreting the importance of HFCs to offspring fitness. However, we offer two arguments to support our results. First, estimates of relatedness and differentiation were most often biased by the number of individuals sampled, rather than number of markers—here, we sampled 130 or more individuals per pond and life stage, a number sufficient to limit bias in the estimation of these parameters (Beaumont & Nichols, 1996; Queller & Goodnight, 1989). In addition, small numbers of markers often increase the uncertainty around estimates of r_{xy} and F_{ST} (Holsinger & Weir, 2009; Lynch & Ritland, 1999)—therefore, significant differences observed here may be conservative, as using relatively few number of markers may have limited our power to detect more subtle differences. Second, a recent examination of microsatellite, single nucleotide polymorphism (SNP), and pedigree data showed that a small panel of microsatellites fared equally well at measuring heterozygosity and detecting HFCs as either more numerous SNPs or inbreeding measured via pedigree (Forstmeier, Schielzeth, Mueller, Ellegren, & Kempenaers, 2012), supporting our conclusion that ontogeny and environment may interact to influence the importance of heterozygosity to individual fitness. Moreover, many of the patterns we observed are supported by existing literature, although examinations

of environmental or ontogenetic effects on HFCs are relatively scant. Negative associations between HFC strength and environmental quality have been observed in several taxa (Chapman et al., 2009), whereas other studies have shown that HFCs should be strongest during juvenile life stages (Pujolar et al., 2006; Cohas et al., 2009; Doyle et al. 2016), suggesting dynamic influences of heterozygosity on individual fitness may be prevalent during early life in many taxa.

In addition to their evolutionary insights, our data have practical ramifications. First, we have shown that population-level genetic structure and diversity can change within a single generation even when individuals are allowed to spawn naturally. Small-scale aquaculture programs are common throughout the world and often supply fish for stocking into natural systems (Subasinghe, Soto, & Jia, 2009). We recommend that such propagation practices take into account potential loss of genetic diversity through drift and selection for captivity, preferably by minimizing the number of generations produced from a single stock, mixing multiple generations within broodstocks, and refreshing stocks with new genetic material often (Frankham, 2008). Second, HFCs may be important to early life success in fish depending on the life stage of interest. These early life dynamics are not well understood in fish, but elucidating patterns and importance of heterozygosity could explain mortality and growth patterns in young fish that ultimately influence reproductive success. Finally, rapid growth and attainment of large size by some individuals could be related to differences in parentage, hatch timing, and genetic diversity, and environmental effects may influence the relative importance of these factors and the potential contribution of these size classes to year class strength. More extensive examinations of heterozygosity influences on fitness, including parentage analysis, the inclusion of more informative genetic markers, and quantitative assessment of environmental effects on HFCs, could better discern the mechanisms driving variability in HFCs and their ontogenetic longevity in fish populations.

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CONFLICT OF INTEREST

None declared.

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