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Evaluation of relative growth performance and genotype by environment effects for cross-bred yellow perch families reared in communal ponds using DNA parentage analyses

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Abstract

From 24 mating sets, 6300 fingerling of yellow perch (*Perca flavescens*) were stocked into one pond and equal numbers of progeny from six representative sets out of the 24 were stocked into each of two other ponds. After communal rearing for 21 months, total length and body weight were assessed for $n = 300$ fish in each of the three ponds and molecular pedigrees were performed for each sampled individual to assign the progeny back to the original parents. The overall average number of alleles per locus was $A = 16.4$ and observed and expected heterozygosities were $H_o = 0.88$ and $H_e = 0.77$ respectively. The mean weight of random samples and the top 10% fast-growing fish from the pond with all the sets was significantly greater than those from either of the two replicate ponds with six crosses. For the two replicate ponds, no significant differences were found in family rankings and assignment of the top 10% fast-growing fish, indicating that families with superior growth performance in one pond also exhibited the same superior growth performance in the replicate pond. However, there were no significant correlations detected in family mean weights of the top 10% fish between any two of the three ponds.

Keywords: yellow perch, DNA parentage analysis, growth, genotype by environment, genetic improvement

Introduction

Yellow perch *Perca flavescens* (Mitchell 1814) is a particularly important aquacultural and ecological species in the Great Lakes Region and the Midwest USA (Craig, 2000). The demand for yellow perch has remained very high in the region because they are the traditional fish species used in local restaurants, social organizations and the Friday night fish fry dinners that are a staple in many Great Lakes states. Because of a mild taste and firm flesh with low fat and phospholipid content (Malison 2000), yellow perch are recognized as one of the finest flavoured species among all panfish and have been widely introduced throughout the southern and western regions of the United States, southern British Columbia and other countries. Historically, the supply of yellow perch largely relied on capture fisheries in the Great Lakes, but during the 1980s and 1990s, wild harvests began to decline from 5 to 8 million

kg year^{-1} to the current limit of <3 million-kg year^{-1}. Except for Lake Erie and Green Bay, commercial fishing of yellow perch has been closed in the Great Lakes due to overfishing, and quotas for sport fishing have also been greatly reduced. Emerging diseases such as viral haemorrhagic septicaemia are expected to further threaten wild yellow perch populations. Yellow perch have been cultured for more than 30 years; however, rapid expansion of the yellow perch aquaculture industry has not yet occurred. One particular reason hindering expansion has been the relatively slow growth of currently cultured populations of this species (Malison, Kestemont & Summerfelt 2003). To improve growth rate and aquaculture production of yellow perch, Ohio State University has undertaken an Ohio Genetic Improvement of Farmed-Fish Traits (O'GIFT) programme.

Genetic improvement in quantitative traits becomes more important in aquaculture by selecting genetically superior broodfish using both phenotypic and genotypic information. In the last decade, substantial genetic improvement and increase in production efficiency have been achieved in farmed fish species such as salmonids and tilapia (Gjerde 1986, 2000; Gjerde & Korsvoll 1999; Hulata 2001). However, traditional or family-based selection for fish is based on the rearing of full-sib families in separate tanks until the fish are large enough to be tagged with physical tags. A sample of a given number of tagged individuals from each full-sib family is then mixed. This mode of rearing is very costly, and the number of full-sib families therefore limits the size of the breeding nucleus. In addition, separate rearing of full-sib families results in common environmental effects. Furthermore, traditional selective breeding programmes incur substantial levels of unintentional inbreeding that can lead to reduced performance.

The development and application of molecular genetic markers provides a feasible solution to overcome these limitations, allowing multiple families to be reared communally and subsequent construction of a genetic pedigree using parentage analysis. For communal rearing in molecular marker-based selection, there has been a need for examining the influence of disparate environments on genotypic expression of production traits. The aims of this study were to (1) evaluate the relative growth performance of yellow perch crosses reared under commercial production conditions using DNA parentage analyses and (2) examine the effects of genotype by environment interactions on family growth performance by identifying whether families with superior growth performance in one pond also exhibited the same superior growth performance in the other ponds.

Materials and methods

Mating and fry nursery

Broodfish were from the base population of the yellow perch broodstock improvement programme at the Ohio State University South Centers. Five females and 10 males were randomly selected from each of five geographic broodstock populations (North Carolina, Pennsylvania, Maine, Michigan and Ohio) and a diallel cross was made among the five populations in April 2004. For each cross-set, one female and two males were placed in a 55 L round tank for spawning. Some fish spawned naturally during the night resulting in fertilization by either one or two males. In other instances, strip spawning was performed and eggs of the female from each set were combined with the milt of both males. Fertilized eggs were incubated in 25 L round tanks with flow-through well water for 11–12 days at the temperature of 11–12 °C. Twenty-four mating sets (four from strip spawning) successfully hatched resulting in, hypothetically, a maximum of 48 half-sib families and a minimum of 28 half-sib and full-sib families for the experiment, with 62 parents being involved in spawning (some males were used twice). Similar numbers of fry from all 24 mating sets were stocked into three ponds for nursery. Six (one from strip spawning) of these sets were stocked into an additional pond. The fish were nursed in the four ponds using the pond-fertilization method for 6 weeks before feed training in 400 L round tanks for 3 weeks.

Communal rearing

Feed-trained fingerlings were stocked in June 2004 and communally reared in three 0.1 ha earthen ponds for 21 months. In the first pond (Pond 11), a total of 6300 fingerlings from all 24 mating sets were stocked to determine the effectiveness of communal rearing in terms of evaluating relative growth performance and fast-growing fish representations across the different families. Into each of two additional ponds (Ponds 4 and 7), a total of 6300 fingerlings from six (nursed in a separate pond above) of the 24 crosses were stocked to examine whether there were differences in mean growth performance of families in the top fish group due to possible environmental dispari-

ties among ponds. Using the data across the three ponds, differences in mean growth performance of families due to both family and environmental disparities were evaluated.

Commercial floating feed (Silver Cup, 45% protein, 16% fat; Nelson and Sons, Murray, UT, USA) was used during the period of communal rearing. Fish were fed 2% of body weight (BW) over the summer, 3% BW in the fall and spring and 1% BW during the winter when water temperature was above 10 °C, based on an assumed survival of 75% and estimated or calculated biomass. Daily ration was distributed over the entire surface of each pond twice daily at 09:00 and 16:00 hours. Dissolved oxygen (DO) and temperature measurements were taken twice daily, morning and afternoon with a YSI 51B DO meter (Yellow Spring Instruments, Yellow Spring, OH, USA). Any pond with DO levels at or $< 5.0\ mg\ L^{-1}$ received aeration with electrical aerators, until the DO levels stabilized above $7.0\ mg\ L^{-1}$.

Samples and harvest

On four separate occasions during the period of communal rearing, 100 fish were sampled from each of the three ponds for BW and length. In each case, the fish were returned to their respective ponds. In March 2006, all three ponds were drained and harvested. All fish from each pond were counted and group weighed (drained weight) close to 1 g to determine total biomass. A random sample of 200 fish was taken from each pond for final weight and length. An additional 100 fish were randomly collected from each pond and their lengths measured and ordered, to determine the size-cut-off points for the top 10% fish. Based on these cut-off points, 10–20 fish were selected from each pond group for testing and setting of the bar gaps of graders. Then, the top 10% of fish were passively graded from the remaining fish from each of the three ponds as a part of selection effects. Finally, a total of 360 fish were randomly sampled from 450 top 10% of fish from Pond 11, and the 100 top 10% of fish were similarly collected from Ponds 4 and 7 for this study. A non-lethal biopsy (fin clip) was taken from each specimen and preserved immediately in 95% ethanol for DNA analyses and subsequent parentage analysis.

Microsatellite analysis

In all, 62 potential parents (24 females and 38 males), and 560 offspring (100 from Ponds 4 and 7, and 360 from Pond 11) were genotyped. Genomic DNA was extracted from fin tissues of the yellow perch using the method described by Li, Wang, Givens, Czesny and Brown (2007), and parents and progeny were genotyped with seven highly polymorphic microsatellite loci (YP49, YP60, YP65, YP73, YP78, YP85 and YP109; Li *et al.* 2007). Amplification of microsatellite loci was performed with the three-primer system where a universal primer having the same sequence as the universal tail had 5′-label of FAM, TET or HEX (Li *et al.* 2007). Polymerase chain reactions (PCRs) were conducted in 6 μL mixes containing 3 μL of JumpStart RedMix (Sigma), 1.5 pmol of both non-tailed and labelled primers and 0.1 pmol of the tailed primer, 25 ng of DNA, in the presence of 100 μM spermidine. Amplification was performed in PTC-200 thermal cyclers (MJ Research, Waltham, MA, USA) using an initial denaturation at 94 °C for 2 min, followed by 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at a locus-specific temperature (Li *et al.* 2007), 30 s extension at 72 °C and a final 5 min extension at 72 °C. Amplification products were separated using an ABI 3130 Prism DNA genetic analyser and the results were analysed using GENEMAP® 4.0 software.

Statistical analyses for genotypic data

Near-complete genotyping was obtained for all progeny. However, DNA from 18 of the 62 parents (six females and 12 males) ultimately was of such poor quality that complete microsatellite genotyping for these individuals was not possible. GENETIC STUDIO (Dyer 2009) was used to calculate allele frequencies and relatedness among individuals (Lynch & Ritland 1999), and determine the theoretical distribution of inbreeding (Ayers & Balding 1998) in each pond. Effective number of breeders for each pond was calculated using the non-Fisherian sex ratio equation (Wright 1931) and estimated from the molecular data using the program LDNE version 1.2 (Waples 2006) using monogamy as opposed to random as the mating model. The software PAPA version 2.0 (Duchesne, Godbout & Bernatchez 2002) was used to estimate heterozygosities and polymorphism information content (PIC) and to assign individuals from the ponds to their most likely family of origin. The genotyping error rate for PAPA was set at 2%. To assess correctness of allocation, simulations were run with the same parameters, with the same sets of parents, and same number of offspring as those used in the allocation procedure. Pre-parental simulation was per-

formed to confirm that the selected loci had sufficient power for the parentage analysis.

Phenotypic data analysis

Absolute growth rate (AGR) and food conversion ratio (FCR) were calculated as follows: AGR = $(W_t - W_0)/t$ where t is the number of rearing days, W_t is the mean BW (g) at day t, W_0 is the mean initial BW (g); FCR = food consumed per fish (g)/$(W_t - W_0)$. Differences in the mean BW, AGR, FCR, water temperature and DO were analysed using two-way analysis of variance (ANOVA) ($P < 0.05$). Duncan's test was followed for mean separation when significant differences were indicated using ANOVA. The differences among the numbers of top 10% progeny assigned to each family in each pond were examined using χ^2 tests. Similarity in family mean weights of the top 10% fish between any two of the three ponds was analyzed by correlating mean family weight using SAS.

Results

Genotype diversity and parentage assignment

A total of 62 potential parents and 560 top 10% offspring were genotyped and analysed. Among the 62 potential parents, 18 (six females and 12 males) failed to yield complete results. It was determined that tissue for these 18 fish had not been adequately preserved. Further attempts using a whole genome amplification technique to improve the PCR amplification were unsuccessful in obtaining complete genotypes for these individuals. The average total number of alleles observed per locus for progeny was 16.4 (ranging from 6 to 21) and the observed heterozygosity ranged from 0.57 to 0.98 (Table 1). Allelic diversity in the broodstock provided good resolving power (mean PIC = 0.74; Table 1) for assigning parentage to progeny. The results of simulated parental assignment based on the seven markers indicated that > 98.37% of offspring could be correctly allocated to a single parental pair. However, in practical parentage determination, only 400 out of 560 top 10% yellow perch offspring (88, 73 and 239 from Ponds 4, 7 and 11 respectively) could be unambiguously assigned to their putative parents, including the same six families from Ponds 4 and 7, and 30 families from Pond 11 respectively. The remaining 160 fish (28.6%) did not exhibit allelic profiles consistent with those of the fully genotyped parents. Given that 18 parents were not successfully genotyped and very high assignment ratios were observed in the simulation tests, it was assumed that the majority of these misclassifications likely derived from the partially genotyped parents; thus these offspring were excluded from all subsequent analyses.

Allele frequency distributions were approximately normal. Gene diversity ($H_e = 0.70$) was significantly lower ($P < 0.001$) for Pond 11 (stocked with all 24 crosses) than for either Ponds 4 or 7 stocked with six of the 24 crosses ($H_e = 0.77$ and 0.78 respectively). Relatedness among the top 10% largest individuals in Pond 4 was -0.15 ± 0.26, and for Pond 7 was -0.15 ± 0.27, whereas for Pond 11, relatedness was 0.00 ± 0.52 (Fig. 1). Average single locus inbreeding estimates for Ponds 4 and 7 indicated moderate inbreeding ($F_{IS} = 0.14$) whereas outbreeding was indicated for Pond 11 ($F_{IS} = -0.23$). Conversely, the theoretical distributions of multilocus inbreeding for the top individuals sampled in each pond peaked at -0.01, 0.00 and 0.00 for Ponds 4, 7 and 11, respectively.

For Ponds 4 and 7, based on the one female:two male breeding strategy, there were 12 possible pairs

Table 1 Number of alleles (*A*), observed heterozygosity (H_o), expected heterozygosity (H_e) and polymorphic information content (PIC) of the yellow perch stocked into three ponds

	Pond 4				Pond 7				Pond 11				Overall			
Locus	*A*	H_o	H_e	PIC	*A*	H_o	H_e	PIC	*A*	H_o	H_e	PIC	*A*	H_o	H_e	PIC
YP49	6	0.97	0.73	0.69	7	0.95	0.72	0.68	7	0.99	0.67	0.61	8	0.96	0.72	0.68
YP60	7	0.90	0.79	0.76	8	0.87	0.76	0.73	12	0.98	0.69	0.64	13	0.93	0.74	0.71
YP65	8	0.95	0.78	0.75	8	0.94	0.86	0.83	16	0.96	0.71	0.66	17	0.94	0.78	0.75
YP73	6	0.89	0.68	0.63	–	–	–	–	7	0.52	0.54	0.44	13	0.59	0.63	0.58
YP78	6	0.69	0.67	0.62	9	0.56	0.59	0.54	12	0.93	0.74	0.70	15	0.83	0.82	0.80
YP85	9	0.89	0.84	0.81	10	0.93	0.85	0.82	14	0.96	0.79	0.76	20	0.94	0.83	0.81
YP109	15	0.98	0.90	0.88	15	1.00	0.90	0.89	21	0.99	0.67	0.62	29	0.98	0.85	0.84
Mean	8.1	0.90	0.77	0.73	9.5	0.88	0.78	0.75	12.7	0.90	0.70	0.63	16.4	0.88	0.77	0.74

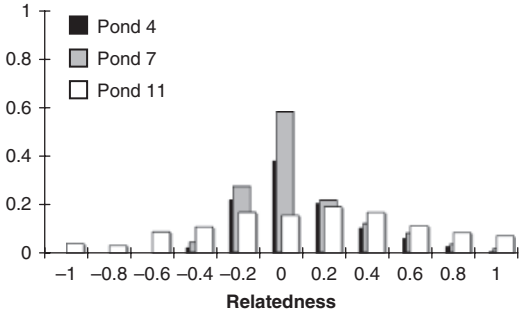

Figure 1 Estimated relatedness among top 10% of yellow perch offspring collected from three ponds.

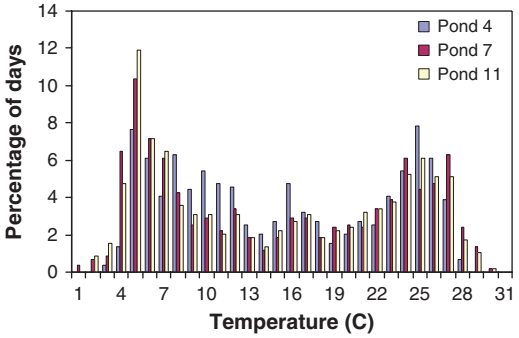

Figure 2 Range and frequency distribution of daily bottom water temperatures, rounded to the nearest whole number, in the three ponds used in communal rearing of yellow perch for the period from June 2004 to March 2006.

of parents. The effective number of breeders, calculated from the known sex ratio of broodfish, was $N_e = 16$ for Ponds 4 and 7. Based on molecular data for Ponds 4 and 7, the estimates of effective number of breeders ($N_e = 17.9 \pm 3$ and 16.4 ± 3, respectively) were similar to the calculated value. For Pond 11, there were 48 possible parental pairs and the calculated effective number of breeders for this pond was $N_e = 59$. However, the observed $N_e = 13.8 \pm 4$ resulting from molecular data was lower than the theoretical value.

Environmental factors associated with pond culture

Temperature and DO

Based on the recorded range and frequency distributions of water temperatures in three ponds for the duration of experiment (mean water temperatures being 14.9, 14.5 and 14.3 °C in Ponds 4, 7 and 11 over the period of experiment respectively), no significant difference ($P > 0.05$) in water temperature was detected among the three ponds for the entire period of the experiment and any month (Fig. 2). Approximately half (55.4%, 45.7% and 47.6% in ponds 4, 7 and 11 respectively) of the total recording days of water temperatures ($n = 588$) during the culture period were in the temperature range required for growth of yellow perch (11–26 °C). The DO levels over the 21-month experiment in Ponds 4, 7 and 11 were 9.72 ± 2.89 (mean $\pm$ SD), 10.67 ± 3.88 and 10.48 ± 3.53 mg L^{-1} respectively, and not significantly different ($P > 0.05$) except for the period from December 2004 to March 2005 (Fig. 3). The DO concentration for the three ponds was < 5.0 mg L^{-1} for only 1.4% of the total 1758 recorded observations. The pH of

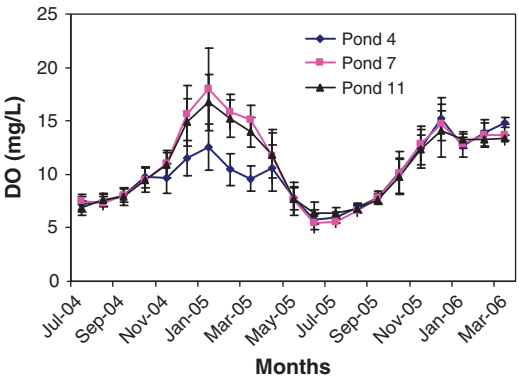

Figure 3 Monthly mean dissolved oxygen (DO) readings in the three ponds used in communal rearing of yellow perch for the period of June 2004 to March 2006.

the water in the ponds ranged from 7 to 8, which was appropriate for growth of yellow perch.

Density and feeding level

All ponds were stocked at the same density of 6300 fingerlings 0.1 ha^{-1} at the beginning of the study. Mean weights were 1.13 g for Ponds 4 and 7 and 0.79 g for Pond 11. By the end of communal rearing, there were 3458, and 3540 and 3729 fish in Ponds 4, 7 and 11 respectively, with corresponding survival of 54.89%, 56.19% and 59.19% (Table 2). Therefore, the densities in all the three ponds were similar during the entire period of communal rearing. All the fish in the three ponds received the same feeding rates for the period of communal rearing.

Table 2 Feed consumption (FC) and feed conversion rate (FCR) of communally reared fish in the three ponds in the different culture periods from June 2004 to March 2006

	FC (g fish^{-1} day^{-1})			FCR		
Period	**Pond 11**	**Pond 7**	**Pond 4**	**Pond 11**	**Pond 7**	**Pond 4**
June 2004–October 2004	0.18	0.15	0.15	1.18	1.26	1.18
October 2004–April 2005	0.27	0.21	0.23	3.98	2.34	4.16
April 2005–July 2005	0.73	0.72	0.64	1.99	2.15	1.72
July 2005–March 2006	0.59	0.64	0.68	2.72	3.77	3.02
Mean	0.44^{a}	0.43^{a}	0.43^{a}	2.47^{z}	2.38^{z}	2.52^{z}

Means within a group of FC or FCR followed by the same superscript letters were not significantly different ($P > 0.05$).

There were no significant differences ($P > 0.05$) in mean feed consumption (FC) and FCR among the three ponds (Table 2).

Growth performance and genotype by environment effects

Fast-growing fish

There was no significant difference ($P > 0.05$) in BW and length (Table 3) for the top 10% fast-growing fish from Ponds 4 and 7 where the same families were stocked. This indicates the environmental effects, which are capable of impeding family growth of aquatic animals (Jerry, Preston, Crocos, Keys, Meadows & Li 2006), were not so great in the current experiment as to override the higher genetic growth capacity of yellow perch communally reared in ponds. However, the top 10% fast-growing fish from Pond 11 grew significantly ($P < 0.05$) larger than those from Ponds 4 or 7, indicating strong genetic and family effects on growth performance. Theoretically, Pond 11 had a larger gene pool.

The same six spawning pairs were identified by parentage analysis in the top 10% fast-growing fish from Ponds 4 and 7. Of these, three brood pairs were overrepresented ($> 80\%$ of offsprings) and three pairs were underrepresented (Table 3, Fig. 4). The numbers of fish allocated to each family were analysed for genotype by environment interactions by testing for differences in family ranking among ponds. No significant difference was detected in family rankings of the top 10% heaviest fish between Ponds 4 and 7 ($P > 0.05$), indicating that the families demonstrating superior mean growth performance in Pond 4 also exhibited superior growth performance in Pond 7. By comparison, in Pond 11 with 24 crosses, 30 families were indentified in the top 10% fast-growing fish. These 30 families included five of the families in Ponds 4 and 7. Nevertheless, no significant correlations ($P > 0.05$; $R^2 < 0.6$) were observed in family mean weights of the top 10% fast-growing fish of the five families between any two of the three ponds. The absence of correlation indicates that there was still variation among family/pond mean weight of the top 10% fish, which reflects mostly environmental effects rather than genetic differences among the families.

Random fish

There was no significant difference ($P > 0.05$) detected in mean BW and length of randomly selected fish ($n = 200$) between Ponds 4 and 7 (Fig. 5), where the same six crosses were stocked. This indicates that environmental effects on phenotypic expression (growth) were not significant across these two ponds. However, the random fish sampled from Pond 11 (stocked with 24 crosses) were significantly larger ($P < 0.05$) than those from either Ponds 4 or 7. Similar results were obtained for AGR for the random fish from the three ponds (Fig. 6). The fish population from Pond 11 also had a broader weight frequency distribution and size variation than that from Pond 4 or Pond 7 (Fig. 7). The BW between females and males was significantly different ($P < 0.05$) within each pond, with females attaining significantly heavier weights than males (Table 4), which conforms to previous observations that female yellow perch grow faster than males.

Discussion

The ability to track individual family performance in communally reared yellow perch was facilitated by parentage analyses using microsatellite DNA markers. Although hypervariable microsatellite markers were used and very high success rate of assignment

Table 3 Mean weight (g) ± SD and length (cm) ± SD of the yellow perch from the top 10% fish of three ponds allocated to their families of origin using seven microsatellite markers

	Pond 4		Pond 7		Pond 11	
Family	**Weight**	**Length**	**Weight**	**Length**	**Weight**	**Length**
12 × 57	–	–	–	–	72.2 ± 3.0(2)	18.5 ± 1.6(2)
13 × 57	–	–	–	–	197.9 ± 60.6(2)	24.2 ± 0.28(2)
14 × 09	–	–	–	–	96.0 ± 0(1)	19.7 ± 0(1)
15 × 01	–	–	–	–	204.0 ± 0(1)	24.3 ± 0(1)
15 × 32	–	–	–	–	183.6 ± 59.3(13)	23.8 ± 2.4(13)
16 × 02	145.9 ± 28.6 (31)	22.3 ± 1.3 (31)	123.6 ± 21.1(27)	21.7 ± 1.0(27)	147.4 ± 34.9(2)	22.1 ± 1.6(2)
16 × 61	–	–	–	–	161.0 ± 0(1)	23.9 ± 0(1)
17 × 03	156.4 ± 34.2 (27)	22.6 ± 1.4(27)	138.4 ± 25.7(20)	22.6 ± 1.2(20)	118.7 ± 56.0(3)	20.8 ± 3.5(3)
17 × 32	–	–	–	–	182.5 ± 14.8(2)	25.4 ± 0(2)
18 × 04	112.0 ± 0(1)	21.5 ± 0(1)	190.3 ± 23.6(4)	24.4 ± 0.8(4)	184.0 ± 0(1)	25.5 ± 0(1)
18 × 61	–	–	–	–	157.2 ± 85.1(4)	22.7 ± 3.5(4)
19 × 01	–	–	–	–	153.4 ± 106.7(3)	22.0 ± 4(3)
20 × 02	143.6 ± 33.5(19)	22.0 ± 1.5(19)	142.5 ± 37.8(12)	22.0 ± 1.7(12)	108.1 ± 47.8(10)	20.2 ± 2.9(10)
20 × 48	–	–	–	–	167.5 ± 6.4(2)	24.0 ± 0.6(2)
22 × 03	142.9 ± 25.0(8)	22.0 ± 1.3(8)	112.6 ± 24.5(7)	21.2 ± 1.2(7)	–	–
23 × 31	–	–	–	–	170.5 ± 50.9(8)	23.5 ± 2.2(8)
26 × 25	–	–	–	–	55.1 ± 0(1)	17.5 ± 0(1)
26 × 07	–	–	–	–	163.9 ± 45.5(75)	23.6 ± 2.4(75)
28 × 58	–	–	–	–	137.1 ± 67.8(4)	22.3 ± 3.3(4)
29 × 05	194.4 ± 17.5(2)	23.7 ± 1.0(2)	183.9 ± 94.5(3)	24.2 ± 2.7(3)	173.0 ± 12.7(2)	23.3 ± 1.5(2)
33 × 42	–	–	–	–	189 ± 0(1)	22.7 ± 0(1)
34 × 08	–	–	–	–	81.9 ± 35.8(5)	18.2 ± 3.0(5)
36 × 06	–	–	–	–	208.4 ± 140.5(3)	23.8 ± 4.0(3)
37 × 08	–	–	–	–	178.3 ± 47.9(13)	24.1 ± 2.6(13)
38 × 11	–	–	–	–	120.9 ± 59.0(16)	21.1 ± 3.0(16)
39 × 10	–	–	–	–	165.0 ± 49.8(55)	23.6 ± 2.5(55)
40 × 11	–	–	–	–	204.3 ± 34.6(3)	24.9 ± 1.3(3)
44 × 53	–	–	–	–	187.0 ± 0(1)	25.3 ± 0(1)
54 × 24	–	–	–	–	204.0 ± 42.0(3)	24.7 ± 1.5(3)
59 × 52	–	–	–	–	76.5 ± 0(1)	19.4 ± 0(1)
59 × 06	–	–	–	–	168.0 ± 0(1)	25.0 ± 0(1)
Unallocated	158.8 ± 36.3(12)	22.9 ± 1.6(12)	151.7 ± 44.7(27)	22.9 ± 1.7(27)	165.6 ± 53.5(145)	23.6 ± 3.1(145)
Fish no.	100	100	360			
Mean	149.74 ± 32.22^{z}	22.38 ± 1.42	140.40 ± 38.33^{z}	22.40 ± 1.55	161.2 ± 54.9^{y}	23.3 ± 2.9
Allocated (*n*)	88		73		239	
Mating sets stocked	6		6		24	
Families identified	6		6		30	
$\chi^2_{d.f.}$	$\chi^2_{11} = 179.02^*$		$\chi^2_{11} = 114.1^*$		$\chi^2_{47} = 1083.25^*$	

Numbers of individuals allocated to each family are provided in parentheses.

Hypotheses of there being no differences in the number of progeny assigned to each family were evaluated by χ^2 analyses for each pond where $^*P < 0.001$.

Means (± SD) within a row followed by different superscript letters were significantly different ($P < 0.05$).

simulation was observed, the seven microsatellite markers selected for this study did not permit assignment of all sampled yellow perch individuals from each pond back to their parents. The percentage of unambiguous assignment was 88.0% for Pond 4, 73.0% for Pond 7 and 66.4% for Pond 11. The performance of microsatellites to allocate offspring to their parents is affected by the allelic diversities of markers, all moderate to high for those utilized in this study, and/or genotype variations among parents (Estoup, Gharbi, Sancristobal, Chevalet, Haffray & Guyomard 1998; Marshall, Slate & Kruuk 1998; Bernatchez & Duchesne 2000). For Pond 11, in particular, we attributed the low assignment ratios to the fact that 29% of parents could not be utilized in the PAPA analyses due to missing genotype information. Although percentage assignment was not complete for any of the three ponds, evaluation of effects of genotype by en-

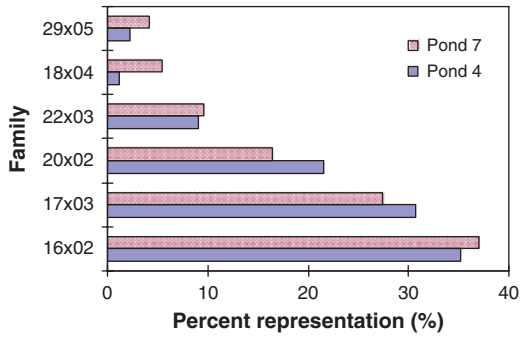

Figure 4 Percentage of the yellow perch from each family represented within the top 10% of heaviest (wet weight) fish from the Ponds 4 and 7.

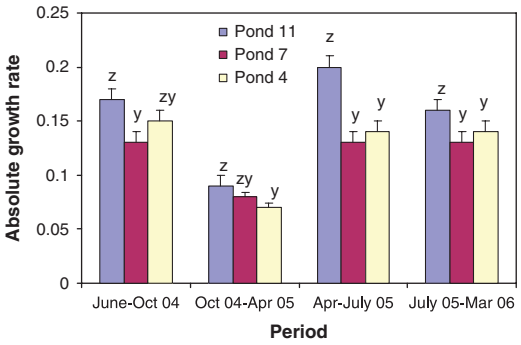

Figure 6 The absolute growth rate of cross-bred yellow perch communally reared in three ponds. The groups with the same letter were not significantly different ($P > 0.05$).

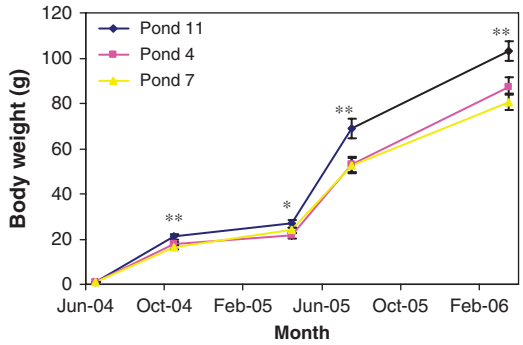

Figure 5 Mean weights of the three treatments over the experimental period. NS, no significant difference ($P > 0.05$) among the three pond groups; *significant difference ($P < 0.05$) between Ponds 11 and 7; **significant difference ($P < 0.05$) between Pond 11 and (Pond 4+Pond 7) groups.

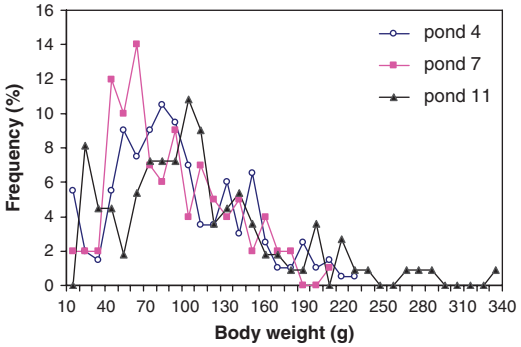

Figure 7 Weight frequency distributions of cross-bred yellow perch sampled from each of three ponds.

vironment interactions on family growth performance of yellow perch in this study was effective, because we used only parents with complete genotype data for all the three ponds. Nevertheless, more molecular markers that promote higher resolution will be useful to further improve the efficiency and accuracy of parentage analysis for the commercial-scale yellow perch breeding programme that requires at least 50 families per generation.

The lower genetic diversity (H_e) observed for Pond 11 was consistent across most of loci and indicates that the larger parental pool (62 potential parents for Pond 11) held a higher proportion of alleles in common than the smaller set of parents (18 parents for Ponds 4 and 7). This is a sign that inbreeding could potentially become an issue of importance in the breeding programme for yellow perch. The combination of a high incidence of shared alleles and the fact that yellow perch exhibit only moderate levels of genetic variation (Brown, Wang, Li, Givens & Wallat 2007), send a strong signal regarding the importance of monitoring inbreeding at the molecular genetic level when undertaking a breeding programme for this species. Furthermore, the larger weights obtained in Pond 11, despite the lower H_e, emphasizes the point that there is more to fitness and genetic variance than simple quantification of heterozygosity.

Examination of the molecular pedigrees and the effective number of breeders yielded important information regarding the breeding strategy. First, most of $n = 3$ spawning sets that were believed to increase the success of mating actually resulted in only pair matings in most cases. This reduction in the number of breeders would have gone undetected without the molecular pedigrees. The reduction became even more apparent when the molecular data were used to estimate N_e for Pond 11, illustrating that the theoretical number of breeders was a grave overesti-

Table 4 Growth performance of randomly selected fish from each pond

	Female			Male			
	Weight (g)	Length (cm)	Sex ratio (%)	Weight (g)	Length (cm)	Sex ratio (%)	Survival (%)
Pond 4	116.55 ± 40.74 (58)^{z}	20.52 ± 2.29^{z}	58	55.34 ± 19.63 (42)^{z}	16.83 ± 1.90^{z}	42.0	54.89
Pond 7	112.19 ± 35.19 (51)^{z}	20.67 ± 1.86^{z}	51	48.00 ± 19.85(49)^{z}	16.08 ± 2.30^{z}	49.0	56.19
Pond 11	134.37 ± 64.14 (63)^{y}	21.30 ± 3.26^{y}	57	60.93 ± 32.61(48)^{y}	17.01 ± 3.30^{y}	43.0	59.19

Numbers of females and males are provided in parentheses.
Means ± SD within a column followed by different superscript letters were significantly different ($P < 0.05$).

mate of the effective number of breeders. The ramification of these results for yellow perch breeding is that a matrix of paired matings would be a more successful strategy for identification of superior broodfish and that attention to shared alleles and relatedness among broodfish (Doyle & Herbinger 1995) is an essential strategy.

In commercial pond culture situations, feeding level, fish density, water temperature and DO are the most important factors relative to fish growth. During the period of communal rearing, we strove to maintain the environmental factors of the three ponds as similar as possible, and are confident that the observed differences in growth rates and BW between Pond 11 and Ponds 4 and 7 were not attributable to differences in environment factors. Typical stocking density is about 50 000 fingerlings ha^{-1} for commercial pond culture of yellow perch (Malison 2000), we maintain that although the high stocking density of 6300 fingerlings for each 0.1-ha pond likely affected the growth rate of fish, this effect should have been similar for all ponds and should not have influenced comparative analysis across the three ponds. Also, temperature might similarly affect fish growth in all ponds. Reported temperature for growth of yellow perch ranged from approximately 11 to 26 °C with optimum temperatures ranging from 23 to 25 °C (Hokanson 1977). Only half of the total recording days of water temperatures during the culture period were in this temperature range.

Over the period of communal rearing, it appeared that the fish were overfed, and FC and FCR were a little higher than expected. This is because we used 75% of survival rate for calculation of feeding rates, but the actual overall survival rates were 54.9–59.2% (Table 4). Therefore, we believe that feed did not limit fish growth in any ponds. There were no significant differences detected in water temperature, DO (in most of months) and feeding level among the communal ponds. This may be one of the reasons that the environmental effects were minor relative to genetic effects during the period of communal rearing in this study. Because of the very similar environments across ponds, and a similarly large population held in each pond, we are confident that the results from this study reflect the effects of genotype by environment interactions on family growth, through identifying that families with superior growth performance in one pond also exhibited the same superior growth performance in the other ponds.

Studies of genotype by environment interactions on phenotype have been widely reported in the past decades in various aquatic animals (Gjerde & Schaeffer 1989; Bagley, Bentley & Gall 1994; Herbinger, O'Reilly, Doyle, Wright & O'Flynn 1999; Fishback, Danzmann, Ferguson & Gibson 2002; Gall & Neira, 2004; Jerry *et al.* 2006; Saillant, Dupont-Nivet, Haffray & Chatain 2006; Wang & Li, 2007). Studies on yellow perch have demonstrated that environmental factors, such as temperature and density, can have a profound influence on growth (Power & Van den Heuvel 1999; Tidwell, Coyle, Evans, Weibel, McKinney, Dodson & Jones 1999; Headley & Lauer 2008). However, no study examined the influence of disparate environments on genotypic expression of family growth for this species. In the present study, no significant differences were found in family rankings of the top 10% heaviest fish and growth performance of the top 10% fish and random samples between the two pond environments, where the same six crosses were stocked. On the other hand, there were no significant correlations detected in family mean weights of the top 10% fast-growing fish between any pairs of the three ponds. These results indicate that although there may have been strong environmental effects across the experimental ponds, they were not so great as to override the higher genetic component of growth.

Both samples from Pond 11 (the top 10% fast-growing fish and random sample) were significantly greater than fish from either Ponds 4 or 7. This difference likely resulted from the larger number of crosses and

hence greater heterosis in Pond 11. This further demonstrates the importance of maintaining and screening large numbers of families for identification of fast-growing bloodstock, and further illustrates the potential utility of molecular marker-assisted selection in this species. This also emphasizes the significance of maintaining a large genetic pool for improving growth and production.

Although the family representation was unequal to the top 10% population within the pond, the same six families were identified in Ponds 4 and 7. In addition, the three overrepresented and three underrepresented families were consistent between Ponds 4 and 7. These findings are relatively consistent with reports about Pacific white shrimp *Litopenaeus vannamei*, where no differences were found in family rankings in growth between round and raceway tanks (Argue, Arce, Lot & Moss 2002). But the studies in rainbow trout *Oncorhynchus mykiss* (Bagley *et al.* 1994), Chinook salmon *Oncorhynchus tshawytscha* (Winkelman & Peterson 1994) and shrimp *Penaeus japonicus* (Jerry *et al.* 2006) showed significant G × E effects on growth performance. The fact that a high percentage of families in all three ponds contributed to the top 10% fish suggests that superior families of yellow perch have the potential to show consistent fast growth performance under different environments with similar conditions and management. These results also indicate that communal rearing of yellow perch in multi-ponds and selection of the top-performing fish as breeding candidates is feasible in terms of maintaining biological fitness and avoiding inbreeding as long as molecular markers are routinely utilized in the breeding programme.

In conclusion, the present study demonstrated that for yellow perch, molecular pedigrees for DNA parentage are useful and essential for tracking individual family performance and examining G × E interactions on trait expression. Our results indicate there were genotype–environment interactions on growth of certain yellow perch families, and that environmental effects were not so great as to override the higher genetic growth capacity of yellow perch communally reared in commercial-scale ponds.

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