DR. QIAN MA (Orcid ID : 0000-0002-5546-3489)

Received Date : 14-Mar-2016
Revised Date : 22-Mar-2017
Accepted Date : 22-Mar-2017
Article type : Original Article

Evaluating spawning performance among captive Florida pompano *Trachinotus carolinus* broodstock using microsatellite based parentage assignment

Qian Ma[1,2*], Seifu Seyoum[3], Michael D. Tringali[3], Matthew J. Resley[1], Nicole R. Rhody[1], Kevan L. Main[1], Kenneth M. Leber[1]

[1]Mote Marine Laboratory, Directorate of Fisheries and Aquaculture, Sarasota, Florida, USA

[2]Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, Shandong, China

[3] Florida Fish and Wildlife Research Institute, St. Petersburg, Florida, USA

Correspondence: Q Ma, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 106 Nanjing Road, Qingdao 266071, China.
E-mail: maqian@ysfri.ac.cn; Fax: 0086-532-85811514; Tel: 0086-532-85836344

Running Head: Spawning performance in captive pompano broodstock

Key words: Florida pompano, parentage assignment, microsatellites, reproductive success, rapid growth trait

Abstract

Florida pompano has been identified as a promising candidate for commercial scale aquaculture production, but to date little information is available regarding captive broodstock spawning characteristics. Genetic markers were tested for their power in monitoring mating outcomes and potential in analyzing heritability of rapid growth trait in *Trachinotus carolinus*. A total of 20 unrelated adults (10 females and 10 males) were chosen for a hormone-induced mass spawning event. The 515 fastest growing and 485 slowest growing fish out of the total 4852 offspring were considered a selected progeny stock, fish were collected at 45 days post-hatch based on their growth traits. Parentage analyses based on the 20 breeders and 1,000 selected progeny were performed using a total of nine microsatellite markers, a 100% assignment rate was achieved and a four marker-set was the minimum number for the parentage assignment. The effective breeding number for the selected progeny was 11 (six females and five males), among which three females and two males were predominant contributors with the total contribution of 95.8% and 94.7%, respectively. The proportion of fast-growing offspring from broodfish and each mating cross (sire/dam) was used for detecting whether variation in growth of the offspring was related to

parental stocks. Results showed that three adults and their mated combination exhibited the greatest fast-growing offspring proportion (69.73% and 55.95%). This research provided new information regarding spawning performance and parental contribution during mass spawning events; both important first steps toward developing improved management strategies for captive Florida pompano broodstock.

Introduction

Florida pompano (*Trachinotus carolinus*) are distributed in coastal waters throughout the Gulf of Mexico and along the eastern United States in the Atlantic Ocean (Gilbert 1986). Males and females are sexually mature at one to three years of age and normally attain a maximum weight ranging from 0.7 to 2.3 kg (Gilbert 1986). In Florida, spawning is thought to occur year round in the Gulf (Berry & Iverson 1967) and on the Atlantic coast from February to October at 22-26 °C, with peaks in April to May and September to October (Fields 1962). Larvae develop at sea, whereas juveniles inhabit the surf zone until temperatures are < 20 °C, when they again migrate offshore (Fields 1962).

Pompano have long been considered a high-value marine food fish, as evidenced by increasing market prices and a rise in consumer demand (Hauville, Zambonino-Infante, Bell, Migaud & Main 2014; Main, Rhody, Nystrom & Resley 2007). Although significant interest in developing commercial culture capabilities began as early as the 1960's and 1970's (Watanabe 1995), efforts to develop reliable protocols for successful commercial scale farming of Florida pompano are ongoing. To date, studies on the culture of *T. carolinus* have focused on feed composition (Hauville *et al.* 2014; Riche & Williams 2011), spawning behavior (Hoff, Mountain, Frakes & Halcott 1978; Reynolds 2010) and improving overall culture conditions (Weirich & Riche 2006; Weirich, Wills, Baptiste, Woodward & Riche 2009). Research has shown pompano can be readily induced to spawn in captivity (Main *et al.* 2007; Weirich & Riley 2007), have a high tolerance to different salinities and stressors (Weirich & Riche 2006), and readily accept pelletized diets (Hauville *et al.* 2014; Riche 2014). These advantages make *T. carolinus* an excellent candidate

87 species for commercial aquaculture. However, further improvements in aquaculture
88 technology are needed to ensure development of a viable Florida pompano industry.
89 The implementation of selective breeding programs for commercially farmed fishes is
90 important for the long-term sustainability of the aquaculture industry. So far, selective
91 breeding programs have been well established for some marine and freshwater species,
92 including red sea bream (*Pargus major*) (Murata, Miyashita, Izumi, Maeda, Kato &
93 Kumai 1996), European sea bass (*Dicentrarchus labrax*) (Vandeputte, Dupont-Nivet,
94 Haffray, Chavanne, Cenadelli, Parati, Vidal, Vergnet & Chatain 2009), Atlantic
95 salmon (*Salmo salar*) (de Mestral & Herbinger, 2013), common carp (*Cyprinus*
96 *carpio*) (Ninh, Ponzoni, Nguyen, Woolliams, Taggart, McAndrew & Penman 2011;
97 Vandeputte 2003) and Egyptian Nile tilapia (*Oreochromis niloticus*) (Rezk, Ponzoni,
98 Khaw, Kamel, Dawood & John 2009). During the past ten years, a summary of
99 measured responses to selection has shown that estimated genetic gains in growth rate
100 could reach 10–20% per generation (Gjedrem & Baranski 2010). However, Gjedrem,
101 Robinson & Rye (2012) estimated that only 10% of aquaculture production
102 worldwide is based on genetically improved stocks. One possible reason is the fact
103 that pedigree information is often difficult and costly to obtain (Vandeputte & Haffray
104 2014).
105 Accurate pedigree information is of paramount importance in selective breeding
106 programs (El-Kassaby, Cappa, Liewlaksaneeyanawin, Klápště & Lstibůrek 2011;
107 Lacy 2012). Molecular tools, such as microsatellite markers, have been used in
108 parentage analysis to provide pedigree information to estimate breeding success,
109 effective population size, individual inbreeding levels and other genetic parameters
110 (Blonk, Komen, Kamstra, Crooijmans & van Arendonk 2009; Dodds, Tate & Sise
111 2005; Kapralova, Morrissey, Kristjánsson, Ólafsdóttir, Snorrason & Ferguson 2011;
112 Ponzoni, Khaw, Nguyen & Hamzah 2010). Additionally, genetic markers have also
113 been used extensively as a management tool, particularly in selective breeding
114 programs involving a number of fish species, including gilthead sea bream (*Sparus*
115 *aurata*) (Antonello, Massault, Franch, Haley, Pellizzari, Bovo, Patarnello, de Koning
116 & Bargelloni 2009; Navarro, Zamorano, Hildebrandt, Ginés, Aguilera & Afonso

2009), large yellow croaker (*Larimichthys crocea*) (Liu, Sui, Wang, Cai, Yao & Chen 2011) and Japanese flounder (*Paralichthys olivaceus*) (Shikano 2005). To date, there is no information on parental contribution to mass spawning in Florida pompano, which is important not only for understanding basic and fundamental data on spawning characteristics, but also for the development of a successful breeding program.

Accurate parentage assignment based on high resolution molecular markers is important for the purpose of conducting future heritability studies. The estimation of heritability and genetic correlations allows operations to design breeding programs and allows for the evaluation of expected genetic gains (Vandeputte & Haffray 2014). As a first step in this direction, we initiated a scoping study employing microsatellite-based DNA profiling to obtain pedigree information within a subset of captive Florida pompano broodstock maintained at Mote Aquaculture Park in Sarasota, Florida, USA. A molecular-based assignment of the selected progeny stock to parents was undertaken, genetic diversity data were compared between broodstock and progeny stock, assignment power of selected markers were evaluated, and individual/parental contributions to larval production were detected and quantified to estimate reproductive success of breeders. In addition to evaluating spawning performance of broodstock, associations between fast-growing progeny and related breeders were investigated to determine whether variation in growth of the offspring was related to parental stocks. Our objective is to demonstrate the potential use of molecular-based parentage assignment as a practical tool for conducting genetic selection of important attributes in evaluating spawning performance using captive Florida pompano, *Trachinotus carolinus*.

Materials and methods

Broodstock collection, spawning and larval rearing

Eight adult Florida pompano were collected from coastal waters in Sarasota (Florida, USA) and transported to Mote Aquaculture Research Park (Sarasota, Florida, USA). Following a 40-day quarantine period, wild caught broodstock were PIT-tagged and

combined with an existing population of F1 generation pompano (captive bred offspring) that were previously spawned and reared at Mote Aquaculture Research Park. A tissue sample (fin clip) was taken from each fish and samples were stored individually in 90% ethanol for later parentage analysis. Prior to spawning, all adult broodstock were screened through genotyping with 15 microsatellite markers to ensure they were unrelated (neither full- or half-sibling). A total of seven F1 and one wild fish were removed from the broodstock population thus excluding them from the study.

In total, 20 adult fish (10 females and 10 males) were held in a single, indoor, photoperiod (11-13 H light) and temperature controlled system (22-28 °C) and maintained at a salinity of 35 ± 1 g L^{-1} (Fig.1). The recirculating system consisted of a 28 m^3 tank equipped with filtration, which included a 0.085 m^3 drop filter (Aquaculture Systems Technologies, New Orleans, LA, USA) for solids removal, a 900-l moving bed for biofiltration containing 0.283 m^3 plastic extruded floating media (AMBTM media, EEC, Blue Bell, PA, USA), a protein skimmer, and two 150-W High Output SMART HO UV® units.

To sample broodstock, individual fish were netted into a 500-l tank containing 200-l of saltwater and anesthetized with Tricaine-S (Western Chemical, Inc., Ferndale, WA, USA) at a concentration of 300 ppm for approximately 1-2 minutes. All male and female pompano were weighed (body mass, weight, g) and measured (standard length, SL, cm) at sampling (Table 1). Fulton's condition factor (K) was calculated following the formula: $K = 10^2 \times \text{body weight} \times \text{standard length}^{-3}$ (Williams 2000).

To ascertain broodstock spawning condition, females were cannulated using a soft plastic tubing (1.0 mm inside diameter) and oocytes were examined under a light microscope. Oocyte staging terminology was used to identify the reproductive condition (stage and step) of each female and to determine the individuals that were suitable for hormonal implantation (Rhody, Neidig, Grier, Main & Migaud 2013). Only females with oocytes in late secondary growth (SGl) or the later stages of oogenesis (≥ 400 μm, n = 10 females) were induced to spawn. A single intramuscular implant containing gonadotropin releasing hormone analogue (sGnRHa) was

administered at a dosage of 50 µg/kg body weight (Ovaplant®, Western Chemical, Inc., Ferndale, WA, USA). Males were not implanted during this study. Spawning occurred approximately 24 hours following hormonal implantation. Following the spawning event, eggs were transferred from the broodstock tank to a 100-l conical hatching tank. At 4–6 h post fertilization (blastula stage), aeration was removed and non-viable (sinking) eggs were discarded. The aeration was then turned back on and three aliquot 5 ml samples were taken and counted to estimate the egg concentration and fertilization rate. Approximately 150 eggs (volumetrically measured) were stocked into individual microcosms. Accuracy of initial stocking ranged from 151 to 168 larvae per microcosm. The microcosms (n = 12) were made of a 100 mm diameter PVC pipe sealed at one end with a 330 µm mesh. The sieves were set on a grid in a 340 L water table equipped with UV sterilization (salinity 34 ± 1 g L^{-1}, dissolved oxygen 5 ± 1 mg L^{-1}, pH 8.5 ± 0.3, temperature 27 °C). Larvae were reared in a 3.3 m^3 tank (temperature 26 ± 1 °C, salinity 35 ± 1 g L^{-1}, dissolved oxygen 6 ± 1 mg L^{-1}) following protocols described by Hauville *et al.* (2014). All progeny were size graded at 45 days post-hatch (n = 4,852) and the following size standards were established and used to separate the pompano fingerlings into three groups: fast-growing (standard length > 4.7 cm, body height > 2.0 cm, body mass > 2.9 g), slow-growing (standard length < 3.5 cm, body height < 1.5 cm, body mass < 1.1 g) and moderate-growing (with the size in between the fast- and slow- growing). All individuals from the fast-growing group (515 fish) and slow-growing group (485 fish) were sampled to form a selected progeny stock (n = 1,000), whose members were of significant different growth traits. Fish were collected from the tank and euthanized with Tricaine-S (Western Chemical, Inc., Ferndale, WA, USA) in accordance with Mote Marine Laboratory's Institutional Animal Care and Use Committee approved protocols (IACUC Approval No. 12-03-KM1). Whole animals were stored in absolute ethanol for further DNA extraction and genotype analysis.

DNA extraction

Total genomic DNA was extracted from caudal fin clips of pompano broodstock and offspring by using PureGene DNA Extraction kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. RNase treatment was performed by adding DNase-free RNase A solution and incubating at 37 °C for 60 min. All DNA samples were quantified using NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA), then diluted to 100 ng/µl and stored at 4 °C prior to PCR amplification.

PCR amplification and microsatellite analysis

Microsatellite markers used in this study were selected from an existing suite of microsatellite DNA markers developed for permit (*Trachinotus falcatus*) and described by Seyoum (2014). Each broodfish was independently genotyped using 15 polymorphic microsatellite markers (Table 2), which were validated for pompano. Each amplification and analysis was run twice to evaluate scores for consistency of the broodstock relation test. Nine polymorphic microsatellite loci (TFl05, TFl07, TFl15, TFl39, TFl51, TFl56, TFl62, TFl64, TFl70) were finally selected for parentage assignment of all 1,000 fingerlings, and these loci were assayed in five optimized PCR multiplexes (Table 2).

Each 12.5 µl PCR reaction consisted of 0.3 U of GoTaq (Promega, Madison, WI, USA), 2.5 µl 5 x GoTaq Buffer, 0.2 mM each of four dNTPs, 3 mM $MgCl_2$, 1.25 mg/ml BSA, 0.8 µM of each primer, and 100 ng DNA template. PCR amplification was performed according to the following protocol: 94 °C for 2 min; followed by 8 cycles of 94 °C for 45 s, 58 °C for 45 s, 72 °C 45 s; 10 cycles of 94 °C for 40 s, 57 °C for 40 s, 72 °C 40 s; 12 cycles of 94 °C for 35 s, 56 °C for 35 s, 72 °C 35 s; 9 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C 30 s; and a final extension of 7 min at 72 °C. One microlitre of each PCR multiplex was combined with 12 µl Hi-Di formamide and 0.5 µl Gene Scan-500 ROX-labeled size standard (Applied Biosystems, Carlsbad, CA, USA) for fragment assay and denatured at 94°C for four minutes, and snap-cooled before loading. Microsatellite alleles were detected and sized on an automated ABI 3130XL genetic analyzer (Applied Biosystems, Carlsbad, CA, USA). Fragment

lengths were analyzed using GeneMapper (version 4.0; Applied Biosystems, Carlsbad, CA, USA).

Genetic diversity, parentage assignment and statistical analysis

Genetic diversity estimators (number of alleles, observed and expected heterozygosity, and polymorphic information content) were assessed for each locus based on the genotypes of 20 broodstock and 1,000 offspring using the genetic parentage analysis software, CERVUS (version 3.0) (Kalinowski, Taper & Marshall 2007).

The effective population size (N_e) was estimated from the microsatellite DNA genotype data using the linkage-disequilibrium of Burrows option (Hill 1981; Waples 2006) implemented in the program NeEstimator version 2 (Do, Waples, Peel, Macbeth, Tillett & Ovenden 2014). This approach generally gives unbiased estimates of linkage-disequlibrium from which estimates of N_e can be derived (Robinson & Moyer 2012) with 95% confidence intervals based on the parametric procedure of Waples (2006). Deviations from Hardy–Weinberg (HW) equilibrium and linkage disequilibrium between all possible pairs of loci in the broodstock were analyzed using GENEPOP (version 4.2) (Rousset 2008). Prior to spawning, a marker-based parentage testing was used to rule-out full-sib or half-sib individuals from the adult broodstock population (Tringali 2006). Assignment rates of the nine markers in all 20 breeders and 1,000 offspring were calculated (with the confidence of 95%, error rate of 0.01 and minimum number of typed loci of 3) using CERVUS (version 3.0) (Kalinowski *et al.* 2007). Markers were then removed in a step-wise fashion in order to exclude the locus with the lowest PIC (removed order: TFl70, TFl51, TFl39 and TFl05, TFl56, TFl62), and assignment rates of the remaining marker sets were tested to evaluate their power.

Subsequently, the number of progeny produced by each parent was determined and used to calculate their contribution as a percentage of the total sampled cohort (the subset of fast- and slow- growing individuals). The number of fast- and slow- growing offspring produced by each parental combination was also calculated. Fast-growing offspring proportion of each broodfish or mating pair was defined as the percentage of

fast-growing offspring in its total progeny.

Growth data were expressed as the mean ± standard deviation (S.D.). Weight (g) and body length (SL, cm) measurements were analyzed by one-way ANOVA to determine significant differences between samples using the Statistical Package for the Social Sciences, SPSS (version16.0). Values were considered statistically significant when $P < 0.05$. The strength of association between parameters (weight, Fulton's condition factor, No. offspring and fast-growing offspring proportion) was evaluated by calculating the Pearson product-moment correlation coefficient (R). Values were considered significantly positively correlated when $R > 0.80$, while moderate positive correlation was determined when $0.50 < R < 0.80$.

Results

Growth characteristics of sample sources

The weight (g) and body length (SL, cm) of male and female pompano broodstock are presented in Table 1. Overall, mean female weight and body length were calculated at 1,682.0 ± 534.7 g and 37.6 ± 4.7 cm, respectively. When compared, the mean weight (891.5 ± 328.5 g) and body length of males (32.1 ± 2.9 cm) was significantly less than in females ($P < 0.01$). Additionally, the average body length and height of the fast-growing progeny (4.8 ± 0.2 cm, 2.1 ± 0.2 cm) was significantly higher ($P < 0.01$) than in slow-growing progeny (2.8 ± 0.3 cm, 1.2 ± 0.1 cm).

Parentage assignment and contribution of breeders

Analyses based on the broodstock (n = 20) and the selected progeny stock (n = 1,000) were performed by using a total of nine microsatellite markers, complete genetic profiles were obtained for each individual with 100% assigned to a single parental pair (Tables 1 and 3). Among all 20 breeders used for the spawning event, the effective breeding number of the selected progeny stock was 11, including six females and five males; however, a limited number of individuals contributed a large proportion of the offspring. As listed in Table 1, three females (F-12, F-13 and F-9) and two males (M-9 and M-10) were predominant contributors to the sampled

offspring. Progeny were identified from three predominant female breeders (95.8%) and two males (94.7%). Low levels of contribution (3-20 offspring) were detected for the other three females and three males (F-16, F-1, F-10, M-12, M-4 and M-6). The mean fertilization and hatch rate of this single mass spawning event measured 35.5 % and 59.5 %, respectively. All the mating crosses (sires x dams) and their contribution to the selected offspring are listed in Table 3. Among the 17 sire/dam combinations represented, three mating pairs (M-9/F-12, M-9/F-13 and M-10/F-12) had the largest contribution with over 100 out of the total 1,000 sampled offspring. An additional six mating pairs produced 7-71 offspring, whereas the remaining eight pairs contributed the least with less than six total offspring (Table 3).

Effects on reproductive success of broodfish

The effects of body size, sample sources (wild versus F1) and condition factor on the reproductive success were evaluated in this study. The largest female broodfish (F-12) contributed 59.4% of the total progeny. However, the third largest contributor was the third smallest female (F-9), which contributed 11.2%. Among the ten male breeders, only the five largest males contributed to the spawning, with the greatest contribution (73.8%) from the largest male (M-9). As shown in Fig. 2A and 2B, there was significant correlation between male body weight and contribution to offspring ($P < 0.001$, $R = 0.88$, $n = 10$), and moderate positive correlation was detected in females ($P = 0.02$, $R = 0.66$, $n = 10$).

In comparison with the F1 individuals (n = 12), the wild broodfish (n = 8) showed greater reproductive success in both females and males. Wild females contributed 86.6% of the offspring, and wild males contributed 99.1% of the total progeny. Additionally, we analyzed the effect of K values on reproductive success. As shown in Table1, F-10 and M-9 exhibited the highest K value in female and male breeders, and M-9 was also the predominant contributor in males; however, F-10 only contributed three offspring to the spawning event. The analysis revealed that there was no significant correlation between condition factor of the broodfish and their contribution to offspring (male regression, $P = 0.10$, $R = 0.56$, $n = 10$; female

regression, P = 0.42, R = 0.29, n = 10) (Fig. 2C and 2D).

Evaluation of broodfish contribution to rapid growth offspring

In this study, we examined the proportion of fast-growing offspring from broodfish and each mating cross (sire/dam). The results revealed that the fast-growing offspring proportion of two females (F-12 and F-13) and one male (M-9) were over 50% (Table 1). Furthermore, four pairs of mating combination were shown to exhibit a high proportion of fast-growing offspring (Table 3). Based on the proportion of fast-growing offspring, only two sire/dam combinations (M-9/F-12 and M-9/F-13) are recommended as candidate broodfish for further studies; the other two mating crosses (M-9/F-16 and M-10/F-10) contributed a small number of total offspring numbers (one and five).

Genetic diversity of breeders and progeny

The 20 broodstock were genotyped at nine microsatellite loci (Table 4). The number of alleles per locus ranged from 5 to 16 (mean = 11.11). The mean observed heterozygosity (H_O) was 0.7833, the mean expected heterozygosity (H_E) was 0.7858, and the mean polymorphic information content (PIC) was 0.7427. In Florida pompano broodstock, no significant departures from HW equilibrium expectations was observed at all the nine loci, and the results showed that P (P-value for HWE) > 0.071, which indicated the data was not affected by Hardy-Weinberg equilibrium.

The genetic characteristics of the nine microsatellite loci of the selected pompano progeny (Table 4) indicated that the average number of alleles per locus was 9.11, the mean H_O was 0.8522, the mean H_E was 0.6996, the mean PIC was 0.6640, and significant departures from HW equilibrium expectations (heterozygosity excess) were observed at all the nine loci. The estimated N_e of the total 1,000 offspring was 3.6 ± 3.3 with used lowest allele frequency of 0.05. When the progeny was divided into fast- and slow-growing groups, the estimated N_e at lowest allele frequency of 0.05 was 3.9 ± 3.4 and 3.5 ± 3.2, respectively.

The power of the markers for parentage assignment in Florida pompano

In this study, the power of the selected markers for parentage assignment in Florida pompano was investigated in the context of the data (genotype data of all 20 parents and 1,000 offspring). Parentage assignment rate was calculated with the full set of 9 markers and was found to be 100% with the 95% confidence. Markers were then removed in a step-wise fashion in order to exclude the locus with the lowest PIC. As a result, TFl70 (PIC = 0.2793), TFl51 (PIC = 0.5813), TFl39 and TFl05 (PIC = 0.6883 and 0.6898, respectively), TFl56 (PIC = 0.6978), TFl62 (PIC = 0.7166) were removed in order and the assignment rates were calculated by using the remained markers. The results showed that 99% of assignment rate could still be determined when TFl56 was removed (only 4 markers left), but the assignment rate dropped to 89% when TFl62 was removed and there were only 3 markers left (TFl07, TFl15 and TFl64).

Discussion

Using a molecular based assessment, this work provides the first description of spawn contribution and mating success of captive pompano broodstock. In this study, nine microsatellites with a combined number of 102 alleles adequately identified the effective breeding number and their relative contribution to the progeny. A high level of accuracy (100%) was found in achieving the assignment success to parental pairs, thus highlighting the usefulness of these markers to retain pedigree information. Identification of the minimum number of microsatellite markers required to assign parentage with a target accuracy rate of 95% correct assignments (i.e., to build a cost-effective system with markers of high assignment power) is of great importance in selective breeding programs. The assignment power of markers was shown to depend on several factors, such as polymorphisms of markers, locations of markers on the genome, the number of parents and offspring, and mating systems (Vandeputte, Rossignol & Pincent 2011). Sefc & Koblmüller (2009) reported that the variability of the markers can be more critical than the number of markers used. In this study, we found that the average non-exclusion probability of each locus was significantly related to their PIC ($P < 0.05$). In other words, the markers with higher PIC exhibited

higher exclusion probability (assignment power). Under this circumstance, we tested the power of marker sets by removing one or two weakest markers from the set, the results showed that removing the weakest markers did not have much effect on the assignment rate until there were only 4 markers left (TFl62, TFl07, TFl15 and TFl64). As a result, with 10 males and 10 females in this study, the four marker-set listed above is the minimum number for parentage assignment in Florida pompano. According to Vandeputte (2014), assignment power > 0.99 can generally be obtained by 8–15 microsatellite markers in fish crosses involving a few tens or hundreds of parents, and a reasonable option when designing a marker set is to include a few more markers than theoretically needed, since there might be small problems of genotyping errors during the assignment due to inbreeding or the presence of null alleles. In this study, only four markers were required to successfully assign parentage with a target accuracy rate of 95%. However, using all nine markers would be optimal in future studies for identifying family structure in mixed family cohorts of *T. carolinus*.

In studies with gilthead seabream, the variance in family size and a large number of non-contributing fish (males), were found to be the main limitations to N_e (Brown, Woolliams & McAndrew 2005). Similarly, genetic diversity data were compared between broodstock and selected progeny in the present study and this significantly decreased from breeders to offspring. For example, PIC was reduced from 0.7427 to 0.6640 due to the limited number of pompano breeders used for the spawning event. Although N_e of broodstock was not estimable since the breeders might be of different age, the low estimated N_e of offspring (3.6) at lowest allele frequency of 0.05 might also be related to the overall limited contribution of total broodfish in the spawning event.

In this study, a theoretical number of 100 full sibs could have been obtained by using 10 males and 10 females as broodstock, but only 17 families were identified instead. As listed in Table 1, nearly 90% of these offspring from 17 families turned out to have been sired by two male breeders (M-9 and M-10). Similar results have also been reported in Japanese flounder where approximately 100% offspring were contributed by a single male (Sekino, Saitoh, Yamada, Kumagai, Hara & Yamashita 2003). In

417 evaluating spawning performance in captive common snook populations, up to 93%
418 of the offspring were assigned to one sire in a single tank (Rhody, Puchulutegui,
419 Taggart, Main & Migaud 2014). Possible explanations for these findings might be
420 reproductive competition among males at the mating event or unsuccessful
421 reproduction of other males (i.e., poor sperm quality) (Rhody *et al.* 2014; Sekino *et al.*
422 2003). More evidence from this study were found that the body weight of male
423 breeders to be significantly related to the contribution to offspring (Fig.2A). Only the
424 largest five males contributed to the spawn with one siring 73.8% of the total progeny.
425 This observation suggested that the larger males might be more competitive in the
426 reproductive event, and the reproductive success of male breeders might be linked to
427 their body size.
428 Beldade, Holbrook, Schmitt, Planes, Malone & Bernardi (2012) suggested there is an
429 important maternal effect of female size on traits of their offspring, where larger
430 female fish contribute more to population replenishment. Nevertheless, no significant
431 correlation was detected between the female body weight and the contribution to the
432 offspring in this study. The unequal contributions of females to differential numbers
433 of offspring have been attributed to other factors, such as age and condition (Hislop
434 1988; Marteinsdóttir & Steinarsson 1998). Brown *et al.* (2005) also indicated that
435 parental contribution was associated with parent weight, which may be explained by
436 the age of fish. Moreover, correlations between parental size (age) and offspring size
437 could be due to egg size, i.e., egg diameter has been shown to increase with
438 broodstock age in some species, and larger eggs normally result in larger larvae (Jerez,
439 Rodríguezb, Cejasa, Martína, Bolañosb & Lorenzo 2012). Parental age was not
440 known in this study since some of the broodstock were wild caught fish. Future
441 research should consider parental age as a factor affecting reproductive success.
442 According to our results, wild broodfish showed greater reproductive success in
443 comparison with F1 individuals. Additionally, all three breeders with the greatest
444 proportion of fast-growing offspring (F-12, F-13 and M-9) were wild caught fish.
445 However, the effect of breeder source (wild versus F1) on fish reproductive success
446 has not been established. In giant freshwater prawns, significant differences in terms

447 of offspring quality between different broodstock sources (pond-reared and wild) was
448 detected by Nhan, Wille, Hung & Sorgeloos (2009), which indicating that broodstock
449 sourcing deserves proper attention in hatchery operations. Despite the potential role of
450 broodstock sources in reproductive success of pompano, most of the wild fish in this
451 study were larger than the F1 broodstock. Since brooder body size exhibited a
452 correlation with their contribution to offspring, the difference between contributions
453 of the wild and F1 brooders might also be related to the body size. In addition to body
454 size, variations in condition factor can reflect the state of sexual maturity and degree
455 of nourishment (Lamas & Godinho 1996; Williams 2000). Previous reports have
456 shown that fertilization success is positively associated with male K value in Atlantic
457 cod (Rakitin, Ferguson & Trippel 1999), which led us to examine the effect of
458 condition factor on the contribution to offspring in the present paper. Although male
459 body weight was significantly correlated with contribution to offspring, no significant
460 correlation was detected between contribution to offspring and the K values of either
461 female or male breeders (Fig.2).
462 Improving growth rate is a major breeding goal for the aquaculture industry, but
463 individual selection has often shown poor responses in fish species (Chevassus,
464 Quillet, Krieg, Hollebecq, Mambrini, Fauré, Labbé, Hiseux & Vandeputte 2004). In
465 this study, all progeny from a single spawning event of Florida pompano were
466 cultured in the same tank and grown to 45 days post-hatch. Offspring of significant
467 differences (fast- and slow- growing) in growth characteristics were collected to form
468 a selected progeny stock and genotyped, the relationship between parentage and
469 growth characteristics of progeny was estimated. Given that growth is heritable in fish,
470 we speculate that certain breeders may have a higher contribution to the fast-growing
471 progeny. Overall, two females (F-12 and F-13) and one male (M-9) produced a higher
472 proportion of fast-growing offspring (> 50%). Interestingly, F-12 and M-9 also turned
473 out to be the largest female and male breeders in our spawning population. However,
474 whether the rapid growth in Florida pompano broodstock is related to their own
475 growth characteristics still needs further studies, since the condition of wild fish in
476 broodstock (age, etc.) was unclear in this study. Both the sire and dam might have

characteristics associated with rapid growth in order to produce more fast-growing progeny. For instance, among all the mating crosses, only M-9/F-12 and M-9/F-13 were shown to produce a large proportion of high growth rate offspring, and all three breeders in these two mating combinations also exhibited individually high evaluation scores. As shown in Table 3, when one of best performing females (F-12) mated with a different male (M-10), the proportion of fast-growing offspring only reached 29.32%.

Selective breeding programs have been well established in agriculture as a means to produce genetically improved animals and seed; however, incorporation of this technology in aquaculture has been slow. This study is the first attempt to select suitable microsatellite loci for parentage assignment of Florida pompano (*T. carolinus*) and to evaluate their assignment power to obtain an effective marker set. As a result, polymorphic and powerful markers were selected for efficiently parentage assignment and obtaining pedigree information. The potential to utilize this practical tool for estimating reproductive success and analyzing heritability of growth related traits in Florida pompano was demonstrated.

Another main finding of this research is that a very small number of breeders were contributed to the spawning. This type of dominance has also been seen in other mass spawning fish species, such as Atlantic cod (Herlin, Delghandi, Wesmajervi, Taggart, McAndrew & Penman 2008), common sole (Blonk *et al.* 2009), gilthead seabream (Chavanne, Parati, Cambuli, Capoferri, Jiménez & Galli 2012) and barramundi (Frost, Evans & Jerry 2006; Loughnan, Domingosb, Smith-Keuneb, Forresterc & Jerry 2013). For instance, broodstock contributions of barramundi were skewed following mass spawning, although there was a high participation rate of broodstock, individual broodstock contribution reached 48% (Loughnan *et al.* 2013). However, selection programs in all livestock require as many families as possible to maintain a strain with sufficient genetic diversity for breeding. From the aforementioned barramundi research, recommendations for further program were monitoring parental contribution over multiple spawning nights, synchronising spawning in multiple tanks, and using more broodfish per spawning group, in order to maximize the transfer of genetic

variation to the next generation of broodstock candidates . As a preliminary research of pompano breeding program, we suggest that further researches need to increase the total number of broodstock population and the contribution of breeders. Employing a genetically rigorous breeding plan, such as setting up mating sets with optimal numbers of dams and sires (i.e., 1-2 females to 3 males), would be the strategy for further breeding program to generate large numbers of families and maximize the genetic variability.

Acknowledgments

Funding for this study was provided by Mote Marine Laboratory; Florida Fish and Wildlife Research Institute; National Oceanographic and Atmospheric Administration (NOAA), National Marine Fisheries Service, Office of Aquaculture; China Postdoctoral Science Foundation (2013M531658); and International Science & Technology Cooperation Program of China (grant no. 2013DFA31410). The views are those of the authors and do not necessarily reflect the view of these organizations. The authors also thank the research teams at the Mote Marine Laboratory's Aquaculture Research Park and Florida Fish and Wildlife Research Institute, as well as Dr. Ernst Peebles at the College of Marine Science, University of South Florida for his support.

References

Antonello J., Massault C., Franch R., Haley C., Pellizzari C., Bovo G., Patarnello T., de Koning D.J. & Bargelloni L. (2009) Estimates of heritability and genetic correlation for body length and resistance to fish pasteurellosis in the gilthead sea bream (*Sparus aurata* L.). *Aquaculture* **298**, 29–35.

Beldade R., Holbrook S., Schmitt R., Planes S., Malone D. & Bernardi G. (2012) Larger female fish contribute disproportionately more to self-replenishment. *Proceedings of the Royal Society B: Biological Sciences* **279**, 2116–2121.

Berry F. & Iverson E.S. (1967) Pompano: Biology, fisheries and farming potential. Gulf Caribbean Fisheries Institute. *Proceedings Nineteenth Annual Session 1966*. 116–128.

Blonk R.J., Komen J., Kamstra A., Crooijmans R.P. & van Arendonk J.A. (2009) Levels of inbreeding in group mating captive broodstock populations of Common sole, (*Solea solea*), inferred from parental

relatedness and contribution. *Aquaculture* **289**, 26–31.

Brown R.C., Woolliams J.A. & McAndrew B.J. (2005) Factors influencing effective population size in commercial populations of gilthead seabream, *Sparus aurata*. *Aquaculture* **247**, 219–225.

Chavanne H., Parati K., Cambuli C., Capoferri R., Jiménez C.A. & Galli A. (2012) Microsatellites markers to depict the reproductive and genetic patterns of farmed gilthead seabream (*Sparus aurata*): illustration by a case study on mass spawning. *Aquaculture Research* **45**, 577–590.

Chevassus B., Quillet E., Krieg F., Hollebecq M.G., Mambrini M., Fauré A., Labbé L., Hiseux J.P. & Vandeputte M. (2004) Enhanced individual selection for selecting fast growing fish: the "PROSPER" method, with application on brown trout (*Salmo trutta fario*). *Genetics Selection Evolution* **36**, 643–661.

de Mestral L.G. & Herbinger C.M. (2013) Reduction in antipredator response detected between first and second generations of endangered juvenile Atlantic salmon *Salmo salar* in a captive breeding and rearing programme. *Journal of Fish Biology* **83**, 1268–1286.

Do C., Waples R.S., Peel D., Macbeth G.M., Tillett B.J. & Ovenden J.R. (2014) NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Molecular Ecology Resources* **14**, 209–214.

Dodds K., Tate M. & Sise J. (2005) Genetic evaluation using parentage information from genetic markers. *Journal of Animal Science* **83**, 2271–2279.

El-Kassaby Y.A., Cappa E.P., Liewlaksaneeyanawin C., Klápště J. & Lstibůrek M. (2011) Breeding without breeding: is a complete pedigree necessary for efficient breeding? *PLoS One* **6**, e25737.

Fields H.M. (1962) Pompanos (*Trachinotus* spp.) of south Atlantic coast of the United States. U.S. Fish and Wildlife Service Fishery Bulletin **207**:189–222.

Frost L.A., Evans B.S. & Jerry D.R. (2006) Loss of genetic diversity due to hatchery culture practices in barramundi (*Lates calcarifer*). *Aquaculture* **261**, 1056–1064.

Gilbert C. (1986) Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (south Florida)--Florida pompano. *Biology of Reproduction* **82**, 24.

Gjedrem T. & Baranski M. (2010) Selective Breeding in Aquaculture: An Introduction. pp 22. Springer, the Netherlands.

Gjedrem T., Robinson N. & Rye M. (2012) The importance of selective breeding in aquaculture to meet future demands for animal protein: A review. *Aquaculture* **350–353**, 117–129.

Hauville M.R., Zambonino-Infante J.L., Bell G., Migaud H. & Main K.L. (2014) Impacts of three different

567 microdiets on Florida Pompano, *Trachinotus carolinus*, weaning success, growth, fatty acid
568 incorporation and enzyme activity. *Aquaculture* **422**, 268–276.
569 Herlin M., Delghandi M., Wesmajervi M., Taggart J.B., McAndrew B.J. & Penman D.J. (2008) Analysis of the
570 parental contribution to a group of fry from a single day of spawning from a commercial Atlantic cod
571 (*Gadus morhua*) breeding tank. *Aquaculture* **274**, 218–224.
572 Hill W.G. (1981) Estimation of effective population size from data on linkage disequilibrium. *Genetical Research*
573 **38,** 209–216
574 Hislop J. (1988) The influence of maternal length and age on the size and weight of the eggs and the relative
575 fecundity of the haddock, *Melanogrammus aeglefinus*, in British waters. *Journal of Fish Biology* **32**,
576 923–930.
577 Hoff F., Mountain J., Frakes T. & Halcott K. (1978) Spawning, oocyte development and larvae rearing of the
578 Florida pompano (*Trachinotus carolinus*). *Proceedings of the World Mariculture Society* **9**, 279–297.
579 Jerez S., Rodríguezb C., Cejasa J.R., Martína M.V. Bolañosb A. & Lorenzo A. (2012) Influence of age of female
580 gilthead seabream (*Sparus aurata* L.) broodstock on spawning quality throughout the reproductive
581 season. *Aquaculture* **350–353**, 54–62.
582 Kalinowski S.T., Taper M.L. & Marshall T.C. (2007) Revising how the computer program CERVUS
583 accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* **16**,
584 1099–1106.
585 Kapralova K., Morrissey M., Kristjánsson B., Ólafsdóttir G.Á., Snorrason S. & Ferguson M. (2011) Evolution of
586 adaptive diversity and genetic connectivity in Arctic charr (*Salvelinus alpinus*) in Iceland. *Heredity* **106**,
587 472–487.
588 Lacy R.C. (2012) Extending pedigree analysis for uncertain parentage and diverse breeding systems. *Journal of*
589 *Heredity* **103**, 197–205.
590 Lamas I.R. & Godinho A.L. (1996) Reproduction in the piranha *Serrasalmus spilopleura*, a neotropical fish with
591 an unusual pattern of sexual maturity. *Environmental Biology of Fishes* **45**, 161–168.
592 Liu X., Sui B., Wang Z., Cai M., Yao C. & Chen Q. (2011) Estimated reproductive success of brooders and
593 heritability of growth traits for large yellow croaker (*Larimichthys crocea*) using microsatellites. *Chinese*
594 *Journal of Oceanology and Limnology* **29**, 990–995.
595 Loughnana S.R, Domingosb J.A., Smith-Keuneb C., Forresterc J.P. & Jerry D.R. (2013) Broodstock contribution
596 after mass spawning and size grading in barramundi (*Lates calcarifer*, Bloch). *Aquaculture* **404-405**,

139–149.

Main K.L., Rhody N., Nystrom M. & Resley M. (2007) Species Profile – Florida Pompano. Southern Regional Aquaculture Center (SRAC), No. 7206.

Marteinsdóttir G. & Steinarsson A. (1998) Maternal influence on the size and viability of Iceland cod *Gadus morhua* eggs and larvae. *Journal of Fish Biology* **52**, 1241–1258.

Murata O., Miyashita S., Izumi K., Maeda S., Kato K. & Kumai H. (1996) Selective breeding for growth in red sea bream. *Fisheries Science* **62**, 845–849.

Navarro A., Zamorano M.J., Hildebrandt S., Ginés R., Aguilera C. & Afonso J.M. (2009) Estimates of heritabilities and genetic correlations for growth and carcass traits in gilthead seabream (*Sparus auratus* L.), under industrial conditions. *Aquaculture* **289**, 225–230.

Nhan D.T., Wille M., Hung L.T. & Sorgeloos P. (2009) Comparison of reproductive performance and offspring quality of giant freshwater prawn (*Macrobrachium rosenbergii*) broodstock from different regions. *Aquaculture* **298**, 36–42.

Ninh N.H., Ponzoni R.W., Nguyen N.H., Woolliams J.A., Taggart J.B., McAndrew B.J. & Penman D.J. (2011) A comparison of communal and separate rearing of families in selective breeding of common carp (*Cyprinus carpio*): Estimation of genetic parameters. *Aquaculture* **322**, 39–46.

Olafsson K., Hjorleifsdottir S., Pampoulie C., Hreggvidsson G.O. & Gudjonsson S. (2010) Novel set of multiplex assays (SalPrint15) for efficient analysis of 15 microsatellite loci of contemporary samples of the Atlantic salmon (*Salmo salar*). *Molecular Ecology Resources* **10**, 533–537.

Ponzoni R.W., Khaw H.L., Nguyen N.H. & Hamzah A. (2010) Inbreeding and effective population size in the Malaysian nucleus of the GIFT strain of Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **302**, 42–48.

Rakitin A., Ferguson M.M. & Trippel E.A. (1999) Sperm competition and fertilization success in Atlantic cod (*Gadus morhua*): effect of sire size and condition factor on gamete quality. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 2315–2323.

Reynolds E.A. (2010) Analysis of spawning behavior and gamete availability of the florida pompano (*Trachinotus carolinus*) in a recirculating aquaculture system. Master thesis. University of Florida.

Rezk M.A., Ponzoni R.W., Khaw H.L., Kamel E., Dawood T. & John G. (2009) Selective breeding for increased body weight in a synthetic breed of Egyptian Nile tilapia, *Oreochromis niloticus*: Response to selection and genetic parameters. *Aquaculture* **293**, 187–194.

Rhody N.R., Neidig C.L., Grier H.J., Main K.L. & Migaud H. (2013) Assessing Reproductive Condition in

627 Captive and Wild Common Snook Stocks: A Comparison between the Wet Mount Technique and
628 Histological Preparations. *Transactions of the American Fisheries Society* **142**, 979–988.
629 Rhody N.R., Puchulutegui C., Taggart J.B., Main K.L. & Migaud H. (2014) Parental contribution and spawning
630 performance in captive common snook *Centropomus undecimalis* broodstock. *Aquaculture* **432**,
631 144–153.
632 Riche M. & Williams T. (2011) Fish meal replacement with solvent-extracted soybean meal or soy protein isolate
633 in a practical diet formulation for Florida pompano (*Trachinotus carolinus*, L.) reared in low salinity.
634 *Aquaculture Nutrition* **17**, 368–379.
635 Riche M. (2014) Development of a semipurified test diet for determining amino acid requirements of Florida
636 pompano *Trachinotus carolinus* reared under low-salinity conditions. *Aquaculture* **420–421**, 49–56.
637 Robinson J.D. & Moyer G.R. (2012) Linkage disequilibrium and effective population size when generations
638 overlap. *Evoltionary Applications* **6**, 290–302.
639 Rousset F. (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux.
640 *Molecular Ecology Resources* **8**, 103–106.
641 Sefc K.M. & Koblmüller S. (2009) Assessing parent numbers from offspring genotypes: the importance of marker
642 polymorphism. *Journal of Heredity* **100**, 197–205.
643 Sekino M., Saitoh K., Yamada T., Kumagai A., Hara M. & Yamashita Y. (2003) Microsatellite-based pedigree
644 tracing in a Japanese flounder *Paralichthys olivaceus* hatchery strain: implications for hatchery
645 management related to stock enhancement program. *Aquaculture* **221**, 255–263.
646 Seyoum S., Puchulutegui C., Guindon K.Y., Gardinal C.M., Denison S.H., & Tringali M.D. (2014) Development
647 of microsatellite markers for Permit (*Trachinotus falcatus*), cross-amplification in Florida Pompano (*T.*
648 *carolinus*) and Palometa (*T. goodei*) and delineation of a new species of Pompano. Florida Fish and
649 Wildlife Conservation Commission FWRI Technical Report TR-XX.
650 Shikano T. (2005) Marker-based estimation of heritability for body color variation in Japanese flounder
651 *Paralichthys olivaceus*. *Aquaculture* **249**, 95–105.
652 Tringali M.D. (2006) A Bayesian approach for the genetic tracking of cultured and released individuals. *Fisheries*
653 *Research* **77**, 159–172.
654 Vandeputte M. (2003) Selective breeding of quantitative traits in the common carp (*Cyprinus carpio*): a review.
655 *Aquatic Living Resources* **16**, 399–407.
656 Vandeputte M., Dupont-Nivet M., Haffray P., Chavanne H., Cenadelli S., Parati K., Vidal M.O., Vergnet A. &

Chatain B. (2009) Response to domestication and selection for growth in the European sea bass (*Dicentrarchus labrax*) in separate and mixed tanks. *Aquaculture* **286**, 20–27.

Vandeputte M., Rossignol M.N. & Pincent C. (2011) From theory to practice: empirical evaluation of the assignment power of marker sets for pedigree analysis in fish breeding. *Aquaculture* **314**, 80–86.

Vandeputte M. & Haffray P. (2014) Parentage assignment with genomic markers: a major advance for understanding and exploiting genetic variation of quantitative traits in farmed aquatic animals. *Frontiers in Genetics* **5**, 432.

Watanabe W.O. (1995) Aquaculture of the Florida pompano and other jacks (Family Carangidae) in the Western Atlantic, Gulf of Mexico, and Caribbean basin: status and potential. In: *Culture of High-Value Marine Fishes* (ed. by Main K.L. & Rosenfeld C.), pp 185–205. Oceanic Institute, Honolulu, Hawaii, USA.

Waples R.S. (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conservation Genetics* **7**,167–184.

Weirich C.R. & Riche M. (2006) Acute tolerance of juvenile Florida pompano, *Trachinotus carolinus* L., to ammonia and nitrite at various salinities. *Aquaculture Research* **37**, 855–861.

Weirich C.R. & Riley K.L. (2007) Volitional spawning of Florida pompano, *Trachinotus carolinus*, induced via administration of gonadotropin releasing hormone analogue (GnRHa). *Journal of Applied Aquaculture* **19**, 47–60.

Weirich C.R., Groat D.R., Reigh R.C., Chesney E.J. & Malone R.F. (2006) Effect of feeding strategies on production characteristics and body composition of Florida pompano reared in marine recirculating systems. *North American Journal of Aquaculture* **68**, 330–338.

Weirich C.R., Wills P.S., Baptiste R.M., Woodward P.N. & Riche M.A. (2009) Production characteristics and body composition of Florida pompano reared to market size at two different densities in low-salinity recirculating aquaculture systems. *North American Journal of Aquaculture* **71**, 165–173.

Williams J. (2000) The coefficient of condition of fish. In: *Manual of Fisheries Survey Methods II: with Periodic Updates* (ed. by Schneider J.C.). Michigan Department of Natural Resources, Ann Arbor, Michigan, USA.

Table 1 Sires/Dams spawn contribution of the Florida pompano (Trachinotus carolinus)

Sires /Dams		Sample Source	Weight (g)	Standard length (cm)	Condition Factor (K)	Total No. Offspring	Total Contribution (%)	Fast-growing offspring No.	Fast-growing proportion (%)
Males	M-2	F1	500	27.0	2.54	0	0	0	N/A
	M-3	F1	785	31.8	2.44	0	0	0	N/A
	M-4	F1	925	32.0	2.82	5	0.5	0	0
	M-5	F1	675	33.0	1.88	0	0	0	N/A
	M-6	F1	995	33.0	2.77	4	0.4	0	0
	M-7	F1	665	31.0	2.23	0	0	0	N/A
	M-8	F1	635	29.0	2.60	0	0	0	N/A
	M-9	Wild	1645	37.5	3.12	738	73.8	453	61.4
	M-10	Wild	1135	35.0	2.65	209	20.9	59	28.2
	M-12	Wild	955	32.0	2.91	44	4.4	3	6.8
Females	F-1	F1	1555	37.0	3.07	19	1.9	2	10.5
	F-2	F1	1080	34.1	2.72	0	0	0	N/A
	F-3	F1	1585	36.0	3.40	0	0	0	N/A
	F-9	F1	1300	36.0	2.79	112	11.2	29	25.9

F-10	F1	1135	29.0	4.65	3	0.3	1	33.3

Locus	Primer sequence (5’−3’)	Primer label	Repeat motif	No. of alleles	Size range of alleles	Relation testing Multiplex	Parentage assignment Multiplex
F-12	Wild	2730	45.5	3.90	594	59.4	337	56.7
F-13	Wild	1820	39.0	3.07	252	25.2	141	56.0
F-14	Wild	2440	44.0	2.86	0	0	0	N/A
F-15	Wild	1465	37.5	2.78	0	0	0	N/A
F-16	Wild	1710	38.2	3.07	20	2.0	5	25.0

Table 2 15 microsatellite DNA loci used in this study

TFl01	F: CGTAAAGGAAAGGAATGAAGTTAATC R: CCTCTTCCTCTTTCTATCTCTCTTTG	FAM	$(GT)_{17}$	7	241-263	1	N/A
TFl05	F: ATTAGGATGAAGAAGGAAAAGCAAA R: TCATTTATGGGGAATAATCTGAATG	HEX	$(CA)_{13}$	14	147-225	3	3
TFl07	F: CGTTTACTTTACTTTGGTCTCTGGT R: AACCAATAAATTAAAGCGGCTCTC	FAM	$(CT)_6CC(CT)_8/(CT)_9$	11	170-208	2	2
TFl15	F: ACACTAAGCAATACAAGAGCACTCC R: TAAACCACAGAAATGCAGACAATTT	NED	$(GT)_{10}$	17	133-221	1	1
TFl26	F: TGTGTTTTACAACTCTCCTCACATT R: TGAGCACCTTTTGTGTGATATTTTA	HEX	$(CA)_7/(CA)_{10}$	3	224-228	5	N/A
TFl30	F: GACAGGTCTCCTCTCTGAGCTG R: CTCGACTCTAAGTCTGGAGTGTTTC	NED	$(GT)_{21}$	5	134-142	7	N/A
TFl39	F: AAACGCATCCTCTCACATACTCAC R: GCAAACACACACTCCACTCTGTTAT	NED	$(CT)_4(AC)_{12}/(TC)_3/(CT)_6$	7	210-238	2	2
TFl43	F: ACAGTGATAGTTCCTGCTACAGTGG R: ACCTTCTCTGCCATCACTCATTTTA	FAM	$(CA)_9$	8	163-181	4	N/A
TFl51	F: GAGAAGAGAGAAAAGAGCAGAGCA	NED	$(GT)_{19}$	5	201-209	6	5

	R: AAGCCTTTATACTTCACTCTCCTGT						
TFl56	F: TAGAGCAGAAAAACAACTTTCAACC	HEX	$(CT)_{10}(TC)_{31}$	13	112-148	2	2
	R: CTGGCAAGCCAAATATATGATCTAC						
TFl62	F: ATAATTCATCCATTCAGCCTACTTG	HEX	$(AC)_{34}$	13	157-195	6	5
	R: ACTAATCCAATTTCTAGCCGAAGAC						
TFl64	F: ACATTGGCGTTGTTGTTATAGTTCT	FAM	$(GCA)_{28}$	17	122-191	5	4
	R: GAGCAGATAACCGTCTAATCATCTG						
TFl65	F: CTTTTCCTGCATCCTGCTATAACC	HEX	$(CA)_{12}$	3	136-148	4	N/A
	R: TGGAGGAATGTGAACAAGTAATACA						
TFl66	F: CTTTCCATTCACACTCTGAACTCC	NED	$(CA)_{10}$	8	187-205	4	N/A
	R: ACTGACTGGCACAGCATAAGAGAC						
TFl70	F: GGCATATTAACAACACACTCACAGA	FAM	$(CA)_{16}$	5	110-122	6	5
	R: CATTTGCACAAAGTGATTTAACGTA						

Table 3 Mating crosses (sires x dams) and their contribution to the offspring

Sires	Dams	Offspring No.			Fast-growing proportion
		Fast-growing	Slow-growing	Total	
M-4	F-16	0	5	5	0.00%
M-6	F-9	0	3	3	0.00%
M-6	F-10	0	1	1	0.00%
M-9	F-1	1	6	7	14.29%
M-9	F-9	27	44	71	38.03%
M-9	F-12	281	122	403	69.73%
M-9	F-13	141	111	252	55.95%
M-9	F-16	3	2	5	60.00%
M-10	F-1	0	1	1	0.00%
M-10	F-9	0	7	7	0.00%
M-10	F-10	1	0	1	100.00%
M-10	F-12	56	135	191	29.32%
M-10	F-16	2	7	9	22.22%
M-12	F-1	1	10	11	9.09%

M-12	F-9	2	29	31	6.45%
M-12	F-10	0	1	1	0.00%
M-12	F-16	0	1	1	0.00%

Table 4 Characterization of 9 microsatellite loci in broodstock and progeny of Trachinotus carolinus

		TFl05	TFl07	TFl15	TFl39	TFl 51	TFl 56	TFl 62	TFl 64	TFl70	Average
N_A	Broodstock	14	11	16	7	5	13	13	16	5	11.11
	Progeny	11	9	13	6	5	11	10	13	4	9.11
H_O	Broodstock	0.7000	0.9000	0.8000	0.7500	0.5500	0.8500	0.8500	1.0000	0.6500	0.7833
	Progeny	0.8592	0.9930	0.9790	0.8870	0.9610	0.7840	0.9870	0.8930	0.3263	0.8522
H_E	Broodstock	0.8269	0.8782	0.8808	0.7974	0.5833	0.7885	0.8897	0.9244	0.5026	0.7858
	Progeny	0.7373	0.7730	0.8250	0.7364	0.6479	0.7255	0.7472	0.8041	0.3000	0.6996
PIC	Broodstock	0.7895	0.8410	0.8476	0.7433	0.5163	0.7564	0.8562	0.8938	0.4401	0.7427
	Progeny	0.6898	0.7414	0.8014	0.6883	0.5813	0.6978	0.7166	0.7797	0.2793	0.6640

F	Broodstock	+0.0776	-0.0271	+0.0442	+0.0206	+0.0359	-0.0604	+0.0058	-0.0547	-0.1738	
	Progeny	-0.0807	-0.1399	-0.0888	-0.0951	-0.2198	-0.0331	-0.1689	-0.0521	-0.0616	
P	Broodstock	0.0716	0.4016	0.0937	0.7060	0.1138	0.8936	0.2991	0.3367	0.6610	
	Progeny	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	
NE-PP	Broodstock	0.1563	0.1180	0.0983	0.2482	0.5004	0.1733	0.0943	0.0597	0.5816	0.00002010†
	Progeny	0.3260	0.2408	0.1709	0.3348	0.4655	0.2683	0.2595	0.1887	0.7320	0.00000013†

N_A, Number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphic information content; F, Null allele frequency estimate; P, Hardy-Weinberg equilibrium test; NE-PP, average non-exclusion probability (parent pair).

* indicates the locus deviated from Hardy–Weinberg proportions; † represents Combined non-exclusion probability (parent pair).

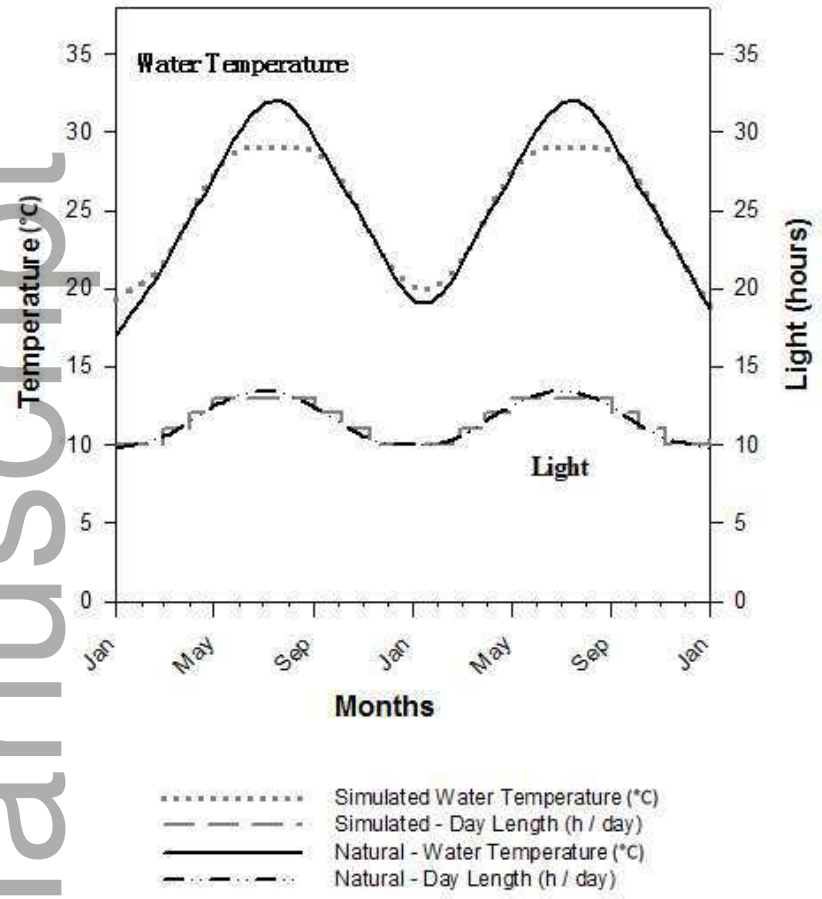

Figure.1. Schematic representation of ambient natural and artificial (simulated) environmental conditions associated with the annual reproductive cycle of wild Florida pompano located on the Gulf coast of Florida and captive broodstock held at Mote Aquaculture Research Park, Sarasota, FL. Natural ambient cycle of day length (light h/day) (—) and water temperature(°C)(—) in Tampa Bay, FL. Imposed photo-thermal cycle used to mature and spawn captive broodstock including day length (light h/day) (− ‥ −) and water temperature (°C) (···).

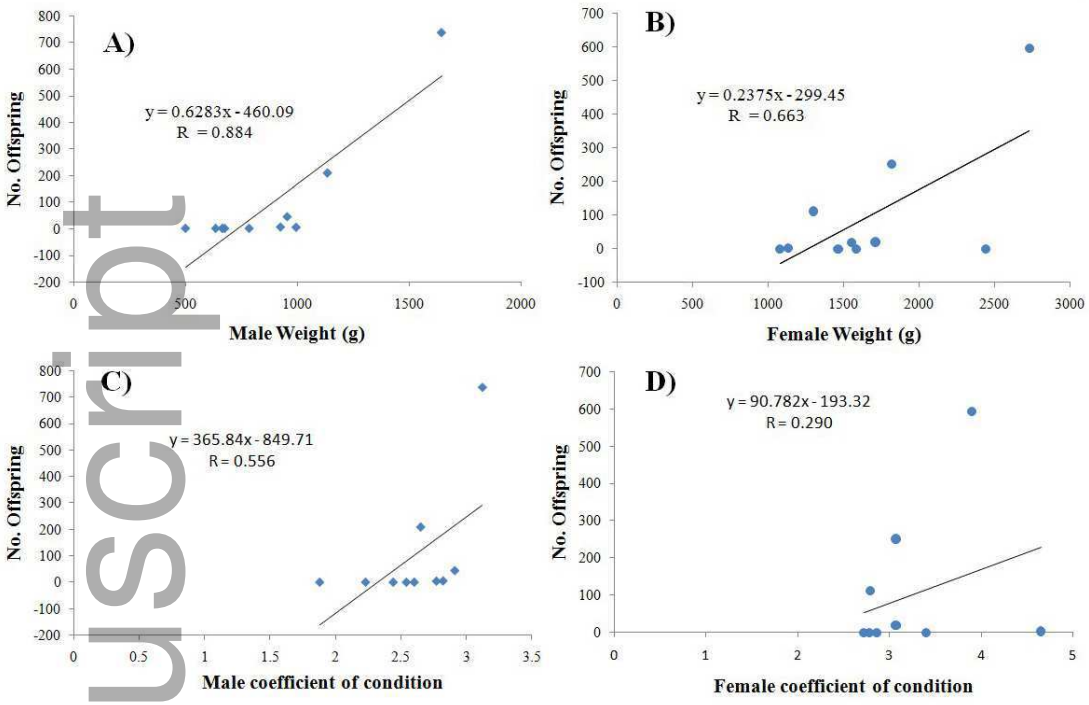

Fig.2. Scatterplot of male (◆) and female (●) body characteristics versus contribution to offspring (n=10). Male (A) and female (B) body weight (g) versus contribution to offspring number. Male (C) and female (D) condition factor versus contribution to offspring number.