Agriculture and Natural Resources 52 (2018) 93–98

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Growth performance and genetic diversity in four strains of Asian sea bass, *Lates calcarifer* (Bloch, 1790) cultivated in Thailand

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ARTICLE INFO

Article history:
Received 23 May 2017
Accepted 1 August 2017
Available online 4 June 2018

Keywords:
Barramundi
Condition factor
Microsatellite diversity
Pond culture
Stock improvement

ABSTRACT

The growth and genetic variation were examined of four strains of Asian sea bass: Chon Buri (CHB), Chumphon (CHP), Chachoengsao (CHS), and Samut Songkhram (SMS). The growth of juvenile fish (90 d post-hatch) was assessed in earthen-pond culture environments for 220 d. Fish, with an average initial weight ±SE ranging from 9.48 ± 0.12 to 9.74 ± 0.16 g, depending on strain, were stocked in four 6 m × 6 m × 1.2 m stationary cages (replicates) in each of four 800 m^2 ponds at 3 fish per m^3 following a randomized complete block design. Strain differences in body weight were highly significant ($p < 0.001$). The CHS strain displayed the highest final mean weight ± SE (730.23 ± 10.48 g) followed by CHP (709.60 ± 14.76 g), CHB (661.66 ± 14.92 g) and SMS (650.60 ± 11.66 g). The absolute growth rates were significantly different among strains, with values ranging from 2.86 to 3.22 g/d for SMS and CHS. Similarly, specific growth rates differed significantly among strains and ranged from 1.88 to 1.97% for SMS and CHS. All four strains had moderate to high levels of microsatellite genetic variation, with average numbers of alleles per locus ranging from 6.8 to 9.4, effective numbers of alleles per locus ranging from 4.33 to 5.99 and observed heterozygosities ranging from 0.75 to 0.81. Overall, the CHS strain, which exhibited the highest numbers of alleles and an effective population size of 55, was the best performing strain.

Introduction

Asian sea bass, or barramundi, *Lates calcarifer* (Bloch, 1790) is a species native to Thailand and is widely distributed in the Indo-West Pacific region from the Arabian Gulf to China, Taiwan, Papua New Guinea and northern Australia (Food and Agriculture Organization, 2017). Culture of Asian sea bass was well established in Thailand long before the 1970s, when the Thai Department of Fisheries (DOF) was the first to succeed in breeding the species (Kungvankij et al., 1984). Sea bass has been widely cultivated in India, Southeast Asia and Australia where the fish is known for its rapid growth rate, attaining a size of 3–5 kg within 2–3 yr, and for its high tolerance to crowding and environmental fluctuations (Kungvankij et al., 1984). Asian sea bass is used for coastal aquaculture in provinces along the Gulf of Thailand and the Andaman Sea, where the fish can grow in sea cages or in brackish and freshwater ponds (Kungvankij et al., 1984). Thailand has been the top producer of both fingerlings and marketable fish for both domestic and export markets, such as China, Malaysia, Singapore and Taiwan (Senanan et al., 2015). During the past decade, the production of sea bass has increased from 9700 t in 2007 to 16,900 t in 2014 (Department of Fisheries, 2015). Global production of sea bass was 71,581 t in 2014 (Food and Agriculture Organization, 2017).

However, the production potential of Asian sea bass is limited by a lack of genetically improved strains; farmers have relied mostly on fish derived from unimproved stock (Senanan et al., 2015). In the long run, sustainability of the industry will rely on a high-quality fish supply from genetically improved strains. Selective breeding programs can be used to improve productivity and increase economic values in aquaculture species, which has been documented for Atlantic salmon (*Salmo salar*; Gjedrem, 1997), rainbow trout (*Oncorhynchus mykiss*; Gjedrem, 2000) and Nile tilapia (*Oreochromis niloticus*; Eknath et al., 1998). The ultimate success for a selective breeding program depends in large part upon the base population; ideally, the population should have high levels of

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https://doi.org/10.1016/j.anres.2018.05.015

genetic variation with large phenotypic variation in production traits (Gjerde, 2004). Advanced selection programs for Asian sea bass have been established in Singapore (Yue et al., 2009) and Australia (Macbeth and Palmer, 2011). Strain evaluation has been suggested as the first step in establishing a breeding program (Gjerde, 2004). Although the varying performance of seeding-stock originating from different Thai strains of Asian sea bass is recognized by growers, a systematic strain performance evaluation has not been executed.

This study evaluated the growth performance of four strains of Asian sea bass which have been used by small-sized and medium-sized farms located along the east coast and the upper Gulf of Thailand. Farming of Asian sea bass in these areas accounts for 65% of total production in the country (Department of Fisheries, 2015). Asian sea bass strains were tested in the pond culture environment. Additionally, to generate baseline population genetic information for variation within each strain, genetic variation was assessed within and between strains using microsatellite markers.

Materials and methods

Origin of sea bass strains

Samples of fingerlings of four strains at approximately 90 d-post-hatch were obtained from private hatcheries—the CHS strain from Chachoengsao province, the CHB strain from Chon Buri province, the CHP strain from Chumphon province and the SMS strain from Samut Songkhram province (Fig. 1). The CHS strain originated from wild populations in the Bang Pakong River and has been domesticated for over 40 yr (Senanan et al., 2015). The strain has been utilized by most private Asian sea bass hatcheries as well as government fisheries stations for production of seeding-stock. On the other hand, the CHB strain was founded from Asian sea bass stocks obtained from cage culture operations along the east coast of the Gulf of Thailand (Senanan et al., 2015). The origin of the CHP fish was the Chumphon River, which runs through the west coast of the Gulf of Thailand. Broodstock of the SMS strain were originally collected from floating cage culture operations in the Meklong River.

Fish culture and growth trials

Prior to the growth trials, the Asian sea bass fry underwent two rounds of size-grading at approximately 30 and 60 d post-hatch at the hatcheries to minimize losses from cannibalism. At 90 d, these juveniles had mean weight ± SE of 9.48 ± 0.12 to 9.74 ± 0.16 g (Table 1); they were transported to the Kasetsart University Fisheries Research Station in Samut Songkram province (13° 42.58′ N, 100° 59.14′ E). Four earthen ponds (14 m × 38 m × 1 m or 800 m^2) in blocks were used, each with four stationary cages (6 × 6 × 1.2 m^3). These cages were fixed in place by fastening a bamboo pole at each corner and then submerging to contain water to a depth of 80 cm, leaving 40 cm of each side above the water surface. Common practice suggests that the use of stationary cages in the pond can minimize handling stress during measurement and eases harvest. Four hundred fish from each strain were randomly divided into four groups of 100 fish, and each group was stocked into a cage at a density of 3 fish per m^2. The strains were assigned to cages following a randomized complete block design. Within ponds, the cage layout was identical but cage assignments of strains were different among ponds. To prevent access by predatory birds during grow-out, the cages were covered with netting. To maintain high levels of dissolved oxygen, aeration was provided through polyethylene pipes on the bottom of the ponds below the cages. Water was supplied from a brackish-water canal connected to the Meklong River. Fresh well water was used to dilute brackish water to maintain the salinity

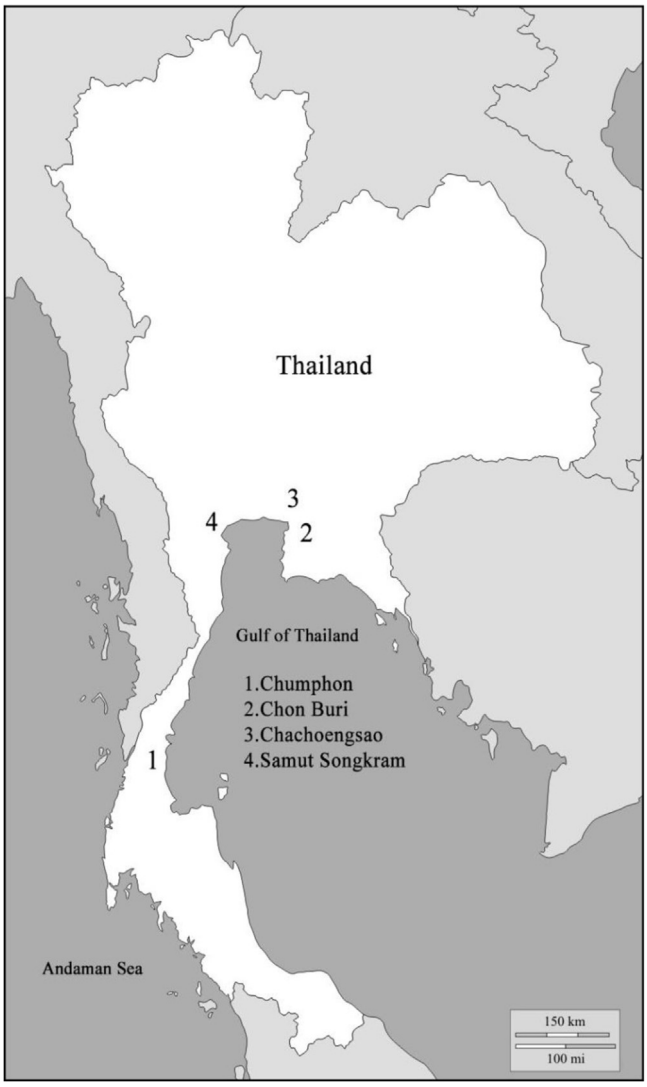

Fig. 1. Locations of strain origin of Asian sea bass.

within the range 10–25 ppt. During grow-out, there was no water exchange, but the ponds were topped off regularly to compensate for evaporation, maintaining the water depth within the net at 80–90 cm. The netting was cleaned regularly to allow better flow of water through the cages.

Fish were fed by hand to apparent satiation with a 40% protein commercial floating diet (Betagro Feed Mills; Bangkok, Thailand) twice daily at 0700 and 1700 h. Feed consumption was closely monitored so that unconsumed feed was collected for waste measurement. Checking for dead fish occurred during feeding and they were removed from cages immediately. The temperature and other water quality parameters were checked twice daily. Dissolved oxygen (DO) was measured using a portable meter model YSI 550A (Yellow Springs Instruments, Inc; Yellow Springs, OH, USA) and pH using a YSI EcoSense pH 100A meter (Yellow Springs Instruments, Inc.; Yellow Springs, OH, USA). Salinity was measured weekly using a portable salinity refractometer (Atago, Co., Ltd.; Tokyo, Japan). Aquatic plants and algae that could interfere with water exchange and fish growth were removed regularly from the ponds. The initial biomass of fish in each rearing unit was assessed before stocking. After that, 50 fish from each stationary cage in each pond were sampled for growth measurements (body weight and standard length) every 4 wk. At the end of the grow-out period

Table 1
Least-squares means and analysis of variance (ANOVA, *p*-values) results of growth traits at harvest among Asian sea bass strains from earthen ponds.

	Strain				ANOVA
	CHB	CHP	CHS	SMS	Strain effect
Start weight (g)	9.69 ± 0.20[a]	9.48 ± 0.12	9.59 ± 0.15	9.74 ± 0.16	NS
Final weight (g)	661.66 ± 14.92[bb]	709.60 ± 14.76[a]	730.23 ± 10.48[a]	650.35 ± 11.66[b]	0.0003
Standard length (cm)	28.99 ± 0.34	29.18 ± 0.34	30.02 ± 0.33	28.83 ± 0.33	NS
AGR (g/d)	2.925 ± 0.071[b]	3.120 ± 0.070[a]	3.221 ± 0.071[a]	2.863 ± 0.070[b]	0.0015
SGR (%)	1.895 ± 0.011[c]	1.942 ± 0.010[b]	1.975 ± 0.011[a]	1.879 ± 0.010[c]	<0.0001
Condition factor	1.51 ± 0.02[b]	1.62 ± 0.01[a]	1.65 ± 0.02[a]	1.51 ± 0.01[b]	<0.0001
FCR	1.71 ± 0.09	1.86 ± 0.41	1.73 ± 0.17	1.74 ± 0.09	NS
Survival	66.40 ± 3.15[b]	71.50 ± 8.41[a]	75.50 ± 2.50[a]	62.40 ± 7.45[b]	0.01
Gross yield (kg/m^2)	2.09 ± 0.01[b]	2.33 ± 0.02[b]	3.17 ± 0.02[a]	1.14 ± 0.01[c]	0.03

CHB = Chon Buri, CHP = Chumphon, CHS = Chachoengsao, SMS = Samut Songkram, AGR = absolute growth rate, SGR = specific growth rate, FCR = feed conversion ratio.
[a] Values are mean ± SE.
[b] Values in rows with different lowercase superscripts differ significantly at $p < 0.05$.

(approximately 220 d), all fish were harvested. Gross yields, feed conversion ratio (FCR) and survival rates were calculated. The culture period was 7 mth from October 2015 to April 2016.

Statistical analysis

Due to the variation in pond size, aeration and water quality, a randomized complete block design was used to evaluate the results for pond culture. Each pond was treated as a block and fish from each strain were randomly stocked in a fixed cage. The statistical model used was: $Y_{ijk} = \mu + S_i + T_j + e_{ijk}$:

where Y_{ijk}, μ, S_i, T_j and e_{ijk} are the data for individual fish, the common mean, the strain effect, the block effect and the residual effect, respectively.

The growth of Asian sea bass was assessed at harvest as follows. The absolute growth rate (AGR), which indicates the absolute increase in weight each day, was calculated as: $AGR = \frac{w_t - w_i}{t}$. The specific growth rate (SGR), which describes the percentage increase in body weight per day, was calculated as: $SGR = \frac{\log(w_t) - \log(w_i)}{t} \times 100$, where *log* is the natural logarithm, w_t is individual weight at time t, and w_i is initial weight. Condition factor, which is used to quantify the growth condition and body shape of fish, was calculated as: $K = 100 \times \frac{W}{L^3}$, where W is the body weight and L is the standard length. The feed conversion ratio was calculated as weight gain/dry feed fed.

The least-squares mean and standard error were estimated for all parameters (final weight, standard length, AGR, SGR, condition factor, survival and feed efficiency) for each strain and were subjected to analysis of variance using PROC GLM implemented in the software package SAS (SAS Institute Inc.; Cary, NC, USA) to determine whether there were significant differences ($p < 0.05$) among strain means. For traits showing a significant *F*-value, Dunnett's procedure (Dunnett, 1955) was used to detect differences among strain means.

Microsatellite genotyping

To assess genetic diversity within and among populations, caudal fin clips of 50 fingerlings (90 d post-hatch) from each of the four strains used in the growth trials were collected and preserved in 95% ethanol. DNA extraction was performed using a standard salting-out protocol (Aljanabi and Martinez, 1997) as described by Senanan et al. (2015). The extracted DNA samples were arrayed into 96-well plates for polymerase chain reaction. Seven microsatellite loci identified by Yue et al. (2002) (*Lca20* and *Lca27*), Zhu et al. (2006) (*Lca70*) and Wang et al. (2008) (*Lca98*, *Lca185*, *Lca260* and *Lca284*) were screened to assess the genetic diversity of Asian sea bass stocks. One primer of each primer pair was labeled with fluorescent dye (6FAM, VIC, NED or PET) at the 5′end. DNA was amplified using the polymerase chain reaction (PCR) conditions described by Senanan et al. (2015). PCR was performed in a thermocycler (MJ Mini Thermal Cycler; Bio-Rad Laboratories; Hercules, CA, USA). Each reaction mixture (10 μL) contained 3 μL of template DNA solution (approximately 20–50 ng template DNA), 0.2 μM of each primer, 1.5 mM $MgCl_2$, 0.02 mM of each dNTP, 1 × reaction buffer (Fermentas; Thermo Fisher Scientific; Waltham, MA, USA) and 0.6 unit of *Taq* polymerase (Fermentas; Thermo Fisher Scientific; Waltham, MA, USA). The PCR profile consisted of: initial denaturation at 94 °C for 2 min; 33 cycles of 94 °C for 30 s, annealing at 50–57 °C (depending on the primer pair) for 30 s, and elongation at 72 °C for 30 s; and 1 cycle of 72 °C for 5 min (adapted from Zhu et al., 2006). The PCR products were submitted to a commercial genetic analysis service (First BASE Laboratory Sdn Bhd; Seri Kembangan, Selangor, Malaysia) for electrophoresis and genotyping on an ABI3730XL DNA analyzer (Thermo Fisher Scientific; Waltham, MA, USA). Allele scores were determined relative to a LIZ 500 internal size standard using the GeneMapper software v. 3.0 (Applied Biosystems; Forest City, CA, USA). To verify allele sizes among runs, two individuals with known genotypes were included within each run.

Genetic data analysis

The program MICRO-CHECKER version 2.2.0 (Van Oosterhout et al., 2004) was used to test for the presence of null alleles, large-allele dropout or scoring errors. Estimates of genetic variation within each of the four strains, including the number of alleles per locus (A), effective number of alleles per locus (A_e) and observed (H_o) and expected (H_e) heterozygosities, as well as *F*-statistics (F_{IS}), were calculated using the GenAlEx 6.5 (Peakall and Smouse, 2012) and Microsatellite Analyzer software (Dieringer and Schlötterer, 2003). Departures from Hardy-Weinberg expectations and non-random association of genotypes between pairs of loci (linkage disequilibrium) were tested for by the exact *p*-values calculated using a Markov chain randomization method (Guo and Thompson, 1992) with the GENEPOP version 3.4 software (Raymond and Rousset, 1995). The *p*-values were estimated from 10,000 dememorization numbers, in 100 batches with 5000 iterations per batch. Probability thresholds for the Hardy-Weinberg equilibrium (HWE) tests were adjusted using a Bonferonni correction (Rice, 1989). Differences in allelic richness, and observed and expected heterozygosities averaged across all loci among samples were tested using a Mann-Whitney *U* rank test (SOFA statistics version 1.6; Paton-Simpson & Associates, Ltd.; Auckland, New Zealand). Among-population genetic variation was evaluated using the analysis of

molecular variance (AMOVA; Excoffier, 2007) implemented in the GenAlEx 6.5 software. In addition, the GENEPOP program was used to calculate global and pair-wise F_{ST} values among samples using exact tests.

Effective population size (N_e) of each strain was also estimated based on linkage disequilibrium among unlinked loci (LDNe version 1.31) (Waples and Do, 2008). Waples and Do (2008) corrected a bias associated with the estimation of expected mean square correlation of allele frequencies at different loci [$E(\hat{r}^2)$]. The program used the jackknife method to generate a 95% confidence interval for each N_e estimate.

Ethics statement

All experimental procedures using animals conformed to the guidelines established by the Animal Care Committee, Kasetsart University, Bangkok, Thailand.

Results and discussion

Growth performance

The growth performance of fish is known to be strongly influenced by both genetic and environmental factors, such as culture method, stocking density and water quality (Moreira et al., 2005; Ridha, 2006). In the present study, water quality parameters did not differ significantly among ponds for any day of measurements during the rearing period and were within the optimal ranges for sea bass growth. DO ranged from 3 to 6 mg/L, temperature from 23 to 30 °C, pH from 5 to 8 and salinity from 10 to 15 ppt. Significant differences in growth metrics were observed among strains of Asian sea bass after 220 d of rearing. Fish growth was highest in the CHS and CHP strains, and differed significantly from that in the CHB and SMS strains (Table 1). The CHS and CHP had the largest final mean weights ± SE of 730.23 ± 10.48 g and 709.60 ± 14.76 g, respectively, compared to 661.66 ± 14.92 g and 650.60 ± 11.66 g for the CHB and SMS strains. Absolute growth rates (AGR) were significantly different among strains, with values ranging from 2.86 to 3.22 g/day for SMS and CHS. Similarly, specific growth rates (SGR) differed significantly among strains and ranged from 1.88 to 1.97% for SMS and CHS. Mean growth curves for sea bass strains showed higher growth rates from days 180–300 (Fig. 2).

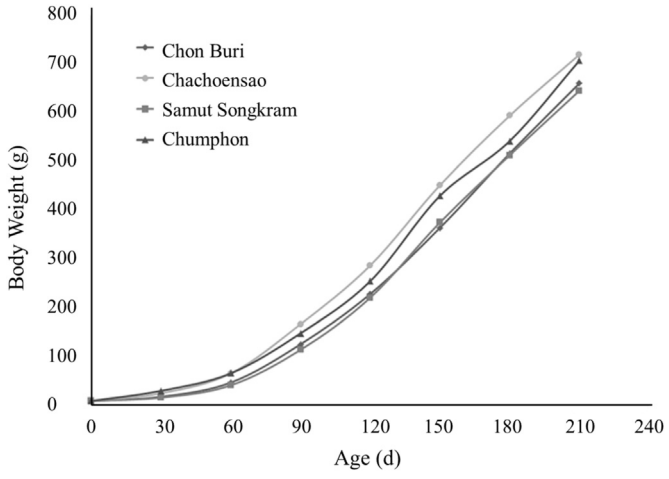

Fig. 2. Average body weight at different ages among four strains of sea bass cultured in earthen ponds for 220 d.

The condition of fish was also significantly different among strains. At harvest, CHP (1.62) and CHS (1.65) fish had higher condition factors than the CHB (1.51) and SMS (1.51) strains ($p < 0.0001$). The value of K can be influenced by the age of fish, food availability and stocking density (Biswas et al., 2011; Solanki et al., 2013). In addition, the condition factor of Asian sea bass has been reported to be influenced by the type of feed. A study of Asian sea bass culture in India using live prey or trash fish reported condition factors of 1.28 and 1.5, respectively, with final weights of 366.43–486.33 g after 330 d of grow-out (Biswas et al., 2011); these measurements of growth performance were lower than those obtained using a commercial diet in the present study. The CHS fish had the highest survival rate (75.5%), similar to CHP (71.5%) and CHB (66.25%), while SMS had the lowest survival rate (62.40%). The gross yield was highest in CHS (3.17 kg/m^2), which was comparable with CHP (2.33 kg/m^2) and CHB (2.09 kg/m^2) but was lower than for the SMS strain (1.14 kg/m^2). The FCR ranged from 1.71 to 1.86 and did not differ among strains. Feed conversion ratios were in the range expected for carnivorous fish fed artificial diets (Biswas et al., 2010; Phromkunthong et al., 2015). Overall, the Chachoengsao (CHS) strain was the best-performing fish in terms of growth, FCR, survival and yield within earthen pond culture.

Genetic variation among and within stocks

In total, 198 Asian sea bass fingerlings (CHB = 49, CHP = 49, CHS = 50 and SMS = 50) were genotyped at seven microsatellite loci (Table 2). All four stocks exhibited similar and moderate genetic variation, with average numbers of alleles per locus of 8.0, 6.8, 9.4 and 7.7, and observed heterozygosities of 0.83, 0.79, 0.75 and 0.81 for the CHB, CHP, CHS and SMS strains, respectively. Within-population genetic variation did not differ among populations using the Mann-Whitney rank test. N_e estimates, however, differed among stocks, with CHS having the highest estimates of 55.0 (95% confidence intervals = 38.5–86.0) as shown in Table 3. The remaining stocks had similar N_e estimates, in the range 11.9–13.5. The genotypic deviations from Hardy-Weinberg equilibrium over all loci were due to heterozygote excess ($H_o > H_e$) in three populations: CHB, CHP and SMS. All HWE tests were not significant after Bonferroni correction. The overall F_{ST} value of 0.049 indicated statistically significant levels of differentiation among stocks. Significant pair-wise F_{ST} values were observed for five of the six strain comparisons ($p < 0.001$, Table 4). The highest genetic differentiation was observed between CHB and CHP ($F_{ST} = 0.079$), while the differentiation between CHB and SMS was not significant ($F_{ST} = 0.015$). The AMOVA results indicated that within-population genetic variance accounted for 95% of total variation, while among-population variance accounted for 5%.

Hence, Thai Asian sea bass strains exhibited similar and moderate genetic variability within populations, as well as significant population differentiation. The amount of genetic variation at seven microsatellite loci detected among these four strains, however, was lower than that for Asian sea bass broodstock populations in Thailand reported previously (Senanan et al., 2015) in terms of the average numbers of alleles (10.24) and the effective number of alleles (5.78), but not in terms of the observed heterozygosity (0.80) because different stocks were tested. Among strains, CHS exhibited the highest number of alleles and effective number of alleles per locus (9.43 and 5.99, respectively), while CHP had the lowest (6.86 and 4.33, respectively). The percent heterozygosity was slightly lower in the CHS ($H_o = 0.75$) than in the other three strains ($H_o = 0.79–0.81$). It has been suggested that heterozygosity may not be a reliable indicator of genetic variation for hatchery stocks because a loss of heterozygosity per generation occurs more slowly than a loss of rare alleles during a population bottleneck (Allendorf,

Table 2
Genetic variation across seven microsatellite loci within Asian sea bass strains for sample size (N), total number of alleles (A), effective number of alleles (A_e), observed heterozygosity (H_o), expected heterozygosity (H_e) and fixation index (F_{IS}) and p value for test of Hardy-Weinberg expectations (HWE). Standard deviation for each index is shown in parentheses.

Pop	Locus							Average
(N)	*Lca20*	*Lca27*	*Lca70*	*Lca98*	*Lca185*	*Lca260*	*Lca284*	across loci
CHB (49)								
A	6	9	4	10	10	9	8	8.00 (0.85)
A_e	3.168	5.482	3.306	3.901	6.253	4.736	5.545	4.63 (0.45)
H_o	1.343	0.755	0.750	0.918	0.837	0.854	0.898	0.831 (0.025)
H_e	0.804	0.818	0.697	0.744	0.840	0.789	0.820	0.770 (0.026)
F_{IS}	0.789	0.076	−0.075	−0.235	0.004	−0.083	−0.096	−0.083 (0.036)
HWE†	0.0140	0.0006*	0.6132	0.148	0.0000*	0.3360	0.0000*	
CHP (49)								
A	5	7	4	7	8	8	9	6.86 (0.67)
A_e	2.677	4.227	3.793	3.972	4.805	5.341	5.475	4.33 (0.37)
H_o	0.571	0.714	0.633	0.959	0.875	0.857	0.939	0.793 (0.058)
H_e	0.626	0.763	0.736	0.748	0.792	0.813	0.817	0.757 (0.025)
F_{IS}	0.088	0.064	0.141	−0.282	−0.105	−0.055	−0.149	−0.042 (0.057)
HWE†	0.2376	0.0383	0.5277	0.0000*	0.0596	0.0173	0.0000*	
CHS (50)								
A	6	14	6	8	10	10	12	9.43 (1.31)
A_e	3.404	6.135	3.064	4.363	6.266	5.896	5.862	5.99 (0.52)
H_o	0.580	0.820	0.640	0.840	0.740	0.780	0.880	0.754 (0.041)
H_e	0.706	0.837	0.674	0.771	0.840	0.830	0.829	0.784 (0.026)
F_{IS}	0.179	0.020	0.050	−0.090	0.119	0.061	−0.061	0.040 (0.036)
HWE†	0.1880	0.4907	0.6844	0.4098	0.0052	0.0787	0.0079	
SMS (50)								
A	5	10	5	8	9	9	8	7.71 (0.75)
A_e	3.027	4.464	2.846	5.599	6.105	4.850	5.400	4.61 (0.48)
H_o	0.840	0.600	0.700	0.920	0.920	0.780	0.920	0.811 (0.047)
H_e	0.670	0.776	0.649	0.821	0.836	0.794	0.815	0.766 (0.029)
F_{IS}	−0.254	0.227	−0.079	−0.120	−0.100	0.017	−0.129	−0.063 (0.057)
HWE†	0.0267	0.0000*	0.7464	0.0000*	0.0005*	0.1214	0.0007*	

CHB = Chon Buri, CHP = Chumphon, CHS = Chachoengsao, and SMS = Samut Songkram.
* p value < 0.0018.
† p values were adjusted for multiple comparisons using the Bonferroni correction: $p < 0.0018$ (0.05/28).

1986). Nevertheless, high levels of heterozygosity suggest the ability of these four hatchery stocks to respond to short-term changes in the environment. Allelic diversity, on the other hand, is a useful measure of a population's ability to respond to long-term environmental changes and is more sensitive than heterozygosity to founder effects (Allendorf, 1986; Falconer and Mackay, 1997). Small heterozygote deficiencies found in the CHB, CHP and SMS stocks may indicate the mixing of families or populations within samples or inbreeding, and while the heterozygote excess found in the CHS strain may indicate a recent population bottleneck, there was no evidence of significant departure from Hardy-Weinberg expectations at all loci. With the use of the group-spawning method for breeding Asian sea bass, the average effective number of breeding individuals over many generations is expected to be less than the actual number of breeders due to unequal sex ratios and unequal family size (Loughnan et al., 2013; Domingos et al., 2014; Senanan et al., 2015). As a result, random genetic drift and, if numbers of breeders are small, inbreeding would be expected to occur in most hatchery populations. Among strains, the CHS had the highest N_e (55), indicating that the stock was founded by many individuals and that genetic variability within the strain has been maintained through the use of a sufficiently large number of breeders for each generation. In contrast, the N_e estimates were much lower (11–13) in the three remaining populations, suggesting that inbreeding and genetic drift were likely to have occurred, at least in later generations.

Hatchery stocks of Asian sea bass in this study exhibited significant population differentiation. Although most hatchery populations of Asian sea bass in the upper Gulf of Thailand originated from the wild, hatchery managers occasionally obtain broodstock from pond and cage culture operations in Chachoengsao province to replace old broodstock. Exchange of broodstock is commonly practiced among sea bass hatcheries in Thailand, with the aim of broadening the genetic base and enhancing performance of the stocks. This practice may increase genetic variation and effective population size, and also may lead to increased genetic similarity between populations in later generations. Significant pair-wise

Table 3
Estimates and 95% confidence interval for estimated effective population size (N_e) based on linkage disequilibrium among unlinked loci within each strain. The analysis included alleles with frequency greater than 0.01.

Strain	N_e estimate	95% confidence interval	
		Lower bound	Upper bound
CHB	13.5	10.8	16.7
CHP	11.9	9.2	15.2
CHS	55.0	38.5	86.9
SMS	12.1	8.8	16.2

CHB = Chon Buri, CHP = Chumphon, CHS = Chachoengsao, and SMS = Samut Songkram.

Table 4
Pair-wise F_{ST} values (above diagonal) and numbers of migrants (N_m) per generation between population pairs estimated based on F_{ST} values (below diagonal). Asterisks indicate highly significant differentiation ($p < 0.001$).

Strain	CHB	CHP	CHS	SMS
CHB	–	0.079**	0.049**	0.008
CHP	2.91	–	0.067**	0.070**
CHS	4.89	3.46	–	0.048**
SMS	31.87	3.32	4.91	–

CHB = Chon Buri, CHP = Chumphon, CHS = Chachoengsao, and SMS = Samut Songkram.

population differentiation was found in five of six comparisons. This may suggest that these hatcheries have maintained the original genetic character of their own broodstocks over the short term with an introduction of only 3–4 individuals per generation. Only the CHB and SMS strains shared high genetic similarity. The close relationship between the two strains was likely due to the exchange of breeders, at a rate equivalent to 32 migrants between populations per generation, based on Wright's island model assumptions (Berg et al., 1998).

In conclusion, this study clearly demonstrated significant effects of strain on the growth performance and survival of Asian sea bass when fish were raised in fixed cages under earthen-pond environmental conditions. The CHS strain was the best-performing strain in terms of growth, survival and gross yield. The strain exhibited substantial genetic diversity and large variation in growth traits, potentially suitable for exploitation in a selective breeding program. It is recommended that the base population be established from a mixture of the best performing strains that are genetically similar to maximize genetic diversity and minimize long-term inbreeding. Despite their best performance, mixing of CHB and CHP strains is not suggested due to the high genetic differences between the two strains. Because Asian sea bass has a long growing period, genetic improvement should target the development of a fast-growing strain. Next steps should include the estimation of genetic parameters for desired traits in order to employ the best selection strategies for future breeding programs.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was part of the first author's Master's thesis. The Kasetsart University Research and Development Institute (KURDI) provided support through research grant number Kor-Sor (Chor) 1.58.

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