

Growth Performance, Genetic Diversity and Morphometric Traits of an Introduced Wild and Hatchery Population of *Clarias macrocephalus* Günther, 1864

Delfina A. Muiocha¹, Saowalak Onming² and Uthairat Na-Nakorn^{2*}

ABSTRACT

Clarias macrocephalus Günther, 1864, is important as a female parent for the production of the hybrid catfish widely cultured in SE Asia. A comparison was made between a hatchery strain (KU) which was thought to have low genetic variation, and a wild population (NE) on genetic diversity, growth performance, and morphometric traits. The information obtained will be used for decision making regarding introduction of NE to expand genetic diversity of KU, which will be used as a base population for selective breeding. Sixty day old fingerlings were stocked in 3 replicates of $1 \times 2 \text{ m}^2$, 0.8 m deep concrete tanks at 70 fish per tank. Fourteen morphometric parameters were measured at 150 days old (90 days of rearing), and the data were SL-standardized prior to analysis using univariate (t-test) and multivariate (PCA and DA). Genetic diversity was analyzed using four microsatellite loci (*Cma-04*, *Cma-05*, *Cma-08* and *Cma-17*). The overall growth trial results showed that KU performed better than NE ($P < 0.05$) based on body weight (BW) and total length (TL) throughout the study; average daily growth (ADG) ($P < 0.05$) in 3 of 5 time points, and specific growth rate (SGR) in the first and last month of the experiment. Survival rate was not significantly different between populations ($P > 0.05$) except during 61-90 days of rearing (KU > NE). Three morphometric parameters were significantly different and were capable to separate the two strains. The two populations were genetically different based on allele frequencies, and genetic distance was 1.0395. The genetic variation was not significantly different between strains ($P > 0.05$). It is recommended that an introduction of NE would compromise the growth performance and some morphometric changes could also occur, although it would increase genetic variation of KU.

Keywords: bighead catfish, strain comparison, genetic diversity, growth, morphometric traits, PCA

INTRODUCTION

Globally, *Clarias* catfishes are among the economically important freshwater fish species having an annual production ranging from 270,944 to 1,164,955.45 tonnes during 2004 to 2014. In Thailand, Clariid catfish are among the most intensively cultured fishes (Areerat, 1987), contributing approximately 15 % of total aquaculture production. This made

Thailand among the top five largest producers of this commodity globally, ranked third following Nigeria and Indonesia in 2014 (FishStatJ, 2014).

Clarias macrocephalus Günther, 1864, commonly known as bighead catfish, has a great economic importance and long history of culture in Thailand (Yuan *et al.*, 2006; Suanyuk *et al.*, 2014). Hence, due to its poor performance when compared

¹ Department of Aquaculture, Fisheries Research Institute, Mao Tse Tung no 389, PO Box 4603, Maputo, Mozambique

² Department of Aquaculture, Faculty of Fisheries, Kasetsart University, 50 Ngamwongwan Road, Ladyao, Chatuchak, Bangkok 10900 Thailand

* Corresponding author, e-mail address: ffishurn@ku.ac.th

Received 21 October 2016 / Accepted 13 January 2017

with the commercial hybrid [female *C. macrocephalus* × male introduced *C. gariepinus* (Burchell, 1822)], culture of *C. macrocephalus* is less practiced nowadays. However, stocks of *C. macrocephalus* are still essential for producing the hybrid and for conservation.

In this context, the Department of Aquaculture, Faculty of Fisheries, Kasetsart University is among a few institutes that keep the broodstock of *C. macrocephalus*. The genetically improved KU (Kasetsart University) strain has been developed by intercrossing two hatchery populations and a wild population followed by five generations of mass selection aiming to improve growth performance (Na-Nakorn and Koonawootrittrirong, 2015). However, the loss of the majority of the brooders during the devastating flood in Thailand in 2011 triggered the need to introduce a new stock to rehabilitate the existing stock.

Introduction of new populations is a common practice to increase genetic variation of a target population (Falconer and Mackay, 1996; Allendorf and Luikart, 2007) for either conservation or genetic improvement. However, in case of genetic improvement, comparative performances of the introduced and the original populations should be studied beforehand (Fjalestad, 2005). Outbreeding depression, a decline of performance resulting from a mating between genetically diverted populations (Frankham *et al.*, 2002), should also be taken into consideration. Moreover, morphometric traits of each population are important because they affect market value (Koumoundouros *et al.*, 1995; Loy *et al.*, 1999; Sarà *et al.*, 1999). Some of the traits relate to adaptability of fish (e.g. Blouin, 1992; Klingenberg, 2002; Von Cramon-Taubadel *et al.*, 2005; Bracciali *et al.*, 2016; Pulcini *et al.*, 2016). Thus, these traits should be studied and considered as criteria for introduction.

Therefore, in an attempt to rescue the existing hatchery population, a wild population was considered for introduction aiming mainly at increasing genetic variation of the hatchery population. The present study was conducted to compare the introduced wild and hatchery populations of *C. macrocephalus* on growth performance, genetic variation within populations as well as genetic distance, and morphometric traits.

The feasibility of such introduction is discussed and recommendations given.

MATERIALS AND METHODS

Growth performance

Fish populations

The experiment was conducted at the Laboratory of Fish Genetics, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand. The strain/population used in this study was composed of: 1) KU strain which originated from crossing two hatchery populations (Pan Panpla Farm, Amphur Muang, Nakornpathom province; Si Roy Farm, Amphur Viset Chaicharn, Angthong province) and a wild population collected from Amphur Muang, Uthai Thani province, and exposed to mass selection for growth for five generations (Na-Nakorn and Brummett, 2009; Na-Nakorn and Koonawootrittrirong, 2015); and, 2) a wild population collected from Udon Thani province in North-East Thailand (NE; $n = 45$). This population gave the highest percentage of traits with favoring heterosis after hybridizing with *C. gariepinus* (Koolboon *et al.*, 2014). The NE population was brought to the Department of Aquaculture 2 weeks prior to the experiment.

Selection of broodfish and induced breeding

Ripe brooders (10 individuals/sex/strain or population) were selected based on swelling of the urogenital papillae of both sexes and swelling bellies of females. The female fish were injected with 30 µg/kg LH-RH analogue (Buserellin acetate, Suprefact®) plus 5 mg/kg Motilium® (Domperidone) while the males were injected with 20 µg/kg LH-RH analogue and the same dosage of Motilium®. Nine hours after injection, eggs were stripped from the injected females while the males were sacrificed for their gonads. Their testes were removed and then minced. Single pair mating was performed within each strain. Then the fertilized eggs were spread on a fine-mesh nylon net immersed in hatching fiber glass tanks (70 × 170 cm², 60 cm in depth) fitted with

a water flow-through system. To avoid confusion the 3 days post hatching larvae were considered as 0 day old larvae.

Larval rearing

After hatching, the larvae of each group were reared in two rectangular fiber tanks ($70 \times 170 \text{ cm}^2$, 60 cm in depth) filled with 40 cm water depth at approximately 2,000 larvae/tank. They were fed to satiation with *Moina* sp. for 14 days followed by 7 days of *Moina* sp. + 30% protein commercial catfish feed (Charoen Phokphand Co. Ltd.), and 39 days of artificial feed alone. Feeding was done twice daily. Water exchanged was at 30% daily. In total, the larvae were reared for 60 days. Then they were pooled within groups and the survivors were enumerated. Body weight (BW) and total length (TL) were measured in 50 fish/group (BW = 0.71 ± 0.28 and 0.48 ± 0.18 g for KU and NE, respectively; $P < 0.05$; TL = 4.1 ± 0.61 and 3.75 ± 0.48 cm for KU and NE, respectively; $P < 0.05$; Survival rate = 65.03 ± 1.87 and $64.88 \pm 2.05\%$; $P > 0.05$). The fingerlings were used in the growth trial described below.

Rearing conditions and experimental design

The growth trial started after stocking the 60 day old fingerlings in 3 replicates of $1 \times 2 \text{ m}^2$, 0.8 m deep concrete tanks at 70 fish per tank following a complete randomized design. The fish were fed 25% protein commercial catfish feed (Charoen Phokphand Co. Ltd.) at 5% of the total biomass throughout the experiment which lasted for 150 days.

Growth parameters

Measurement was done monthly on 25-30 fish/replicate. Fish samples were individually weighed to the nearest 0.1 g with an electronic balance, and total body length was measured to the nearest millimeter using a ruler. Fish survival was noted at the end of the month. Then the survivors were pooled within groups and reallocated at random to each pond with equal stocking density across groups. Fish were not fed for 24 hours before the samples were collected. The growth performance parameters were estimated according Steffens (1989) and De Silva and Anderson (1995) as shown below:

$$\text{Survival Rate (SUR) [\%]} = \frac{(\text{number of fish at } X^{\text{th}} + Y \text{ days})}{\text{number of fish at } X^{\text{th}} \text{ days}} \times 100$$

$$\text{Condition Factor (K)} = 100 \times W/L^3$$

$$\text{Daily Weight Gain or Average Daily Growth (ADG) [g. day}^{-1}] = \frac{(\text{Weight of fish at } X^{\text{th}} + Y \text{ days} - \text{Weight of fish at } X^{\text{th}} \text{ days})}{t^{-1}}$$

$$\text{Instantaneous or Specific Growth Rate (SGR) [\% day}^{-1}] = 100 \times [\text{Ln (Weight of fish at } X^{\text{th}} + Y \text{ days}) - \text{Ln (Weight of fish at } X^{\text{th}} \text{ days)}] \times t^{-1}$$

Where,

W = body weight (g); L = total body length (cm); Ln = Natural logarithm; t^{-1} = rearing days; X^{th} day = the 1st day of experiment or the day when the fish were reallocated after each measurement, and Y = rearing period

Morphometric traits

At 90 days of rearing, five fish were randomly taken from each replicate and 14 morphometric characters were measured to an accuracy of 0.01 mm (Figure 1). Measurement was done using a digital caliper on the left side of the specimens. To remove the bias due to size difference, the data of each trait were standardized as percentages of standard length (Agnès *et al.*, 1997; Çakmak and Alp, 2010).

Genetic Diversity

Fin-clip collection and DNA extraction

A caudal fin clip from the broodfish, with approximately 50 mg was taken from 50 individuals per population; the tissue was then kept in 70% alcohol until DNA extraction. Total genomic DNA was extracted from individual fin clip using phenol/chloroform protocol (Taggart *et al.*, 1992). Then the DNA was precipitated in cold ethanol, re-suspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), and stored at 4°C until PCR amplification. The quality of the DNA was tested using 1% agarose gel electrophoresis and the quantity was determined *via* spectrophotometry (NanoDrop™ ND-2000c spectrophotometer, Thermo Fisher Scientific, Inc.). Then the DNA concentration was adjusted to 10 ng/μL.

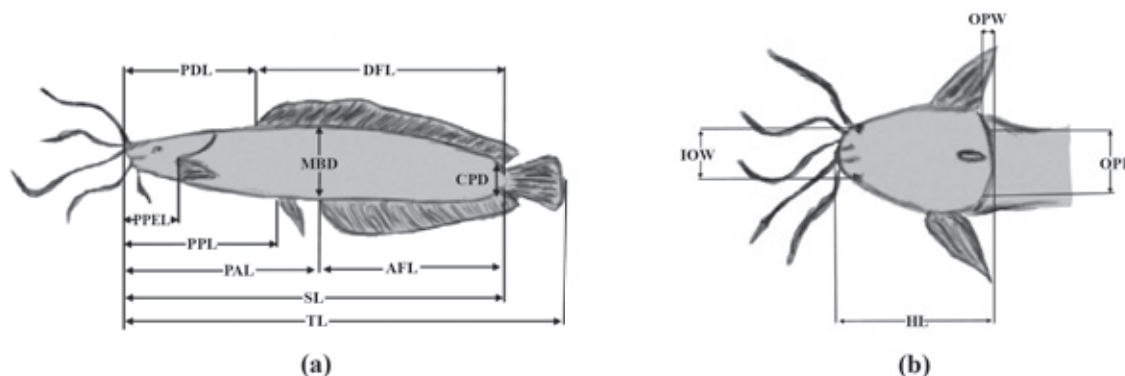


Figure 1. Schematic illustration of morphometric characters measured on *Clarias macrocephalus*, (a) Body measurements: SL, standard length; PDL, pre-dorsal length; PAL, pre-anal length; PPL, pre-pelvic length; PPEL, pre-pectoral length; DFL, dorsal fin length; AFL, anal fin length; CPD, Caudal peduncle depth; MBD, Maximum body depth; (b) Head measurements: HL, head length; IOW, inter-orbital width; OPL, occipital process length; OPW, occipital process width

Polymerase chain reaction

Four microsatellite loci (*Cma-04*, *Cma-05*, *Cma-08* and *Cma-17*) developed from DNA of *C. macrocephalus* were selected based on their high degree of polymorphism in other studies (Na-Nakorn *et al.*, 1999; Sukmanomon *et al.*, 2003). The genomic DNA was amplified in PCR reactions with a total reaction volume of 10 μ L consisting of 20 ng of genomic DNA (2 μ L), 1 \times PCR buffer $[(\text{NH}_4)_2\text{SO}_4]$, 1.5–2.5 mM MgCl_2 , 100 μ L of each dNTPs, 0.25 μ M of forward and reverse primer, 0.2U of *Taq* DNA polymerase and sterile deionized water to adjust to final volume. The thermal cycler (Takara PCR Thermal Cycler Dice™, TP600) was used and the temperature profile was as follows: 94°C for 5 min of an initial denaturing cycle followed by 35 cycles of 94°C denaturation for 30 s, a 50–60°C annealing for 30 s (varied according to primers), a 72°C extension cycle for 1 min; followed by a final extension step at 72°C for 5 min. Then the PCR products were kept at 4°C until used. Then the PCR products were separated on polyacrylamide denaturing gels along with an M13 sequence ladder and the bands were visualized by silver staining (Bassam *et al.*, 1991).

Statistical analysis

Growth trial and morphometric data

Descriptive statistics (means and standard deviations) of each trait were calculated. The homogeneity of variances of means was tested by Levene's test. When data were normally distributed, one-way analysis of variance (ANOVA) was performed (Steel and Torrie, 1980). When significant difference between treatments was found ($P < 0.05$), the means were compared using Student's "t" test (Steel and Torrie, 1980). All analyses were performed using the statistical package SPSS 21.0 (IBM SPSS Version 21.0).

Morphometric data were subjected to univariate and multivariate statistical analyses using the statistical package SPSS 21.0 (IBM SPSS Version 21.0). The SL-standardized data of the 14 morphometric measurements of the two strains were compared using Mann-Whitney test (Steel and Torrie, 1980). Characters that presented significant differences between populations was retained for Principal Component Analysis (PCA). PCA was used

to estimate morphometric variation among strains and to identify variables contributing substantially to the variation. Discriminant Factorial Analysis (DFA) was run to test the effectiveness of the characters for predicting morphological differences between populations using stepwise analysis to reduce the number of traits (Jain *et al.*, 2000; Poulet *et al.*, 2005) and to identify the combinations of traits that best separate the populations.

Microsatellite data

Genetic diversity was analyzed within and among populations. The computer program Micro-checker 2.2.3 (Van Oosterhout *et al.*, 2004) was used to identify the presence of genotyping error due to the presence of null alleles, stuttering or large allele drop out. The average number of alleles per locus (N_A), effective number of alleles per locus (A_e) and observed (H_o) and expected (H_e) heterozygosity were analyzed using Popgene 1.32 (Yeh *et al.*, 2000). Allelic richness (AR) was calculated using FSTAT 2.9.3 (Goudet, 2001). Deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium and population differentiation were estimated using Genepop 4.5 (Raymond and Rousset, 1995; Rousset, 2008) using Markov chain method with dememorization = 1000, batches = 1000, iterations per batch = 1000. Meanwhile the Bonferroni correction was used to adjust probability level for multiple tests. The F -statistics (F_{IS} , F_{ST} , and F_{IT}) and Nei's unbiased genetic distance between populations (Nei, 1978) were estimated using the software Popgene 1.32 (Yeh *et al.*, 2000).

RESULTS

Growth Performance

Mean \pm SD of body weight (BW), total length (TL), average daily weight gain (ADG), specific growth rate (SGR) at each time point are in Table 1, wherein BW and TL of KU was significantly greater ($P < 0.05$) than NE throughout the rearing period. At harvest (150 days of rearing, 210 days old), BW of KU was 103.85 ± 21.06 g, and that of NE was 69.48 ± 16.92 g, and TL was 23.71 ± 1.42 cm for KU which was longer than that of NE (20.91 ± 1.66 cm).

A slight decrease in body weight and total length was observed during 150 days of rearing (210 days old).

The average daily gain (ADG) varied from 0.25 ± 0.03 g. day⁻¹ and 0.50 ± 0.07 g. day⁻¹ in first 30 days (61-90 days old) for KU and NE respectively to negative values during day 121-150 of rearing (-0.35 ± 0.06 g. day⁻¹ for KU and -0.02 ± 0.07 g. day⁻¹ for NE) (Table 1). The ADG was significantly different ($P < 0.05$) in the first two months of rearing (KU > NE), becoming not significantly different in the 90 and 120 days of rearing (150 and 180 days old, respectively) ($P > 0.05$), and it became again significantly different ($P < 0.05$) in the 150 days (210 days old).

Specific growth rate (SGR) declined as the fish grew, from a maximum of $10.32 \pm 0.37\%$ day⁻¹ during the first month of study to as low as $-0.32 \pm 0.04\%$ day⁻¹ in KU and 9.35 to -0.04% day⁻¹ in NE (Table 1). The SGR of KU and NE strain was significantly different ($P < 0.05$) for the 30 and 150 days of rearing (90 days and 210 days old, respectively) but during the experiment it showed no significant difference ($P > 0.05$) assuming the same perceptual growth performance between the strains during this period of experiment.

Condition factor (K) was always lower than 1 in all growth period for both populations except for NE strain at 60 days of trial (Table 1). No significant differences ($P > 0.05$) were observed for K during the trial except for 90 days (150 days old) and 150 days of rearing (210 days old).

Survival rate was not significantly different between populations ($P > 0.05$) with values above 95%, except between 91-120 days when a big mortality was observed and decreased survival rate of NE to less than 75% (Table 1).

Strain differences based on morphometric traits

The mean values and standard deviations of 14 standardized morphometric traits measured on *C. macrocephalus* are presented in Table 2. Overall, the KU strain showed higher mean value in head morphometric parameters than NE except for occipital process length (OPL). For body measurements, the

Table 1. Growth parameters (mean \pm SD) of body weight (BW, g), total length (TL, cm), survival rate (SUR, %), average daily gain (ADG, g. day⁻¹), specific growth rate (SGR, % . day⁻¹), condition factor (K) of the introduced wild (NE) and the domestic (KU) strains of *Clarias macrocephalus* cultured for 150 days. Note: Asterisks denote significant difference ($P < 0.05$) between strains at each time point.

| Traits | 30 days (90 days old) | | 60 days (120 days old) | | 90 days (150 days old) | | 120 days (180 days old) | | 150 days (210 days old) | |
|---------------------------------|--------------------------|------------------|---------------------------|--------------------|---------------------------|--------------------|----------------------------|--------------------|----------------------------|--------------------|
| | KU | NE | KU | NE | KU | NE | KU | NE | KU | NE |
| BW (g) | 15.76 \pm 3.32* | 7.91 \pm 2.50* | 81.35 \pm 16.65* | 40.34 \pm 10.49* | 106.43 \pm 22.37* | 56.05 \pm 18.07* | 118.64 \pm 26.25* | 70.46 \pm 18.29* | 103.85 \pm 21.06* | 69.48 \pm 16.92* |
| TL (cm) | 11.79 \pm 0.85* | 9.26 \pm 1.57* | 20.32 \pm 1.04* | 16.64 \pm 1.46* | 22.74 \pm 1.21* | 18.71 \pm 2.27* | 23.91 \pm 1.38* | 20.55 \pm 1.79* | 23.71 \pm 1.42* | 20.91 \pm 1.66* |
| ADG (g . day ⁻¹) | 0.50 \pm 0.07* | 0.25 \pm 0.03* | 2.11 \pm 0.25* | 1.05 \pm 0.10* | 0.87 \pm 0.21 | 0.54 \pm 0.20 | 0.30 \pm 0.28 | 0.35 \pm 0.03 | -0.35 \pm 0.06* | -0.02 \pm 0.07* |
| SGR (% . day ⁻¹) | 10.32 \pm 0.37* | 9.35 \pm 0.36* | 5.30 \pm 0.17 | 5.26 \pm 0.53 | 0.94 \pm 0.27 | 1.13 \pm 0.42 | 0.27 \pm 0.25 | 0.56 \pm 0.08 | -0.32 \pm 0.04* | -0.04 \pm 0.10* |
| SUR (%) | 98.57 \pm 1.43 | 99.05 \pm 1.65 | 100 \pm 0.00 | 99.05 \pm 1.65 | 100 \pm 0.00* | 71.93 \pm 7.86* | 98.53 \pm 2.55 | 99.24 \pm 1.31 | 100 \pm 0.00 | 100 \pm 0.00 |
| K | 0.95 \pm 0.02 | 1.08 \pm 0.40 | 0.96 \pm 0.04* | 0.85 \pm 0.02* | 0.90 \pm 0.04 | 0.83 \pm 0.00 | 0.86 \pm 0.02* | 0.80 \pm 0.03* | 0.77 \pm 0.02 | 0.75 \pm 0.01 |

KU strain had smaller fins than NE strain except for fins close to head such as pre-pectoral length (PPEL) and pre-dorsal length (PDL). KU strain also showed robust bodies with higher measurements for caudal peduncle depth (CPD) and maximum body depth (MBD). The two tails t-test showed that five of the traits, namely, inter-orbital width (IOW), occipital process width (OPW), pre-pectoral length (PPEL), dorsal fin length (DFL) and maximum body depth (MBD) were significantly different ($P < 0.05$) between strains (Table 2). The KU strain showed higher mean values in all but one of the five morphometric parameters (NE > KU for DFL).

Two components explain 62.65% of the variance of which 41.10 and 21.55% were accounted for by the component 1 (PCA1) and 2 (PCA2) respectively (Figure 2). The four traits loaded in the PCA1 comprised IOW, OPW, MBD and PPEL. The PCA2 was loaded with only DFL. These five traits can separate most individuals of these two strains (Figure 2), however with some overlapping. By the results of PCA analysis, it is also possible to see that the shape of NE wild strain was more variable than KU domestic strain.

The discriminant analysis (DA) selected three of the five morphometric traits ($P = 0.000 - 0.006$) to separate the two strains of *C. macrocephalus*. The Wilk's Lambda values were moderate (0.524 - 0.678) and thus showed that each trait moderately contributed to the difference between the strains based on these three traits. The tolerance values were high (0.804 - 0.944) which meant that there was no problem on collinearity of the three most discriminating traits. The discriminant equation that can be used to identify individuals was as follows:

$$\text{Dscore} = 6.063 + 1.244\text{OPW} + 1.039\text{PPEL} - 0.558\text{DFL}$$

The discriminant scores undertake the same task as multiple linear regression by predicting an outcome. The linear equation will predict which group each individual belongs to. The box plot showing discrimination of the two strains based on the three most discriminating traits is shown in Figure 3. Each represents the variables selected in stepwise analysis in each population; the central line indicates the central tendency or location (i.e. the mean of each population; box indicates variability around the

Table 2. Descriptive statistics of morphometric measures of the introduced wild (NE) and the domestic (KU) strains of *Clarias macrocephalus* (n = 15 fish/strain), including mean \pm SD, ranges, SL-standardized ratios (as percentage of standard length or head length) and ANOVA P -value; Standard length and weight measures are unstandardized. Note: Morphometric variables abbreviations are explained in Figure 1.

| | NE | | | | KU | | | | t | P -value |
|--------------------------------|-------|-------|--------|-------|-------|-------|--------|-------|--------|------------|
| | Min | Max | Mean | S.D. | Min | Max | Mean | S.D. | | |
| Standard length (SL) (mm) | 146 | 210 | 182.3 | 18.23 | 195 | 255 | 223.3 | 18.23 | 0.567 | 0.000 |
| Head length (HL) (%SL) | 21.15 | 24.90 | 22.98 | 0.84 | 21.56 | 23.89 | 23.15 | 0.93 | -0.521 | 0.607 |
| Head width (%HL) | 1.35 | 1.42 | 1.385 | 0.01 | 1.29 | 1.41 | 1.388 | 0.03 | -0.330 | 0.744 |
| Inter-orbital width (%HL) | 8.95 | 10.46 | 9.566 | 0.35 | 8.99 | 10.34 | 9.886 | 0.39 | -2.357 | 0.026 |
| Occipital process width (%HL) | 8.54 | 11.01 | 9.514 | 0.67 | 9.42 | 10.61 | 10.059 | 0.47 | -2.560 | 0.016 |
| Occipital process length (%HL) | 1.86 | 2.87 | 2.293 | 0.32 | 1.42 | 2.83 | 2.113 | 0.43 | 1.296 | 0.205 |
| Pre-pectoral length (%SL) | 14.42 | 17.26 | 15.632 | 0.64 | 15.42 | 16.90 | 16.218 | 0.52 | -2.745 | 0.010 |
| Pre-dorsal length (%SL) | 25.22 | 28.25 | 26.999 | 0.98 | 25.87 | 28.25 | 27.336 | 0.69 | -1.083 | 0.288 |
| Pre-pelvic length (%SL) | 36.10 | 40.02 | 38.366 | 1.63 | 35.15 | 40.75 | 37.423 | 1.47 | 1.662 | 0.108 |
| Pre-anal length (%SL) | 42.78 | 48.04 | 46.197 | 1.63 | 41.88 | 48.24 | 45.087 | 1.84 | 1.747 | 0.092 |
| Dorsal fin length (%SL) | 59.54 | 64.85 | 63.218 | 1.46 | 59.02 | 64.82 | 61.437 | 1.77 | 3.006 | 0.006 |
| Anal fin length (%SL) | 39.34 | 44.84 | 42.336 | 1.76 | 39.10 | 45.67 | 41.722 | 1.83 | 0.938 | 0.356 |
| Maximum body depth (%SL) | 12.65 | 16.23 | 13.823 | 0.94 | 13.69 | 16.43 | 14.799 | 0.84 | -3.005 | 0.006 |
| Caudal peduncle depth (%SL) | 3.48 | 4.81 | 4.247 | 0.44 | 3.48 | 3.71 | 4.406 | 0.32 | -1.140 | 0.264 |

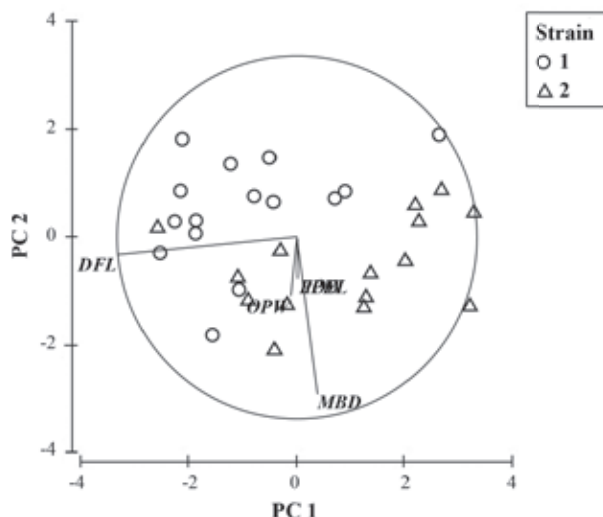


Figure 2. Principal component analysis biplot based on five morphometric traits (IOW, OPW, PPEL, DFL and MBD) of the introduced wild (1 = NE) and domestic (2 = KU) strains of *Clarias macrocephalus*

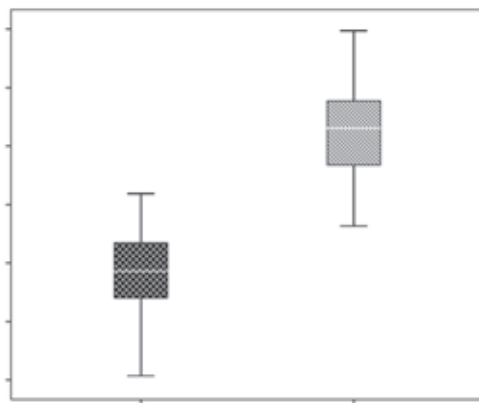


Figure 3. The box plot showing discrimination of the introduced wild (NE) and the domestic (KU) strains of *Clarias macrocephalus* based on the three most discriminating traits (DFL, OPW and PPEL)

central line (standard deviations) and the whiskers around the box indicate the range of variable.

In summary, the variables that better contributed substantially to the morphometric variation were DFL and MBD, with PPEL, IOW and OPW having minor effect (Figure 2). Although, DFL and MBD were the variables that better contributed to the morphometric variation in PCA, MBD was found not favorable discriminant variable in predicting the

morphological differences between populations.

Genetic diversity

Departure from the Hardy-Weinberg equilibrium and linkage disequilibrium

The test based on original data showed that both populations were not in Hardy-Weinberg equilibrium (HWE) towards heterozygous deficiency.

Therefore, the test for presence of null alleles was performed and detected at *Cma-05* and *Cma-08* in NE, and *Cma-05* and *Cma-17* in KU. Then the genotypic data were adjusted according to the suggestion from the program. However, after the adjustment, both populations still deviated from HWE ($P < 0.025$, Bonferroni correction) towards heterozygous deficiency and the deviation was showed in a majority of loci (3 of 4 loci in each population). There was no linkage disequilibrium between loci in NE, while significant disequilibrium was observed between *Cma-08* and *Cma-05* in KU ($P = 0.000$).

Allele frequencies

Regarding the original data, before null allele adjustment, all loci showed polymorphism in both populations, with number of alleles ranging from 5 to 16 in NE and 5 to 8 for KU strain (Table 3). The population differentiation test which based on allele frequencies showed that the two populations

were significantly different ($P < 0.025$). Private alleles were observed in all loci being KU with 11 and NE with 19 private alleles. Differences of allele frequencies between populations were significant for all loci.

Genetic variation within populations

The number of alleles per locus (N_A) tended to be higher for 2 of the 4 loci (*Cma-05*, *Cma-08*) in NE wild population and the other two for KU domestic strain (*Cma-04*, *Cma-17*). However, the overall average N_A across loci showed non-significant difference ($P > 0.05$) between populations/strains (8.00 ± 5.47 for NE and 6.75 ± 1.50 alleles/locus for KU). Similarly, effective number of alleles per locus (A_e) which is numbers of alleles/locus weighed with allele frequencies, and allelic richness (A_r) which is average number of allele/locus based on smallest sample size showed the same trend with that of N_A ($A_e = 5.31 \pm 4.44$ for NE, 3.85 ± 0.51 for KU; $A_r =$

Table 3. Genetic variation based on four microsatellite loci of the introduced wild (NE) and the domestic (KU) strains of *Clarias macrocephalus*, N = Sample size; N_A = number of alleles per locus; N_{PA} = Number of private alleles, A_e = effective number of alleles, A_r = allelic richness; H_O = Observed heterozygosity; H_e = expected heterozygosity; F_{IS} = Fixation index; $*P_{HWE}$ = P-value for Hardy-Weinberg Equilibrium (Bonferroni correction $P < 0.025$)

| Population | | <i>Cma-04</i> | <i>Cma-05</i> | <i>Cma-08</i> | <i>Cma-17</i> | All Loci |
|------------|-----------|---------------|---------------|---------------|---------------|-----------------|
| NE | N | 40 | 34 | 34 | 30 | |
| | N_A | 5 | 16 | 7 | 4 | 8.00 ± 5.47 |
| | N_{PA} | 1 | 16 | 3 | 1 | |
| | A_e | 2.8752 | 11.7556 | 4.6468 | 1.9527 | 5.31 ± 4.44 |
| | A_r | 4.580 | 16.000 | 6.920 | 3.847 | 7.84 ± 5.60 |
| | H_O | 0.7179 | 0.7391 | 0.6800 | 0.5000 | 0.66 ± 0.11 |
| | H_e | 0.6607 | 0.9353 | 0.8008 | 0.4944 | 0.72 ± 0.19 |
| | F_{IS} | -0.088 | 0.213 | 0.154 | -0.012 | 0.090 |
| | P_{HWE} | 0.0974 | 0.0000 | 0.0145 | 0.3001 | High. sign |
| KU | N | 39 | 27 | 41 | 38 | |
| | N_A | 6 | 8 | 5 | 8 | 6.75 ± 1.50 |
| | N_{PA} | 2 | 8 | 1 | 5 | |
| | A_e | 3.3092 | 4.5217 | 3.9253 | 3.6443 | 3.85 ± 0.51 |
| | A_r | 5.500 | 7.643 | 4.676 | 7.760 | 6.39 ± 1.55 |
| | H_O | 0.5250 | 0.7308 | 0.6176 | 0.6000 | 0.62 ± 0.09 |
| | H_e | 0.7066 | 0.7941 | 0.7564 | 0.7404 | 0.75 ± 0.04 |
| | F_{IS} | 0.259 | 0.081 | 0.186 | 0.193 | 0.258 |
| | P_{HWE} | 0.0000 | 0.0822 | 0.0240 | 0.0036 | High. sign. |

*Markov chain method (dememorization=1000, batches=1000, iterations per batch=1000)

7.84 ± 5.60 for NE, 6.39 ± 1.55 for KU). The average observed heterozygosity per locus (H_o) was moderate for both populations (0.66 ± 0.11 for NE and 0.62 ± 0.09 for KU) and they were significantly lower than the expected heterozygosity (H_e) (0.72 ± 0.19 for NE and 0.75 ± 0.04 for KU) (Table 3).

Genetic diversity between populations

The overall F_{ST} was 0.184. However, the small numbers of populations did not allow for the significant test. The F_{IS} and F_{IT} were 0.130 and 0.290 respectively. Among loci, *Cma-08* contributed little to differentiation while *Cma-04* and *Cma-05* showed moderate differentiation and *Cma-17* indicated large differentiation among populations. Likewise, the test for population differentiation was not allowed due to small number of loci studied. Genetic distance between the two populations was 1.0395.

DISCUSSION

Growth performance

Overall growth of the fish in the present study was comparable with the results of previous studies (Coniza *et al.*, 2003). The present results showed that growth performance of KU and NE was different. This could be due to difference in genetic background (e.g. Besnier *et al.*, 2011; Arechavala-Lopez *et al.*, 2013; Benhaïm *et al.*, 2013; Bicskei *et al.*, 2014). A decrease at 180 days in body weight in both populations caused the ADG and SGR values to be negative. A slight decrease was also observed for total length that could be mainly caused by sampling errors.

The KU strain had better overall growth performance than the NE strain. This is not surprising because KU has been domesticated, and as such, it already adapted well to captive environments. Many other studies reported that domesticated strains usually perform better in the aquaculture environment than wild strains (Burnside *et al.*, 1975; Fleming and Einum, 1997; Thodesen *et al.*, 1999; Handeland *et al.*, 2003; Glover *et al.*, 2009; Wolters *et al.*, 2009). This is due to natural and artificial selection (either

intentional or un-intentional) occurring during domestication processes (Doyle, 1983; Einum and Fleming, 2001; Fraser *et al.*, 2010) that enhance adaptability of the domesticated population to captive environment. Nevertheless, domestication may lead to unfavorable traits (e.g. slow growth rate), if the broodstock were not properly managed (e.g. Brummett *et al.*, 2004), or no alteration of traits (e.g. Osure and Phelps, 2006). Moreover, the KU strain was subjected to mass selection to improve growth for five generations. Even though the selection response of the KU strain has not been evaluated, mass selection has been effective in improving growth of many fish species, e.g. Atlantic salmon (Wolters *et al.*, 2009), Nile tilapia (Hulata *et al.*, 1986; Huang and Liao, 1990), shrimp (Goyard *et al.*, 2002; De Donato *et al.*, 2005), common carp (Ankorian *et al.*, 1992), channel catfish (Dunham *et al.*, 1987) and so on. Therefore, mass selection could have contributed to the improved growth rate of the KU strain.

It should be noted that the initial weight of KU was greater than NE, and hence may affect growth difference. Owing to the fact that the environment during the nursing period (e.g. stocking density, feeding and water quality management) was the same for both groups, it was surmised that the size difference was accounted mainly by genetic difference.

The growth rates (ADG and SGR) of the two strains followed a normal pattern, high in the early life stages and decline as the fish grew. High initial specific growth rates that decrease with increasing age and individual fish biomass (and consequent increased metabolic cost) followed typical pattern of fish growth (Kerby *et al.*, 1987). Overall, similar results were observed for ADG and SGR throughout the experiment, except for the period of 61-90 days of rearing when ADG showed the difference between strains but not for SGR. In fact, ADG could be biased by different initial sizes of the experimental fish. This was the case in our study where initial weight of KU was greater than NE. However, the SGR which is standardized with the initial weight, supported the ADG results. Thus, this verified the faster growth rate of KU over NE during the early stage. The unexpected sharp decline of growth rate of KU comparing to NE in the last 3 months of rearing eventually led to significant lower

ADG and SGR of KU than NE. The last 4 months of the experiment was in winter (November – February) when daily temperature was occasionally lower than normal (mean = 28°C; range = 18.5–34.5°C, Thai Meteorological Department, 2015). Therefore, the slower growth rate of KU than NE might indicate the different temperature tolerance of these two strains. The NE strain which originated from northeastern Thailand where winter is much colder (mean = 24.4°C; range = 18.7 – 30.6°C, Thai Meteorological Department, 2015) may have better ability to tolerate low temperatures than KU. Furthermore, despite limited supporting evidence, it is also possible that cold tolerance of the KU strain might have been compromised by selection for growth as has been reported for Nile tilapia (Rezk and Kamel, 2011). Nevertheless, controversial result was reported in rainbow trout whereas selection for growth either increased (Molony *et al.*, 2004) or compromised (Roze *et al.*, 2013) heat tolerance in rainbow trout. Although it is not conclusive, this factor should not be overlooked.

The condition factor (K) normally reflects the health and shape of fish, and hence it varies with species. It is based on the assumption that heavier fish of a given length are in better condition (Barnham and Baxter, 2003; Froese, 2006). The K values of *C. macrocephalus* in this study fall in a normal range for this species (e.g. 0.8 to 0.9, Coniza *et al.*, 2003).

The survival of the two strains was not different throughout the study except during days 61–90 of rearing (survival of NE < KU) when both groups were infected with disease. This implied that KU strain was more tolerant to the disease than NE strain. Our result was in line with some studies which suggested that each strain was best adapted to its own rearing environment; that is to say, hatchery strains showed poor survival in natural environments and wild strains in hatchery environment (Lachance and Magnan, 1990; Reisenbichler and McIntyre, 1977).

Differences of morphometric traits between strains

Morphometric differences have been reported among strains (stocks) (Corti *et al.*, 1988; Shepherd, 1991; Haddon and Willis, 1995; Bembo *et al.*, 1996) and between domesticated and wild populations,

e.g. in salmonid fishes (Fleming and Gross, 1994; reviewed by Araki *et al.*, 2008; Blanchet *et al.*, 2008; Bailey *et al.*, 2010), gilthead seabream, *Sparus aurata* and European seabass, *Dicentrarchus labrax* (Arechavala-Lopez *et al.*, 2011; El-Zaeem *et al.*, 2012; Arechavala-Lopez *et al.*, 2013), Atlantic cod, *Gadus morhua* (Wringe *et al.*, 2015). The changes in the latter case were mainly the result of adaptation to captive environments (Vehanen and Huusko, 2011; Pulcini *et al.*, 2013). In the present study, at least three (DFL, OPW and PPEL) of 13 measured traits after SL-standardization showed high discriminating power between KU and NE strains.

The difference of these strains might have been caused by different genetic backgrounds or effects of domestication acting on the KU strain or a combination of both factors. Effects of domestication on alteration of morphometric traits have been reported, e.g. bigger head of the domestic Atlantic salmon strains than the wild strain (Balbontin *et al.*, 1973; Blanchet *et al.*, 2008); robust bodies and smaller fins of domestic strain of the same species than those of the wild strain (Fleming and Einum, 1997). These might be the result of adaptability to hatchery environment. Although the differences of these morphometric traits are useful for monitoring morphometric changes resulting from the introduction of NE to KU strain, they also raised concern on slight retreat of adaptability that might occur after the introduction.

Genetic diversity

Departure from the Hardy–Weinberg equilibrium and linkage disequilibrium

Both NE and KU showed highly significant departure from Hardy – Weinberg Equilibrium (HWE) towards heterozygous deficiency, even after the adjustment for presence of null alleles. This can be attributed to population admixture (Wahlund effect) or inbreeding (Chakraborty and Leimar, 1987; Li *et al.*, 2004). We observed a weak sign of population admixture in KU (linkage disequilibrium at a loci pair) but not in NE. This aligns with the history of KU population which originated from crossing of populations different origins (Na-Nakorn *et al.*, 2004; Na-Nakorn and Brummett, 2009; Na-Nakorn and

Koonawootrittriron, 2015). Reports from other studies also showed departures from HWE in natural populations of a wide range of fish species (Castric *et al.*, 2002; Yue *et al.*, 2004). The heterozygote deficit in natural populations may emerge through many other factors that differ from Wahlund effect, such as inbreeding and nonrandom sampling (Castric *et al.*, 2002), genetic drift, null alleles, natural selection against heterozygotes (Pérez-Ruzafa *et al.*, 2006), fishing pressure (Bergh and Getz, 1989), migration-drift disequilibrium (Gillespie, 2010) or combined impacts of the afore mentioned factors. Consequently, due to limited available information in the present study, the causes of deviation from HWE in the wild population were beyond the capacity of our data.

Genetic variation within populations

It is well known that wild populations represent the primary source of genetic variability for aquaculture stocks. Therefore, it is always used to rehabilitate deteriorated stocks (Beardmore *et al.*, 1997). However, it is important to quantify genetic variation within populations and also genetic differences between the introduced and the original hatchery populations for effective monitoring of the upcoming genetic alteration (Hindar *et al.*, 1991; Alarcón *et al.*, 2004).

The average number of alleles per locus (N_A) of NE was in a normal range for freshwater fishes ($N_A = 7.5$, DeWoody and Avise, 2000) while that of KU was slightly lower. However, the t-test did not show significant difference between N_A of the two populations which might be due to the large variation of N_A at each locus and small number of loci included in this study. A similar trend was observed for effective numbers of alleles per locus (N_e) and allelic richness (A_r). It should be noted that N_e was much smaller than N_A in both populations, which implied that there were many alleles with low frequencies in each population. Thus, they might be easily lost in the succeeding generations. Observed heterozygosities (H_o) of the two populations were not different and were slightly higher than the average for freshwater fishes ($H = 0.46$, DeWoody and Avise, 2000). The overall genetic variation of *C. macrocephalus* revealed here fell within the ranges reported in other studies based on microsatellites

[$N_A = 8.42 - 13.6$; $A_e = 6.0 - 8.5$; $H_o = 0.64 - 0.73$; $H_e = 0.84 - 0.89$ (Sukmanomon, 2003); $N_A = 6.0 - 10$; $A_e = 3.6 - 6.0$; $H_o = 0.62 - 0.718$; $H_e = 0.718 - 0.810$ (Na-Nakorn *et al.*, 1999)].

Although both populations showed similar genetic variation within populations, there was a remarkable number of private alleles in each population. Therefore, the introduction of NE would surely increase genetic variation especially in terms of average number of alleles per locus which is very important for population adaptability (Allendorf and Luikart, 2009).

Genetic diversity between populations

F_{ST} is one of the most widely used measures for evaluating genetic differentiation between and/or among populations, which can provide important insights into the evolutionary processes that cause genetic differentiation among populations (Holsinger and Weir, 2009). The longer the separation of the two populations, the more variation could be observed (Ardestani *et al.*, 2014). Whilst the magnitude of F_{ST} values is to some extent species- or even group-specific. As a general rule F_{ST} values of 0 – 0.05 represent little differentiation, values of 0.05 – 0.25 indicate moderate differentiation, and values higher than 0.25 indicate very great differentiation among populations (Wright, 1978). In the present study, the average value of F_{ST} between two populations for all loci was moderate. The genetic distance between the two populations was very high but still within range of the results reported in previous studies [0.230 - 0.535 among 4 populations (Na-Nakorn *et al.*, 1999); 0.2973 - 1.454 among 5 populations (Sukmanomon, 2003)]. All these parameters indicated sufficient genetic differentiation between these populations.

Overall, based on genetic diversity data, NE and KU were genetically different and possessed sufficient genetic variation within populations. Furthermore, they possessed remarkable allelic differences. Therefore, an introduction of NE to KU would surely enhance genetic variation of the resulting population.

When outbreeding depression is considered, the theory says that the best fitness peak is achieved

at intermediate outcrossing distance between parents (Neff, 2004). Although the results reported in literature are inconclusive, with some studies reporting better performance at intermediate genetic crossing distances (Neff, 2004), others found performance to increase with distance (Jagosz, 2011), decrease with distance (Houde *et al.*, 2011; Pekkala *et al.*, 2012) or no effect of genetic distance was observed at all (Hung *et al.*, 2012). Moreover, Clark *et al.* (2013) reported no evidence of a relationship between genetic distance based on F_{ST} and the expression of early-life history traits. Therefore, the impact of outbreeding depression following the cross breeding of KU and NE is not known until the cross is made. As such, we recommend the crossing between these two populations.

Feasibility of the introduction

Regarding the ultimate goal of this study, which is to expand the genetic diversity of the hatchery population-KU, we found that in terms of genetic variation, introduction of NE to KU would increase genetic variation. Despite the large genetic distance between populations, the impact of outbreeding depression is not yet conclusive for the cross between KU and NE. However, in terms of growth performance, such introduction may cause decline in growth and survival in succeeding generations due to poor performance of NE. This is a cost that should be considered. However, it will be compensated by enhancement of selection potential (Caro and Laurenson, 1994; Frankham *et al.*, 2002; Frankham, 2005).

According to previous reports (Swain *et al.*, 1991; Blanchet *et al.*, 2008; Vehanen and Huusko, 2011; Pulcini *et al.*, 2013), hatchery populations showed morphometric changes which are related to adaptation to the captive environment. It is therefore of concern that after the introduction of NE to KU strain, the morphometric change towards less adapted characters would occur and might affect the adaptability of the new population. However, artificial selection towards growth and survival enhancement would solve the problem (Doyle *et al.*, 2001; Tallmon *et al.*, 2004; Edmands, 2007; Tymchuk *et al.*, 2007; Hill *et al.*, 2015). Therefore, we would recommend introducing NE into KU population providing that the impacts of outbreeding depression and morphometric changes are closely monitored.

LITERATURE CITED

- Agnèse, J.-F., G. Teugels, P. Galbusera, R. Guyomard and F. Volckaert. 1997. Morphometric and genetic characterization of sympatric populations of *Clarias gariepinus* and *C. anguillaris* from Senegal. **Journal of Fish Biology** 50:1143-1157.
- Alarcón, J., A. Magoulas, T. Georgakopoulos, E. Zouros and M. Alvarez. 2004. Genetic comparison of wild and cultivated european populations of the gilthead sea bream (*Sparus aurata*). **Aquaculture** 230:65 - 80.
- Allendorf, F.W. and G. Luikart. 2007. **Conservation and the genetics of populations**. Blackwell Publishing, UK.
- Ankorion, Y., R. Moav and G. Wohlfarth. 1992. Bidirectional mass selection for body shape in common carp. **Genetics Selection Evolution** 24:43-54.
- Araki, H., K. Tsukamoto, T. Kawamura, T. Takeuchi, T. Beard Jr and M. Kaiser. 2008. **Hatchery stocking for restoring wild populations: A genetic evaluation of the reproductive success of hatchery fish vs. wild fish**. Proceedings of the 5th World Fisheries Congress 2008:153-167.
- Ardestani, G., D.R.O. Rogelio, R.C. Reyes and R.P. Laude. 2014. Genetic diversity of two philippine native freshwater goby species (Perciformes: Gobiidae): Implications for conservation. **Aquatic Conservation: Marine and Freshwater Ecosystems** 24: 592-600.
- Arechavala-Lopez, P., D. Fernandez-Jover, K.D. Black, E. Ladoukakis, J.T. Bayle-Sempere, P. Sanchez-Jerez and T. Dempster. 2013. Differentiating the wild or farmed origin of Mediterranean fish: A review of tools for sea bream and sea bass. **Reviews in Aquaculture** 5:137-157.
- Arechavala-Lopez, P., P. Sanchez-Jerez, J.T. Bayle-Sempere, D.G. Sfakianakis and S. Somarakis. 2011. Morphological differences between wild and farmed mediterranean fish. **Hydrobiologia** 679: 217-231.
- Areerat, S. 1987. *Clarias* culture in Thailand. **Aquaculture** 63:355-362.

- Bailey, M.M., K.A. Lachapelle and M.T. Kinnison. 2010. Ontogenetic selection on hatchery salmon in the wild: Natural selection on artificial phenotypes. **Evolutionary applications** 3:340-351.
- Balbontin, F., S. De Silva and K. Ehrlich. 1973. A comparative study of anatomical and chemical characteristics of reared and wild herring. **Aquaculture** 2:217-240.
- Barnham, C.A. and A.F. Baxter. 2003. **Condition factor, k, for salmonid fish**. ISSN 1440-2254. Department of Primary Industries, Victoria.
- Bassam, B.J., G. Caetano-Anollés and P.M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. **Analytical Biochemistry** 196:80-83.
- Beardmore, J., G. Mair and R. Lewis. 1997. Biodiversity in aquatic systems in relation to aquaculture. **Aquaculture Research** 28:829-839.
- Bembo, D.G., G.R. Carvalho, N. Cingolani, E. Arneri, G. Giannetti and T.J. Pitcher. 1996. Allozymic and morphometric evidence for two stocks of the european anchovy *Engraulis encrasicolus* in Adriatic waters. **Marine Biology** 126:529-538.
- Benhaïm, D., R. Guyomard, B. Chatain, E. Quillet and M.-L. Bégout. 2013. Genetic differences for behaviour in juveniles from two strains of brown trout suggest an effect of domestication history. **Applied Animal Behaviour Science** 147:235-242.
- Bergh, M.O. and W.M. Getz. 1989. Stability and harvesting of competing populations with genetic variation in life history strategy. **Theoretical Population Biology** 36:77-124.
- Besnier, F., K. Glover and Ø. Skaala. 2011. Investigating genetic change in wild populations: Modelling gene flow from farm escapees. **Aquaculture Environment Interactions** 2:75-86.
- Bicskei, B., J.E. Bron, K.A. Glover and J.B. Taggart. 2014. A comparison of gene transcription profiles of domesticated and wild Atlantic salmon (*Salmo salar* L.) at early life stages, reared under controlled conditions. **BMC Genomics** 15:884.
- Blanchet, S., D.J. Páez, L. Bernatchez and J.J. Dodson. 2008. An integrated comparison of captive-bred and wild Atlantic salmon (*Salmo salar*): Implications for supportive breeding programs. **Biological Conservation** 141:1989-1999.
- Blouin, M.S. 1992. Genetic correlations among morphometric traits and rates of growth and differentiation in the green tree frog, *Hyla cinerea*. **Evolution** 735-744.
- Bracciali, C., G. Guzzo, C. Giacomia, J.M. Dean and G. Sarà. 2016. Fish functional traits are affected by hydrodynamics at small spatial scale. **Marine Environmental Research** 113: 116-123.
- Brummett, R.E., D.E. Angoni and V. Pouomogne. 2004. On-farm and on-station comparison of wild and domesticated cameroonian populations of *Oreochromis niloticus*. **Aquaculture** 242:157-164.
- Burnside, M.C., J.W. Avault and W.G. Perry. 1975. Comparison of a wild and a domestic strain of channel catfish grown in brackish water. **The Progressive Fish-Culturist** 37:52-54.
- Çakmak, E. and A. Alp. 2010. Morphological differences among the mesopotamian spiny eel, *Mastacembelus mastacembelus* (Banks & Solander 1794), populations. **Turkish Journal of Fisheries and Aquatic Sciences** 10:87-92.
- Caro, T. and M.K. Laurenson. 1994. Ecological and genetic factors in conservation: A cautionary tale. **Science** 263(5146):485-486.
- Castric, V., L. Bernatchez, K. Belkhir and F. Bonhomme. 2002. Heterozygote deficiencies in small lacustrine populations of brook charr *Salvelinus fontinalis mitchill* (pisces, salmonidae): A test of alternative hypotheses. **Heredity** 89:27-35.
- Chakraborty, R. and O. Leimar. 1987. **Genetic variation within a subdivided population**. In: Population genetics and fishery management (eds. N. Ryman and F. Utter), pp. 89-120. Washington Sea Grant Program, University of Washington Press, Seattle.
- Clark, E.S., R.B. Stelkens and C. Wedekind. 2013. Parental influences on pathogen resistance in brown trout embryos and effects of outcrossing within a river network. **PLOS ONE** 8(2): e57832.

- Coniza, E.B., M.R. Catacutan and J.D. Tan-Fermin. 2003. Growth and yield of Asian catfish *Clarias macrocephalus* (Günther) fed different grow-out diets. **Israeli Journal of Aquaculture–Bamidgeh** 55:53-60.
- Corti, M., R. Thorpe, L. Sola, V. Sbordoni and S. Cataudella. 1988. Multivariate morphometrics in aquaculture: A case study of six stocks of the common carp (*Cyprinus carpio*) from Italy. **Canadian Journal of Fisheries and Aquatic Sciences** 45:1548-1554.
- De Donato, M., R. Manrique, R. Ramirez, L. Mayer and C. Howell. 2005. Mass selection and inbreeding effects on a cultivated strain of *Penaeus (Litopenaeus) vannamei* in Venezuela. **Aquaculture** 247:159-167.
- De Silva, S.S. and T.A. Anderson. 1995. **Fish nutrition in aquaculture**. 1st ed. London: Chapman & Hall, UK.
- DeWoody, J.A. and J.C. Avise. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. **Journal of Fish Biology** 56:461-473.
- Doyle, R.M., R. Perez-Enriquez, M. Takagi and N. Taniguchi. 2001. Selective recovery of founder genetic diversity in aquacultural broodstocks and captive, endangered fish populations. **Genetica** 111:291-304.
- Doyle, R.W. 1983. Genetics in aquaculture an approach to the quantitative analysis of domestication selection in aquaculture. **Aquaculture** 33:167-185.
- Dunham, R.A., R.O. Smitherman and R.K. Goodman. 1987. Comparison of mass selection, crossbreeding, and hybridization for improving growth of channel catfish. **The Progressive Fish-Culturist** 49:293-296.
- Edmands, S. 2007. Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. **Molecular Ecology** 16:463-475.
- Einum, S. and I. Fleming. 2001. Implications of stocking: Ecological interactions between wild and released salmonids. **Nordic Journal of Freshwater Research** 75:56-70.
- El-Zaeem, S.Y., M.M.M. Ahmed, M.E.-S. Salama and W.N.A. El-Kader. 2012. Phylogenetic differentiation of wild and cultured Nile tilapia (*Oreochromis niloticus*) populations based on phenotype and genotype analysis. **African Journal of Agricultural Research** 7:2946-2954.
- Falconer, D.S. and T.F. Mackay. 1996. Introduction to quantitative genetics. **Harlow, UK: Longman**.
- FishStatJ, F. 2014. Fisheries and aquaculture department. **Statistics and Information Service FishStatJ: Universal software for fishery statistical time series. Copyright**.
- Fjalestad, K.T. 2005. **Breeding strategies**. In: Selection and breeding programs in aquaculture (ed. T. Gjedrem), pp. 145-158. Springer, Netherlands.
- Fleming, I. and S. Einum. 1997. Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. **ICES Journal of Marine Science: Journal du Conseil** 54:1051-1063.
- Fleming, I.A. and M.R. Gross. 1994. Breeding competition in a Pacific salmon (Coho: *Oncorhynchus kisutch*): Measures of natural and sexual selection. **Evolution** 48:637-657.
- Frankham, R. 2005. Genetics and extinction. **Biological Conservation** 126:131-140.
- Frankham, R., D.A. Briscoe and J.D. Ballou. 2002. **Introduction to conservation genetics**. Cambridge University Press, UK. 617pp.
- Fraser, D.J., C. Minto, A.M. Calvert, J.D. Eddington and J.A. Hutchings. 2010. Potential for domesticated-wild interbreeding to induce maladaptive phenology across multiple populations of wild Atlantic salmon (*Salmo salar*). **Canadian Journal of Fisheries and Aquatic Sciences** 67:1768-1775.
- Froese, R. 2006. Cube law, condition factor and weight-length relationships: History, meta-analysis and recommendations. **Journal of Applied Ichthyology** 22:241-253.
- Gillespie, J.H. 2010. **Population genetics: A concise guide**. The Johns Hopkins University Press. Baltimore. 181pp.
- Glover, K.A., H. Otterå, R.E. Olsen, E. Slinde, G.L. Taranger and Ø. Skaala. 2009. A comparison of farmed, wild and hybrid Atlantic salmon (*Salmo salar* L.) reared under farming conditions. **Aquaculture** 286:203-210.

- Goudet, J. 2001. **Fstat, a program to estimate and test gene diversities and fixation indices (version 2.9.3)**. Institute of Ecology. Switzerland.
- Goyard, E., J. Patrois, J.M. Peignon, V. Vanaa, R. Dufour, J. Viallon and E. Bédier. 2002. Selection for better growth of *Penaeus stylirostris* in Tahiti and New Caledonia. **Aquaculture** 204:461-468.
- Haddon, M. and T.J. Willis. 1995. Morphometric and meristic comparison of orange roughy (*Hoplostethus atlanticus*: Trachichthyidae) from the Puysegur Bank and Lord Howe Rise, New Zealand, and its implications for stock structure. **Marine Biology** 123:19-27.
- Handeland, S., A. Arnesen and S. Stefansson. 2003. Seawater adaptation and growth of post-smolt Atlantic salmon (*Salmo salar*) of wild and farmed strains. **Aquaculture** 220:367-384.
- Hill, E., B.A. Ingram, M. Rourke, J. Mitchell and J.M. Strugnell. 2015. Genetic diversity and population structure of the threatened freshwater catfish, *Tandanus tandanus*, in Victoria, Australia. **Conservation Genetics** 16:317-329.
- Hindar, K., N. Ryman and F. Utter. 1991. Genetic effects of cultured fish on natural fish populations. **Canadian Journal of Fisheries and Aquatic Sciences** 48:945-957.
- Holsinger, K.E. and B.S. Weir. 2009. Genetics in geographically structured populations: Defining, estimating and interpreting F_{st} . **Nature Reviews Genetics** 10:639-650.
- Houde, A.L.S., D.J. Fraser, P. O'Reilly and J.A. Hutchings. 2011. Relative risks of inbreeding and outbreeding depression in the wild in endangered salmon. **Evolutionary Applications** 4(5): 634-647.
- Huang, C.-M. and I.C. Liao. 1990. Genetics in aquaculture (iii) response to mass selection for growth rate in *Oreochromis niloticus*. **Aquaculture** 85:199-205.
- Hung, H.Y., C. Browne, K. Guill, N. Coles, M. Eller, A. Garcia, N. Lepak, S. Melia-Hancock, M. Oropeza-Rosas, S. Salvo, N. Upadyayula, E.S. Buckler, S. Flint-Garcia, M.D. McMullen, T.R. Rocheford and J.B. Holland. 2012. The relationship between parental genetic or phenotypic divergence and progeny variation in the maize nested association mapping population. **Heredity** 108(5): 490-499.
- Hulata, G., G.W. Wohlfarth and A. Halevy. 1986. Genetics in aquaculture (ii) mass selection for growth rate in the Nile tilapia (*Oreochromis niloticus*). **Aquaculture** 57:177-184.
- Jagosz, B. 2011. The relationship between heterosis and genetic distances based on RAPD and AFLP markers in carrot. **Plant Breeding** 130(5): 574-579.
- Jain, A.K., R.P.W. Duin and J. Mao. 2000. Statistical pattern recognition: A review. **IEEE Transactions on pattern analysis and machine intelligence** 22(1): 4-37.
- Kerby, J.H., J.M. Hinshaw and M.T. Huish. 1987. Increased growth and production of striped bass \times white bass hybrids in earthen ponds. **Journal of the World Aquaculture Society** 18:35-43.
- Klingenberg, C.P. 2002. Morphometrics and the role of the phenotype in studies of the evolution of developmental mechanisms. **Gene**. 287 (1): 3-10.
- Koolboon, U., S. Koonawootrittriron, W. Kamolrat and U. Na-Nakorn. 2014. Effects of parental strains and heterosis of the hybrid between *Clarias macrocephalus* and *Clarias gariepinus*. **Aquaculture** 424:131-139.
- Koumoundouros, G., Z. Kiriakos, P. Divanach and M. Kentouri. 1995. Morphometric relationships as criteria for the evaluation of larval quality of gilthead sea bream. **Aquaculture International** 3(2): 143-149.
- Lachance, S. and P. Magnan. 1990. Performance of domestic, hybrid, and wild strains of brook trout, *Salvelinus fontinalis*, after stocking: The impact of intra- and interspecific competition. **Canadian Journal of Fisheries and Aquatic Sciences** 47:2278-2284.
- Li, Q., C. Park, T. Endo and A. Kijima. 2004. Loss of genetic variation at microsatellite loci in hatchery strains of the Pacific abalone (*Haliotis discus hannai*). **Aquaculture** 235: 207-222.

- Loy, B.A., C. Boglione and S. Cataudella. 1999. Geometric morphometrics and morpho-anatomy: A combined tool in the study of sea bream (*Sparus aurata*, Sparidae) shape. **Journal of Applied Ichthyology** 15(3): 104-110
- Molony, B.W., A.R. Church and G.B. Maguire. 2004. A comparison of the heat tolerance and growth of a selected and non-selected line of rainbow trout, *Oncorhynchus mykiss*, in Western Australia. **Aquaculture** 241:655-665.
- Na-Nakorn, U. and R.E. Brummett. 2009. Use and exchange of aquatic genetic resources for food and aquaculture: *Clarias* catfish. **Reviews in Aquaculture** 1:214-223.
- Na-Nakorn, U., W. Kamonrat and T. Ngamsiri. 2004. Genetic diversity of walking catfish, *Clarias macrocephalus*, in Thailand and evidence of genetic introgression from introduced farmed *C. gariepinus*. **Aquaculture** 240: 145-163.
- Na-Nakorn, U. and S. Koonawootrittriron. 2015. Challenges for genetic improvement of livestock and aquatic animals. **Research & Knowledge** 1:18-25.
- Na-Nakorn, U., N. Taniguchi, E. Nugroho, S. Seki and W. Kamonrat. 1999. Isolation and characterization of microsatellite loci of *Clarias macrocephalus* and their application to genetic diversity study. **Fisheries science** 65:520-526.
- Neff, B.D. 2004. Stabilizing selection on genomic divergence in a wild fish population. **Proceedings of the National Academy of Sciences of the United States of America** 101(8): 2381-2385.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. **Genetics** 89:583-590.
- Osure, G.O. and R.P. Phelps. 2006. Evaluation of reproductive performance and early growth of four strains of Nile tilapia (*Oreochromis niloticus*, L.) with different histories of domestication. **Aquaculture** 253:485-494.
- Pekkala, N., K. E. Knott, J.S. Kotiaho, K. Nissinen and M. Puurtinen. 2012. The benefits of interpopulation hybridization diminish with increasing divergence of small populations. **Journal of Evolutionary Biology** 25(11): 2181-2193.
- Pérez-Ruzafa, Á., M. González-Wangüemert, P. Lenfant, C. Marcos and J.A. García-Chartron. 2006. Effects of fishing protection on the genetic structure of fish populations. **Biological Conservation** 129:244-255.
- Poulet, N., Y. Reyjol, H. Collier and S. Lek. 2005. Does fish scale morphology allow the identification of populations at a local scale? A case study for rostrum dace *Leuciscus leuciscus burdigalensis* in river viaur (sw france). **Aquatic Sciences** 67(1): 122-127.
- Pulcini, D., P. Wheeler, S. Cataudella, T. Russo and G. Thorgaard. 2013. Domestication shapes morphology in rainbow trout *Oncorhynchus mykiss*. **Journal of Fish Biology** 82:390-407.
- Pulcini, D., K. Christensen, P. Wheeler, T. Russo and G. Thorgaard. 2016. The genetic basis of morphometric and meristic traits in rainbow trout (*Oncorhynchus mykiss*). **Annals of Aquaculture and Research** 3(3): 1023.
- Raymond, M. and F. Rousset. 1995. Genepop (version 1.2): Population genetics software for exact tests and ecumenicism. **Journal of Heredity** 86:248-249.
- Reisenbichler, R. and J. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. **Journal of the Fisheries Board of Canada** 34:123-128.
- Rezk, M.A. and E.A. Kamel. 2011. Cold tolerance of selective breeding of *Oreochromis niloticus* and *Oreochromis aureus*. **Egyptian Journal for Aquaculture** 1(1):43-53
- Rousset, F. 2008. Genepop'007: A complete re-implementation of the genepop software for Windows and Linux. **Molecular Ecology Resources** 8:103-106.
- Roze, T., F. Christen, A. Amerand and G. Claireaux. 2013. Trade-off between thermal sensitivity, hypoxia tolerance and growth in fish. **Journal of Thermal Biology** 38:98-106.
- Sarà, M., E. Favaloro and A. Mazzola. 1999. Comparative morphometrics of sharpsnout seabream (*Diplodus puntazzo* Cetti, 1777), reared in different conditions. **Aquacultural engineering**. 19(3): 195-209.

- Shepherd, G. 1991. Meristic and morphometric variation in black sea bass North of Cape Hatteras, North Carolina. **North American Journal of Fisheries Management** 11:139-148.
- Steel, R.G. and J.H. Torrie. 1980. **Principles and procedures of statistics: A Biometrical Approach**, second edition. McGraw-Hill, New York. 633 pp.
- Steffens, W. 1989. **Principles of fish nutrition**. Ellis Horwood Limited, UK.
- Suanyuk, N., M. Rogge, R. Thune, M. Watthanaphiromsakul, N. Champhat and W. Wiangkum. 2014. Mortality and pathology of hybrid catfish, *Clarias macrocephalus* (Günther) × *Clarias gariepinus* (Burchell), associated with *Edwardsiella ictaluri* infection in Southern Thailand. **Journal of Fish Diseases** 37:385-395.
- Sukmanomon, S. 2003. **Development of microsatellites primers for *Clarias macrocephalus* and their applications to population genetic studies**. Master of Science, Kasetsart University. 105 pp.
- Sukmanomon, S., S. Poompuang and M. Nakajima. 2003. Isolation and characterization of microsatellites in the asian walking catfish *Clarias macrocephalus*. **Molecular Ecology Notes** 3:350-351.
- Swain, D., B. Riddell and C. Murray. 1991. Morphological differences between hatchery and wild populations of coho salmon (*Oncorhynchus kisutch*): Environmental versus genetic origin. **Canadian Journal of Fisheries and Aquatic Sciences** 48: 1783-1791.
- Taggart, J.B., R.A. Hynes, P.A. Prodöhl and A. Ferguson. 1992. A simplified protocol for routine total DNA isolation from salmonid fishes. **Journal of Fish Biology** 40:963-965.
- Tallmon, D.A., G. Luikart and R.S. Waples. 2004. The alluring simplicity and complex reality of genetic rescue. **Trends in Ecology & Evolution** 19:489-496.
- Thai Meteorological Department. 2015. **Average high and low 2014/2015**. Climatological Center. Meteorological Development Bureau, Bangkok.
- Thodesen, J., B. Grisdale-Helland, S.J. Helland and B. Gjerde. 1999. Feed intake, growth and feed utilization of offspring from wild and selected Atlantic salmon (*Salmo salar*). **Aquaculture** 180:237-246.
- Tymchuk, W.E., L.F. Sundström and R.H. Devlin. 2007. Growth and survival trade-offs and outbreeding depression in rainbow trout (*oncorhynchus mykiss*). **Evolution**. 61: 1225-1237.
- Van Oosterhout, C., W.F. Hutchinson, D.P.M. Wills and P. Shipley. 2004. Micro-checker: Software for identifying and correcting genotyping errors in microsatellite data. **Molecular Ecology Notes** 4:535-538.
- Vehanen, T. and A. Huusko. 2011. Brown trout *Salmo trutta* express different morphometrics due to divergence in the rearing environment. **Journal of Fish Biology** 79:1167-1181.
- Von Cramon-Taubadel, N., E.N. Ling, D. Cotter and N.P. Wilkins. 2005. Determination of body shape variation in irish hatchery-reared and wild Atlantic salmon. **Journal of Fish Biology** 66(5): 1471-1482.
- Wolters, W.R., F.T. Barrows, G.S. Burr and R.W. Hardy. 2009. Growth parameters of wild and selected strains of Atlantic salmon, *Salmo salar*, on two experimental diets. **Aquaculture** 297:136-140.
- Wright, S. 1978. **Variability within and among natural populations**. Evolution and the genetics of populations, Vol. 2. University of Chicago Press, Chicago, 511 pp.
- Wringe, B.F., I.A. Fleming and C.F. Purchase. 2015. Rapid morphological divergence of cultured cod of the Northwest Atlantic from their source population. **Aquaculture Environment Interactions** 7:167-177.
- Yeh, F., Y. Rongcal and T. Boyle. 2000. Popgene-1.32: A free program for the analysis of genetic variation among and within populations using co-dominant and dominant markers. Department of Renewable Resources at the University of Alberta, Canada.
- Yuan, D., Y. Yi and J.S. Diana. 2006. Walking catfish production in Thailand hybrid species helped fuel culture boom. **Global Aquaculture Alliance** 210:59-61.

- Yue, G.H., Y. Li, L.C. Lim and L. Orban. 2004.
Monitoring the genetic diversity of three
Asian arowana (*Scleropages formosus*)
captive stocks using AFLP and microsatellites.
Aquaculture 237:89-102.