Improving biomass gain using crossbreeding of distinct farmed population of African catfish *Clarias gariepinus*

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Abstract. African catfish, *Clarias gariepinus* was introduced to Indonesia, firstly in 1985 and subsequently in 2002, 2005 and 2011, both as farmed and wild population. However, limited number of early parental and uncontrolled broodstock utilization led to deterioration of production performance. One possible method that can produce immediate improvement is crossbreeding between distinct introduced populations. The present study examined the performance and heterosis of crossbreeding of farmed African catfish. Three populations (the Indonesia, Netherlands and Thailand populations) were reciprocally crossbred to form three purebred and six crossbreed populations. Body weight, survival, biomass, feed conversion and growth rate were calculated at the nursing stage (81 days after hatching) and the grow-out stage (172 days after hatching). At the grow-out stage, the Netherlands female x Thailand male (NT) population achieved the highest biomass (22.59 kg) and body weight (241.39 g) and had a higher survival rate (93.67%). The NT population obtained the highest mid-parent heterosis on survival (19.71%) and biomass (52.31%) and the highest best-parent heterosis on biomass (25.34%) at the grow-out stage. These results emphasized the preference of the crossbreeding between separated population for enhancing of the production performance. Further studies and implication of our result to *Clarias* production in Indonesia are proposed.

Key Words: *Clarias gariepinus*, body weight, growth, survival, heterosis.

Introduction. In Indonesia, as an introduced species, African catfish *Clarias gariepinus* (Burchell, 1822) production has increased rapidly from 56,000 tons in 2004 to 678,000 tons in 2014 (FAO 2016). All African catfish production can be recorded generated from aquaculture activities and mostly marketed locally as fish consumption. African catfish which was introduced from Taiwan in 1985 is the most preferred to cultivated in fish farmer. The fish has been augmented through intergenerational backcross (F2 female x F6 male) in 2004 (hereinafter referred as the Indonesia population). In addition to the Indonesia population, there were other populations with different historical introductions, namely: from Thailand in 2002, from Egypt in 2005, from the Netherlands in 2011 and from Kenya in 2011 (Sunarma et al 2016). The Indonesia, Netherlands and Thailand populations were identified as well-domesticated populations. The Egypt and Kenya populations were wild populations that were newly introduced into the aquaculture system. These five populations of African catfish reared separately in government agencies and private company.

The Indonesia population has experienced a growth decline due to inbreeding depression (Nurhidayat et al 2003). Although intergenerational backcross as an genetically improvement have been made, due to a limited number of early parental, the loss of genetic variation and inbreeding might have been unavoidable (Imron et al 2011). Furthermore, decreasing in the genetic quality was also related to uncontrolled broodstock utilization. Many farmers use the broodstock resulting from own production that does not consider genetic management instead of using the broodstock produced by government agencies. In fish farmer level, deteriorating performance of African catfish has been observed, such as increasing of abnormal bodies, for example occurrence of albino progenies, and decreasing of growth, size uniformity and disease resistance.

The disadvantages effects of inbreeding may be recovered by crossbreeding as reported in the Pacific blue shrimp *Penaeus stylirostris* (Goyard et al 2008). Crossbreeding may increase heterozygosity and can produce immediate improvement. Crossbreeding is also performed to incorporate desired traits from different populations/species to single populations/species with the aim that the progeny will perform better than their parents (hybrid vigor or heterosis). Desired traits can include growth rate, disease resistance, production of sterile or single-sex fish and increased adaptability to the cultivation environment (Hulata 2001; Vandeputte et al 2014). Genetically improvement through crossbreeding has been reported in several aquaculture species, such as Pacific blue shrimp (Goyard et al 2008), common carp *Cyprinus carpio* (Zak et al 2007), and tilapia *Oreochromis niloticus* (Thoa et al 2014).

Crossbreeding of African catfish could be conducted by utilizing available varied populations. In nursing stage, our previous work showed crossbreeding between wild and farmed African catfish resulted better body length, survival and over-size fish than their purebreed (Sunarma et al 2016). In the present study, we aimed to improve the African catfish through crossbreeding of farming population, i.e. the Indonesia, Netherlands and Thailand populations in grow-out stage. The body weight growth, survival, biomass and feed conversion of crossbreed population were examined compared to their purebreed populations.

Material and Method

Fish. The study was conducted in December 2014 – August 2015 in the National Freshwater Aquaculture Center, Ministry of Marine Affairs and Fisheries, located in Sukabumi, West Java, Indonesia. Three farming populations of African catfish, each 20 fish, were used, namely: Indonesia, Netherlands and Thailand populations. The fish were marked using PIT-tag and maintained in three tanks (each 4 m^3 in volume) using flow-through water system under indoor natural photoperiod and temperature (ranged 22-25^oC). They were hand-fed once a day at feeding rate of 1% of biomass with commercial pellets (45% protein) enriched with *Spirulina platensis* powder (3% kg^{-1} feed). Broodstocks have been reared for three months before used in the experiment.

Three female parents and three male parents of each population were selected and spawned artificially. Final oocyte maturation and ovulation in the female broodstocks were induced using a single injection 0.2 mL kg^{-1} female of OVAPRIM solution (Syndel Laboratories Ltd, Nanaimo, BC Canada; Each 1 mL contained 0.02 mg salmon gonadotropin-releasing hormone + 110 mg domperidone). At 10-12 hours after injection, the egg from each female were stripped and separated to equally three portions. The milt was obtained from squeezed testes of each sacrificed male. The milt was diluted 1:100 in 0.9% NaCl solution and separated to equally three portions. Each egg portion (ca. 5 mg) fertilized with the milt solution (ca. 10 mL) from a male either the same or different populations. The fertilized eggs from each full sib family were spread in individually aerated 100 L aquarium. Egg hatching occurred at about 30-36 hours after fertilization (temperature of 24-25^oC). After hatching, water replacement was conducted to remove un-hatching egg and other debris. At day 3rd, larvae were counted to adjust the stocking density.

After yolk absorption (4 days after hatching, dah), larvae were fed tubificid worms in the first week, a combination of tubificid worms and powdered feed (40% protein) in the second week, and granulated feed (39-41% protein) in the third to eleventh weeks. Tubificid worms were provided all time (ad-libitum) while artificial feed given as much as the fish can consume (at-satiation) at feeding rate of four times per day. Since artificial feeding was provided, gradually water replacement was conducted every two days to provide suitable water quality. Fish were harvested and sorted in the third, seventh and eleventh weeks. Fish with the closest size to each population mean were saved and further reared. The stocking density reduced consecutively: 15, 8, and 4 fish L^{-1} at 1st-3rd week, 4th-7th week, and 8th-11th week, respectively.

At the grow-out stage, each fish population was reared on constructed of netting which has a mesh size of 3.0 to 4.0 mm, separately. A total of 27 nets (1.5 m x 1.0 m x 1.0 m, water depth of 0.8 m) were installed in one pond (300 m^2, water depth of 1.0-1.2 m). Stocking density was 100 fish per nets. Artificial feed (30-31% protein) was given at-satiation with a feeding frequency of three times per day. Grow-out stage was terminated after 13 weeks rearing.

Experimental design and statistical analysis. The three parental populations used were represented by Indonesia population, Netherland population and Thailand population. In the reciprocal crosses, the female parents were named first. Purebred progeny populations consisted of Indonesia (II), Netherlands (NN), and Thailand (TT) populations. Crossbreed progeny populations involved Indonesia x Netherlands (IN), Indonesia x Thailand (IT), Netherlands x Indonesia (NI), Netherlands x Thailand (NT), Thailand x Indonesia (TI), and Thailand x Netherlands (TN) populations. Three families in the same purebred or crossbreed reared separately and considered as replicates.

Body weight was measured on a sample of 30 fish at the end of the nursing stage (81 dah) and all live fish at the end of the grow-out stage (172 dah). Survival was calculated based on the number of fish at the beginning and end of each stage. Biomass was measured from total live fish at the end of each stage. The growth rate (SGR) was expressed as 100 × (LnBWt - LnBWo) / T, where BWt = body weight at t day, BWo = body weight at o day, and T = the period in the day (Bhujel 2008). Both mid-parent heterosis (MPH) and best-parent heterosis (BPH) (Gjedrem & Baranski 2009) were calculated. MPH was expressed as 100 x ((crossbreed performance – mean of both purebred performances) / mean of both purebred performances), and BPH was expressed as 100 x (crossbreed performance – best of purebred performance) / best of purebred performance) (Guy et al 2009).

Normality of the data was assessed using the Shapiro-Wilk test. The effect on the population of each trait performance was determined using either analysis of variance ($p < 0.05$) or using the Kruskal-Wallis when not normally distributed. The Tukey post hoc test was used to compare populations.

Results. At the nursing stage, the body weight, survival and biomass were not different significantly among populations, while the growth rate was different significantly between the II population (10.13%) and the TN or NN populations (11.23%, 11.21%, respectively). The TN, IB and IT populations achieved higher body weight, survival and biomass, respectively, than other populations (Table 1).

Table 1

Trait performance at the nursing stage (81 days after hatching) of crossbreeding of African catfish

Crosses	*Body weight (g)*	*Survival (%)*	*Biomass (g)*	*Growth rate (%)*
II	5.00 ± 1.35^a	76.66 ± 17.33^a	155.42 ± 59.29^a	10.13 ± 0.33^b
NN	11.42 ± 2.47^a	39.99 ± 13.24^a	179.82 ± 60.83^a	11.21 ± 0.29^a
TT	7.96 ± 2.08^a	72.34 ± 9.87^a	235.63 ± 94.60^a	10.74 ± 0.32^{ab}
IN	8.90 ± 1.13^a	78.08 ± 12.80^a	278.71 ± 58.58^a	10.90 ± 0.16^{ab}
IT	10.59 ± 2.41^a	65.12 ± 24.94^a	287.78 ± 168.41^a	11.11 ± 0.31^{ab}
NI	6.91 ± 2.43^a	56.13 ± 15.18^a	159.15 ± 76.44^a	10.52 ± 0.52^{ab}
NT	9.76 ± 2.88^a	66.08 ± 8.74^a	262.46 ± 106.36^a	10.99 ± 0.41^{ab}
TI	8.34 ± 1.98^a	76.17 ± 4.01^a	252.30 ± 47.74^a	10.80 ± 0.29^{ab}
TN	11.90 ± 4.04^a	47.00 ± 7.40^a	220.87 ± 78.54^a	11.23 ± 0.49^a

Data were presented as mean±SD of three replications. Different superscripts in the same column show significant differences ($p < 0.05$). I = Indonesia, N = Netherlands, T = Thailand. Crossbreed notations represent the dam and sire of population names.

At the grow-out stage, body weight, survival and biomass were different significantly among populations, while the feed conversion and growth rate were not significantly different. The NT population obtained a higher body weight (241.39 g) than other populations and was significantly different from the TI (124.15 g), IT (101.97 g), IN (101.49 g), II (100.11 g), and NI (77.06 g) populations. The NT population achieved a higher survival rate (93.67%) than other populations and was significantly different from the IT (75.33%), IN (67.67%), NN (63.67%), TI (63.33%), TN (58.33%), and NI (39.00%) populations. The NT population reached the highest biomass (22.59 kg) and was different significantly from all populations (Table 2).

Table 2

Trait performance at the grow-out stage (172 days after hatching) of crossbreeding of African catfish

Crosses	*Body weight (g)*	*Survival (%)*	*Biomass (g)*	*Growth rate (%)*	Feed conversion
II	100.11 ± 19.42^{bc}	78.00 ± 10.44^{abc}	7.93 ± 2.61^{cd}	3.30 ± 0.09^{a}	1.10 ± 0.26^{a}
NN	196.87 ± 33.96^{ab}	63.67 ± 10.26^{cd}	12.72 ± 4.19^{bc}	3.14 ± 0.20^{a}	1.24 ± 0.06^{a}
TT	186.86 ± 54.16^{ab}	93.00 ± 2.65^{ab}	17.47 ± 5.61^{b}	3.46 ± 0.48^{a}	1.17 ± 0.32^{a}
IN	101.49 ± 39.74^{bc}	67.67 ± 2.31^{cd}	6.89 ± 2.83^{cd}	2.63 ± 0.28^{a}	1.25 ± 0.09^{a}
IT	101.97 ± 59.14^{bc}	75.33 ± 3.79^{bcd}	7.75 ± 4.67^{cd}	2.40 ± 0.85^{a}	1.14 ± 0.16^{a}
NI	77.06 ± 13.58^{c}	39.00 ± 5.29^{e}	2.97 ± 0.35^{d}	2.69 ± 0.62^{a}	1.23 ± 0.09^{a}
NT	241.39 ± 14.09^{a}	93.67 ± 2.52^{a}	22.59 ± 0.91^{a}	3.56 ± 0.40^{a}	1.01 ± 0.04^{a}
TI	124.25 ± 23.46^{bc}	63.33 ± 3.21^{cd}	7.82 ± 1.15^{cd}	2.97 ± 0.09^{a}	1.23 ± 0.28^{a}
TN	225.05 ± 18.72^{a}	58.33 ± 9.07^{d}	13.20 ± 2.81^{bc}	3.28 ± 0.34^{a}	1.16 ± 0.08^{a}

Data were presented as mean±SD of three replications. Different superscripts in the same column show significant differences ($p < 0.05$). I = Indonesia, N = Netherlands, T = Thailand. Crossbreed notations represent the dam and sire of population names.

Both mid-parent (MPH) and best-parent heterosis (BPH) were generated in this study. However, the MPH and the BPH showed high variation (Figure 1). At the nursing stage, the MPH was not different significantly among populations for all traits, while at the grow-out stage, the MPH was significantly different for body weight, survival and biomass, and not different for feed conversion and growth rate. The NT population generated positive MPH for all traits except body weight at the nursing stage. The NT population achieved the highest MPH in survival (19.71%) and biomass (52.31%), while body weight (27.73%) was different significantly from the NI population (-47.93%). Although not different significantly, the NT population obtained a higher MPH in feed conversion and growth rate than other populations at the grow-out stage.

The BPH was not different significantly among populations in growth rate at the nursing stage and body weight, survival and biomass at the grow-out stage. The NT population generated positive BPH for all traits at the grow-out stage and for biomass at the nursing stage. The NT population reached the highest BPH for biomass (36.43%), whilst body weight (25.72%) was significantly different from the NI population (-60.40%), and survival (0.77%) was significantly different from the IT (-18.97%), TI (-31.85%), TN (-37.38%), and NI (-49.69%) populations. Compared to Indonesia population, the NT population reached higher of body weight, survival, biomass up to 145%, 21%, 202%, respectively, and lower feed conversion rates up to 5.5%.

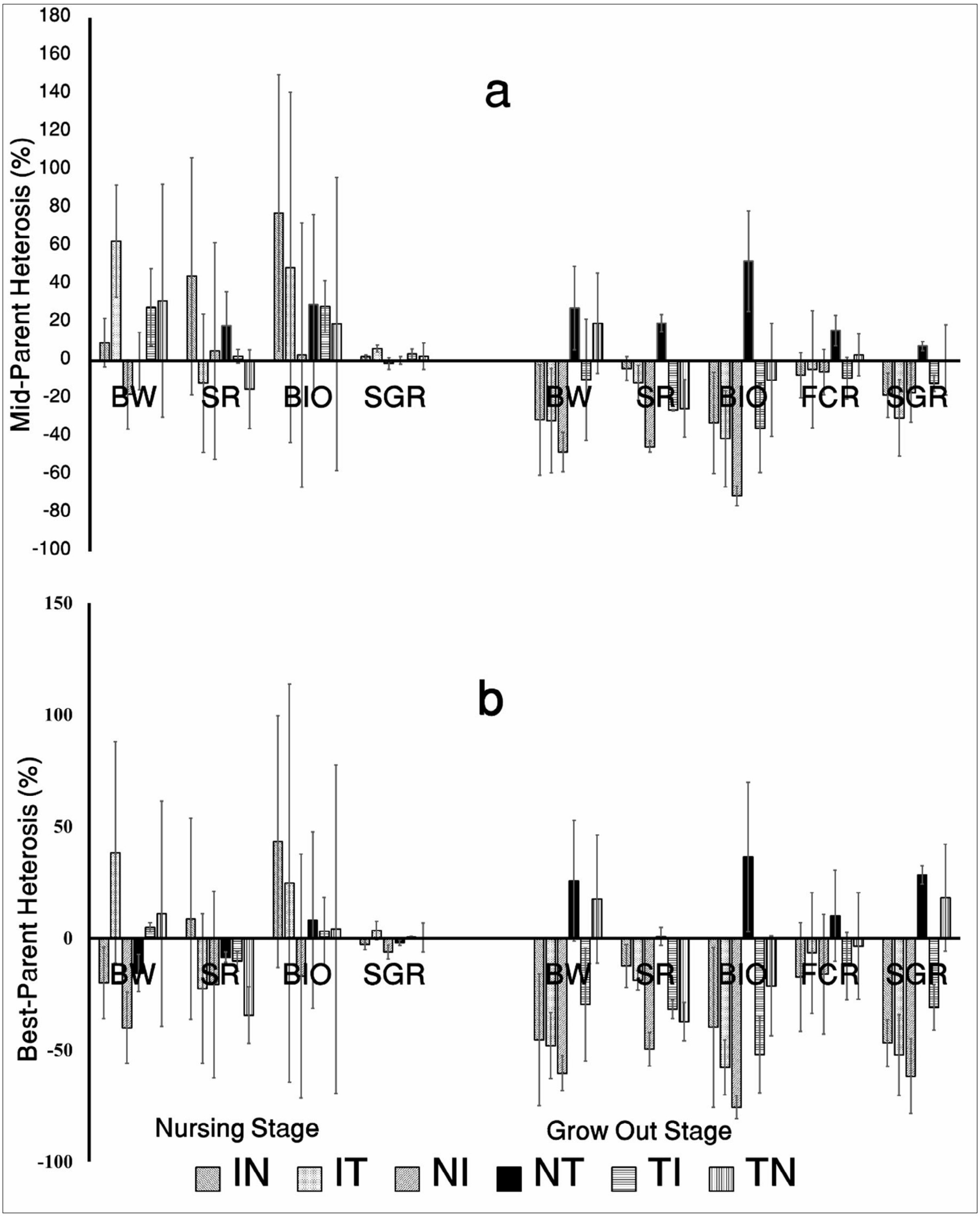

Figure 1. Mid-parent (a) and best-parent (b) heterosis of crossbreeding of African catfish. Data were presented as mean±SD of three replications. BW = body weight, SR = survival rate, BIO = biomass, SGR = growth rate, FCR = feed conversion, I = Indonesia, N = Netherlands, T = Thailand. Crossbreed notations represent the dam and sire of population names.

Discussion

Crossbreeding performance. This research showed the NT population as the best crossbreed based on biomass at the grow-out stage that is in line with heterosis on body weight, survival, biomass, feed conversion and growth rate. The results of crossbreeding of introduced African catfish to Indonesia were different than that to Thailand (Wachirachaikarn et al 2009). This difference in results corresponds to differences in the origins of the fish used in both studies. Based on the microsatellite DNA variation, Wachirachaikarn et al (2009) revealed genetic differentiation between populations of African catfish in Thailand which introduced in 1987 and hypothesized each population was originated from different founder population, but the crossbreed experimental showed no differences between the crossbreds in body weight and length, the specific immune response to *Aeromonas hydrophilla* and phagocytic index. In our study, fish showed a different history of introduction and domestication, although this is not yet corroborated based on DNA variations.

Unfortunately, there are no accurate data on the level of generation of African catfish since they were domesticated. However, by considering the productive age of African catfish > 1.5 years (Fleuren 2008) and the generation interval of one generation per year (Koolboon et al 2014) as well as our experience and the domestication history of about 35-36 years, the domestication of the African catfish was expected to reach about 20 generations. These long-term domestications can lead to a decrease in genetic diversity and the occurrence of inbreeding of fish populations, as shown in the common carp (Kohlmann et al 2005), brown trout *Salmo trutta* (Aho et al 2006) and introduced African catfish to Thailand (Wachirachaikarn et al 2009) and Indonesia (Imron et al 2011). However, the introduced African catfish was fully separated and there was no gene flow expected between populations. The crossbreeding between separately inbred population could improve performance of their progenies, as demonstrated in Pacific blue shrimp (Goyard et al 2008).

At the nursing stage, the only different performance rate among populations was the growth rate between the II populations and the NN or TN population. At relatively equal stages, there were no differences in body weight, survival and growth rates at 78 days of age on the crossbreeding of *Heterobranchus longifilis* (Nguenga et al 2000); body weight, survival and growth rates at 72 days of age on the crossbreeding of the silver perch *Bidyanus bidyanus* (Guy et al 2009); and body weight at 100 days of age on the crossbreeding of tilapia (Thoa et al 2014). There were different results reported on the crossbreeding, for example crossbreeding of black bream *Acanthopagrus butcheri* in body weight at 90 days of age (Doupe et al 2003). However, at almost the same age, body weight of crossbreeding populations in this study (5-11 g) was lower than crossbreeding African catfish in Thailand (31-57 g) (Wachirachaikarn et al 2009). This is allegedly associated to differences in stocking density between this study (consecutively 1500, 800 and 400 fish per 100 liters) and that report (200 fish per 90 liters). Higher stocking density in our research was conducted to representatively similar density that used in fish farmer.

At the grow-out stage, the NT population showed better body weight and survival than other populations and these lead to produce the highest biomass. The highest performance of the NT population expected transferred from the Netherlands and Thailand population as maternal and paternal, respectively. The Netherlands and the Thailand populations obtained higher body weight and survival, respectively, than other purebreds. These results are in line with the crossbreed of Pacific blue shrimp, which produces higher growth and survival than the purebred population (Goyard et al 2008); the crossbreed of the Hungarian common carp and the Israel population (Zak et al 2007); the crossbreed of the scaly common carp (Buchtova et al 2006); and the crossbreed of tilapia (Thoa et al 2014). Although statistically not significant, the NT population showed a lower feed conversion and a higher growth rate than other populations. These results strengthen the evidence of prominence of the NT population.

Our current results showed the population that acts as dam or as sire can obtain some different performances on reciprocally crossbred population. The TN population showed a survival rate and biomass significantly lower than the NT population. Similar results were also found with the survival rate between the IN population and the NI population, but not between the IT population and the TI population. In several fish crossbreedings, the different breeding schemes (i.e. one population acts as a male or as female) can produce a different performance, such as those found with the crossbreeding of Chinook salmon *Oncorhynchus tshawytscha* (Bryden et al 2004), tilapia (Bentsen et al 1998), giant freshwater prawn *Macrobrachium rosenbergii* (Thanh et al 2010), brook trout (Crespel et al 2012) and blunt snout bream, *Megalobrama amblycephala* (Luo et al 2014). This reciprocal effect may be associated to a maternal or paternal effect, cytoplasmic inheritance or genetic linkage between sex genes and performance genes (Bentsen et al 1998; Crespel et al 2012).

Our research also showed the better performance of inbred population does not necessarily indicate similar performance on different crossbreeding. At the grow-out stage, on the crossbreed of the Netherlands population as the dam, the NT population achieved significantly higher body weight, survival and biomass than the NI population. Similar results were also found on the crossbreed of the Thailand population as the sire; the NT population obtained a significantly better performance than the IT population. Also, the Netherlands population could not improve NI population to exhibit better performance than the II population as well as Thailand population on the IT population to make better than II population performance. These showed the effect of the origin of parent on their progenies could not be considered in our research. Instead, the specific crossbreed combination, involving particular dam and sire population, was needed to improve the performance in African catfish, as reported in tilapia (Bentsen et al 1998) and common carp (Zak et al 2007).

Positive mid-parent heterosis (MPH) can be obtained at the nursing stage and the grow-out stage. In the nursing stage, the IN population and the TI population showed a positive MPH for all traits. However, there were no populations that consistently had a higher MPH than other populations. At the grow-out stage, the NT population obtained positive MPH on all traits, while most other populations obtained negative MPH. Heterosis in crossbreeding has been observed in a number of fish species, including *H. longifiliis* (Nguenga et al 2000), guppy *Poecilia reticulata* (Nakadate et al 2003), Chinook salmon (Bryden et al 2004), common carp (Zak et al 2007); Pacific blue shrimp (Goyard et al 2008), silver perch (Guy et al 2009), giant freshwater prawn (Thanh et al 2010), brook trout (Crespel et al 2012) and tilapia (Thoa et al 2014).

In the best-parent heterosis (BPH), the NT population showed positive BPH for all traits on the grow-out stage. From the point of view of fish production, BPH is more favorable than MPH, due to the fact that BPH shows the crossbreeds that have better performance than the best parent, instead of the average parent. Bentsen et al (1998) suggested that the exploitation of heterosis for aquaculture production should be compared to the best-performing purebred and not the mean of the parent purebred, to assess the gain in performance. In our current research, the NT population achieved a BPH of body weight, survival, biomass, feed conversion and growth rate as follows: 25.72%, 0.77%, 36.43%, 10.08% and 3.10%, respectively.

***Implications for African catfish aquaculture in Indonesia*.** Biomass and feed conversion are important factors that determine the efficiency of production of African catfish at the fish farmers level. Higher biomass and lower feed conversion can increase business profits. Our results show superior performance by a crossbreed among newly introduced populations in Indonesia. The NT population reached higher of body weight, survival, biomass up to 145%, 21%, 202%, respectively, and lower feed conversion rates up to 5.5% than the Indonesia population. Therefore, replacing the Indonesia population with the NT population is highly recommended.

This research has not revealed genetic improvement through crossbreeding between the Indonesian population and newly introduced populations, as suggested when the African catfish was introduced to Thailand (Wachirachaikarn et al 2009). Either Indonesia population as dam or as sire, their crossbreed progenies were no exhibit better performances than the Indonesia population. However, maintaining each population separately should be conducted. Separated populations could be a viable option to reconstitute allelic and haplotype variation without new introductions from wild or other genetically improved populations in the future (Knibb et al 2014). Other ways, availability

of different population could be utilized to forming a synthetic base population and applying a proper selection program, as demonstrated in tilapia (Eknath et al 2007).
Considering the African catfish production in Indonesia, mostly conducted on a small scale, it is necessary to conduct further research that examines the performance of the NT population in multi-locations of on-farm scale. Also, many fish farmers can produce their own fish broodstock in small number fish, it is important to research the performance of the next generation of the NT population (at least in F2 hybrids) to determine the potential of outbreeding depression, although there is a report indicate no presence of outbreeding depression in F2 hybrids of Chinook salmon (Lehnert et al 2014). In addition, combining the results of this study with the results of other studies (e.g. Azis et al 2015) is also advisable.

Conclusions. This research has shown the NT population as the best crossbreed based on biomass at the grow-out stage. Although the body weight, survival, feed conversion and growth rates of the NT population were not different from other populations, this population consistently performed better. The research also succeeded in obtaining heterosis on body weight, survival, biomass and growth rates, both in the nursing and grow-out stages. The NT population generated positive mid-parent and best-parent heterosis for all traits in the grow-out stage. From these results, the use of the NT population could increase the production of high-biomass African catfish up to 200% and decrease the feed conversion by 5%, compared to using the present Indonesia population.

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