EVALUATION OF HYBRIDIZATION BETWEEN *PANGASIUS DJAMBAL* BLEEKER 1846 AND *PANGASIANODON HYPOPHTHALMUS* (SAUVAGE 1878): BIOMETRIC CHARACTERIZATION AND GROWTH ANALYSIS

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ABSTRACT

Possible use of pangasiid hybrids in aquaculture might generate potential impacts on wild populations. Therefore, rapid identification tools in the field such as growth rate are urgently needed. This study examines morphological characters and growth performance of *P. djambal* and *P. hypophthalmus* and their reciprocal hybrids. A detailed morphological study analysed 32 morphometric measurements and 5 meristic counts on hybrids of Pangasius *djambal* and *P. hypophthalmus*. Morphometric analysis and meristic counts showed that the reciprocal hybrids have intermediate characters except for gill rakers number which were lower than that of parental species. In general, the hybrids have tendency to be like *P. hypophthalmus* rather than *P. djambal*. The only typical character *P. djambal* appearing in hybrids is teeth shape, both vomerine and palatine. It was shown that the true hybrids have seven pelvic fin rays. Eight months of growth comparison in earthen ponds showed that the hybrids have a better performance for specific growth rate than the parental stock.

KEYWORDS: genetic, hybridization, biometric, growth, Pangasiidae

INTRODUCTION

There have been many breeding program efforts to increase aquaculture production such as interspecific cross-breeding in fish which may lead to hybrids with valuable characteristics for aquaculture (sterility, monosex population, heterosis or growth etc.). In contrast with the abundant literature on the hybridization in other cultured fish families, in particular cichlids, salmonids, cyprinids and ictalurids (for review see Sneed 1971; Moav 1976; Wohlfart and Hulata 1981; Chevassus 1979; 1983), reports on hybridization of Pangasiid catfish is rather scarce. Catfishes of the family Pangasiidae are of great economic importance in Southeast Asia region such as *P. djambal* in Indonesia (Legendre *et al.* 2000; Gustiano *et al*. 2003), *P. bocourti* in Vietnam (Hung *et al*. 1999) and *P. hypophthalmus* (senior of *P. sutchi*) (Tarnchalanukit 1986). Therefore, it was decided to evaluate the effect of hybrid vigor through artificial hybridization in Pangasiid catfishes.

Enzymatic system (protein total) allowed easy and quick differentiation of *Pangasius djambal* from *P. hypophthalmus*: allele 100 for locus Prot1 and allele 105 for locus prot2 for the first species, while allele 100 for Prot1; allele 100 and 150 for Prot2) (Legendre *et al*. 2000). However, there are no genetic data available for the hybrids so far. Hence, it is important to provide quick identification tools in the field since the possible use of these pangasiid hybrids in aquaculture faces the problem of potential impact such as, genetic deterioration on wild populations. Additionally identification tools will be useful to evaluate the growth performance of the hybrids. This study investigated biometrical characters and growth performance of *P. djambal* and *P. hypophthalmus* and their hybrids.

MATERIALS AND METHODS

Biometrics. The following specimens examined in biometric analysis are *Pangasius djambal*: 114 specimens, 142 to 635 mm,

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collected from the main rivers in Java, Sumatra and Kalimantan in Indonesia. *Pangasianodon hypophthalmus*: 31 specimens, 147 – 630 mm, originated from fish culture in Mekong River, Vietnam and fish culture in West Java in Indonesia. Specimens of *P. djambal* and *P. hypophthalmus* are deposited in the Museum Zoologicum Bogoriense (MZB), Cibinong, Indonesia and in the Muséum National d'Histoire Naturelle (MNHN), Paris, France. Hybrid of female *P. djambal* x male *P. hypophthalmus:* 45 specimens, 133 – 490 mm. Hybrid of male *P. hypophthalmus* x female *P. djambal*: 45 specimens, 129 – 473 mm.

All of the hybrid specimens were from artificial breeding performed at Research Institute for Freshwater Fisheries (RIFF) Station in Sukamandi, Indonesia. Specimens of *P. djambal* and *P. hypophthalmus* were identified following Gustiano (2003).

The following anatomical abbreviations are used: SL, standard length, HL, head length. Body length was measured using a one meter graduated ruler. Thirty two measurements were made using a dial calliper following Pouyaud *et al*. (1999). Three additional measurements were done: width of pectoral spine, measured at base of second dorsal spine; anterior width of snout, taken between the borders of anterior nostril; posterior width of snout, taken between the borders of posterior nostril. Five counts were noted: total number of gill rakers on the first branchial arch, number of dorsal, anal, pectoral and pelvic fin rays. Morphological observations include the shape of the swim bladder, the shape of palatine and vomerine tooth patches.

Growth. *Pangasius djambal*, *Pangasianodon hypophthalmus* and their reciprocal hybrid were examined for growth analysis. The growth comparison study were performed in a 200 m^2 earthen pond at Research Institute for Freshwater Fisheries (RIFF) station in Sukamandi, Indonesia. Initial size of the fish was 30 ± 2.0 gram. The stocking density was 100 fish per pond. The fish were fed 3% of total biomass daily using artificial feed containing 28% protein. During eight month rearing period, monthly random sampling was done on 20% of population and body weight measured.

Formula for the specific growth rate following Huisman (1976):

$$SGR = \frac{\frac{\ln W_t}{\ln W_o} \times 100\,\%}{t}$$

SGR = Daily individual spesific growth (% day^{-1})
Wt = body weigth at the end of experiment (g)
Wo = initial body weight (g)
t = rearing period (day)

RESULTS AND DISCUSSION

A principal component analysis performed on the 235 specimens using the covariance matrix for 30 measurements enable to separate *P. djambal, P. hypophthalmus* and their hybrid *Pangasius djambal* was located on the positive sector of factor 2, *P. hypophthalmus* was on the negative sector of factor 2, and their hybrids were in between parental species (Fig. 1).

Factor loading revealed that the second component of PCA was defined by vomerine length, palatine width, mandibular barbell length, palatine length, post-ocular length, anal fin length, maxillary barbell length, caudal peduncle depth, anal fin height (in decreasing order of importance). Further analysis showed that *P. djambal* differs from *P. hypophthalmus* by having a longer vomerine length (3.8 – 14% HL Vs 0.6 – 2.5%HL) and a larger palatine width (1.9 – 8.8%HL Vs 0.7 – 1.8%HL). While on hybrids, those two characters were intermediate, 1.2 – 9.4%HL for vomerine length and 1.2 – 4%HL for palatine width. Morphometric analysis of the specimens demonstrated clearly the presence of two species as defined by Robert and Vidthayanon (1991); Vidthayanon (1993); and Gustiano (2003) as well as hybrids in between of them. This intermediate shape of hybrids suggest that the product of present hybridisation were ''true'' hybrids resulting from the fusions of both parents and not parthenogenesis as observed on general occasions in fish (Chevassus 1983). The important characters revealed from PCA of parental species and reciprocal hybrids are listed on Table 1.

Based on the morphometric data, even though the reciprocal hybrids have intermediate characters, in general their morphology were relatively hypophthalmus-like except the teeth (vomerine and palatine) that was relatively djambal-like. Plot of the second principal component (factor 2) referring to standard

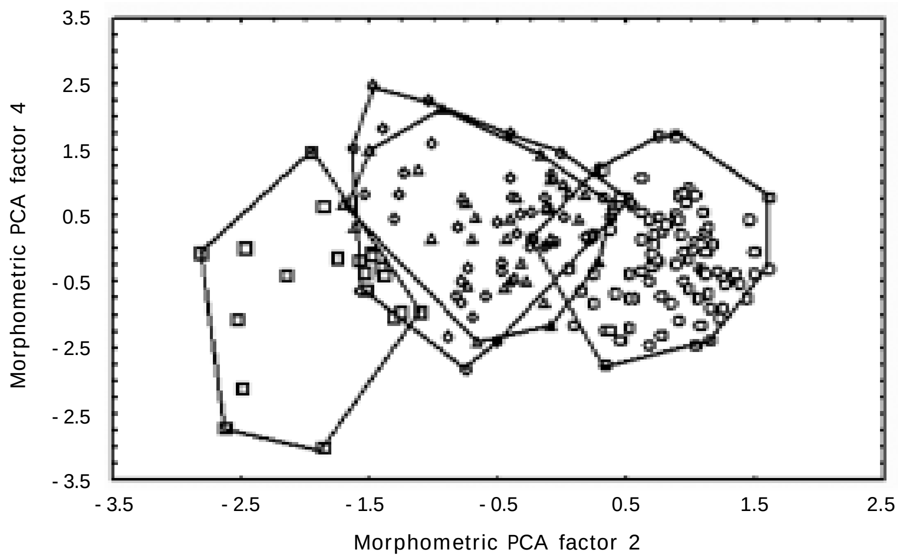

Figure 1. Plot of the second principal component (factor 2) versus the fourth principal component (factor 4) taken from a principal component analysis of 30 metric measurements on 245 specimens. ± *Pangasius djambal*; 9 *P. hypophthalmus*; " *P. hypophthalmus* x *P. djambal*; △ *P. djambal* x *P. hypophthalmus*

length (Fig. 2) supported this phenomena in which the tendency of importance character of hybrids was similar to that of *P. hypophtalmus*. Figure 2 also showed that the overlap between hybrids and parental species related to the size. For meristic observation, all of *P. djambal* had 6 pelvic fin ray, while there were 8 on *P. hypophthalmus*. Of the hybrids, more than 97% of hypophthalmus x djambal had 7 pelvic fin rays. On the other side, the percentage was lower on the djambal x hypophthalmus where it was about 17% of the hybrids had 6 or 8 pelvic fin rays. It is also clear that the true hybrids can be defined on the pelvic ray count. However, the case was contrary for gill raker number, the reciprocal hybrids had lower number than that of the parental species (Fig. 3). The gill raker number was probably due to the recessive evidence.

Based on the results of the present study, several characters enable separation between the hybrids and parental species, especially for pelvic fin rays. The results are very useful in providing a cheap and quick identification tool in the field rather than depending on other genetic analyses, such as enzymatic and DNA. Hence, the results from this study can also be used as a model to analyse other intergeneric hybridisation in which there is not much concern over homozygosity. Most of intergeneric hybridisation is successful under artificial breeding but in future studies should investigate the fertility of hybrids.

For the growth comparison among the different groups, average body weight during eight months rearing period is given on Table 2. At the end of experiment, the hybrids of female *P. hypophthalmus* x male *P. djambal* reached 852.3 g body weight, followed by female *P. djambal* x male *P. hypophthalmus* (764.7 g), *P. hypophthalmus* x *P. hypophthalmus* (665.5 g), *P. djambal* x *P. djambal* (524.7 g).

Body weight is influenced by genetics, environmental variables (such as dissolved oxygen, temperature, feed etc.) and their interaction (Tave 1993). During the first six months of the study, dissolved oxygen concentration ranged between 3 to 6 ppm, and temperature was 26 to 31°C. However, thereafter water quality became worse indicated by low response of feeding, colour and odour of the water in the ponds. To avoid further negative effects, the water in the bottom part was pumped out and then substituted by fresh water. Specific growth rate of the different group based on monthly sampling is shown in Table 3.

Table 1. The important measured characters for specimens of *P. djambal*, *P. hypophthalmus,* and their reciprocal hybrids

% Standard length	***P. jambal***	***P. hypoph.***	***hypo x jambal***	***jambal x hypo***
Caudal peduncle depth				
N	114	30	43	45
Mean	8.18±0.59	8.64±0.53	9.00±0.53	9.32±0.65
Range	6.70-10.60	7.40-9.50	7.67-10.05	8.22-11.77
Anal fin height				
N	110	30	45	45
Mean	13.09±1.28	14.86±1.39	15.03±1.16	13.80±1.30
Range	9.82-18.89	11.99-18.25	11.24-17.66	9.93-16.24
Anal fin length				
N	114	30	45	45
Mean	27.53±1.80	31.53±1.45	32.14±1.51	29.96±1.86
Range	23.78-39.27	29.07-34.18	27.00-34.37	22.27-32.91
% Head length				
Post ocular length				
N	112	29	45	45
Mean	8.71±1.36	10.72±0.85	9.49±0.70	9.95±0.83
Range	6.58-17.56	9.74-14.32	8.02-11.86	7.92-11.65
Maxillary barbel length				
N	108	26	45	45
Mean	56.23±6.60	38.79±10.61	47.70±8.65	49.56±13.03
Range	31.81-88.32	21.95-57.16	24.76-63.05	21.10-67.44
Mandibulary length				
N	109	25	45	42
Mean	37.41±6.94	20.80±8.66	30.45±6.94	29.48±9.05
Range	7.64-65.69	7.11-33.91	18.33-44.56	15.60-42.70
Vomerine length				
N	113	27	45	45
Mean	6.32±1.86	1.40±0.39	2.48±0.98	2.89±1.33
Range	3.79-14.54	0.61-2.50	1.22-5.79	1.46-9.38
Palatine length				
N	113	25	45	45
Mean	13.33±1.62	7.81±1.51	9.24±1.66	9.70±1.59
Range	8.29-16.97	5.25-10.87	6.06-13.14	7.20-13.61
Palatine width				
N	112	25	45	45
Mean	3.94±0.89	1.36±0.31	2.25±0.49	2.35±0.65
Range	1.94-8.76	0.69-1.82	1.45-3.31	1.27-4.00

Table 3 shows normal phenomena in the fish growth such that young fish have a higher specific growth rate compared to the older fish. Among tested groups, the hybrids performed better specific growth rates after the six month rearing period in which the water quality deteriorated. This result indicates that there is a correlation with the genetic and environmental interaction factors. It is clearly defined that *P. hypophthalmus* is an air breathing catfish but not for *P. djambal.* Therefore, *P. hypophthalmus* is more adaptable to the poor water quality than *P. djambal.* Hybridization has improved performance of the hybrids compared to *P. djambal* indicating there is a gene from *P. hypophthalmus* transfered to the hybrids. Evenmore, the hybrids are better than both parental species. This study show similar

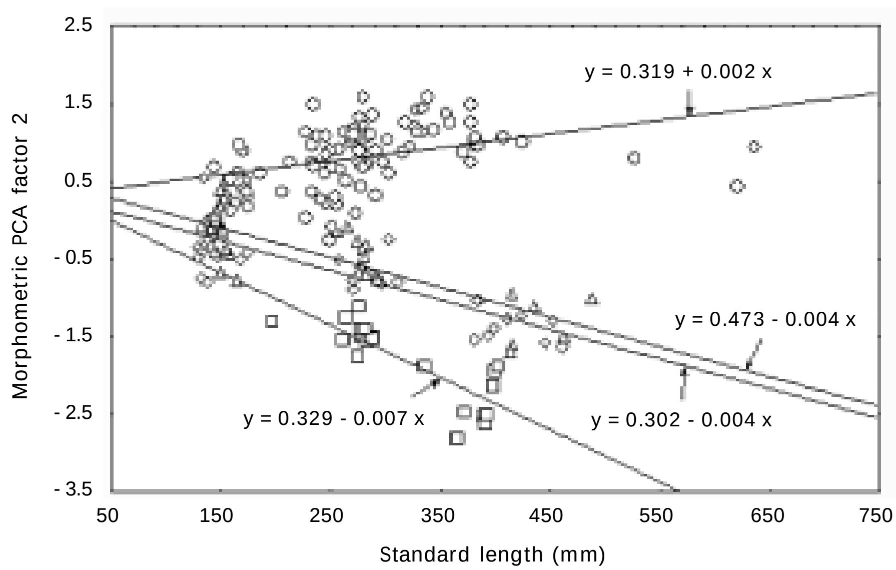

Figure 2. Plot of the second principal component (factor 2) referring to standard length of 235 specimens. ± *Pangasius djambal*; 9 *P. hypophthalmus*; " *P. hypophthalmus* x *P. djambal*; △ *P. djambal* x *P. hypophthalmus*

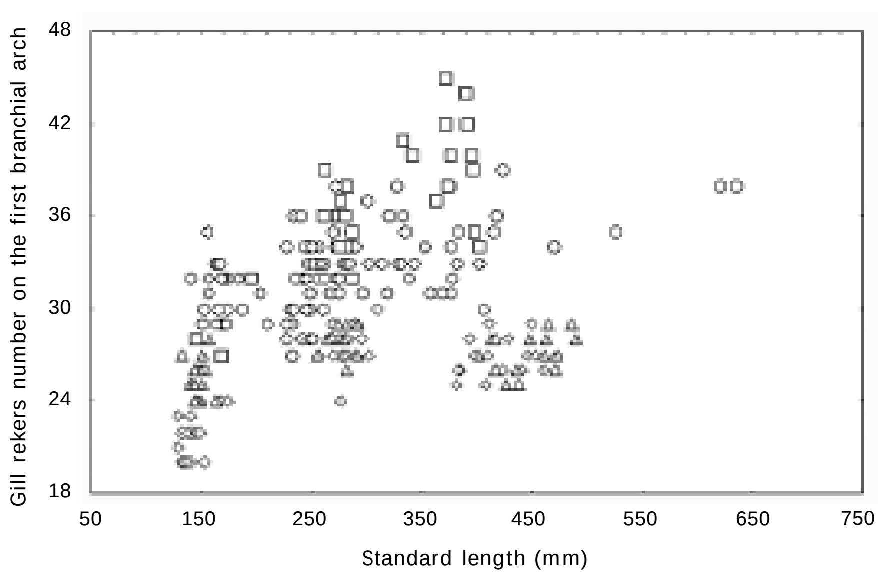

Figure 3. Scatter plot showing the gill raker number on the first branchial arch referring to standard length for ± *Pangasius djambal*; 9 *P. hypophthalmus*; " *P. hypophthalmus* x *P. djambal*; △ *P. djambal* x *P. hypophthalmus*

Table 2. Average body weight (g) during eight month rearing period

Rearing (m)	♀ hypo x ♂ hypo	♀ djambal x ♂ hypo	♀ djambal x ♂ djambal	♀ hypo x ♂ djambal
Initial time	30.0 ± 2.00	30.0 ± 2.00	30.0 ± 2.00	30.0 ,± 2.00
1	52.2 ± 17.45	167.5 ± 24.58	175.6 ± 59.80	125.4 ± 12.31
2	124.1 ± 34.03	241.1 ± 55.96	254.1 ± 52.89	195.5 ± 33.59
3	178.4 ± 49.61	332.2 ± 72.45	330.1 ± 05.01	254.6 ± 38.72
4	310.0 ± 94,87	509.8 ± 125.49	496.5 ± 160.63	429.9 ± 65.19
5	373.0 ± 123.99	550.8 ± 102.45	497.5 ± 55.31	497.7 ± 79.66
6	418.3 ± 131.27	651.1 ± 179.12	439.4 ± 152.92	687.8 ± 96.29
7	469.4 ± 92.30	672.6 ± 169.80	492.5 ± 147.83	752.6 ± 135.27
8	665.5 ± 128.13	764.7 ± 157.36	524.7 ± 151.96	852.3 ± 123.63

Table 3. Spesific growth rate (%/day) based on monthly sampling

Month	♀ hypo x ♂ hypo	♀ djambal x ♂ hypo	♀ djambal x ♂ djambal	♀ hypo x ♂ djambal
1	3.87	5.00	5.00	4.73
2	2.36	2.68	2.71	2.58
3	1.69	1.89	1.89	1.81
4	1.40	1.55	1.52	1.48
5	1.16	1.24	1.21	1.21
6	0.98	1.05	0.99	1.06
7	0.86	0.91	0.86	0.93
8	0.79	0.81	0.76	0.82

results to a study conducted in floating net cages using one month old fry for six weeks (Utami *et al.* 2005),

CONCLUSION

The reciprocal hybrids have intermediate characters, except lower number of gill rakers than that of parental species. It is clearly defined that the true hybrids have seven pelvic fin rays. The results of growth comparisons showed that hybrids have a better specific growth rate than that of parental species.

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