Genetic improvement
of the herbivorous blunt snout bream (*Megalobrama amblycephala*)

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Abstract

Selection experiments with the herbivorous blunt snout bream or Wuchang bream (*Megalobrama amblycephala*) were started in 1985. Mass selection for size and length/depth ratio resulted in a significant increase in growth and better shape, while inbreeding led to a significant decrease in growth. The total selection ratio from fry to mature brooders was about 0.03 per cent per generation. In the grow out stage, the average daily body weight gains of two lines of fifth generation (F_5) fish were 29 per cent and 20 per cent respectively more than the control group, with an average of 5.8 per cent and 4 per cent improvements per generation, respectively. The body was 4 per cent deeper in ratio of standard length/body depth. The effects of inbreeding were examined by crossing full-sibs, the offspring of which were kept without selection. The third generation inbred fish showed 17 per cent lower growth as compared to the control group, with an average of 7.5 per cent per generation. The results demonstrate that selection is a powerful tool to improve the economic traits of the blunt snout bream, but inbreeding can rapidly lead to a reduction in performance. In 2000, the 6th generation of selected bream was certified by the Chinese Ministry of Agriculture as a good breed for aquaculture.

Introduction

The herbivorous blunt snout bream (*Megalobrama amblycephala*), also known as the Wuchang fish, is distributed in a few medium and large-sized lakes of the middle reaches of the Yangtze River. Because of its desirable qualities for aquaculture such as herbivorous feeding habit, general hardiness, resistance to disease, good seinability, and reproductive performance, the species has been domesticated since 1964 and has become a major species for freshwater aquaculture in China (Ko 1975). The overall production of *M. amblycephala* and other related breams in 2000 was estimated at 531 730 t (China Fisheries Bureau 2001), ranking sixth among freshwater fishes cultured in China.

After domestication, the aquaculture performance of many hatchery populations of *M. amblycephala* deteriorated as indicated by slower growth rate, earlier maturity and thin and longer body. Poor management of broodstocks and inbreeding depression were thought to be the major causes for this deterioration. In view of this, a systematic selection program was started in 1986 for faster growth rate and deeper body shape (measured as the ratio of body length to body depth).

Materials and methods

Establishment of selected and control groups

The founder population was established in the winter of 1985, comprising 102 adult fish collected from the Yuni Lake. Of these 102, only 15 males and 15 females survived on arrival in Shanghai. Subsequently, 46 females and 112 males were collected and transported successfully in late 1986. Based on these fish, two selection groups (Line I of 1985 and Line II of 1986) and one unselected control group (mix of 1985 and 1986) were established. In order to prevent a possible loss of genetic diversity in captive environment, 2 000 one-year old fish were introduced from the same lake in 1992 and their offspring were mixed into the control group in 1994. The control group was maintained by random breeding without selection.

M. amblycephala was bred by hypophysation. Usually 15-20 pairs of fish were placed in one tank as one batch of spawners. For each generation there were four batches for the selected groups, and two to three batches for the control group.

Developing the selected strain (positive-selection)

Mass selection was practiced. The high fecundity of the species with an average of 158 000 eggs in three-year old females (Li 1998) permits an intense selection. The major steps followed in selection for each generation were: selection of the top 5 per cent of 3-4 cm fry 20-30 days in age, the top 10 per cent of 12-15 cm fingerlings at about 120 days of age, the top 12 per cent of two-year old pre-mature fish weighing 400-500 g, at 18 months of age, and finally the top 50 per cent before first spawning at a weight of 750-1 000 g, at 30 months of age. The overall selection ratio was about 0.03 per cent of the starting number. Criteria used for selecting the breeders was length (see the four steps above) and increased body depth individuals (2.1-2.2 of ratio of standard length/body depth). *M. amblycephala* normally mature in their third year. However, it was found that the breams could be made to mature under intensive culture conditions and to reproduce through hypophysation at

2^+ year of age. Earlier maturing fish on average are smaller (514 g vs. 620 g) with slightly lower fecundity (11 900 eggs vs. 25 800 eggs) than the 3^+ year-old fish, but have the same size eggs (1.04-1.05 mm in diameter) (Li 1998). To accelerate selection, both 2^+ and 3^+ year brood fish were used separately for selection experiments after 1992. Selection lines I and II reached the sixth generation, (F_6) (Fig. 1) in 1998 and 1999 and as the seventh generation (F_7) in 2000 and 2001, respectively.

Building inbreeding group (negative-selection)

A full-sib mating experiment, designed to quantitatively estimate the effects of inbreeding has been carried out since 1989. The offsprings from one pair of the original stock (1985 and1986) were randomly culled down to only three pairs. These fish were stripped. The fry from only one full sib mating were maintained. The other two crosses had been produced to ensure at least one successful batch, after which they were discarded.

Comparison of aquaculture performance

Performance of fish under captive, commercial culture conditions were monitored in successive generations. Fishes from the four genetic groups, the selected lines I and II, the inbred line and the control group, were tagged by fin clipping when they were 5-6 cm in total length and 45-50 days in age. These fish were then combined and raised communally in tanks (28 m^2) during the fingerling stage, and in ponds (1 400 m^2) during the grow out stage. The experimental design was a complete randomized block design (four tanks, four genetic groups for one-year old fish, and two ponds, four genetic groups for two-year old fish). Absolute body weight gain per day, and the mean generational response to selection were calculated as follows.

Absolute body weight gain (g/d) =

$$\frac{\text{Final weight } (W_2) - \text{Initial weight } (W_1)}{\text{No. of days } (d = t_2 - t_1)}$$

Selective response (R) =

$$\frac{\text{Average weight of selected strain} - \text{Average weight of control group}}{\text{Generation number of selection}}$$

Statistical analysis

Analysis of variance and Duncan's multiple range testing were conducted according to Zar (1974).

Results

Growth

Comparisons of standard length, and body weight among the fifth generation (F_5) fish of selected lines I and II, the control group, and the inbred line at one, two and three years of age are shown in Table 1. Length and weight in the two selected lines were significantly greater ($P<0.01$) than that of control and inbred groups at all three ages. Further, the inbred line was significantly smaller than the control group.

Growth in the selected lines was significantly greater than growth in the control group, no matter whether they were one-year old (Table 2) or two-year old fish (Table 3). The performance of

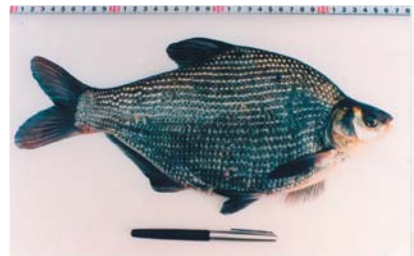

Fig. 1. Selected line of Megalobrama amblycephala (F_6)

Table 1. Standard length (cm) and body weight (g) of selected line (F_5), control group and inbred line (IB_3) of Megalobrama amblycephala at different ages (mean±SD).

Genetic groups	Age (year)	Standard length (cm)	Body weight (g)
Selected line I	1	12.8 ± 1.4^{a}	49 ± 19^{a}
Selected line II	1	12.5 ± 1.1^{b}	45 ± 13^{b}
Control group	1	11.0 ± 1.7^{c}	33 ± 16^{c}
Inbred line	1	10.6 ± 1.2^{d}	26 ± 11^{d}
Selected line I	2	24.0 ± 3.0^{a}	430 ± 106^{a}
Selected line II	2	23.4 ± 1.5^{b}	400 ± 68^{b}
Control group	2	21.9 ± 2.2^{c}	328 ± 85^{c}
Inbred line	2	21.2 ± 1.7^{d}	272 ± 74^{d}
Selected line I	3	33.8 ± 1.4^{a}	1043 ± 394^{a}
Selected line II	3	33.1 ± 1.1^{b}	941 ± 667^{a}
Control group	3	31.8 ± 1.5^{c}	817 ± 120^{b}
Inbred line	3	32.1 ± 2.0^{c}	786 ± 93^{b}

Different superscripts indicate significant ($P<0.01$) differences between genetic groups

Table 2. Average absolute weight gain (g/d) of selected lines (F_{4-6}), control group and inbred line (IB_{2-4}) of Megalobrama amblycephala during the one-year old stage.

Year	Selected line I	Selected line II	Control group	Inbred line	Mean of Selected group/ Control group (%)	Inbred line/ Control group (%)
1995[*1]	0.42 (F_4)	0.408 (F_4)	0.34	0.31 (IB_2)	+21	-9
1997[*2]	0.52 (F_5)	0.47 (F_5)	0.35	0.27 (IB_3)	+41	-23
1999[*3]	0.58 (F_6)	0.55 (F_6)	0.33	0.19 (IB_4)	+71	-42

**1. Experimental dates: 30.7.1995 – 4.11.1995 (93 days total); The gain is the mean of four replicates.*
**2. Experimental dates: 31.7.1997 – 5.11.1997 (95 days total); The gain is the mean of four replicates.*
**3. Experimental dates: 26.7.1999 – 26.10.1999 (92 days total); The gain is the mean of four replicates.*

Table 3. Average absolute gain in weight (g/d) of selected line (F_5), control group and inbred line (IB_3) of Megalobrama amblycephala during the two-year old stage (mean±SD).

	Selected line I, F_5	Selected line II, F_5	Control group	Inbred Line, IB_3	Selected/ control (%)	Inbred/ Control (%)
Mean Initial weight (g)	49.1±19.1	44.5± 13.4	32.9±16.3	26.4±9.6		
Mean Final weight (g)	430±106	400± 68	328±85	272±74	Line I +31 Line II+22	-17
Average daily weight gain (g/d)*	1.91	1.78	1.48	1.23	Line I +29 Line II+20	-17

** The experiment in the fingerling stage was conducted in eight tanks under a communal stocking and randomized block design. At the end of the year, fishes from eight tanks were pooled into a 1 400 m^2 earthen pond for grow out. Testing lasted 420 days from 3.12.1997 to 28.1.1999. However, the total growing days at over 20°C water temperature was 200 days only. The average daily weight gain was calculated for 200 days.*

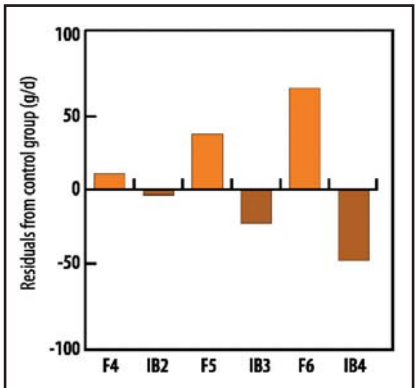

Fig. 2 The average daily weight gain difference between the selected lines and control groups during the one-year old stage of selected lines (F_4-F_6) and inbred lines (IB_2-IB_4) by generation.

the inbred line was significantly worse than the control group (Tables 2 and 3). The difference in growth rate between the selected lines and the control group, as well as between the control group and the inbred line increased with each generation of selection. Fig. 2 indicates the trend by positive and negative selection clearly. There was significant difference among selected lines (F_5), control group and inbred line (IB_3) when fish were two years old (Table 3). The average growth rate of two selected lines was 24.5 per cent higher than the control group, and the inbred line was 17 per cent lower than the control group.

Body shape

The ratio of standard length/body depth was 2.10 for adult selected fish F_6, i.e. four per cent deeper than that of the founding population of the Yuni lake (Li 1998).

Response to selection

The response to selection when the fish reach marketable size was calculated from data in Table 3. Results showed that the average absolute weight gain for the two-year olds from the selected lines I and II were 29 per cent and 20 per cent, respectively. The control group had an average absolute weight gain of only 5.8 per cent and 4 per cent per generation. The inbred line's growth was 17 per cent less than that of the control group, with an average absolute weight gain reduction of 5.7 per cent per generation.

Discussion

Selection response per generation

The average genetic gain per generation in the present study was 5.4 per cent over five generations as compared to 10.1 per cent for the coho salmon (*Oncorhyncus kisutch*) (Hershberger et al. 1990); 13 per cent for the rainbow trout (*Oncorhyncus mykiss* or *Salmo gairdneri)* (Gjerde 1986); 10.6-14.2 per cent for the Atlantic salmon (*Salmo salar*) (Gjedrem 2000); 12.2-20.0 per cent for the channel catfish (*Ictalurus punctatus*) (Bondari 1983; Dunham 1987) and 15 per cent for the Nile tilapia (*Oreochromis niloticus*) (Gjedrem 2000).

M. amblycephala has a rather narrow natural distribution, occurring only in a few lakes in the middle reaches of the Yangtze River. Based on the investigation of populations from four lakes, it can be said that *M. amblycephala* has a relatively low genetic variation, e.g., 13.3-20.0 per cent of mean proportion of polymorphism, 0.055-0.085 of heterozygosity, and 0-0.022 of Nei genetic distance. In comparison, other carps which are widely distributed, e.g. the grass carp (*Ctenopharyngodon idellus)* which is distributed in major rivers such as the Yangtze River, Pearl River and Helongjiang River, has a higher polymorphism (20.0-33.3 per cent) and heterozygosity (0.0454-0.1076) (Li 1998). The lower level of genetic diversity of *M. amblycephala* might be the reason for the relatively low response to selection for size in this species. Hence, the increase in growth achieved over the five to six generations is commercially very significant.

The low response also could be due to the mass spawning system used, which is likely to produce unequal family sizes, and it is likely that some parents did not participate in the mating, with a potential reduction in the effective population size (Ne) and some accumulated inbreeding.

Most of the observed genetic gain per generation (see above) were obtained through family selection studies.

It appears that the response is greater at the fingerlings stage, which might be due to the application of selection pressure at this stage, and hence the responses decrease with the age of the fish.

Prevention of inbreeding effects during long-term selection

A breeding program is a long-term investment, and it should be designed to avoid excessive loss of genetic variation and to prevent rapid accumulation of inbreeding. This problem could be resolved by maintaining a minimum effective breeding number in a population where random mating is used. In our study, annually 500 pairs of brooders are maintained and about 50-100 females and 60-120 males are used in breeding. In this case, the estimated inbreeding rate per generation of mating should be 0.23-0.46 per cent (Tave 1993). Since no critical inbreeding rate (*F*) has been determined for fish, Tave (1993) suggested using 5 per cent as a conservative value and 10 per cent as a liberal estimate. For the Ne value, he summarized that the Ne recommended to prevent inbreeding varies from 50 to 500 in the literature (Tave 1993). We consider that our actual values of 50-100 females and 60-120 males as effective breeders in our study would be sufficient to prevent deleterious rates of inbreeding.

Even though the effects of inbreeding are well known to biologists and agricultural breeders, they are not well understood or taken seriously by fish farmers. There have been relatively few inbreeding studies of fish (e.g. Kinkaid 1976a, 1976b, 1983) and farmers, particularly in China, often ignore the detrimental effects of inbreeding in aquaculture. The high fecundity of fish increases the risk of inbreeding as large quantities of offsprings can be produced by a small number of fish. Inbreeding tends to increase homozygosity and leads to the loss of genetic diversity. The loss of genetic diversity in populations used in aquaculture can lead to the loss of favorable alleles at the loci that control production traits. In this study, we found that the average selection response per generation was +4-5.8 per cent in positive selection and –5.7 per cent with inbreeding. These values are very useful benchmarks for fish farmers.

On the other hand, inbreeding could sometimes be used to produce strains for specific characters. Therefore, the use of inbreeding requires an assessment of the balance between the gain of stability in a character and the loss of genetic diversity.

Illustration and extension of a new breed

In the year 2000, the Pujiang No.1 bream (selected sixth generation *M. amblycephala*) was certified by the National Certification Committee of Aquatic Wild and Bred Varieties as a new "improved variety", and was approved for use in aquaculture by the Ministry of Agriculture. These breams are widely used by the fish farmers because of their rapid growth, better shape and high survival. The economic benefits for the industry by using improved bream are obvious: increased production and income. For instance, in the Lake Gehu area, where pen culture is well developed and *M. amblycephala* is the principal species comprising 80 per cent of the volume and 90 per cent of the value of total production, the Pujiang No.1 bream reaches marketable size before October, yielding a 20 per cent higher price than normal breams that are usually harvested much later. It is estimated that if the Pujiang No.1 Bream could be cultured across the country, the economic benefit would be 1 billion RMB (120 million USD) annually (Shanghai Economic Newspaper 2001).

Acknowledgement

This study was funded by the AGNA project of International Development Research Center (IDRC) of Canada from 1986-1992, the Carps Genetic Improvement Project of INGA (International Network of Genetics in Aquaculture), funded by the Asian Developmental Bank from 1997-2000, and the Agriculture Committee of Shanghai City from1998-2000.

During the 16 years of study, we greatly appreciate valuable support and encouragement received from many friends, particularly Drs. B. Davy (IDRC, Canada), R. Doyle and G. Newkirk (Dalhousie University, Canada), R. Dunham (Auburn University, USA), M.V. Gupta, S. Grant and R. Ponzoni (WorldFish Center). The final manuscript was improved upon by Dr. Jeffrey Silverstein (USDA/ARS Catfish Genetic Research, USA) and Dr. M.V. Gupta.

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