Thesis for the Degree of Master of Fisheries Science

Age and Growth of Amur Barbell *Hemibarbus labeo,* in Goe-san Lake South Korea

by

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KOICA-PKNU International Graduate Program of Fisheries Science

The Graduate School

Pukyong National University

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Hemibarbus labeo, in Goe-san Lake

South Korea

한국 괴산호에 서식하는 누치의 연령

및 성장에 관한 연구

Advisor: Prof. Chul-Woong OH

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A thesis submitted in partial fulfillment of the requirements for the degree of

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Abstract

This study evaluates the age and growth of Amur barbell (*Hemibarbus Labeo*) in Geo-san Lake, South Korea via vertebra reading methods. The samples were collected between March and October 2011, 201 specimens ranging in total length (TL) from 125.5 to 421.0 mm, total weight (TW) from 14.6 to 599.2 gm and the maximum estimated age was 10 years. The samples were collected by gillnets and trap nets. Vertebrae of 65 (56.5%) female and 50 (43.5%) male were analyzed. Age determinations were made by vertebrae microscopic observations. The index of average percentage error (IAPE) estimated from two reading (ranged from 0.02 to 0.39 and mean 0.18. And coefficient of variation (CV) ranged from 0.04 to 0.78 and mean 0.23. The monthly marginal increment (MIR) ratio revealed a tendency toward band pair deposition between autumn and winter which indicate that ring marks form once a year, Length frequency distributions showed differences in the range of sizes between the

sexes, all specimens larger than 387.3 mm in total length were female. The obtained results were compared with those of the previous studies, The relationship between total weight (W) and total length were expresses as W= 1E-05L$^{2.974}$ (n=65, r^2=0.9885, $p < 0.05$) for female and W = 9E-06L$^{2.9991}$ (n=50, r^2= 0.9834, $p < 0.05$) for male, Based on the MIR analysis, growth band pairs (including translucent and opaque bands) in vertebrae formed once a year and were counted up to 10 and 8 for females and males, respectively. Growth parameters were derived using the Von Bertalanffy Growth Function (VBGF), Parameters derived from observed lengths, K = 0.08, L_∞ = 692, r^2=0.92 cm and t_0 = -0.127 year for female and K = 0.1, L_∞ = 641, r^2=0.93 cm and t_0 = -0.120year for male. Parameters derived from back-calculation length, K = 0.054, L_∞ = 744.39, r^2=0.99 cm and t_0 = -0.176 year for female and K = 0.060, L_∞ = 708.11, r^2=0.98 cm and t_0 = -0.164 year for male, were considered to best describe growth

1. Introduction

Family Cyprinidae is the most diverse and widespread freshwater fishes in the world, with at least 210 genera and over 2010 species (Nelson, 1994). Cyprinid fishes are nearly worldwide in distribution, its range encompasses all continents with the exception of south America, Australia, and Antarctica.; contain many culturally and economically important species (Mayden, 1991). The limited ability for these species to migrate give to this family an obvious biogeographical interest since their distribution closely reflects the geological evolution of the landscape (J.-D. Durand et.el, 2002). (they are primary freshwater fish, which are very strictly confined to freshwater based on their perceived physiological intolerance to salinity. In addition, secondary freshwater fish are those that are generally restricted to freshwater (Myers 1938)., but in some cases enter the sea voluntarily for short periods

Classifying cyprinid freshwater species at the subfamily level remains challenging, because of the lack of homologous morphological characters that can be used for appropriate classification (Saitoh et al., 2006; Sasaki et al., 2007).

The largest cyprinid in this family is the Giant Barb (*Catlocarpio* *siamensis*), which may grow up to 3 m (9.8 feet). On the other hand, many species are smaller than 5 cm (0.2 in), the smallest known freshwater fish is a cypriniform, *Danionella translucida,* reaching 12 mm (0.47 in) at the longest (Kottelat et al., 2006)

Cyprinids are highly important food fish; they are fished and farmed across Eurasia. In land-locked countries in particular, cyprinids are often the major species of fish eaten because they make the largest part of biomass in most water types except for fast flowing rivers. Cyprinids also play big role on fish studies. One particular species is the Zebrafish (*Danio rerio*). Over the past twenty year it has become the standard model species for studying developmental genetics of vertebrates, in particular fish. (Fishman, 2001).

Fish of the order Cypriniformes are the largest group of primary freshwater fish in the rivers system (Wang, Hsu, & Chiang, 2000; Chen, Wu, & Hsu, 2008) and the order contains more than 50 species. One of this order is *Hemibarbus Labeo* (amur barbell or barbell steal) distributed in the regions of Amur, River-Campagna, and East-Pacific (Zhang 1954). (Fig. 1).

The study of Amur river fish began in 1772 when petr-simon pallas visited the Onon river, in that year he collected various Amur fish and in 1776 published the first list of Amur fish including three new species: European bitterling *(rhodeus sericeus),* Amur asp (*Pseudaspius leptocephalus)* and barbell steed *Hemibarbus labeo* (Novomodny et al., 2004).   .

H.labeo occurs all over East Asia, from Vietnam to Russia. The populations are distributed in the major drainage of east mainland China, (Yang et al. 2006; Yue 1998). Taiwan, Japan and Korea (Chung et al.2007). (Fig. 2).

Amur barbell can be one of the most economically important fish species not only in South Korea, but also in many regions all over the world because of it is quite appreciated for human consumption. Most individuals of the species are bottom-dwellers in streams at the depth of 5–20 m. It feeds on some aquatic insects, shrimps and snails (Chung et al. 2009). The effect of food and temperature on the growth of the weight was significantly affected by the change of food and temperature. **(**XU Wei et al., 2007).

Fig. 1. ***Hemibarbus labeo*** **Pallas, 1776.**

H.labeo is well-defined and can be distinguished from its sister, *Hemibarbus Medius*, based on broad and thick lateral lobes of lower lip with folds (Yue 1998)., *H.Labeo* spawns every year when the water temperature is about 12-24℃. The ripe eggs are round and orange, and their diameters are between 1.60 -1.90 mm and 1.75 mm averagely. After absorbing enough water their diameters are 2.40-2. 70 mm and a layer of glue cover themselves. The total length of newly hatched larvae is between 6.50 - 6.80 mm and 6.62 mm averagly (He Jisheng et al., 1999). Juveniles of *H.labeo* were obtained from the brood fish by artificial propagation successfully. After being injected the hormone mixture which was composed of HCG, LRH-A2 and DOM, at ambient temperature 22-26℃.(XU Wei1 et al., 2009). Distant hybridization between *Hemibarbus labeo* (♀) and *Hemibarbus Maculates*(♂) were carried out, The main characteristics of embryonic development of crossbreed F1 were similar to those of its maternal fish.(MI Guo-qiang1 et al., 2010).

Fig. 2. Distribution of *H.labeo* in the world

A little attention has been paid on the biological aspect of *H.Labeo*. Morphological characters (LV Yao-ping., 2008), also one study on the population structure of *H.labeo* (Tong et al., 2011). The only puplished data on the biological aspects of *H. labeo* about age and growth were published on the population dynamics of this fish, scale and growth Characteristics of *H.labeo* in the Wusulijiang River in China, (XU Wei et al., 2008)

Studies on the age and growth of fish are crucially important contributions to research regarding population dynamics. Both topics are required when dealing with fisheries forecasts and management (Dwyer et al., 2003).

Age information is ranked among the most important biological variables as it forms the basis for the calculations of growth rate, mortality rate and productivity (Campana, 2001), making it essential for decisions concerning fisheries management (Casselman, 1987; Ragonese and Reale, 1992; Cailliet et al., 2001). Studies on the age and structure of the population of *H. labeo* will greatly contribute to a better understanding of the biology of this species. The most commonly used ageing technique is to count the annuli in the hard part of fishes such as

Sagittal Otolith, scale, fin rays, opercular bones, cleithra, and vertebrates (Devries and Fire, 1996; Michael and Zachary, 2007, Casselman, 1990). Comparison of age estimates from various ageing structures have been reported to be undertaken in a number of fishes with a view to identify the most suitable structure for a fish population (Reid, 2007; Phelps et al., 2007; Maceina and Sammons, 2006).

Conventional methods of age determination are based on the estimation of growth increments in vertebrae and annuli counted. In general, the vertebrae from fish is directly removed to determine the age and growth pattern, count of the annual growth increments, direct observation and count the annuli is difficult by the naked eye. While UV light observation and the morphological measurement of vertebrae have been used for more accurate age determination., these are contributed to cost, effort and time saving.

The present study aim to three fold: (i) to determine the age pattern of the population of Amur barbell *(H.Labeo)* in the Geo-san lake in South Korea; (ii) to estimate its growth parameters via vertebrae reading methods; and (iii) study the growth rate and the environmental factors of this fish can assess the possibility and feasibility of culture in fish farms, not only in South Korea but also in many other regions of the world such as Egypt.

2. Materials and Methods

2.1 Location of the study

A total of 201 of *H.Labeo* were obtained for this study from the Goe-san lake located in chungcheong buk-do, South Korea (Tabe1) (36°45`19,34``N, 127°50`24,05``E) (Fig. 3) divided into upstream (6 stations), midstream (8 stations) and downstream (10 stations). This lake has optimal conditions including extensive shallow areas (<6 m depth) around the upstream that support submerging vegetations as well as areas deep enough (3-15 m depth) around the downstream near a dam (Fig.7a) to overwinter successfully (Carlander, 1977).

2.2 Sampling and data collection

Sampling was carried out monthly from the lake. The samples were collected by gill nets and trap nets from March to November in 2011(Fig. 7b.), since water in the lake was mostly frozen during January and February. In December and March, it was difficult to catch fish for the low activities at lower temperature

Fig. 3. Map of The sampling area in The Goe-san lake, South Korea

Table 1. The specimens used in age determination.

Year	Month	Sex		Total length	Total weight
2011		Female	Male		
	Mar	4	11	210.1 - 370.1	80.3 - 414.9
	Apr	10	20	170.0 – 387.3	43.5 – 323.0
	May	26	22	168.8 - 380.4	42.4 – 436.9
	Jul	18	10	148.0 – 397.2	25.4 – 551.7
	Aug	31	5	148.0 - 421.0	27.1 – 599.2
	Sep	10	18	125.0 - 383.0	14.6 – 465.2
	Oct	10	6	168.0 – 362.0	40.6 – 436.9
Total		109	92	125.0 – 421.0	14.6 – 599.2

In the field, the water environmental factors were measured. In the laboratory, total length (*TL*), fork length (*FL*) and standard length (*SL*) were measured to the nearest 1 mm (Garrick, 1982). (S_L) often use when the caudal fin damaged before landing. total weight, body weight, gonads weight and liver weight were recorded with an electronic analytical balance (Sartorius CPA224S) to the nearest 0.01 g.

2.3 Vertebrae selection and preservation

Spine of *H.labeo* consisted of 42 vertebrae (1-13 abdominal vertebrae, 14-42 caudal vertebrae), were processed according to the methods of (Cailliet et al. 1983). Vertebrae columns were thawed and connective tissue and muscle were cut away (Fig. 6a). Block of six vertebrae (1-6) was removed from each specimen (Fig.4). Cleaned and rinsed in potassium hydroxide (KOH) solution 8-10% for 24 hours to remove connective tissues, washed in running water (Maraldo, 1979), and store in tube using alcohol 70% until further observation (Joung et al., 2005).

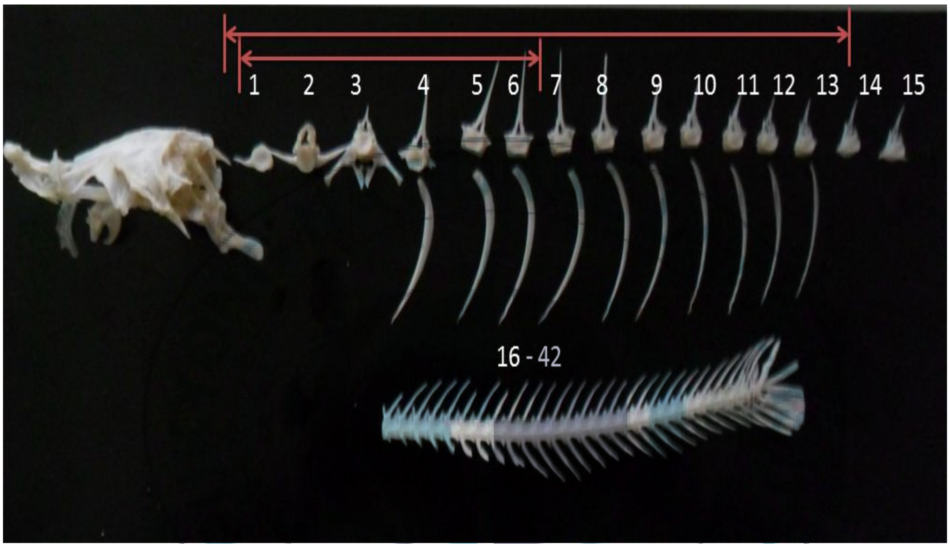


Fig. 4. Vertebra of *H.labeo*., 1-13 abdominal vertebra, 14-42 caudal vertebrae, 1-6 the vertebrae used in this study for age determination

2.4 Age determination

2.4.1 Vertebrae separation and observation

The vertebrae were used for age estimation (Cailliet, 1990), were removed from each fish and viewed using an image processing system consisting of a computer (Fig. 6b), a video camera Microscope (Carl zeiss Discovery v.8) and the Optical Pattern Recognition System software package of Image-Pro Plus Version4.1. with UV, reflected and transmitted light. Each growth band pair (GB) – consisting of one wide band (opaque) and one narrow band (translucent) (Fig. 5) was examined and measured using a stereomicroscope at a magnification of 10× (Cailliet and Goldman, 2004; Cailliet et al., 2006). The narrow bands would appear light and wide bands would appear dark, radius of each centrum (R) was measured to 0.1mm. The distance from the focus (F) to the outer margin of the translucent band of each ring mark (ring radius, r_n) and the vertebra radius (R) were measured a straight line from the focus to the posterior edge of the vertebra, vertebrae that were damaged in preparation or that had unclear ring marks were removed from this analysis.

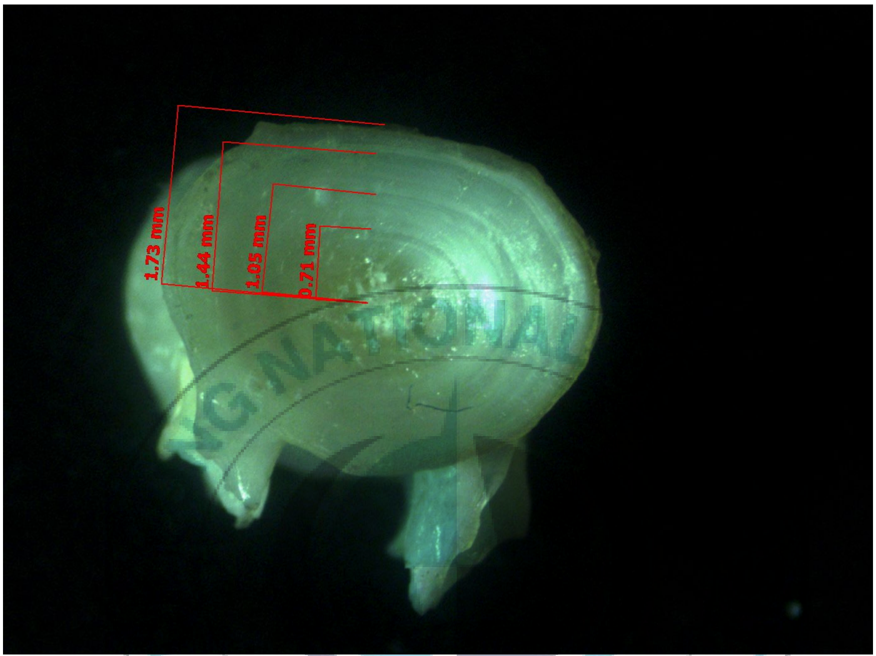


Fig. 5. Measurements of growth bands pairs and radii via vertebra of *H.labeo*

2.4.2 Determination of aging precision

Growth band pairs (including translucent and opaque bands) were counted without perior knowledge of the sex and length of the specimens. The index of average percentage error (IAPE) (Beamish and Fournier, 1981), and coefficient of variation (CV) (Kimura & Lyons, 1991) were calculated to compare reliability of age determination between readings:

$$[\mathrm{IAPE}] = \frac{1}{N}\sum\left(\frac{1}{R}\sum\left(\frac{|X_{ij}-X_j|}{X_j}\right)\right)\times 100$$

$$\mathrm{CV} = \frac{1}{N}\sum_{j=1}^{N}\frac{\sqrt{\sum_{i=1}^{R}(X_{ij}-X_j)^2/R-1}}{X_j}\times 100\%$$

Where N is the number of fish, R the number of readings, X_{ij} the number of growth rings of the jth fish at the ith reading, and X_j the mean number of GRs calculated for the jth fish.

2.4.3 Ring marks formation

To define the period of ring mark formation, monthly changes in both the frequency of appearance of the translucent band on the outer margin of the vertebrae, and the marginal increment of the vertebrae (MI) was used to validate the periodicity of growth (Lai et al., 1996; Liu et al., 1999). The MI was examined based on the following equation:

$$MI = \frac{R - r_{max}}{r_{max} - r_{max-1}}$$

Where R is the vertebra radius (mm), r_{-max} the distance (mm) between the focus and the outer margin of the last translucent band and r_{max_1}. the distance (mm) between the focus and the outer margin of the penultimate translucent band. All ring groups were pooled in this examination. Additionally, vertebra that had been designated as having a just formed ring mark on the margin was reviewed and their time of collection recorded.

MI (Mean ±S.D.) was calculated monthly, tested for normality and for significant differences using analysis of variance (ANOVA) to locate periodic trends in band formation.

2.5. Total weight – total length relationships

The relationship between total weight and the total length was determined by fitting the data to a potential relationship for males and females in the equation:

$$W = aL^b$$

Where W is the weight in grams, L is the total length in millimetre, a and b are the parameters to be estimated. Differences in W-TL relationship between males and female were analyzed using analysis of covariance (ANCOVA) on log-log transformed data.

2-6 Estimation of growth

2-6-1 Back-calculation length at GR

Measurements were used for back-calculation of size at growth rings. To estimate length at previous age based on the linear regression between total length and vertebrae radius using the Fraser–Lee method (richer, 1969; Francis, 1990; Natanson et al., 1995).

$$[\mathrm{TL}]_n = \left(\frac{R_n}{\mathrm{VR}}\right)([\mathrm{TL}]) - a) + a$$

where $[\mathrm{TL}]n$ is the back-calculated length-at-GR n, Rn the vertebral radius at the time of the ring n, VR the vertebral radius-at-capture, TL the length-at-capture and a the intercept on the length axis of linear regression between total length and vertebrae radius

2.6.2 Growth function

Growth curves were fitted using the von Bertalanffy growth equation (von Bertalanffy,1938).

$$L_t = L_\infty\{1 - \exp[-^{K(t - t0)}]\}$$

Where L_t is length at age t, L_∞ the asymptotic length, K the growth coefficient, t is age (years from birth), and t_0 is the age at length zero. All growth rates were calculated using the VBGE based on observed length-at-age and back-calculated values.

2.6.3 Growth Performance

The growth performance (φ' ') of a species canbe captured by the growth index (Munro and Pauy, 1983), which can be used to compare growth rates between species and to evaluate growth performance potential under various environmental stresses (pauly, 1994).

$$\varphi' = \log_{10}K + 2\log_{10} L_{\infty}$$

2.6.4 Overall growth performance (OGP)

Calculated overall growth performance (OGP) indices were useful to compare the VBGFs, OGP is proportional to maximum rate of body mass increase during lifetime, in this study the OGP of *H. labeo* derived from observed and back-calculation length-at-age was calculated as:

$$OGP = \log (k L_{\infty})^3$$

Where k is coefficient of growth, L_{∞} is asymptotic length.

(a) 

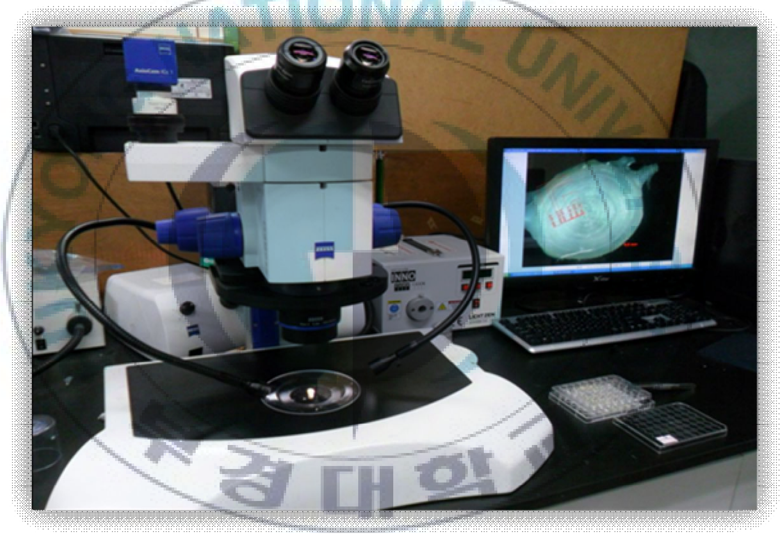
(b) 

Fig.6. (a) Vertebrae separation from the samples.(b) a video camera Microscope (Carl zeiss Discovery v.8) used for vertebrae observation

(a) 

(b) 

Fig.7. Fish sampling area (a) Goe-san lake and the dam located in the north of the lake, (b) fish nets used in collecting samples

2-5 Data analysis

Differences in W-TL relationship between males and female were analyzed using analysis of covariance (ANCOVA) on log-log transformed data. MI was calculated monthly, tested for normality and for significant differences using analysis of variance (ANOVA) to locate periodic trends in band formation, when ANOVA was significant, differences in means of variables were tested by the posterior tukey-HSD method. The parametric paired *t*-test was used to compare assigned ages between twice readings of two readers

All statistical analyses were carried out using SAS (v.9.13) and MINITAB (V.16) software package. Differences were considered significant at the 0.05 probability level ($P<0.05$) for all tests (zar, 1999).

3. Results

3-1 Environmental factors

Water temperature ranged from 11.00 to 29.67 ºC. It increased from April to August (Fig. 8) and attained the peak 29.67 ºC in August, then, decreased until November. The range of dissolved oxygen (DO) is 6.18-10.97 mg l^{-1}. It's the highest with 11.60 mg l^{-1} in April, decreased until August, then, increased until November, and reached the minimum 6.87 mg l^{-1} in August. The pH in April is 0.8 higher than that in May. It increased from May to August, and reached the maximum 8.5 in August. The value in September is 0.6 lower than that in August, and increased slightly until November. The pH value ranged from 7.1 to 8.8. The rainfall in April was lower than that in May, but higher than the value in June. From June to August, it increased and attained the peak 433.2 mm in August, then, decreased until November. The water temperature differed significantly among seasons (Spring: April and May; Summer: June, July and August; Autumn: September, October and November) ($F = 34.715$, $P < 0.05$). The value in spring was significantly lower than summer's, and had no difference with autumn. The DO also differed significantly among seasons ($F = 9.529$, $P < 0.05$). The DO in spring was significantly higher than that in summer and autumn, and in summer was significantly lower than autumn's. The pH had no significant difference among seasons ($F = 3.304$, $P > 0.05$), and the rainfall also had no significant difference among seasons ($F = 2.964$, $P > 0.05$). By the correlation analysis among months, the temperature was significantly correlated with DO and rainfall, and not correlated with pH. The DO was significantly correlated with other factors. The pH wasn't correlated with rainfall.

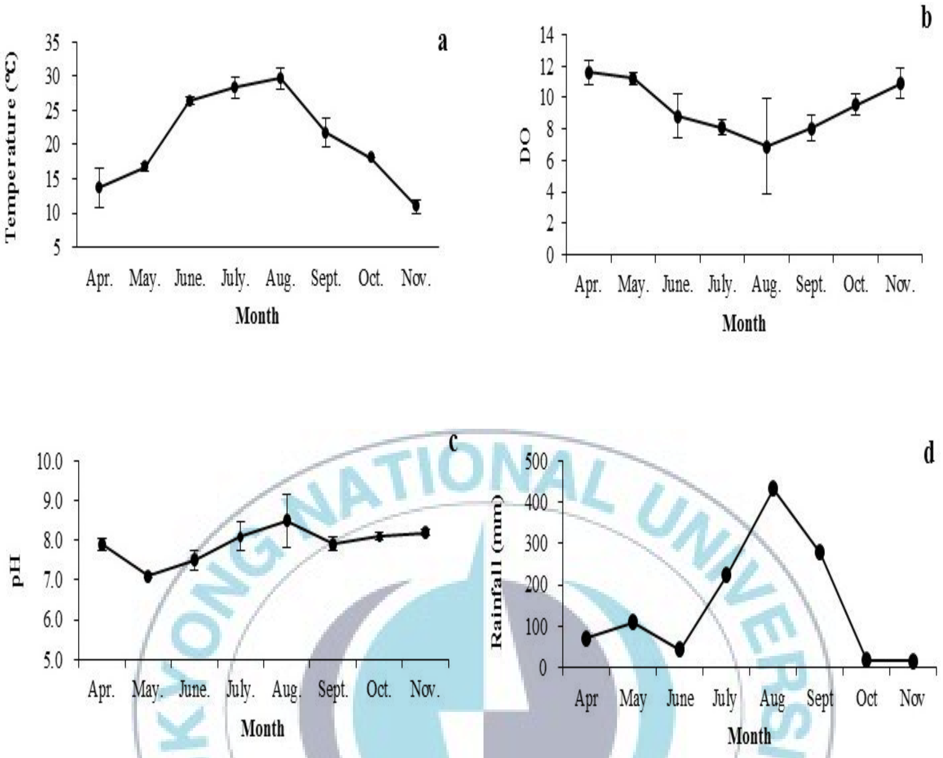


Fig. 8. Environmental factors distribution from April to November in 2010 in Goe-san Lake. (a:Water temperature,b: DO, c pH and d: Rainfall)

3.2 Length-age relationship

Logarithmic relationship between age and TL, FL and BL were estimated the regression of total length (TL) and age was best fitted relationship (Fig.9). There was highest value for the adjusted coefficient of determination (R^2 =8158), therefore, we used TL as estimate parameter for length in this study.

3.3 Length – frequency distribution

Among the overall sample length–frequency distributions (Fig.10) clearly show differences in the range of sizes for each of the sexes. Most males (72%) samples ranged from 209 to 298 mm (TL) and females (76%) from 269 to 388 mm TL (Fig. 9). All specimens larger than 400 mm TL were females. The maximum size of the males was 387.3 mm TL, and a females was 421 mm TL.

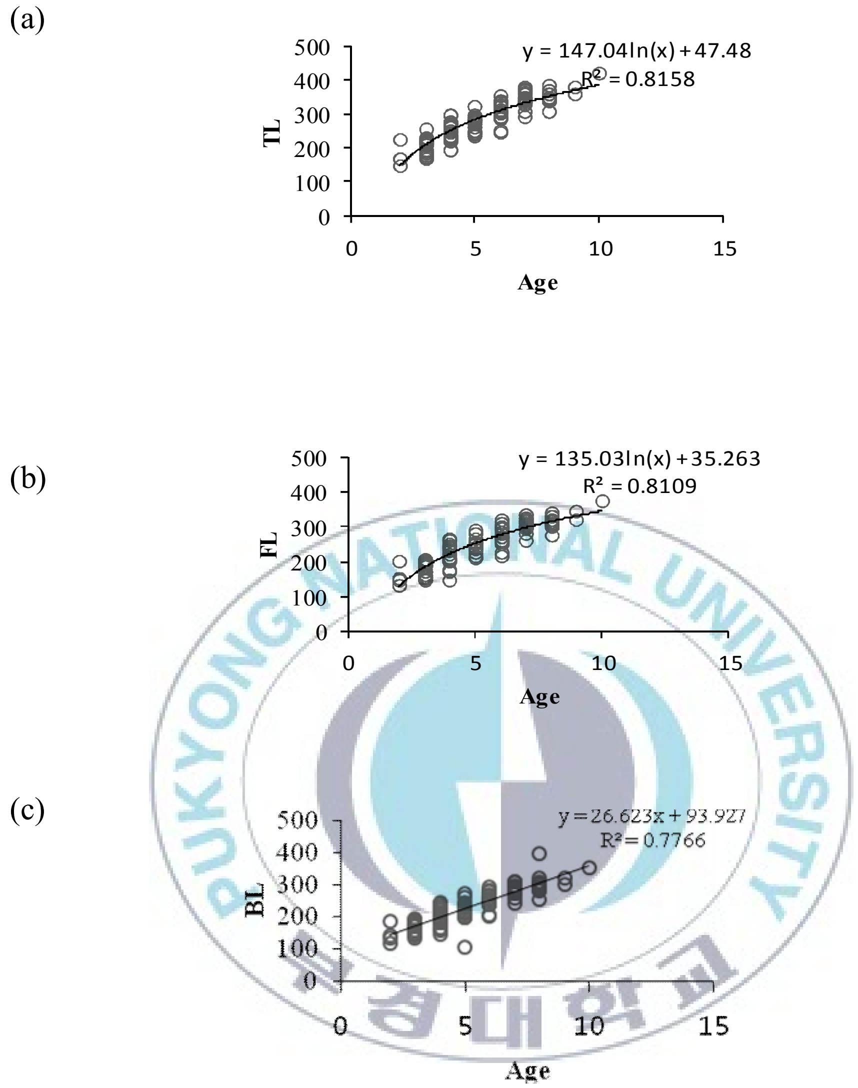


Fig. 9. The relationship between age and (a): TL, (b): FL, (c): BL in *H.labeo*

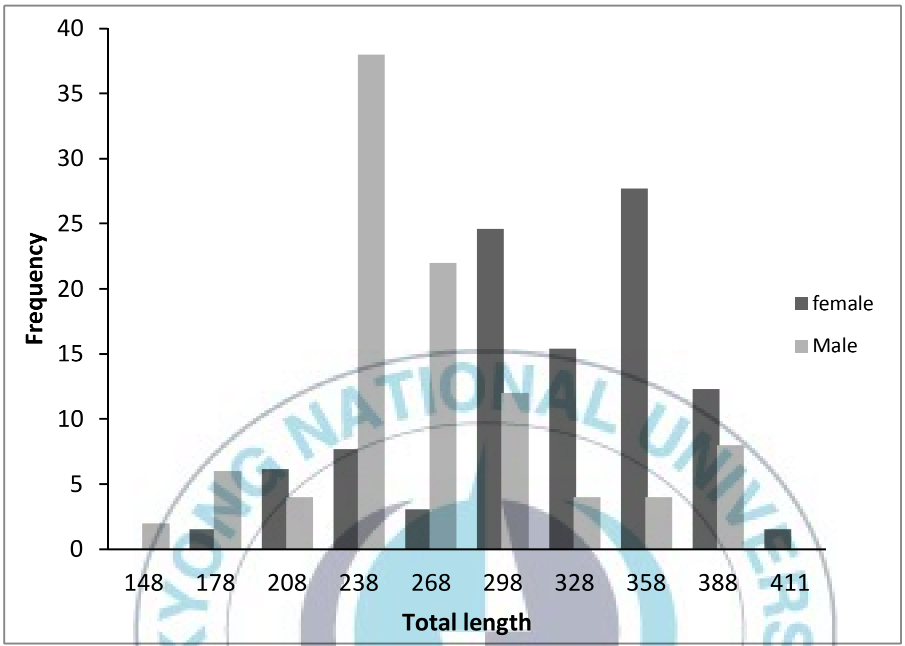


Fig. 10. Length-frequency distribution of *h.labeo* sampled from TheGoe-san lake from March to October in 2011.

3-4 Relationship between total length and total weight

The samples of Amur barbell (*Hemibarus Labeo)* ranged from 148-421mm in total length and from 25.4 – 599.26 gm in total weight (Tables 2, 3) Out of 115 individuals used for age estimation in this study, 65 (56.6%) were identified as female ranging from 168 - 421 mm and from 40.85 to 599.26 g and 50 (43.4%) were identified as male ranging from 148 to 387.3 mm and from 25.4 to 550.98 g. Mean total length (±S.D) for male and females were 249.83 (±53.51) mm, and 306.82 (±54.19) mm, respectively. Mean total weight (±S.D) for male and females 157.18 (±110.34) and 270.42 (±120.26) respectively.

TL–*W* relationships were evaluated for the combined sex and separately evaluated for females and males (Fig. 11) are given below:

Total $W = 1E\text{-}05L^{2.965,}$ ($R^2 = 0.9887$)

Female $W = 1E\text{-}05L^{2.974,}$ ($R^2 = 0.9885$)

Male $W = 9E\text{-}06L^{2.99\,9,}$ ($R^2 = 0.983$)

Table 2a
Average values (±S.D.) of size corresponding to combined sex, females and males

	N	TL (mm)	
		Mean±S.D.	Min-Max
Total	115	282.05±60.70	148-421
Females	65	306.82±54.19	168-421
Males	50	249.83±53.51	148-387.3

Table 2b
Mean length-at-age (±S.D.) and sample size (N) corresponding to total population, females and males

Age	Total		Females		Males	
	Mean TL±S.D	N	Mean TL±S.D	N	Mean TL±S.D.	N
2	158.50±33.98	4	159.57±36.77	2	159.00±15.57	2
3	210.50±22.84	19	176.00±15.21	4	213.12±23.32	15
4	245.41±28.36	23	255.68±35.02	8	237.14±21.80	14
5	273.44±24.04	17	284.49±20.79	10	260.33±19.97	8
6	308.40±28.56	20	314.94±23.19	14	293.13±36.21	6
7	345.07±22.03	17	334.00±21.93	15	353.10±29.84	2
8	353.62±19.89	12	348.23±18.85	9	369.76±15.22	3
9	371.50±16.26	2	371.50±16.26	2	0	0
0	421	1	421	1	0	0

Table3a
Average values (±S.D.) of weight corresponding to total population, females and males

	N	TW(g)	
		Mean±S.D.	**Min-Max**
Total	115	221.18±128.58	25.4-559.97
Females	65	270.42±120.26	40.85-599.26
Males	50	157.18±110.34	25.4-550.98

Table 3b
Mean Total wieght (±SD) at-age determined by vertebrate and sample size (N) corresponding t o total population

Age	Total		Females.		Males	
	Mean TW±S.D	**N**	**Mean TW±S.D**	**N**	**Mean TW±S.D.**	**N**
2	53.18±34.12	4	71.90±43.90	2	34.47±12.82	2
3	84.31±25.53	19	68.90±25.38	4	88.43±24.77	15
4	137.07±49.68	23	154.21±63.02	8	123.32±37.67	14
5	188.21±57.32	17	213.43±57.27	10	157.22±35.91	8
6	255.60±66.07	20	266.99±56.20	14	229.02±84.65	6
7	356.68±64.13	17	356.55±67.06	15	357.69±53.23	2
8	384.48±77.96	12	356.87±60.44	9	463.30±77.93	3
9	446.34±26.74	2	446.34±26.74	2	0	0
10	599.26	1	599.26	1	0	0

(a)
(b)
(c)

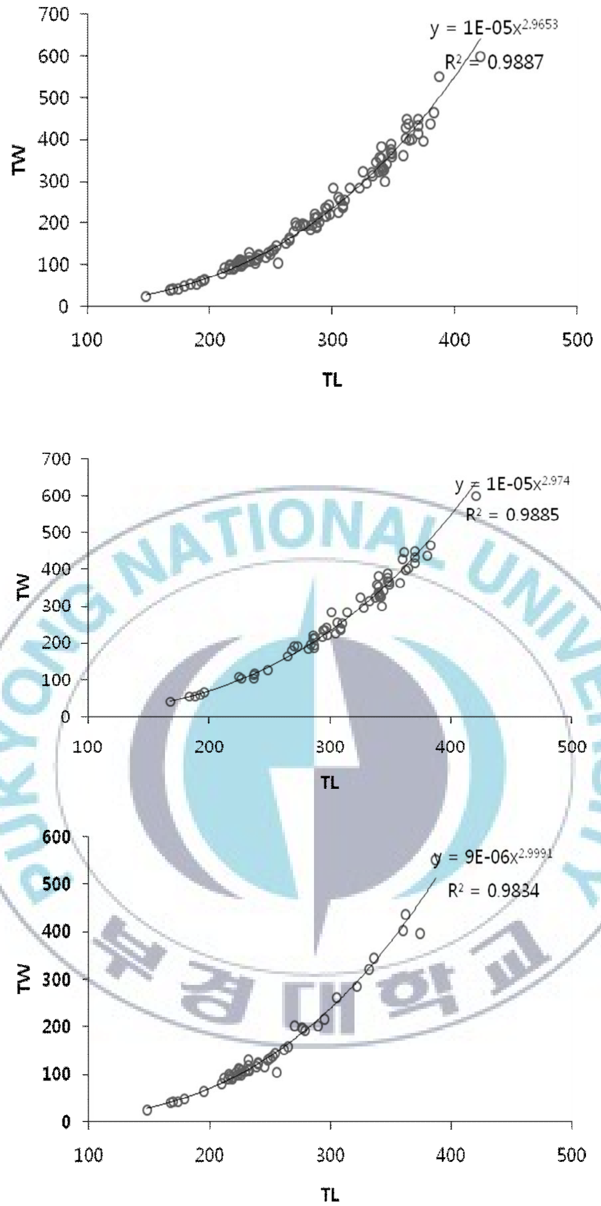


Fig. 11. TL–BW relationship of *H. Labeo* for combined (a) sex,(b) females, (c). males

3-5 Ageing and ageing precision

From the examination of vertebra, clear alternating areas were observed, one of active growth on which circulli were deposited, followed by a mark indicative of growth interruption (annulus). One year of life was assigned to each of these interruption marks.

Age estimates ranging from 2 to10 for females and 2 to 8 for males, based on the 201 vertebra centra examined. The vertebrae was read twice (Fig. 12). The index of average percentage error (IAPE) estimated from the twice reading ranged from 0.02 to 0.39 and mean 0.18 and coefficient of variation (CV) ranged from 0.04 to 0.78 and mean 0.23. In total, 38 vertebra centra (19%) were rejected because either the vertebra centra was unreadable or the third band pair counts differed from the previous two counts.

3-6 Period of ring mark formation

Marginal increment rate (MIR) analysis (Fig. 13) showed a trend of increasing marginal increment widths from spring to autumn. Starting from March and then showed a trend of decreasing MIR in April then increase again in May. The highest MIR means occurred in October for both sexes. We assumed that the opaque band represented a one year annulus which formed in December. The standard deviations of monthly samples were large because annulus growth for each individual or age groups was different, even in the same month (Chen et al., 1990).

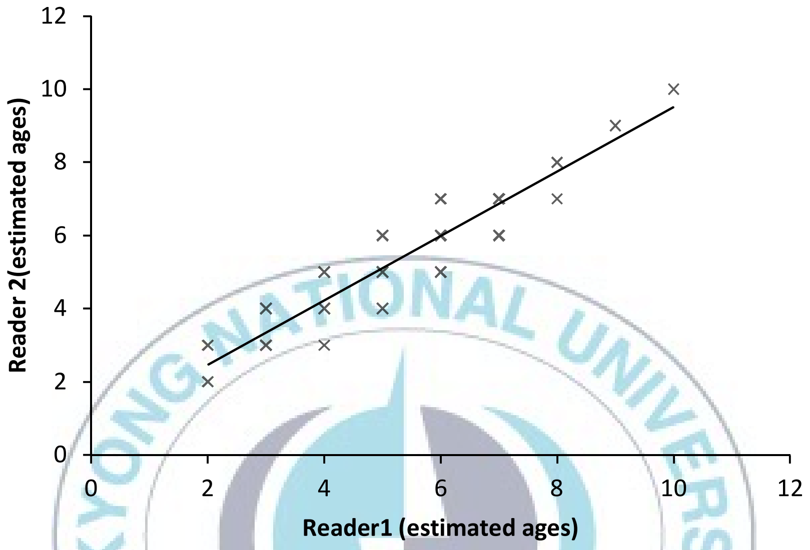


Fig.12. Agreement plot comparison between ages assigned twice reading by two readers

(a) (b) (c)

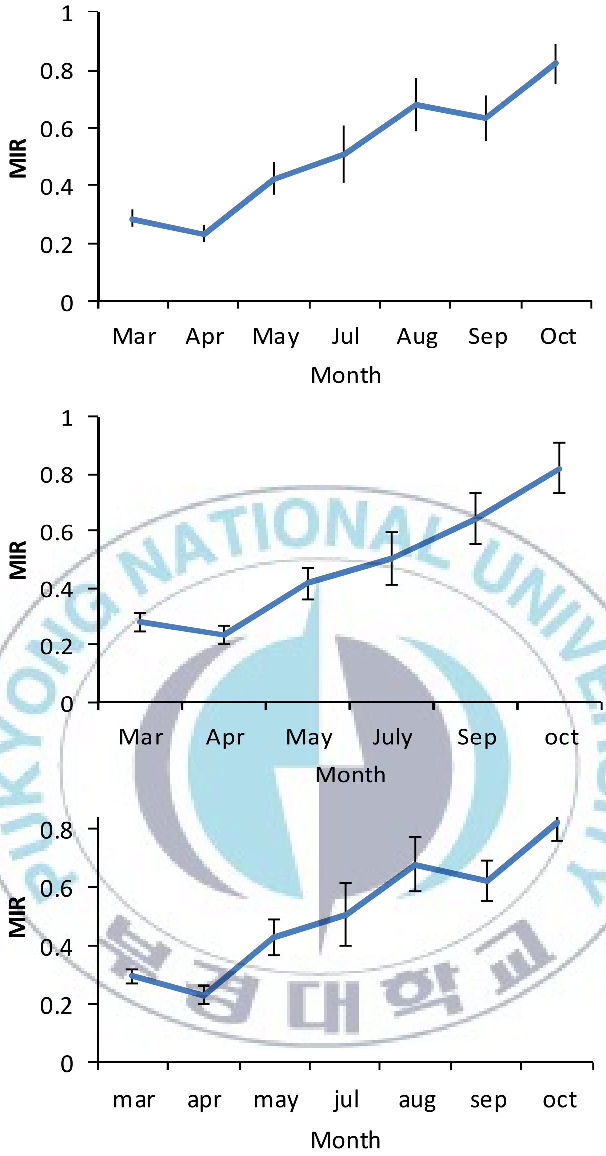


Fig. 13. Monthly change of marginal increment of *H. Labeo* from goe-san lake Female(a), Male(b), Total(c).

3.7 Relationship between radius (R) and length (L)

According to the size range of fish examined, there was a significant and positive linear correlation between total length (mm) and the radius (mm) of the vertebra (Fig. 14). An analysis of covariance was used to compare the homogeneity of *R*–TL relationships between sexes, indicating that significantly difference could be observed on R-TL for sexes (ANCOVA, F= 128.01, $P < 0.05$). The *R*–TL relationships for females and males and combined sex as follows:

Female: TL = 71.72*R* + 101.81 ($n = 65$; $r^2 = 0.837$)

Male: TL = 73.82*R* + 91.57 ($n = 50$; $r^2 = 0.860$)

Combine: TL= 74.157R+93.153(n=115; r^2 =0.878)

Graphical comparison between vertebrae radius and total length in the present study and scale radius and body length with the other study in Wusulijiang River (XU Wei *et al.*2008) show that significantly deferrance in results (Fig.14).

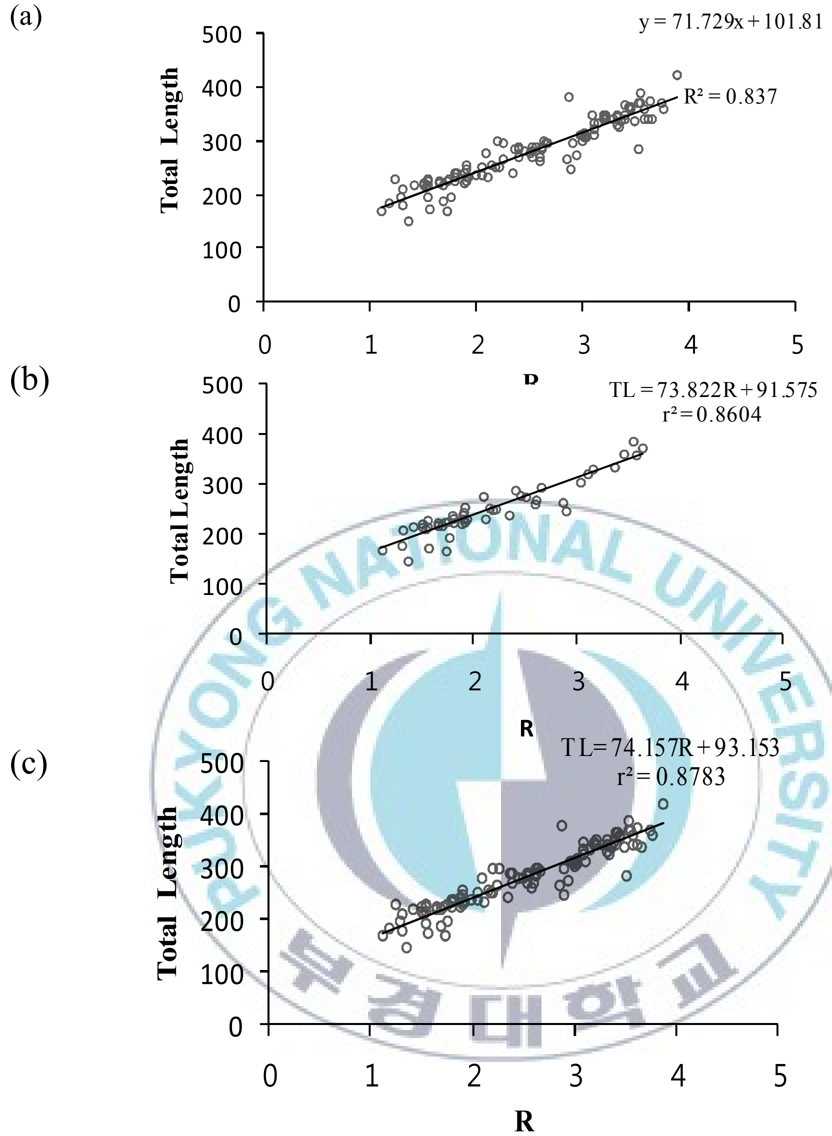
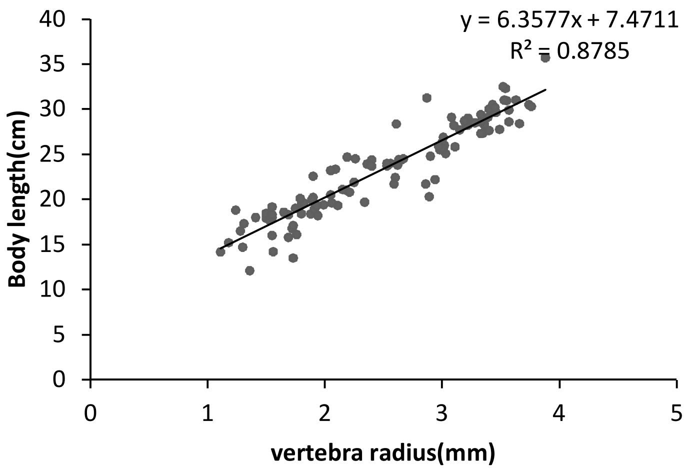
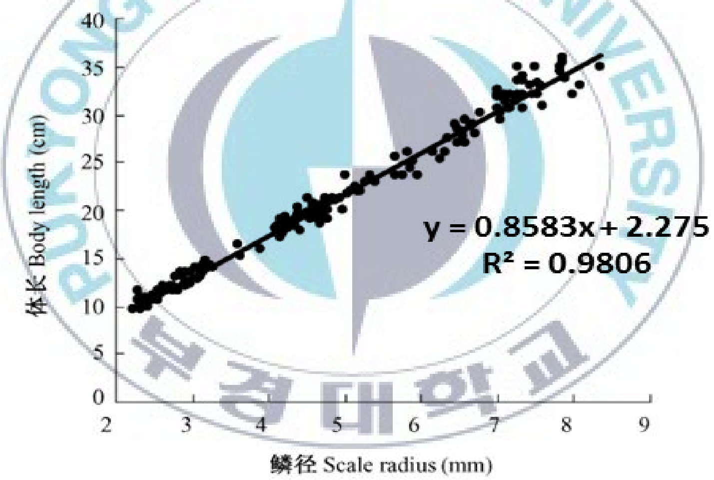


Fig.15. Relationship between TL-R in *H. labeo* (a): female, (b): Male, 9(c) combine sex



(a)



(b)

Fig. 15. comparison of (a) Relationship between V. radius and body length of *H.labeo* in the Goe-san Lake. (b) The relationship between scale radius and body length in *H.Labeo* in Wusulijiang River

3.8 Back-calculated length at the time of annulus formation

Samples were composed of individuals ranging from 2 to 10 GRs for females and from 2 to 8 for males. Mean annulus radius were calculated for combined and each sex separately (Tables 4,6 and 8). The mean radii for each annulus were measured to determine the intercept(a), back-calculated total lengths at the time of annulus formation were calculated and are presented in Tables 5,7 and 9.

Von Bertalanffy Growth Equation Observed lengths at different ages were used to calculate the VBGE predicted lengths. The VBGE of both sexes were compared by ANOVA-test, indicating that the two equations are significantly different (F= 36.48, p<0.05 for male and F= 40.44, P<0.05 for female). The parameteres of VBGE estimates were K =0.079 year^{-1}, L_{∞} = 653 cm, and t_0 =−0.139 year for both sexes. The growth performance and the overall growth performance index were 4.52 and 4.99 respectively

Back-calculated lengths by ultimate annulus formation at different ages were also used to calculate the predicted lengths of VBGE. The ANOVA-test indicating that the VBGE of both sexes was significantly different. (F = 212.38, P < 0.05 for male and F=228.19, P<0.05 for female). The estimated parameteres were $K = 0.161$ year^{-1}, $L_\infty = 683$ cm, and $t_0 = -0.167$ year for combine sex. The variances of parameters between observed and back calculation methods for males and females and for both sex shown in (Table. 10).

3.9 Growth equations

The von Bertalanffy Growth Equations were determined from the back-calculated and observed length at age for combined sex of *H.Labeo*(Fig.15) the equation for observed legth at age was

$$\mathbf{L_t = 653(1\text{-}e^{-0.079(t+0.139)})}$$

while the equation for back-calculated length at GR was:

$$\mathbf{L_t = 683(1\text{-}e^{-0.061(t+0.167)})}$$

.

Table 4. Mean ring radius and standard deviation at estimated age for female of *H.Labeo*

Age (year)	N	R	v1	v2	v3	v4	v5	v6	v7	v8	v9	v10
2	2	1.55±0.26	0.92±0.01	1.3±0.11								
3	4	1.49±0.22	0.88±0.18	1.18±0.20	1.40±0.20							
4	8	2.16±0.51	0.76±0.16	1.19±0.31	1.66±0.34	2.01±0.47						
5	10	2.53±0.45	0.76±0.13	1.17±0.20	1.59±0.36	2.07±0.47	2.39±0.40					
6	14	2.92±0.35	0.80±0.10	1.16±0.20	1.57±0.26	2.03±0.29	2.40±0.36	2.77±0.34				
7	15	3.25±0.28	0.82±0.10	1.18±0.15	1.52±0.15	1.90±0.21	2.3±0.28	2.74±0.25	3.10±0.27			
8	9	3.36±0.24	0.78±0.96	1.13±0.26	1.15±0.25	1.88±0.26	2.24±0.29	2.54±0.31	2.82±0.32	3.15±0.25		
9	2	3.64±0.17	0.83±0.12	1.12±0.18	1.56±0.28	2.05±0.12	2.36±0.09	2.73±0.49	2.99±0.15	3.24±0.21	3.48±0.19	
10	1	3.88	0.72	0.99	1.28	1.76	2.18	2.49	2.71	3.01	3.21	3.59
mean radius(mm)		2.75	0.8	1.16	1.47	1.96	2.31	2.65	2.9	3.13	3.34	3.59
SD		0.82	0.057	0.55	0.17	0.08	0.67	0.104	0.14	0.063	0.191	0

Table 5. Back-calculation of standard length at age for female of *H.Labeo*

Age (year)	N	TL (mm)	R	Back-calculated standard length (mm)									
				L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
2	2	159.57	1.55	136.09	150.25								
3	4	176.00	1.49	145.63	160.56	171.52							
4	8	255.68	2.16	155.95	186.58	220.06	244.99						
5	10	284.49	2.53	156.69	186.29	216.62	251.28	274.38					
6	14	314.94	2.92	160.2	186.48	216.4	249.98	276.99	303.99				
7	15	334.00	3.25	160.39	186.11	210.4	237.55	266.13	297.56	323.28			
8	9	348.23	3.36	159.01	184.68	186.15	239.69	266.09	288.09	308.63	332.83		
9	2	371.5	3.64	163.31	184.79	217.39	253.7	276.66	304.08	323.34	341.86	357.65	
10	1	421.00	3.88	161.04	183.25	207.11	246.6	281.15	306.65	324.75	349.43	363.88	397.14
	Measured mean			155.36	178.77	205.70	246.25	273.56	300.07	320	341.37	360.76	397.14
	SD			8.84	13.54	17.53	5.99	6.17	7.49	7.61	8.31	4.40	0

Table 6. Mean ring radius and standard deviation at estimated age for male of *H. labeo*

Age (year)	N	R	v1	v2	v3	v4	v5	v6	v7	v8
2	2	1.24±0.18	0.76±0.21	1.12±0.19						
3	15	1.63±0.23	0.78±0.12	1.10±0.18	1.50±0.21					
4	14	1.98±0.30	0.8±0.12	1.2±0.16	1.5±0.25	1.86±0.27				
5	8	2.21±0.36	0.78±0.16	1.15±.27	1.54±0.37	1.78±0.42	2.07±0.37			
6	6	2.86±0.39	0.78±0.13	1.16±0.15	1.57±0.21	1.95±0.24	2.33±0.36	2.71±0.39		
7	2	3.39±0.34	0.75±0.15	0.97±0.19	1.38±0.34	1.91±0.35	2.33±0.45	2.87±0.46	3.2±0.41	
8	3	3.52±0.06	0.71±0.11	1.07±0.17	1.54±0.18	1.96±0.75	2.35±0.6	2.69±0.16	3.03±0.10	3.34±0.06
mean radius(mm)		2.4	0.77	1.11	1.5	1.89	2.27	2.76	3.11	3.34
SD		0.88	0.029	0.075	0.066	0.074	0.133	0.099	0.12	0

Table 7. Back-calculation of standard length at age for male of *H.Labeo*

Age (year)	N	TL (mm)	R	Back-calculated standard length (mm)							
				L1	L2	L3	L4	L5	L6	L7	L8
2	2	159	1.24	132.9	152.47						
3	15	213.12	1.63	149.74	173.6	203.42					
4	14	237.14	1.98	150.39	179.79	201.85	228.32				
5	8	260.33	2.21	151.13	179.39	209.17	227.49	249.64			
6	6	293.13	2.86	146.54	173.32	202.22	228.99	255.78	282.56		
7	2	353.1	3.39	149.43	166.4	198.03	238.92	271.32	312.98	338.44	
8	3	369.76	3.52	147.68	176.13	213.28	246.47	277.29	304.16	331.03	340.53
	Measured mean			146.83	171.58	204.66	234.03	263.50	299.9	334.73	340.53
	SD			6.34	9.55	5.54	8.35	12.94	15.65	5.239	0

Table 8. Mean ring radius and standard deviation at estimated age for combined sex of *H. labeo*

Age (year)	N	R	v1	v2	v3	v4	v5	v6	v7	v8	v9	v10
4	2	1.39±0.26	0.83±.0.15	1.2±0.14								
19	3	1.6±0.23	0.79±0.13	1.12±0.18	1.47±0.20							
23	4	2.04±0.38	0.78±0.13	1.196±0.21	1.56±0.28	1.91±0.34						
17	5	2.4±0.43	0.77±0.13	1.16±0.22	1.56±0.35	1.94±0.46	2.25±0.45					
20	6	2.9±0.35	0.79±0.11	1.15±0.18	1.56±0.23	2.00±0.27	2.38±0.34	2.75±.034				
17	7	3.27±0.26	0.8±0.10	1.15±0.15	1.5±0.16	1.9±0.21	2.3±0.28	2.75±0.25	3.11±0.27			
12	8	3.39±0.22	0.761±0.10	1.11±0.23	1.51±0.22	1.89±0.22	2.26±0.25	2.57±0.27	2.87±0.28	3.19±0.23		
2	9	3.64±0.17	0.83±12	1.11±0.17	1.55±0.27	2.05±0.12	2.35±0.09	2.72±0.04	2.98±0.15	3.24±0.21	3.47±0.19	
1	10	3.88	0.72	0.99	1.28	1.76	2.18	2.49	2.71	3.01	3.21	3.59
mean radius(mm)		2.72	0.78	1.13	1.5	1.92	2.29	2.66	2.92	3.15	3.34	3.59
SD		0.91	0.034	0.061	0.95	0.916	0.73	0.119	0.169	0.12	0.184	

Table 9. Back-calculation of standard length at age for combined sex of *H.Labeo*

age (year)	n	TL (mm)	R	Back-calculated standard length (mm)									
				L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
2	4	158.5	1.39	143.7	166.53								
3	19	210.5	1.6	150.46	174.67	200.34							
4	23	245.4	2.04	151.36	182.19	209.75	235.82						
5	17	273.4	2.4	150.68	179.97	210.71	239.11	262.59					
6	20	308.4	2.9	151.45	178.52	209.23	241.66	269.51	297.13				
7	17	345.1	3.27	154.81	181.31	208.49	239.29	270.42	305.08	332.49			
8	12	353.6	3.396	151.07	178.2	208.58	238.49	266.93	290.57	313.12	338.03		
9	2	371.5	3.64	156.13	177.97	211.7	249.64	273.01	301.37	321.3	340.84	357.35	
10	1	421	3.88	153.47	176.33	200.88	241.52	277.08	303.32	321.95	347.34	362.78	396.45
Measured mean				151.45	177.29	207.46	239.05	267.79	298.5	324.2	339.03	360.65	396.45
SD				3.52	4.65	4.36	4.37	4.97	5.80	7.94	4.77	3.84	

Table10. Von Bertalanffy growth parameters from estimated age models of *H.Labeo*

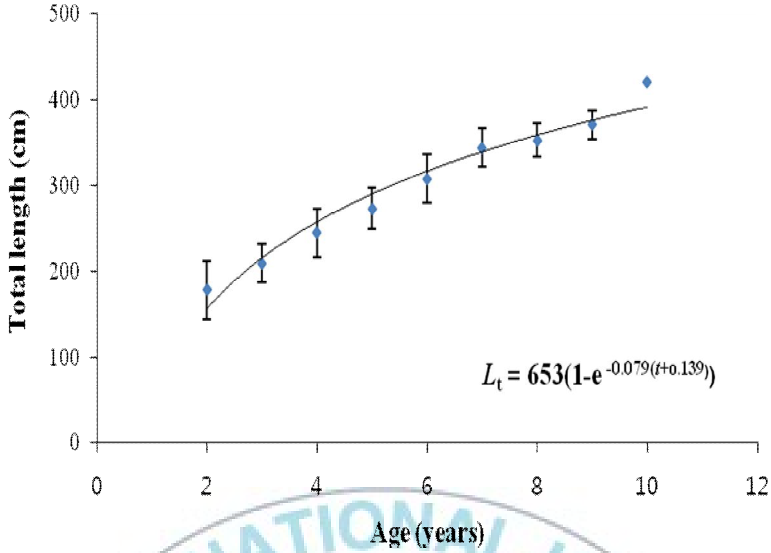
(a) observed length

	N	L_∞	k	t_0	r^2	φ'	OGP
Female	65	692	0.083	-0.127	0.92	4.6	5.43
Male	50	641	0.095	-0.120	0. 93	4.59	5.67
Total	115	653	0.079	-0.139	0.98	4.52	4.99

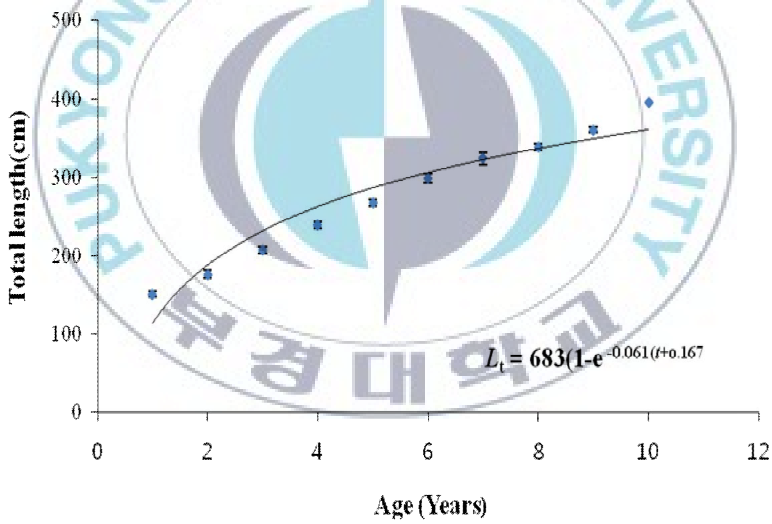
(b) back-calculation length

	N	L_∞	k	t_0	r^2	φ'	OGP
Female	65	744	0.054	-0.176	0.99	4.47	4.27
Male	50	708	0.060	-0.164	0.98	4.82	4.10
Total	115	683	0.061	-0.167	0.96	4.46	4.35

Where L_t: L at age t, L_∞: the asymptotic length, K: growth coefficient, t: the age (year from birth), t_0:the age at length 0, r^2: coefficient of determination, φ': growth performance, OGP: overall growth performance



(a)



(b)

Fig.16. Plot of the Von Bertalanffy growth curves of *Hemibarbus Labeo* , observed length at-GR (a). back calculated length–at GR (b).

4. Discussion

4.1. Age determination

4.1.1. Aging methodology

This study report on age and growth of *Hemebarbus Labeo* in the Goe-san lake using vertebrae as an age determinant marker. Wang and Milton (2000) and Katsanevakis and Maravelias (2008) state that the choice of the best growth model is subjective and should be in some cases based on the decision of the researcher, founded on experience with the species and previous studies in order to interpret the viability in the estimated parameters and goodness of fit. Cailliet and Goldman (2004) mentioned that there has been an increase in the use of both verification and validation methodologies in fish growth studies, such as marginal increment analysis, centrum edge analysis, size mode analysis, tag–recapture analysis, captive growth analysis, OTC marking, and bomb carbon. Using combinations of verification and validation approaches is most likely to produce convincing results. Many calcified structure have been used for aging (Yole, 1989). In this study the age determined based on the vertebrae band pair counting because scales of *H.Labeo* is easy to strip and remove by fish nets while sampling and otoliths easy to destroyed while removed and preserved, also the clarity of band pair counted from otiliths was not the same comparing by vertebra.

4.1.2. Vertebra rings formation

Vertebra rings occur systematically as length increases. Their formation is probably more directly related to factors other than size (Pratt and Casey, 1983). In several age studies, year marks form in summer (Allen and Wintner, 2002; Natanson et al., 1999; Simpfendorfer et al., 2002;Wintner et al., 2002). Our analysis indicates that the year marks are deposited once a year in October. All of the factors influencing formation of the opaque zone are not clear cut, but several hypotheses explaining its deposition have been reported. A shortage of food, food deprivation caused by migration and spawning, (Stevens, 1973; Wang and Chen, 1982; Campana, 1983; Pratt and Casey, 1983). Seasonal changes in temperature, and fluctuation of body condition associated with spawning (Blake & Blake, 1978; Nekrasov, 1980; Yosef & Casselman, 1995), may all be factors affecting their formation. in this study, the water flow rate of the dam directly affect on the increase of food availability (Aquatic insects, shrimps and snails) in March and April corresponding to the time of opaque band formation suggests that the fast growth of the *h.Labeo* might be correlated to its feeding behavior. However, the underlying mechanisms governing band deposition still need to be determined.

4.1.3. Vertebra bands estimation

Several techniques to enhance vertebra bands were tried, the simplest and most efficient technique that rinsed the vertebrae with KOH (8-10%) then preserve by soaking in alcohol 70% to enhance centrum bands. This technique may prove suitable for similar assessments in other species. Vertebrae were read twice at an interval of 20 days. They were read randomly to avoid bias in assigning ages (Murieand Parkyn, 2005), however, we sometimes saw "pairs" of dark zones where two dark bands were closer than normal. If these bands coalesced at the margin of the vertebrae we counted them as part of the same increment. If they did not coalesce, they were counted as two increments. In order to avoid underestimation of total ages, mean annular measurements were used to estimate, specially the location of bands nearest the foci that might be difficult to detect. Counts were only accepted if both counts by the same examiner were in agreement. If the estimated numbers of band pairs from the two counts were different, then the centrum was recounted and the final count then accepted as the agreed age. If the third count had no consensus with one of the previous two counts, the sample was discarded, about 19% of the whole vertebrae were considered “unreadable” and excluded from the data set and subsequent analyses

MIR used for validate growth data and suggested that one opaque band and one translucent band formed annually. Campana (2001) mentioned that MIR can only be used to validate the periodicity of band formation. Hence, the precision of ageing in this study needs to be further validated with tagging in the future

4.1.4 Aging precision

The vertebrae from *H.Labeo* proved useful since high reliability was evident by the low average percentage of error (APE) and coefficient of variation (CV). Although the vertebrae of older specimens are more prone to reading errors due to the overlapping of growth bands pairs as a result of the approximation to asymptotic length, the rate of reading errors for these specimens as well as for the entire sample was below the limit proposed value for the CV was 7.6% and for APE was 5.5% (Campana, 2001).

4.2. Length-weight relationship

The estimated total length of *H.Labeo* at each age is larger for females than for males, and the observed longevity for females is greater than for males. These findings are consistent with the sex-specific length–frequency distributions. Length-weight relationship gives information on the condition and growth pattern fish (Bagenal and Tesch, 1978). Although distinct growth by sex has not yet been clearly ascertained, the estimation of separate equations is a common practice for the species (Cailliet et al., 1983). This is perhaps due to the assumption that biological differences determine dissimilar growth by sex (Natanson and Kohler, 1996). S.D of length and weight is significantly high, even in the same age. It maybe related to variety conditions of the lake (depth – food –change of water flow from the dam. etc).

Curvilinear relation was detected, the growth was found to be allometric since the *b* value was 2.974, 2.999 for female and male, suggesting that weight increase about the cube of their standard length, the value is little bit faster than length in males, for combined sexes *b* value was 2.965 lower than that estimated by (XU Wei et al., 2008) (b=3.086). This relative variation in the exponents could be related to the difference of geographic location and associated environmental conditions such as season (date and time of capture), disease, parasite and the nature of the artificial lake in this study and river in the other study can affect the value of "b".

4.3. Comparsion with previous study

Comparison between the relationship of scales radius and body length in *H.Labeo* in the wusulijiang river (r^2= 0.9806), and Vertebra radius and total length of in our study (Fig.15) (r^2=0.8785) show siginificant differance in results which are substantially lower than our estimates, nevertheless we can not evaluate the efficiency of use vertebrates in estimating the age because of several factors affected the results, the comparsion show *H.labeo* extremely affected by change of food, water depth, water flow and other factor related to the nature of the artificial lake in our study,

4.4. Von Bertalanffy parameters

4.4.1. Observed length at age

The von Bertalanffy growth equation is frequently used in life history and fisheries research to describe the relationship between the length or weight of a fish and its age (Ratkowsky, 1986). Cailliet and Goldman (2004) reported that growth model estimates are greatly affected by the lack of very young or old individuals. The L_∞ of the VBGF 653.mmTL, was larger than the maximum observed size of 461 cm TL for females and much larger for of 387.3 cm TL for males. It was likely that the lack of large or small specimens due to the fish nets size used in collecting the samples may affect our estimations of the maximum observed size and L_∞ for both sexes.

4.4.2 Back-calculated length at GR

The back-calculation of size at GR was estimated because recreational-fishing in the Goe-san Lake practices don't allow to catch small sized fishes as a conservation measure, therefore, the small specimens (0 to 2 years) remain underrepresented in samples.

Differences in vertebra diameter by sex gave rise to significant differences in VR-TL equations, implying that growth analysis had to be carried out separately by sex, a procedure that Schwartz (1983) and Yudin cailliet (1990) followed.

Among the VBGF parameters generated, observed lengths provided higher K and r^2 and lower L_{∞}, in comparison with back-calculated length-at-GR showed that the back-calculated age was older than the observed age. it was also testified that the age by vertebrae was under-estimated. It is obvious that Lee's phenomenon (the differences between calculated length and true length at earlier annuli is greater at younger ages (Lee, 1912; Ricker, 1958) does exist in the *H.labeo.*

4.4.3 Growth performance (φ')

The growth performance (φ') of the fish reflects the ability of a species to meet nutritional requirements and its adaption to prevailing abiotic conditions such as the temperature regime (Beamesderfer and North, 1995). There are not any previous results for this fish or fishes of the same family to compare and evaluate the results obtained, so this point need more study in the future

4.4.4. Overall growth performance

Several authors (e.g. Pauly, 1979; Munro and Pauly, 1983; Moreau,et al. 1986; laudien et al., 2003 Defeo and Cardoso, 2004) demonstrated the suitability of composite indices for OGP for inter and intraspecific comparisons., Marko. et al., (2009) shows that the overall growth performance OGP is habitat - specific; species populating tropical-subtrobical regions show lowerest OGP (2.84-3.68), temperate species have intermittent OGP (4.17 – 4.91) while species of upwelling areas show the highest OGP (5.06-5.65). the results from the present study (OGP = 4.35, 4.99 form observed and back calculation parameters respectively) indicated that *H.labeo* grow well in moderate temperature

5. Conclusion

H.Labeo one of the order Cypriniformes family cyprinadae occurs all over East Asia, from Vietnam to Russia, it can be one of the most economically important fish species not only in South Korea, but also in many regions all over the world because of it is quite appreciated for human consumption. This study aim to determine the age and estimate the growth parameters via vertebrae methods and assess the feasibility of culture in fish farms.

To achieve these tasks 201 samples were obtained from Goe-san lake located in chungcheong buk-do, South korea from March to October 2011 (36°45`19,34``N, 127°50`24,05``E). Vertebrae columns were separated and cleaned. Block of sex vertebrae (1-6) was removed for age determination by count the growth band pairs (including translucent and opaque bands) using optical microscope. The index of average percentage error (IAPE) and coefficient of variation (CV) calculated to compare reliability of age determination between readings. 19% of the whole vertebrae were rejected due to the difference between two readers . Marginal increment (MI) used To define the period of ring mark formation. Our analysis indicates that the marks are deposited once a year in mid-winter. The estimation of curvilinear relationship between total weight and the standard length suggested that weight increase about the cube of their standard length, Von Bertalnffy growth parameters were estimated from observed and back-calculation length at age, length–frequency distributions showed that most males sampled (72%) ranged from 209 to 298 mm TL and females (76%) from 269 to 388 mm TL. Relationship between total length and total weight given as W = 1E-05L$^{2.9653}$. Mean radii for each annulus were summed and back-calculated total lengths at the time of annulus formation were calculated. The parameter of VBGE estimates for observed length were K =0.079 year−1, L_∞ = 653 cm, and t_0 =−0.139 year for both sexes.the growth performance and the overall growth performance index were 4.52 and 4.99 respectively. On the other hand, the parameter estimates from back-calculated length at age were K = 0.161 year−1, L_∞ = 683 cm, and t_0 = −0.167 year for both sex. The growth performance and the overall growth performance index were 4.46 and 4.35 respectively

Through the present study and the previous studies that have been referred in the introduction, suggested that *h.labeo* could have a promising future in aquaculture field in many region all over the world due to it characterized by:

1 – It can grow will in moderate temperature (OGP = 4.35, 4.99 form observed and back calculation barameters respectively), and spawns in water temperature range from 12 to 24℃.

2- Juveniles can obtaine from the brood fish by artificial propagation also Distant hybridization with other species (*Hemibarbus Maculates*) were carried out successfully.

3- The weight significantly affected by the change of food, water depth, water flow, temperature and other factors.

Finally, this study provides the first detailes estimates of age and growth for *H.labeo* (amur barbell), which can be used as biological input parameters in further stock evaluations and conservation in this region. However, additional validation of the size composition and stock structure, also feeding and growth behavior are needed for future studies.

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