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Seasonal variation of giant gourami (*Osphronemus goramy*) spawning activity and egg production in aquaculture ponds

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Abstract

The giant gourami *Osphronemus goramy* Lacépède, 1801, spontaneously spawns in captivity, making nests for egg deposition when suitable nest supports and nesting material are made available in rearing facilities. The seasonal variation in reproductive performance of the giant gourami was characterized during a 20-month experiment in two compartmentalized ponds (about 550 m^2 each) at a fry production center in West Java, Indonesia. A total of 208 giant gourami broodfish were introduced in the two experimental ponds, with a sex-ratio of one male to three females in each of the 52 spawning compartments delimited by vertical nets. Nesting supports in each spawning compartment were examined every two days for the presence of eggs resulting from natural reproduction. Three performance indicators were quantified from the collected nests: spawning frequency (monthly number of spawns per female), effective fecundity (number of eggs per spawn) and egg quality (estimated from the proportion of clear living eggs in each spawn). The seasonality of giant gourami reproduction was analyzed as a function of seasonal variation in rainfall, rearing water temperature and day length. Based on a total of 413 collected spawns, the multiple linear regression models did not show any significant relationship between giant gourami spawning frequency and the three environmental variables studied. In contrast, the number of eggs per spawn was highly and positively related to rainfall ($p < 0.0001$), moderately and negatively related to water temperature ($p < 0.001$), and weakly and negatively related to day length ($p < 0.01$). The proportion of clear eggs was a positively related to day length ($p < 0.01$). Overall, this study represents one of the first comprehensive datasets on the reproductive phenology of giant gourami, and as such will be helpful in formulating broodfish management strategies for the species.

Keywords: Reproduction control; Breeding cycle; Natural spawning; Freshwater aquaculture; Indonesia

1. Introduction

Native to Southeast Asia, notably from Indonesia, the Malay Peninsula, Thailand and the Mekong basin, the giant gourami *Osphronemus goramy* Lacépède, 1801, has been introduced to several other countries for aquaculture purposes (Welcomme, 1988). This fish is omnivorous, with a strong vegetarian tendency, and shows relative robustness and adaptability to unfavorable environmental conditions (aerial respiration), which make it a very attractive candidate for low-input aquaculture (Caruso et al., 2019). In Indonesia, the giant gourami is one of the main freshwater commodities of economic importance owing to its high price and local demand (Rimmer et al., 2013). Since 2000, giant gourami aquaculture production has grown by a factor of 10 in Indonesia, the main country farming this species (145,000 tons in 2017; FAO, 2019). The Indonesian national production is ensured by approximately 100,000 small-scale fish farmers mainly located on Java Island (79%; BPS, 2013). Although the giant gourami has been reared for decades and has now reached a significant production level, there are still important gaps in our knowledge on several aspects of its biology.

The production of giant gourami fry relies on the natural spawning of captive broodfish held in ponds, but the commercial-scale propagation of the species still needs to be standardized (Amornsakun et al., 2014a; Arifin et al., 2019; Prakoso et al., 2019). The overall recruitment of this multiple spawning species (i.e. number of fry produced per broodfish and per spawn) in the breeding ponds remains generally low, resulting from variable spawning frequency or success, and variable egg number and quality (Azrita, 2015). Therefore, ensuring a regular and sufficient supply of fry through reliable production methods remains a major challenge for the expansion of gourami aquaculture (Amornsakun et al., 2014a, b; Slembrouck et al., 2019). Among the various biotic and abiotic factors influencing the reproductive performance of broodfish, the effects of pond management strategies, sex-ratio and duration of egg production periods were specifically investigated in a recent study (Arifin et al., 2020). Nevertheless, the seasonality of reproduction has also been mentioned as an important limitation to a regular supply of giant gourami fry (e.g. Arfah et al., 2006; Kristanto et al., 2019; Wijayanti et al., 2009). In some instances, this seasonality has prompted studies to test hormonal treatments that induce oocyte maturation and ovulation of giant gourami broodfish (Arfah et al., 2006). Nevertheless, to our knowledge, an accurate description of the seasonal variation in reproductive performance of captive giant gourami broodfish is still lacking in the scientific literature. Such information would help to rationalize and optimize hatchery operations, as in many intensively farmed finfish species (Migaud et al., 2013). Because there are considerable interspecific variations in the patterns of reproductive seasonality (Wootton and Smith, 2014; Paugy et al. 2017), such investigation would also be useful to further document the influence of the environment on the reproductive phenology of freshwater tropical fishes for which, with the exception of some families (mostly cichlids and clariids), availability of accurate datasets remains limited.

The objective of the present study was to assess seasonal fluctuations in the reproduction of giant gourami (spawning frequency, number of eggs in a spawn and egg quality), based on nearly two years of follow-up in ponds at the Tasikmalaya Center for the Development of Giant Gourami Culture (BPPSIGN), the largest giant gourami fry production center in the West Java Province, Indonesia. The seasonality of giant gourami reproduction was analyzed in function of seasonal variation in rainfall, rearing water temperature and day length, three environmental parameters considered as major cues for the reproductive phenology of most tropical fishes (Lowe-McConnell, 1979; Legendre and Jalabert, 1988; Paugy et al., 2017).

2. Materials and Methods

2.1. Experimental facilities and pond water quality

This study was carried out at the BPPSIGN Tasikmalaya Center (7°19′37.992″N, 108°6′101.155″E; altitude 489 m). The experiment was performed over a period of 20 months from May 2016 to December 2017. Experimental structures were two earthen-bottom ponds of 527 and 563 m^2 in surface area with vertical concrete banks and an average water depth of 0.6 m. The two ponds were divided into spawning compartments of about 20 m^2 each (52 compartments in total), delimited with nets embedded in the sediment and held vertically on bamboo poles. Ponds were supplied from a conduit carrying spring water from the Galunggung Mountain located less than 10 km from the BPPSIGN Center.

Before starting the experiment, the spawning ponds were dried, cleaned, rid of all other species and limed before being refilled. Pond water pH, dissolved oxygen, conductivity, and turbidity were measured at quarterly intervals between 07:00 and 08:00 AM with direct measurements using a multi-parameter probe (HI 9829 Hanna). Water concentration in ammonia (NH_3), nitrite (NO_2^-) and nitrate (NO_3^-) was measured at the same time using spectrophotometry analysis (Hanna HI83399). The average and range of variation of these parameters are provided in Table 1 and indicate that the pond water quality remained stable during the experiment and within appropriate standards for tropical freshwater fish (Colt, 2006).

2.2 Fish maintenance and assessment of reproductive performance

The mature broodfish (n = 208) used in this study were descendants of giant gourami belonging to the "Galunggung" strain (Arifin et al., 2018). The females were 3-5 years old and weighed ~3 kg, whereas males were 5-7 years old and weighed ~4 kg. Identification of fish sex and assessment of their sexual maturity were done on the basis of morphological criteria, milt emission following gentle abdominal massage or examination of oocytes obtained by intraovarian biopsies as described by Slembrouck et al. (2019). One sexually mature male and three sexually mature females were placed in each of the 52 spawning compartments of the two experimental ponds.

The giant gourami is a nest builder; therefore, nest supports and nesting material were provided in each compartment for spawning. Nest supports were baskets made of braided bamboo strips (total length: 25 cm, including an opening of about 30 cm in diameter), attached to bamboo stakes, and placed about 15 cm below the water surface with the opening positioned slightly downward (angle of about 30°). Plant fibers from palm trees (*Arenga* sp.) were piled on a bamboo table positioned at the surface of the water in the middle of every compartment, so that the broodfish could easily grasp the fibers with their mouths and build their nests (see Arifin et al., 2020, for picture of the nest supports).

Every two days, all nest supports were examined and closed nests containing eggs were collected, transferred to the hatchery, then immersed in a water tank to release the eggs by carefully opening the palm fiber material as detailed in Arifin et al. (2020). All the eggs from each nest were manually counted, separating live (transparent/clear) eggs from dead (opaque/white) eggs. During the experiment, reproductive performance was assessed by monitoring spawning frequency (the number of spawns per female per month), the number of eggs in a spawn (i.e., numbers of eggs in a nest) and egg quality (estimated by the proportion of clear eggs in each spawn). In some instances, the eggs were newly hatched at the moment of nest collection; however, this had no or little incidence on the evaluation of progeny number and spawn quality because white (dead) eggs take more than one day to break up and newly hatched larvae, unable to swim, remain inside the nest.

During the egg production periods, the broodfish were fed with leaves of elephant ear plants (*Alocasia macrorrhizos*) and commercial floating pellets (32% proteins, 5% lipids) distributed at a daily feeding rate of 2% and 1% of fish biomass, respectively. The egg production periods lasted for 3 or 7 months depending on spawning compartments, two durations that lead to equivalent results in terms of egg production over the long term (Arifin et al., 2020). Between two successive egg production periods, the broodfish were reconditioned for 1 month (separating males and females in different ponds and using reverse daily feeding rates of elephant ear leaves and floating pellets). The broodfish fish were examined every three months and weighed with a digital scale for adjustment of feeding rations.

2.3. Monitoring of environmental conditions

Throughout the 20-month experiment, fluctuations of three environmental parameters were considered: rainfall, pond water temperature and day length. Pond water temperature was monitored continuously using a data logger (Onset HOBO) placed in the center of the ponds at 20 cm depth. A rain gauge was installed in the immediate vicinity of the ponds to record the daily rainfall and the information related to day length was collected from Time and Date (2019).

2.4. Data treatment and statistical analysis

Prior to statistical analysis, fish reproduction and environmental data were compiled and, for each pond and each variable, monthly means of the recorded values were calculated. Statistical analyses were then performed on these average monthly values.

The non-significance of the variable “pond”, initially included in general linear models (GLM) with quantitative environmental variables was confirmed. Then, stepwise multiple linear regression models (MLR) were used to test for relationships between female spawning frequency, egg numbers or clear egg proportion in spawns and environmental variables (rainfall, water temperature and day length). The assumptions underlying the MLR were checked by visual observation of the model residuals and by statistical analysis (Goldfeld-

Quandt test, Rainbow test and Shapiro-Wilk test). Variance inflation factors (VIF) were used to estimate collinearity of the explanatory variables, with VIF values close to 1 indicating the absence of strong multi-collinearity (Hair et al., 2010). Statistical analyses were performed using R freeware version 3.5.2 using the packages "car", "lmtest" and "stats" (R Development Core Team, 2019).

3. Results

At the BPPSIGN Tasikmalaya Center, day length varied annually between 11 h 41 min and 12 h 33 min, and pond water temperature ranged from 22.6°C to 32.7°C. The cumulative monthly rainfall ranged from 5 to 528 mm during the experiment, from May 2016 to December 2017. Overall, the monthly mean of the pond water temperature showed limited variation (between 25.4°C and 28.0°C), whereas the monthly rainfall was much lower during the second than during the first half of the experimental period (from 331 to 528 mm vs. 0.5 to 288 mm, respectively; Figure 1A).

During this 20-month period, as determined from the 413 spawns collected in the two experimental ponds (206 and 207 spawns per pond), the reproduction of giant gourami occurred continuously. Because no effect of the duration of egg production periods (successive periods of 3 or 7 months) was detected for any variable (spawning frequency, egg number and quality), the data were pooled for analyses. The mean monthly spawning frequency of giant gourami varied between 0.07 and 0.26 spawn $female^{-1}$ $month^{-1}$ without a clear seasonal rhythm in the intensity of spawning activity (Figure 1B). The mean monthly number of eggs per spawn collected from the nests varied between 2020 and 5920 eggs $spawn^{-1}$ and peaked (more than 4000 eggs $spawn^{-1}$) during the period between July and November 2016 (Figure 1B). The mean monthly proportion of clear (live) eggs in the spawns varied between 79 and 96% (Figure 1B).

The MLR did not reveal any significant relationship between giant gourami spawning frequency and any of the environmental variables (Table 2). Observed monthly variation in spawning frequency had no clear seasonal rhythm; it was not related to simultaneous variations in rainfall, water temperature or day length. In contrast, variation in the number of eggs produced per spawn was clearly related to variation in environmental variables (multiple R^2 = 0.71; $p < 0.0001$). Egg number was highly and positively related to rainfall (t-value = 7.777, $p < 0.0001$), moderately and negatively related to water temperature (t-value = -4.387, $p = 0.001$), and weaklier and negatively related to day length (t-value = -2.983, $p < 0.01$) (Table 2). For egg quality, as evaluated through the proportion of clear eggs, there was a positive but relatively weak relationship with day length (t-value = 3.126, $p < 0.01$), but not with rainfall or water temperature.

4. Discussion

In the present study, giant gourami clearly reproduced all year round in the ponds at the BPPSIGN Tasikmalaya Center. No clear seasonal rhythm was revealed in the spawning frequency of broodfish, which was not related to concurrent variations in rainfall, water temperature or day length. Although the spawning activity of the giant gourami is often considered as continuous all year round (Bhimachar et al., 1944), seasonal variations in the spawning frequency have been noted, depending on the region or authors (Arfah et al., 2006; Bhimachar et al., 1944; Wijayanti et al., 2009), but relationships between these variations and environmental variables were not documented. In a recent study performed in the West Java Province, targeted questionnaires and interviews also highlighted that fish farmers from Bogor districts report seasonal variation in the reproductive intensity of their giant gourami broodfish, with rain events (possibly together with associated variation in water temperature) leading to a reduction in spawning frequency (Kristanto et al., 2019). In the same study, farmers from the

Tasikmalaya district observed no clear seasonality in giant gourami spawning activity, which the present investigation confirms. In contrast to spawning frequency, the number of eggs collected from the nests were strongly related to environmental factors; it was positively related to rainfall, intermediately and negatively related to water temperature and weakly, and negatively related to day length. To our knowledge, such temporal variation in egg number and their strong relationship with rainfall and water temperature has not been reported previously in the giant gourami. However, seasonal variation in fecundity is known to occur in several tropical species reproducing all year round. In the African catfish *Heterobranchus longifilis*, maximal egg production is observed during the rainy season, whereas in the lagoon tilapias *Sarotherodon melanotheron* and *Tilapia guineensis*, fecundity is highest during the hot, dry season (Legendre, 1992). In *Oreochromis niloticus*, the peak of fecundity corresponds with maximum resource availability and flooding imminence (Duponchelle et al., 2000). Tropical fish species are generally very plastic and can allocate resources to reproduction according to rainfall regimes (Chellappa et al., 2009). In the present study, the giant gourami egg quality was positively, although weakly, related to day length. Prayogo et al. (2018) claimed that experimentally controlled long photoperiods (18L-6D) have a positive effect on the expression levels of vitellogenin genes. It is well known that fish vitellogenins are critical for providing an adequate nutritional reserve for embryonic development and are also involved in other complex functions that influence egg quality (Reading et al., 2018). Nevertheless, further studies are needed to better understand the mechanisms underlying the positive influence of day length observed here on giant gourami egg quality.

As a whole, the present study provides the first detailed long-term investigation on the seasonal variation of the spawning performance of captive giant gourami. We found no clear spawning rhythms in this species in the environmental conditions of the Tasikmalaya area. On the same location and using identical rearing procedures, it was observed that a same female spawns in average every 5 months and that spawning frequency was strongly influenced by broodfish sex-ratio (Arifin et al, 2020). In Tasikmalaya ponds, fluctuations of environmental parameters were limited (particularly temperature) and, as a matter of fact, remained during the whole study in a range favorable to giant gourami gametogenesis and spawning. Nevertheless, in other areas, several studies (*op. cit.*) reported the occurrence of seasonal variation in giant gourami reproduction, probably as a consequence of a locally less stable environment. Further investigation conducted in such area with the same methodology as in the present study would provide complementary view on the environmental control of giant gourami reproductive phenology. By contrast to spawning frequency, the number of eggs per spawn showed greater sensitivity to environmental changes and was strongly related to variations of environmental variables (particularly rainfall and, to a lesser extent, water temperature and day length). Egg quality was also affected by day length. These findings should be taken into consideration when establishing egg production plans in commercial hatcheries. For instance, egg production periods of 6–7 months separated by resting/reconditioning periods of 1 month are recommended in the management of giant gourami spawning ponds (Arifin et al., 2020). As far as possible, it is therefore recommended for optimizing egg production to synchronize the broodfish resting/reconditioning periods with the periods of the year during which rainfall and broodfish fecundity are at their lowest.

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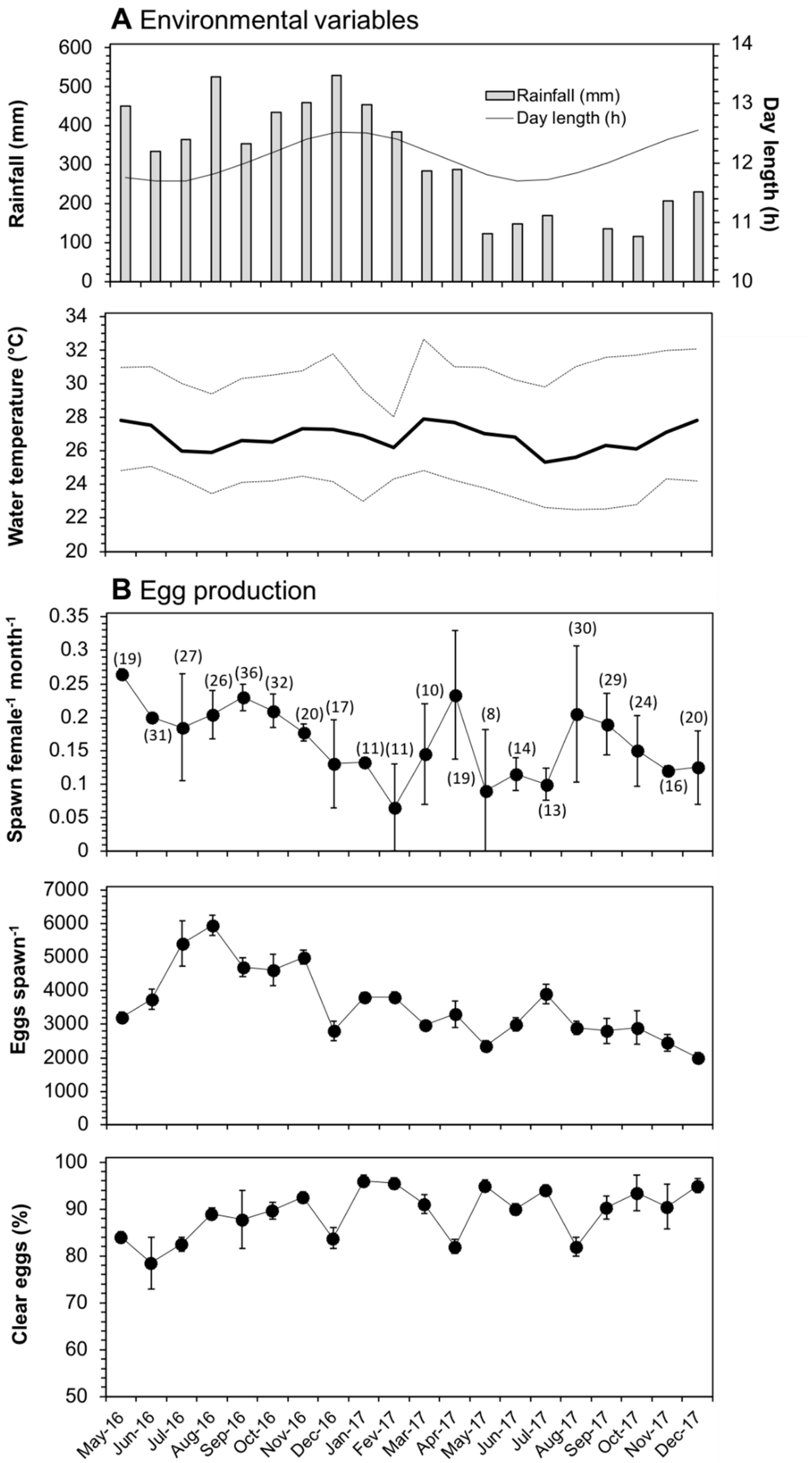
Captions to figure

Figure 1. Mean monthly variation in environmental variables (A) and egg production characteristics of giant gourami in ponds (B) at the BPPSIGN Tasikmalaya Center. A_1: rainfall and day length, A_2: pond water temperature (mean ± extreme instantaneous temperature), B_1: female spawning frequency, B_2: number of eggs in a spawn and B_3: egg quality according to the proportion of clear eggs. In graphs B_1, B_2 and B_3, vertical bars indicate the range between the means recorded in the two compartmentalized ponds. Due to the occurrence of periodic reconditioning periods, the number of females actually involved in reproduction each month was not constant and varied between 72 and 156 individuals (127 females per month on average). The total number of spawns collected per month is given in parentheses (graph B_1).


A Environmental variables
Rainfall (mm)
Day length (h)
Water temperature (°C)
B Egg production
Spawn female-1 month-1
Eggs spawn-1
Clear eggs (%)
(19)
(31)
(27)
(26)
(36)
(32)
(20)
(17)
(11)
(11)
(10)
(19)
(8)
(14)
(13)
(30)
(29)
(24)
(16)
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May-16
Jun-16
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Fev-17
Mar-17
Apr-17
May-17
Jun-17
Jul-17
Aug-17
Sep-17
Oct-17
Nov-17
Dec-17

364 **Figure 1**

Table 1. Summary of water quality parameters measured in the ponds during the 20-month experiment.

Parameters[1]	Mean ± SD (Range)
DO (mg L^{-1})	5.2 ± 1.3 (3.8-7.3)
pH	7.6 ± 0.2 (7.5-7.9)
Conductivity (µS cm^{-1})	180 ± 8 (171-188)
Turbidity (NTU)	9 ± 3 (9-13)
NH_3 (mg L^{-1})	<0.0065
NO_2^- (mg L^{-1})	0.010 ± 0.005 (0.006-0.020)
NO_3^- (mg L^{-1})	0.41 ± 0.12 (0.26-0.59)

[1]DO: dissolved oxygen

Table 2. Results of multiple linear regression models assessing the effects of environmental variables (i.e. daily rainfall, water temperature and day length) on the reproductive performance (i.e. female spawning frequency, egg number and egg quality) of captive giant gourami broodfish.

A - Spawning frequency				
Multiple R^2: 0.08	Estimate	SD	t-value	p-value
Intercept	0.7356	0.6163	1.194	0.241
Daily rainfall	0.0032	0.0027	1.183	0.245
Water temperature	0.0093	0.0180	0.520	0.606
Day length	-0.0707	0.0459	-1.541	0.132
B - Number of eggs per spawn				
Multiple R^2: 0.71	Estimate	SD	t-value	p-value
Intercept	34172.81	5151.12	6.634	**< 0.0001**
Daily rainfall	180.07	23.15	7.777	**< 0.0001**
Water temperature	-678.61	154.70	-4.387	**< 0.001**
Day length	-1172.40	393.08	-2.983	**< 0.01**
C - Proportion of clear eggs				
Multiple R^2: 0.24	Estimate	SD	t-value	p-value
Intercept	14.356	42.061	0.341	0.735
Daily rainfall	-0.217	0.189	-1.148	0.260
Water temperature	-1.678	1.263	-1.328	0.194
Day length	10.034	3.210	3.126	**< 0.01**