SKELETOCHRONOLOGY OF ASIAN GRASS FROG
Fejervarya limnocharis (GRAVENHORST, 1829) FROM JAVA
TO SUPPORT MANAGEMENT CONSERVATION

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ABSTRACT
Asian grass frog Fejervarya limnocharis is being utilized as pets, for laboratory experiments,
for a mixture of traditional medicine and for cuisine. The harvest of F. limnocharis in high volume can
threat its population. Biological data such as the age when the specimens are harvested is valuable
information to manage the harvesting system in sustainable way. We conducted the skeletochronology
technique using paraffin methods and hematoxylin staining from 69 samples (46 males, 21 females, 2
juveniles). The results showed that the age harvested male ranged from 1 to 3 years old, while the
female ranged from 2 to 3 years old. The snout-vent length (SVL) of harvested specimens ranges
between 39.84−52.37 mm for both sexes. We propose an intervention in the harvesting system by
limitation of the size for harvested specimens to at least 46 mm. In this minimum size, individuals of
F. limnocharis have reproduced several times and have contributed to the population in the wild.

Keywords: Age determination, Asian grass frog, Fejervarya limnocharis, Java, skeletochronology

INTRODUCTION
Asia is known as one of the world largest exporters of frogs. They are used as
pets, for laboratory work (Weldon et al., 2007), traditional medicine (Rowley et al., 2010),
and cuisine (Kusrini, 2005; Kusrini & Alford, 2006a). The export of frogs’ legs from
India for consumption is the highest in the world (see Fig. 1), followed by China, Taiwan
and Vietnam (Altherr et al., 2011). The largest species being harvested are Fejervarya
cancrivora, F. limnocharis (see Fig. 2) and Limnonectes macrodon (Kusrini & Alford,
2006a).

The Indonesian Scientific Authority recommended the harvest quota for Fejervarya
cancrivora legs in 2018 was 80,096,450 individuals. For the F. limnocharis was 700
individuals while for L. macrodon is not allowed being harvested due to a significant decrease
of wild population (E. Sulistyadi, pers.com. 2018). In the case of F. limnocharis the real
harvest of wild population are much higher than the recommendation (Kusrini, 2005). The

Although under the International Union for Conservation of Nature (IUCN) red list
data, the species is listed as the least concern, high exploitation might decrease its natural
population and therefore may threaten the existence of the species (Kusrini & Alford, 2006a).
Thus, conservation management is needed to protect this species from extinction.

Age status related to reproductive conditions of any species being exploited plays a
 crucial point in balancing the natural ecosystem. There are three common methods used for
Figure 1. Frog legs: A. for consumption, B. the frog legs are sorted based on the size (Photograph: A. Hamidy).
age determination i.e. age and body size correlation, mark and recapture, and skeletochronology. Skeletochronology is the most appropriate and accurate method in several species (Eden et al., 2007). This method is based on analyzing the number of lines of arrested growth (LAG) in the cross-section of the frog’s bone (Khonsue et al., 2000; Eden et al., 2007). The method has been used to determine the age of several amphibian species in the temperate zone (Guarino et al., 2003), subtropical region (Guarino et al., 1998), and tropical region (Khonsue et al., 2000; Kumbar & Pancharatna, 2001).

Information regarding age status to body size of *Fejervarya limnocharis* in Indonesia was unavailable. Therefore it is important to have this data to support the management of the conservation program in Indonesia.

![Figure 2](image_url). Life specimen of *Fejervarya limnocharis* (Photograph: A. Hamidy).
MATERIALS AND METHODS

Sampling method

The specimens were obtained from the Dramaga village, Bogor, West Java. Frogs were collected in a paddy field in December 20–23, 2013, in the middle of the wet season, coinciding with the annual peak of reproductive activity of this species. Sampling was performed using encounter visual survey. We collected 69 alive individuals (46 males, 21 females, 2 juveniles) of *Fejervarya limnocharis* and preserved them in 96% ethanol. For each specimen, sex, total snout-vent length (SVL) to nearest mm were analyzed using a digital caliper (Mitutoyo, Japan). All samples were cut at the third phalanges of the third finger and fixed in the 70% ethanol for skeletochronology.

Skeletochronology

Skeletochronology processes were carried out according to Liao & Lu (2010) using the paraffin method. The modification was done on the decalcification process, substituting nitric acid to formic acid. The samples were washed in running tap water overnight and then were decalcified in 10% of formic acid for eight hours. Later, samples were rinsed in running tap water overnight, followed by staining in hematoxylin for 75 minutes, and then washed in tap water for 30 minutes. The samples were dehydrated in serial ethanol concentrations (70, 80, 90%, absolute, respectively), each for 1 hour and were cleared in xylene for an hour and were infiltrated in liquid paraffin for 75 minutes. The samples were embedded in paraffin and hardened in room temperature and sectioned in 15 um thickness with a rotary microtome (Yamato RV-240). Samples were deparaffinized and mounted by enthelan and then were observed under a compound microscope at 200x magnification (Nikon Optiphot 2, Japan) attached to a computer for picture analysis. Age was determined by counting the number of lines in the phalangeal bone, where a single LAG was considered as one year old.

RESULTS

The histological performance of *Fejervarya limnocharis* in this study showed distinct faint lines in the bone which can be used to estimate the age of the individual (Fig. 3B–D). However, discontinued patterns of LAG from several samples were noticed, presumably because their habitat changes from the wet rice field to dry field (Fig. 3B–D).

Of the 69 samples being analyzed, the distribution age of harvested individuals showed a dominance (66%) in the age of two years old and followed by the age of three. The SVL measurement was slightly larger in female than in the male, with the range of SVL size wider in both sexes as they aged (Fig. 4). Wider variation was prominent from the samples of 4 and 5 years old.

There was a tendency of linear relation between the increases of SVL to the number of LAG in both sexes (Figs. 4 & 5), though the correlation was considered low in both sexes and
Figure 3. Histology of adult *Fejervarya limnocharis* phalanges: A. No LAG, B. 1 LAG (arrow).
Figure 3. Histology of adult *Fejervarya limnocharis* phalanges: C. 2 LAGs (arrows), D. 3 LAGs (arrows).
Figure 4. Growth curve of male and female *Fejervarya limnocharis* (two samples from the juvenile group were dropped from the analysis).

Figure 5. Overall growth curve of *Fejervarya limnocharis*. 
when it was combined. Sexual maturity in males was detected at one year old, based on the visibility of vocal sac, and in the female at two years old by examination of the gonad.

**DISCUSSION**

Indonesia is the primary exporter of frogs’ legs. In 1992, the export reached 5,600 tonnes and progressively declined to 3,800 tonnes in 2002. The largest harvest area were in the paddy field of Karawang, West Java (Kurniati & Sulistyadi, 2016). The exported specimens was determined by the size. However, capture for local consumption does not distinguish the body sizes (Kusrini & Alford, 2006a).

Age determination for skeletochronology can be used to understand the correlation between age and body size. This study showed that the skeletochronology figures of *F. limnocharis* phalanges from Indonesia were reliable but needed to be treated with caution. The finding is similar to Kusrini & Alford (2006b) for *F. limnocharis* and *F. cancricivora* and to Khonsue et al. (2000) for *Rana nigrovittata*. The formation of line arrested growth (LAG) in the bone is caused by calcium accumulation. The calcium accumulation is influenced by the availability of environmental calcium and the physiological process of each individual. Another factor that influences LAG formation is the annual life activity cycle (hibernating and activating; Eden et al., 2007). Since tropical frogs do not experience hibernating processes in their lifecycle, this has resulted in faint lines in the tropical frog’s bone. However, early studies suggested that lines forming in tropical frogs might occur in a dry season when food availability declines so the foraging activity of frogs also decreases (Reagan & Waide, 1996). Low activity of frogs during the dry season can produce line formation in frog’s legs. Genetic factors also support the LAG formation in the bone (Federico et al., 2001).

The results showed that the female SVL was larger than male at the same age. Females *F. limnocharis* grow faster than males (Shou et al., 2005; Liu et al., 2012). The large females breed explosively for extremely prolonged periods, so they will survive in the reproduction cycle (Woolbright, 1983). Geographical variation, climate, pollution, and altitude can also influence the SVL (Thammachoti et al., 2012; Lu et al., 2012).

The relation between age and body size can predict the size of reproductive stages. The current study was concordant with *F. limnocharis* in India (Pancharatna & Deshpande, 2003). The dominant LAG numbers in the current study were three, reflecting in age as three years old. Pancharatna & Deshpandhe (2003) reported that the natural lifespan of *F. limnocharis* in southern India was only up to 4 years in both sexes, slightly different from the subtropical *F. limnocharis* in China, which had a lifespan of three years old for males and four years old for females (Liao et al., 2011). These differences are attributed to many factors such as habitat or environmental condition.
Monitoring and management of harvested populations are required to maintain the stability of frog populations in the wild. The maximum SVL of the frog that were commercially harvested in Indonesia was 78 mm, with a maximum lifespan of this species of four years (Kusrini & Alford, 2006a). In our study, the maximum age was three years with a maximum SVL of 52.37 mm. Based on the current results, it was estimated that the reproductive body size of this species in Indonesia is between 42.46–46.01 mm or coincides with the age of 2 years.

Taking into account the reproductive age of this species is between 2–4 years (Phancharatna & Deshpande, 2003), there should be a benchmark of the maximum size the frog can be harvested. For Indonesia, a minimum harvest size of SVL 46 mm is suggested as a recommendation.

CONCLUSION

Skeletochronology can be used to estimate the age of Fejervarya limnocharis in the tropical region. To maintain the sustainability of wild population, harvest size can be recommended from 46 mm.

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