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Reproductive Characteristics of Female Egg-carrying Buntingi, *Xenopoecilus oophorus*, an Endemic Fish to Lake Poso in Central Sulawesi

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Abstract

Reproduction characteristic of female egg-carrying buntingi, *Xenopoecilus oophorus* had been studied. This research was conducted at Lake Poso, Central Sulawesi, Indonesia. Specimens were collected monthly from August 2012 to July 2013 at four sampling stations around the lake. Macroscopic observations of ovarian maturity level and gonadosomatic index revealed a long reproductive period during the rainy season, with four spawning peaks in November, January, February and April. The highest total fecundity was 135 oocytes, and the highest batch fecundity was 36 oocytes. Analysis of the oocyte diameter frequency distribution showed *X. oophorus* is a multiple spawner. Batch fecundity was correlated (r = 0.78) with body weight.

Abstrak

Karakteristik Reproduksi Ikan Endemik Rono, *Xenopoecilus oophorus* betina, di Danau Poso, Sulawesi Tengah. Penelitian ini mempelajari karakteristik reproduksi ikan betina endemik rono, *Xenopoecilus oophorus*, betina di Danau Poso, Sulawesi Tengah. Penelitian dilakukan di Danau Poso Sulawesi Tengah, Indonesia. Sampel dikumpulkan setiap bulan mulai bulan Agustus 2012 sampai bulan Juli 2013 di empat stasiun. Berdasarkan pengamatan tingkat kematangan ovarium secara makroskopis dan pengukuran indeks kematangan gonad, diketahui ikan rono memiliki waktu reproduksi yang panjang yang terjadi selama musim hujan. Pada periode reproduksi ini didapati empat puncak waktu pemijahan yakni bulan November, Januari, Februari dan April. Fekunditas tertinggi 135 oosit, sedangkan gugus fekunditas tertinggi 36 oosit. Berdasarkan analisis sebaran frekuensi ukuran diameter oosit diketahui tipe pemijahan ikan rono pemijah berulang. Hubungan gugus fekunditas dengan bobot tubuh tanpa gonad berkorelasi positif (r = 0,78).

Keywords: batch fecundity, egg-carrying buntingi, Lake Poso, multiple spawner, Xenopoecilus oophorus

Introduction

Sulawesi Island is located at the center of the Indonesian Archipelago and is also known as the heart of the Wallacea area. Lake Poso and the Malili Lakes system at Sulawesi Island are known as the ancient lakes of Southeast Asia [1]. Inhabited by many species of endemic fishes, these lakes are home to many shrimp and gastropod species as well [2,3].

Lake Poso is an oligotrophic lake formed through tectonic processes [4]. Several endemic fishes are found in this lake [5-9], such as *Xenopoecilus* spp, a genus that includes four species. Two of these species are reported from Lake Poso – *Xenopoecilus poptae* (Weber & de Beaufort, 1922) and *Xenopoecilus oophorus* (Kottelat, 1990) (Figure 1) – while the remaining two species from

Figure 1. *Xenopoecilus oophorus* 76 mm TL

Lindu Lake are *Xenopoecilus bonneorum* (Parenti, 2008) and *Xenopoecilus sarasinorum* [9]. Even so, *X. poptae* and *X. oophorus* had been listed as critically endangered and endangered species in the IUCN Red List [32].

Xenopoecilus oophorus species is still commonly found in Lake Poso. A sought-after food fish for the communities living around the lake, *X. oophorus* has been found near the northern, southern, eastern and western shores of Lake Poso [10].

This fish has unusual reproductive characteristics, including its form of parental care. Females incubate their eggs under their pelvic fins until they hatch [5,7,11]. Gundo, *et al.* [11] reported sexual dimorphism, which can be used to distinguish male and female *X. oophorus* based on the pelvic fins of females being longer than those of males. The largest ovarian size is about 5 mm long and 3 mm wide. The length-weight relationship in *X. oophorus* has also been studied. The fish range from 41 to 86 mm in length and from 0.46 to 6.14 g in weight [12].

An ovary with oocytes at several stages of development can be classified as asynchronous [13,14]. Fish with asynchronous oocyte development patterns can spawn several times during one season [15]. Thus the fecundity of fish with this multiple spawning can be determined by counting the number of eggs in the batches that released during a single spawning event. According to Murua *et al.* [16], an estimate of batch fecundity can also be obtained by counting the number of maturing oocytes currently undergoing hydration.

Mekkawy and Hassan [17], Gutiérrez-Estradaet, *et al.* [18] stated that fish reproductive parameters are vital tools for fisheries management. Fish life-cycle theory is based on the study of aspects such as fecundity, egg size, sex ratio, length at maturity and gonad maturation patterns. Studies on the biological reproduction of *X. oophorus* were very limited, even though such information is crucial to determining the form and direction of management needed to ensure the continued survival of this endemic species. In order to address this lack of information, this study aimed to analyze the reproductive characteristics of the *X. oophorus* in Lake Poso.

Materials and Methods

This research was conducted from August 2012 to July 2013 at Lake Poso, situated in Poso District, Central Sulawesi, Indonesia, at 1^{o}44’-2^{o}04’ South and 120^{o}32’-120^{o}43’ East. Four sampling stations were chosen: in the northwestern corner, the northeastern corner (close to the outlet), and along the east and west banks of Lake Poso (Figure 2).

Specimens were collected monthly during the 12-month study period. Sampling was carried out at night using traditional light-fishing gear. Small traditional boats were equipped with pressurized kerosene lamps firmly attached to the bow. Due to positive phototaxis, *X. oophorus* were attracted to the light [5]. Once enough fish had gathered, they were collected using a scoop net. A sample of about 50 fish was selected at random from the catch at each station and preserved in bottles containing a 5% formalin solution. These samples were transported to the laboratory for further analysis.

For each specimen, the body weight (digital scales with 0.01 g precision) and total length (ruler with 0.1 mm precision) were measured. The fish were then dissected to remove the ovaries, which were weighed using digital scales (0.0001 g precision). Gonad maturity of female specimens was determined macroscopically based on the descriptive scale in Gundo, *et al*. [11] (Table 1). The gonadosomatic index (GSI) was determined as follows: GSI = gonad weight/body weight x 100.

Table 1. Descriptive Ovarian Maturity Scale Macroscopically of *Xenopoecilus oophorus*

Gonads Maturity Scale	Description
Initial Development Phase (Phase I)	The ovary is very small, milk white in color and occupy about 1/6 of the abdominal cavity. The surface is smooth, and eggs are not yet clearly visible. The ovary is shaped like a slightly elongated oval sack; the anterior end is more pointed than the posterior end.
Development Phase (Phase II)	The ovary is larger than in the initial development phase, occupy about 1/3 of the abdominal cavity. Color is somewhat yellowish, and egg granules are clearly visible. The ovarian envelope has begun to fill out, and the anterior end is somewhat more rounded but still more pointed than the posterior end. Blood vessels start to become visible in the outer wall of the gonad.
Maturation Phase (Phase III)	The ovary is larger than in the development phase, occupy almost half of the abdominal cavity and dark yellow in color. The eggs (oocytes) are very clearly visible, the ovarian envelope appears full and distended, the anterior part of the gonads is oval-shaped (rounded), and blood vessels in the outer wall of the gonad are clearly visible.
Spent Phase (Phase IV)	The ovary is smaller than in the maturation phase, and it appears shrunken and pale yellow. The anterior end of the gonad is again somewhat pointed. A few eggs are generally left behind.

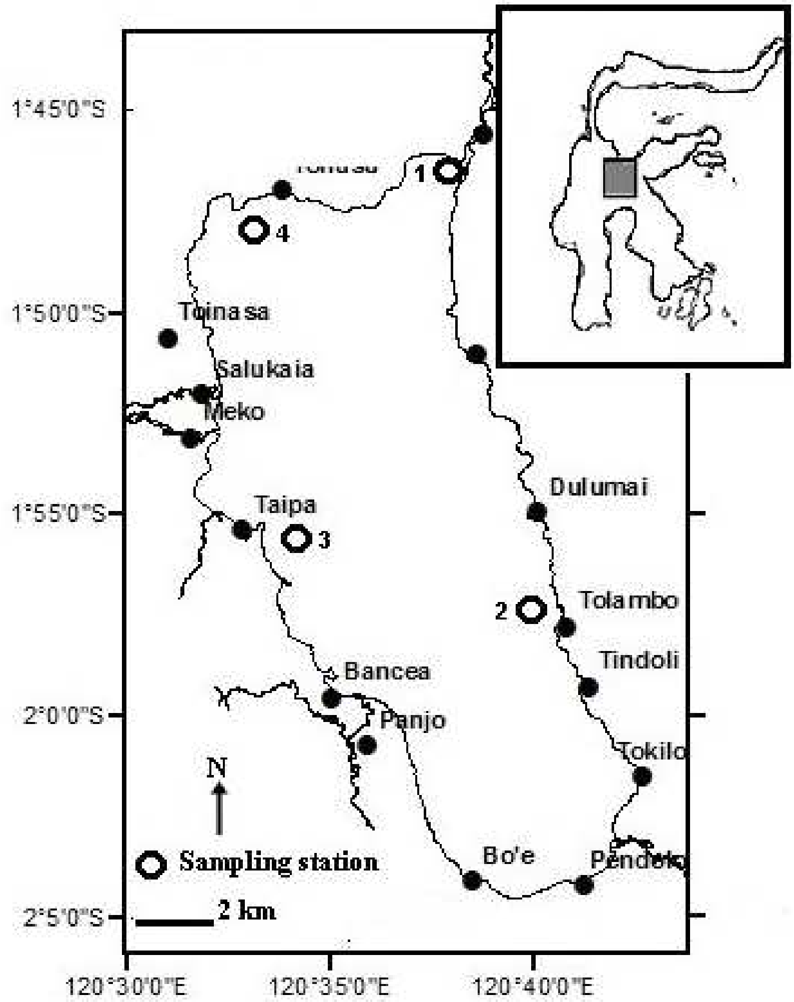

Figure 2. Map of the Lake Poso Study Site and Sampling Stations

Total fecundity was measured directly by counting all the oocytes in each mature ovary. Batch fecundity was estimated by counting the number of eggs considered likely to be discharged in a single spawning using the oocyte size-frequency distribution (OSFD) method in Hunter, *et al.* [15]. All oocytes in the selected ovary were separated from the ovigerous folds (ovarian lamellae) and placed on a glass slide for counting and measuring. The eggs were then arranged in long, straight and narrow rows, and counted under a binocular microscope with a magnification of 40x.

Egg diameter was measured using an ocular micrometer, and the measurements were converted into mm, producing an egg diameter frequency distribution. Oocytes in the group around the foremost (largest diameter) mode were counted to provide the current batch fecundity, or number of eggs to be spawned in the forthcoming season. To investigate this species' spawning pattern, a total of three ovaries were selected representing three phases: development, maturation and spawning/spent.

Data on the number of eggs per batch (Fi) and the ovary-free weight (Wi) measured for the ith fish were used to fit a linear model, which explained the data satisfactorily in terms of residual properties and co-efficients of determination [19]: $Fi = a + bWi + i$.

Rainfall data were obtained from two stations sited at the northern and southern extremities of Lake Poso.

Results and Discussion

Gonad maturity, GSI, and spawning season. The total number of female *X. oophorus* specimens collected and analyzed during this study was 566. The gonad maturity of these fish ranged from early development to the spent phase. However, there were four peak months with a high percentage of female fish with maturing gonads (Phase III). These were November 2012 and January, February, and April 2013, with percentages of 59.4%, 51.8%, 56.3%, and 58.3%, respectively (Figure 3).

The GSI of female *X. oophorus* fluctuated during the year (Figure 4), ranging from 0.03 to 1.94. The highest average GSI value (1.00) occurred in November 2012 and the lowest in October 2012 (0.55). In general, GSI values were higher in the rainy season than in the dry season. There were at least four peak months for GSI during the rainy season (November to April), with GSI values of 1.00, 0.85, 0.86, and 0.86, respectively.

The *X. oophorus* designation as a multiple spawner is further supported by the gonad maturity analysis and GSI data. These data show that *X. oophorus* has a long reproductive period during the rainy season, with peaks in November, January, February and April. Similar long reproductive seasons are common in other multiple spawners such as *Encrasicholina punctifer* [20], *A. presbyter* [21], *Hippichthys spicifer* [22], and *Odontesthes regia* [23]. The *X. oophorus* reproduction peaks coincide with Lake Poso's rising surface level during the rainy season. The lake's water level is directly influenced by precipitation, and these peaks may be related to the increase in water mass and depth. This could provide additional habitat for the young and reduce the impact of predators.

Observation at the study site revealed that *X. oophorus* larvae tend to be seen in the surface layers of more than 10 meters water depth close to gravel or rocks. Many other tropical fish species utilize the rainy season as their spawning period, because various phenomena during this season can promote reproductive success. Crampton [24] reports that fish of the genus *Symphysodon* spawn when water levels rise in the Amazon flood plain to ensure abundant food sources for their offspring and protection from predators.

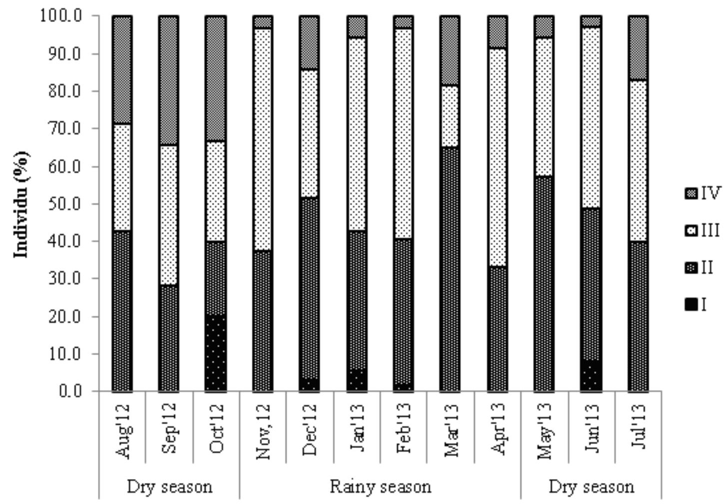

Figure 3. Seasonal Variation in the Gonad Maturity of Female *X. oophorus*: I) Initial Development Phase; II) Development Phase; III) Maturation Phase; IV) Spent Phase

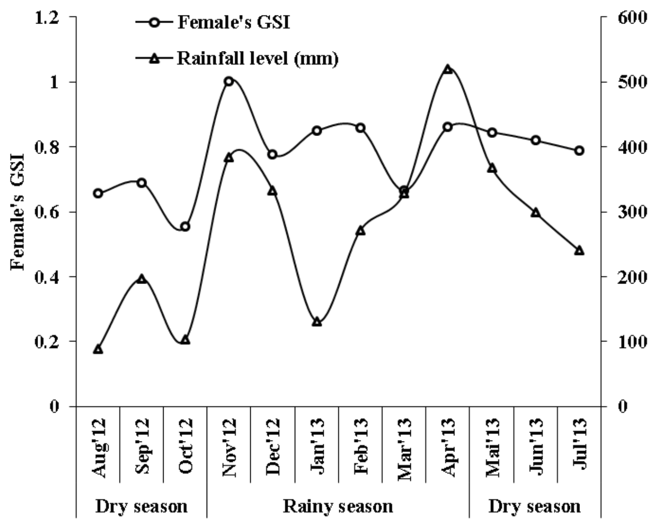

Figure 4. Seasonal Fluctuation in the GSI of Female *X. oophorus* and Rainfall Level

Moreover, it was suggested that runoff from the catchment area and periodic flooding during the rainy season increase food availability. In the case of Lake Poso, rising waters also tend to flood littoral areas where aquatic plants can flourish. Observation has shown that this seasonal vegetation around the banks of the lake can provide important ecological functions as a spawning ground, as well as shelter from predators and a foraging place during larval and juvenile phases. These processes could have particular ecological importance because Lake Poso is an oligotrophic lake, which is poor in nutrients. According to Kolding and van Zwieten [25], the littoral or riparian zone of a lake can become a highly productive transition zone or ecotone due to interactions between aquatic and terrestrial processes associated with fluctuations in water level.

Fecundity and spawning type. The fecundity analysis was conducted on data from 267 female *X. oophorus* with ovaries in the development or maturation phases. The standard length range of the fish analyzed was 60 to 86 mm with a body weight range of 1.77 to 4.98 g. Total fecundity (F) ranged from 33 to 135, and batch fecundity (F_g) from 20 to 36. The number of eggs incubated by brooding females ranged from 23 to a maximum of 33. The highest average fecundity and batch fecundity were observed in April with F = 82 ± (19.5 = SD), and F_g = 24± (5.7= SD). The relation of batch fecundity (F_g) with body weight (W_b) was: $F_g = -10.539 + 8.1372Wb$ (r = 0.78) (N =267).

The frequency distribution of oocyte diameter in the three ovaries selected (representing the development, maturation and spawning/spent phases) had three distribution modes for the development and maturation phase but two modes for the spent phase (Figure 5). The oocytes' measured diameter ranged from a minimum of 0.13 mm to a maximum of 1.32 mm (Figure 5). Hunter, *et al.* [15] stated that oocytes are usually found at almost all stages of development in the ovaries of multiple-spawner fish that have reached maturity. Sizes range from immature oocytes to oocytes with well-developed yolks. In terms of diameter distribution, there is rarely any great distance between oocyte maturity classes, except for a short-term gap between hydrated oocytes and oocytes with yolks. These fish usually have a long reproductive period and spawn multiple times during one season.

The frequency distribution of *X. oophorus* oocyte diameter (Figure 5) shows little distance between maturation classes. Ripe oocytes ready to be released were in class intervals from 0.93-1.02 mm to 1.23-1.32 mm (Figure 5b). Based on these data, it can be concluded that *X. oophorus* females can produce eggs (spawn) several times in a single spawning season. This finding reinforces the previous finding by Gundo *et al.* [9] that this fish's ovaries belong to the asynchronous type, and leads to the conclusion that this fish can be classified as a multiple spawner.

According to Hunter, *et al*. [15], results from measuring batch fecundity using oocyte diameter frequency distributions indicate that the total number of oocytes within the most advanced modal group is considered to be the spawning batch. The distribution pattern of oocyte diameters shows that *X. oophorus* spawns in batches. This conclusion is further reinforced by the observation that batch fecundity (20 to 36 eggs per female) was similar to the number of eggs incubated (23 to 33 eggs per female).

Some other species of endemic freshwater fish of similar size to *X. oophorus* are also partial spawners; for

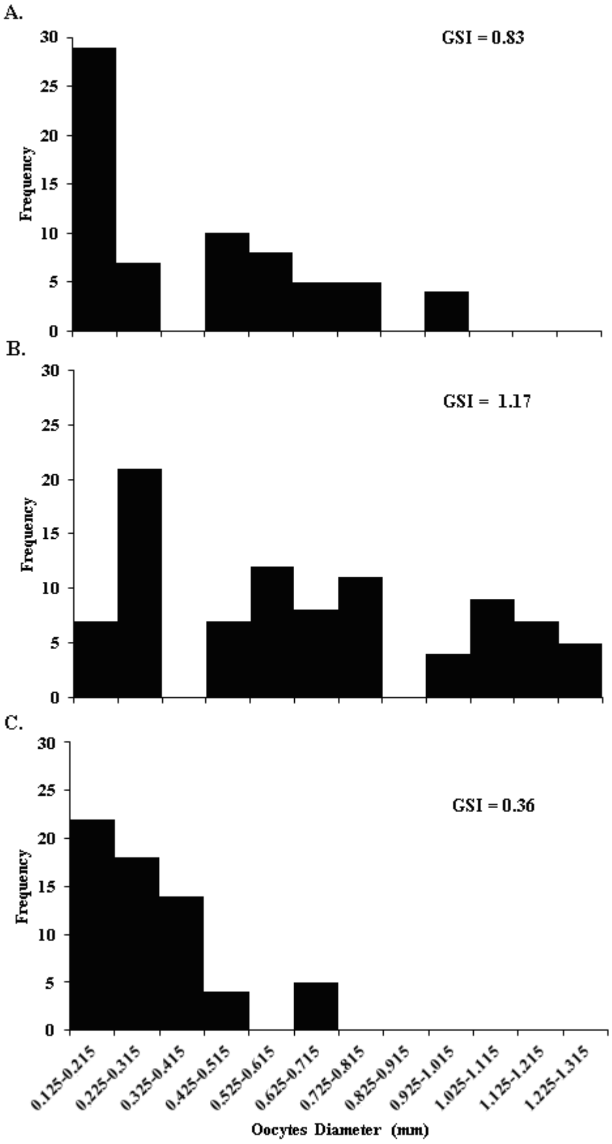

Figure 5. Frequency Distribution of Oocyte Diameter in Female *X. Oophorus*: A) Development Phase; B) Maturation Phase; C) Spent Phase

example, *Melanotaenia seachemensis, M. splendina* [26], *T. celebensis* [27], and *Glossolepis incisus* [28]. These species have much higher fecundity (thousands of eggs per individual) compared to *X. oophorus*. This relatively low fecundity is thought to be related to the reproductive pattern of *X. oophorus*, a bearer fish incubating embryos under the pelvic fins.

According to Lowe-McConnell [29], many tropical fish that guard their eggs and larvae, including mouth-brooders, tend to have smaller egg clusters compared to fish that simply release their eggs. Mature *X. oophorus* oocyte diameters ranged from 0.93 to 1.32 mm, larger in proportion to body size than other egg bearers such as *Aidablennius sphynx* [30] and *Microphis brachyurus* [31], which have mature oocytes measuring 0.7 to 0.9 mm and 0.8 mm, respectively. Thus the relatively large oocyte diameter in *X. oophorus* is part of the reproductive strategy of this fish to ensure that the larvae can survive and reproduce in their turn.

Xenopoecilus oophorus has a long reproductive period during the rainy season and can be classified as a multiple spawner that releases mature oocytes in batches. This species also exhibits parental care, as the females bear the eggs under their pelvic fins, incubating them until they hatch. Batch fecundity was strongly correlated with body weight. As females with mature and maturing ovaries grow and gain weight, their batch fecundity also increases. Thus in *X. oophorus,* the body weight can be used to estimate batch fecundity.

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