



REPRODUCTIVE BIOLOGY OF *GARRA REGRESSUS* AND *GARRA TANA* (CYPRINIFORMES: CYPRINIDAE) FROM LAKE TANA, ETHIOPIA

Akewake Geremew¹, Abebe Getahun² & Eshete Dejen³

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¹ Department of Biology, Dilla University, P.O. Box 419, Dilla, Ethiopia

² Department of Zoological Sciences, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia

³ Agriculture and Environment Division (AED), Inter-Governmental Authority on Development (IGAD), Djibouti

¹ khaliger@yahoo.com (corresponding author), ² abebe12002@yahoo.com, ³ eshete.Dejen@igad.int

Abstract: The reproductive biology of *Garra regressus* and *Garra tana* was investigated by collecting monthly samples (January to December 2006) from the southern Gulf of Lake Tana, where these species are endemic. *Garra regressus* has an extended breeding time from April to October while *G. tana* breeds throughout the year with a peak from March to July. The mean size at maturity in both the species was not significantly different between the sexes, but *G. tana* had a significantly lower mean size at maturity than *G. regressus* in female specimens. Absolute fecundity estimates for *G. regressus* ranged from 580.8–1800, while those for *G. tana* ranged from 538.9–2968. Egg size frequency distribution revealed that *G. regressus* is a multiple spawner, while *G. tana* is a single spawner. The sex ratio in the total catch of *G. regressus* was found to be skewed in favour of females (Chi-square, $P < 0.05$), while those for *G. tana* was not significantly different from 1:1. The mean size at maturity was not significantly different between the sexes for *G. tana*.

Keywords: Conservation, cyprinid, endemism, spawning, threatened.



Garra regressus



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For Author Details and Amharic Abstract see end of this article.

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INTRODUCTION

Lake Tana, an exceptional hotspot of freshwater fish diversity and endemism in Ethiopia, is an important natural laboratory for evolutionary studies (Sibbing et al. 1998). The lake contains an ichthyofauna represented by seven genera, viz.: *Barbus*, *Clarias*, *Garra*, *Labeobarbus*, *Nemacheilus*, *Oreochromis* and *Varicorhinus*. Out of the 28 species currently recognized, 20 are endemic to the lake and its catchments (Vijverberg et al. 2009). However, most endemic species are facing serious population declines as a result of habitat degradation and overfishing (de Graaf et al. 2006; Darwall et al. 2011). An important group of fish threatened by the above stressors in Lake Tana is the genus *Garra* (Getahun 2010a,b; Darwall et al. 2011), represented by four species (*Garra tana*, *G. regressus*, *G. dembecha* and *G. dembeensis*), of which two (*Garra tana* and *G. regressus*) are endemic to the lake (Stiassny & Getahun 2007).

While *G. regressus* mainly inhabits the sub-littoral zones with rare occurrences in the pelagic and littoral zones, *G. tana* occurs in the pelagic zone with rare occurrences in the littoral zone of the lake (Geremew 2007). Both species are, however, absent from the

tributary rivers of Lake Tana indicating its lacustrine adaptation (Geremew 2007). The two species are heavily impacted by overfishing primarily through by-catch, and have been listed as 'Vulnerable' in the IUCN Red List of Threatened Species (Getahun 2010a,b).

The purpose of this paper is to investigate the various aspects of the reproductive biology of *G. regressus* and *G. tana* from Lake Tana, including sex ratio, gonadosomatic index, fecundity, spawning season, and maturity so as to generate baseline information for conservation action.

MATERIAL AND METHODS

Study Area

Lake Tana (12°N & $37^{\circ}21'\text{E}$) is Ethiopia's largest lake (3150km^2 that accounts for 50% of the lacustrine area in Ethiopia). It is formed by the volcanic blocking of the Blue Nile and is situated in the northwestern highlands, 560km north of the capital, Addis Ababa, at an altitude of 1830m (Nagelkerke et al. 1994). It is a shallow, oligo-mesotrophic lake (Dejen et al. 2004) fed by seven big perennial rivers with the Blue Nile being its only out-flow (Fig. 1). The Lake Tana basin is isolated from the lower parts of the Nile basin by a 40m high waterfall

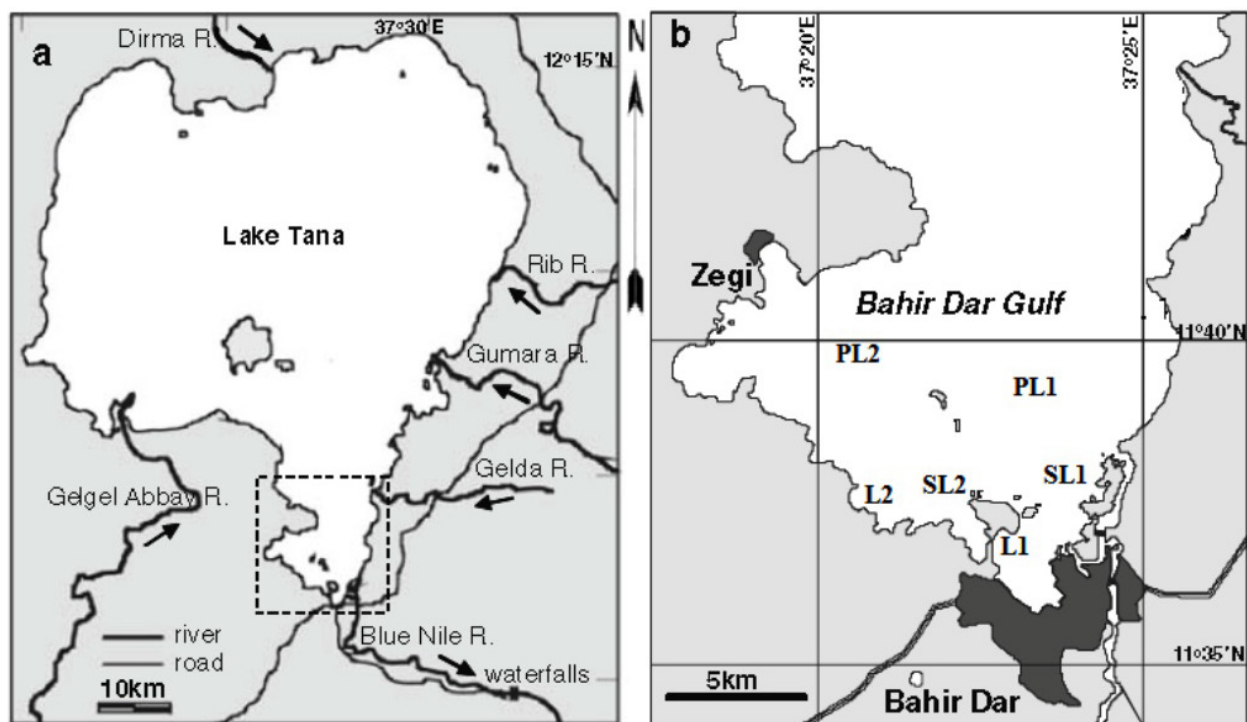


Figure 1. (a) Map of Lake Tana and (b) Sampling stations in the Southern Gulf of Lake Tana with three macro-habitats Littoral (L1: Gerima and L2: Airport), Sub-littoral (SL1: Debremariam and SL2: Bahita) and pelagic (PL1: Kentefami and PL2: Kibran-Zegie) selected for sampling (Modified after Eshete Dejen et al. 2009).

30km downstream from the Blue Nile out-flow. The waterfall interestingly not only isolates the Lake Tana basin from the lower Blue Nile basin but also isolates its ichthyofauna (Thieme & Brown 2013). The Lake Tana basin with a catchment area of 16,500km² is known to be a dendritic type of drainage network (Dejen 2003).

The main rainy season is between June and October, with a peak in August (Dejen 2003), although exceptionally minor rains occur in February and March. The water temperature in 2006 (study period) was moderately warm with a mean of above 20°C throughout the year.

The mean maximum and minimum air temperature was above 23°C and below 15°C (Fig. 2), respectively. Differences between the mean maximum and the mean minimum air temperature were less pronounced during the rainy season.

Sampling and measurements

Sampling was conducted in three macro-habitats, littoral (0–4 m depth), sub littoral (4–8 m depth) and pelagic (8–10 m), respectively. Within these three macro-habitats, six sampling stations (Fig. 1) were selected (two stations for each macro-habitat) in the Southern Gulf of Lake Tana.

Monthly fish samples were obtained using ‘NORDEN’ multi-mesh monofilament gill nets (Lundgrens Company Sweden) of mesh sizes 4, 6, 10, 14 and 15.5 mm. Samples of *G. regressus* (n=149) and *G. tana* (n=745) were collected and measured for their standard length (SL) to the nearest 0.1mm, total weight (TW) and gonad weight (GW) to the nearest 0.1g. The sex of each specimen was recorded and stage of gonad development was also

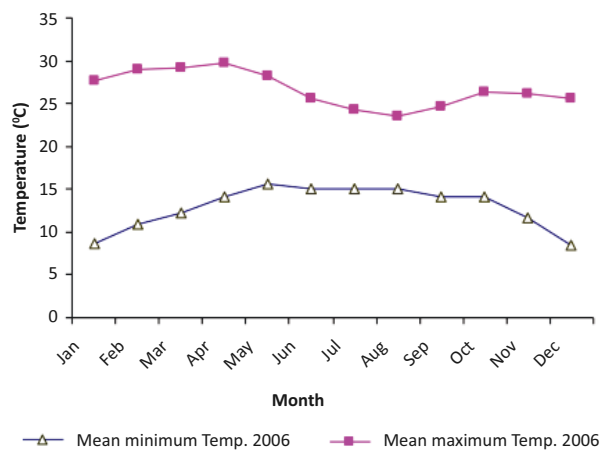


Figure 2. Seasonal variation in mean maximum and minimum air temperature (°C) of Lake Tana (Bahir-Dar) from January to December 2006 (Data from Ethiopian National Meteorological Agency).



Image 1. *Garra tana*



Image 2. *Garra regressus*

determined (Images 1 & 2).

As both species are listed as threatened in the IUCN Red List, collections were made responsibly following IUCN 2008 guidelines for studying species at risk (IUCN 2009). The passive gear used in this study was set only for two hours at each sampling station once per month to collect the fish samples. Furthermore, the fecundity of both species is high and hence, the scientific sampling is not likely to affect the population of either species significantly.

Breeding season, sexual maturity, fecundity, egg size determination and sex ratio

The breeding season was determined from monthly

frequency of female fish with ripe gonads (stage IV) and the gonadosomatic index (GSI). A five-stage gonad maturity scale was considered following Weyl & Booth (1999). Accordingly, the fish were categorized as immature (I), recovering spent or developing virgin (II), ripening (III), ripe (IV) and spent (VI). Gonadosomatic index, which is the gonad weight as a percentage of total body weight, was calculated for ripe stage females.

The mean size at first sexual maturity, i.e., the standard length at which 50% of the males and females mature ($L_{50\%}$), was determined using the software package called "pasgear 2" (Kolding & Skålevik 2010) for both the sexes. Sexual maturity in stage III were considered mature. The percentage of mature fish per length class was calculated and SL_{50} was estimated following Gunderson et al. (1980).

$$P_m = \frac{1}{1 + e^{-(aL+b)}} \times 100$$

Where,

P_m is percent mature (= % Mature) at length L, and bare fitted constants.

The relationship between the percentage of mature fish (P_m) per length class and fish length (Standard Length, SL in mm) was described using a logistic curve:

$$\ln P_m / (1 - p_m) = a + bSL_i$$

where, a is the intercept and b the slope of the curve. From the sigmoid curve $SL_{50\%} = -a/b$ was determined.

Ripe (stage IV) ovaries preserved in Gilson's fluid were considered for fecundity and egg-size determination. Fecundity estimation was carried out using the gravimetric method (Bagenal & Braum 1978). For ova diameter estimation, eggs having a diameter ≥ 0.75 mm were measured to the nearest 0.01mm using an ocular micrometer.

Chi-square test was used to determine general and seasonal variations in sex ratio.

All statistical analyses were performed using Minitab v14 and the results were reported as mean \pm SE (standard error).

RESULTS

Breeding season, sexual maturity, fecundity, egg size determination and sex ratio

Although there was insignificant variation in the mean monthly GSI of female *G. regressus* (ANOVA, $F_{6,36} = 0.9$, $P = 0.507$), the mean values ranged from 7.22 to 14.98 from April to October. Initiation of breeding activity is closely tied to the beginning of the rainy

season, followed by the breeding during the rains (see Fig. 3a and Fig. 4a).

Seasonal variation in the GSI was evident for female specimens of *G. tana*. The mean monthly GSI values ranged from 7.61–17.88. The GSI varied significantly between sampling periods (ANOVA, $F_{11,172} = 3.77$, $P < 0.001$) with higher values recorded between March and June (Fig. 3b). After June, the GSI values progressively dropped, and lower values were recorded during January, October and November. The highest individual GSI (40.45%) as well as the maximum mean monthly GSI (17.94%) in *G. tana* was observed during June. The cycle in GSI was also reflected in the monthly variation in the frequency of females with ripe ovaries (Fig. 4b) with low frequencies encountered during periods of low GSI values. Although, female *G. tana* in breeding conditions were caught throughout the sampling period, considerable proportions were found in the catches from March to July.

There was no noticeable sexual dimorphism in both species. Though it was difficult to observe the spawning behavior, it is possible to indicate the preferred spawning location from the distribution patterns of stage IV females. A higher proportion (78.8%, $n=41$) of stage IV females of *G. regressus* occupied the sub-littoral zone than other regions.

For *G. tana*, the preferred spawning location as seen through the distribution patterns of stage IV females was the pelagic area (87%, $n=269$), and were rarely seen in the littoral habitats.

The mean size at first maturity depicted in Fig. 5 was significantly different between the species only for females, since the confidence interval of males overlapped at SL_{50} (Table 1). Only individuals in the third or higher stages of gonadal maturity have been considered to be mature.

Garra regressus females start maturing earlier (82.6mm) than males (85.3mm). All *G. regressus* males and females over the lengths 124.4mm and 125.0mm respectively are mature.

Table 1. Mean length at first maturity estimated for *Garra tana* and *G. regressus*. Number of specimen (n), Parameter values for b from logistic regression, mean length at maturity (SL_{50} in mm, $-a/b$) and 95 % confidence limit of SL_{50} .

Species	Sex	n	L50	Lower	Upper	b
<i>Garra regressus</i>	M	45	100.9	98.1	103.1	2.81
<i>Garra regressus</i>	F	64	103.6	101.4	106.6	2.63
<i>Garra tana</i>	M	282	100.5	98.6	101.5	2.3
<i>Garra tana</i>	F	351	100.0	98.5	100.9	2.24

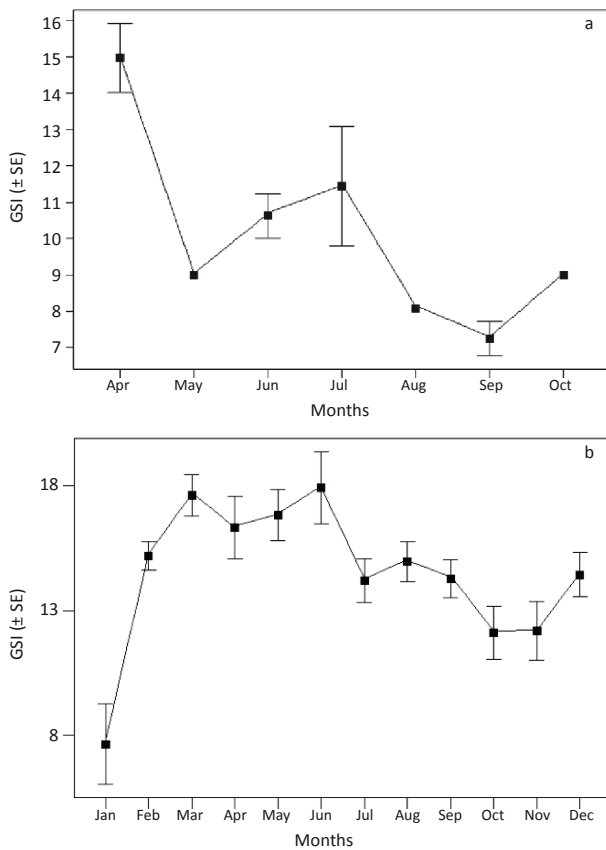


Figure 3. Temporal variation in gonadosomatic index (GSI ± SE) for females of (a) *Garra regressus* and (b) *G. tana* from Lake Tana.

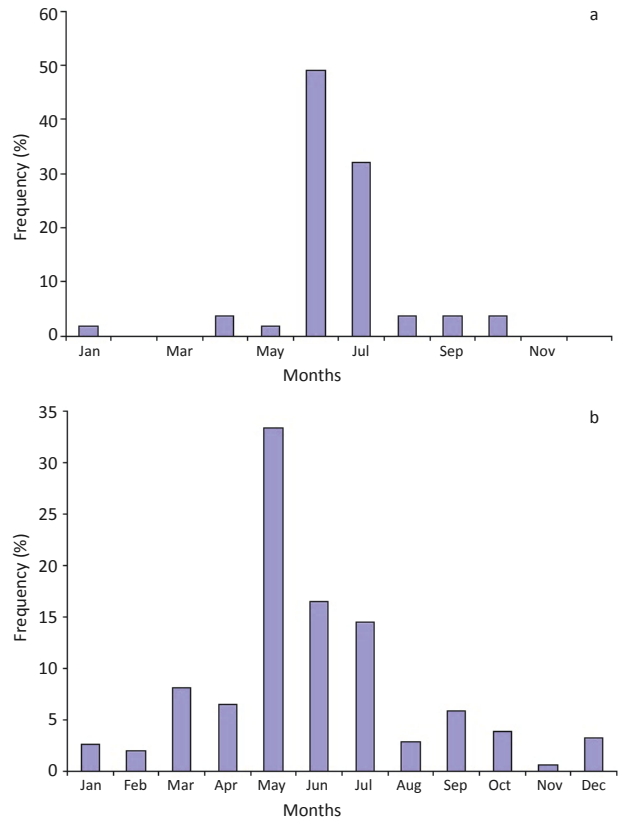


Figure 4. Temporal variation in frequency (%) of ripe female occurrence for (a) *Garra regressus* (b) *G. tana* from Lake Tana.

In case of *G. tana*, both sexes start maturing at approximately the same size (males at 74.4mm and females at 74.6mm) in which the percentage of mature individuals increase with length until all males over 118.2mm and all females over 123.8mm are mature.

Fecundity of *G. regressus* ranged from 581 to 1800 eggs (mean of 1060.8 ± 90.3) (n= 18) for females between 89.3 to 125.9 mm. In this species, fecundity was curvilinearly related to standard length (SL), and linearly related to both total weight (TW) and gonad weight (Fig. 6). The average relative fecundity of *G. regressus* was found to be 55.5 ± 4.3 .

Fecundity of *G. tana* ranged from 539 to 2968 eggs (mean of 1402.8 ± 54.7) (n=93) for females whose length was between 80.4 to 132 mm. Fecundity was curvilinearly related to SL, and linearly related to TW and gonad weight (Fig. 6). The average relative fecundity of *G. tana* was found to be 102.7 ± 3.38 . The relative fecundity of *G. tana* remained more or less the same for all size classes (Table 2). The relative fecundity of the two species was significantly different (ANOVA, $F_{1, 130} = 18.07$, $P < 0.001$).

Table 2. Mean absolute and relative fecundity of *Garra regressus* and *G. tana* in relation to their lengths. N= number of specimens in the particular standard length class from Lake Tana.

Species	Size class	Absolute fecundity	Relative fecundity	N	mean TW
<i>Garra regressus</i>	87.8–94.9	998.4	60.7	3	17.0
	94.9–102.1	987.5	61.2	4	16.5
	102.1–109.2	1274.9	70.3	2	18.2
	109.2–116.3	1088.1	61.2	6	18.0
<i>Garra tana</i>	79.5–92.6	1407.2	102.6	15	12.6
	92.7–105.8	1404.2	101.2	44	12.6
	105.8–118.9	1419.2	102.6	28	12.7
	118.9–132.1	1538.6	103.2	7	13.3

The mean diameter of fully mature ova calculated by measuring a sub sample of (at least 15) the largest eggs showed significant differences (T-test: T-Value = -32.79, P-Value = 0.000, df = 1171) between the two species. The mean diameter of fully mature eggs of *G. regressus* was 1.81 ± 0.016 (n= 178) while that of *G. tana* was found to be 1.22 ± 0.006 (n= 995). In *G. regressus* the size

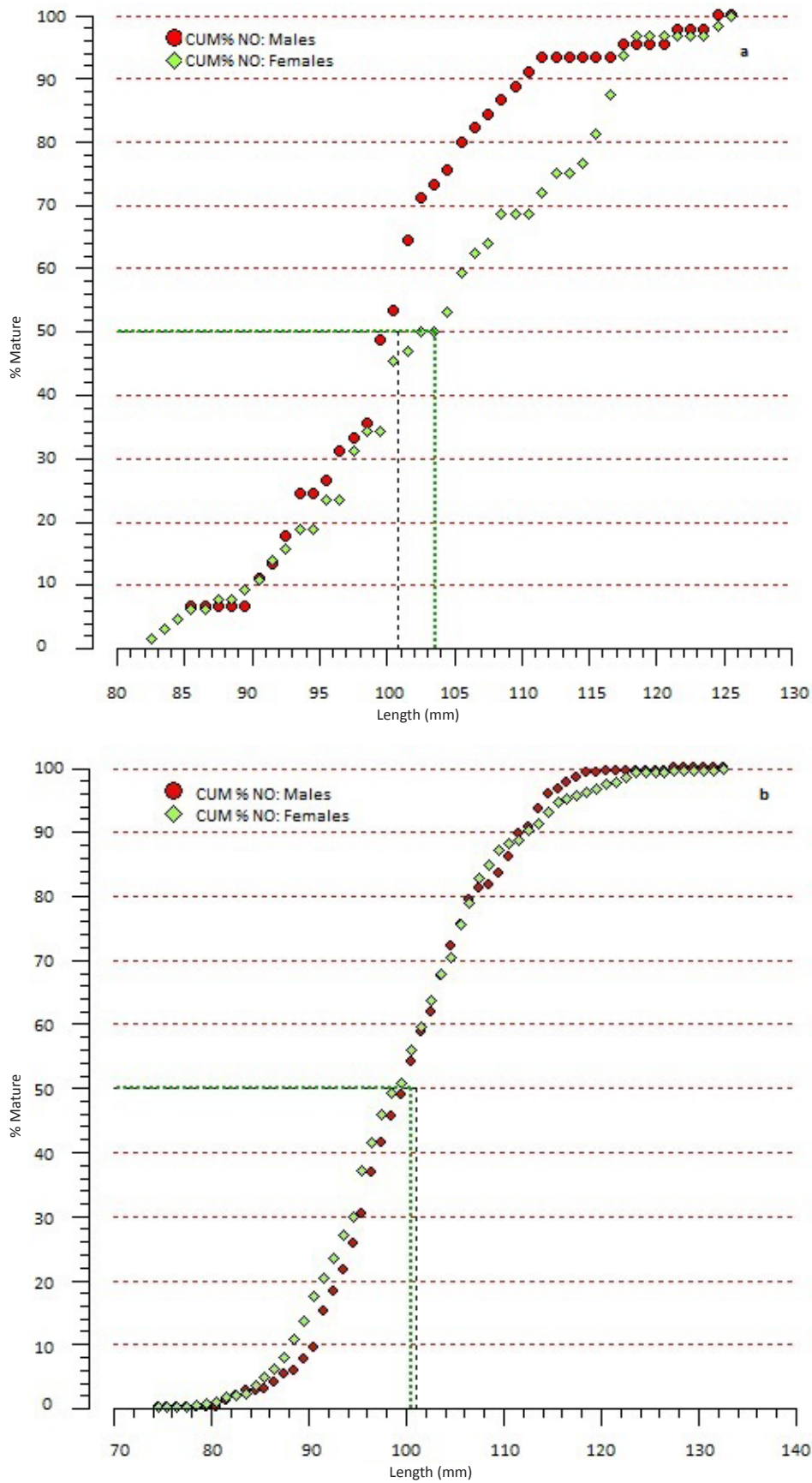


Figure 5. Mean length at first maturity (SL_{50%}) curves for (a) *Garra regressus* and (b) *G. tana* from Lake Tana

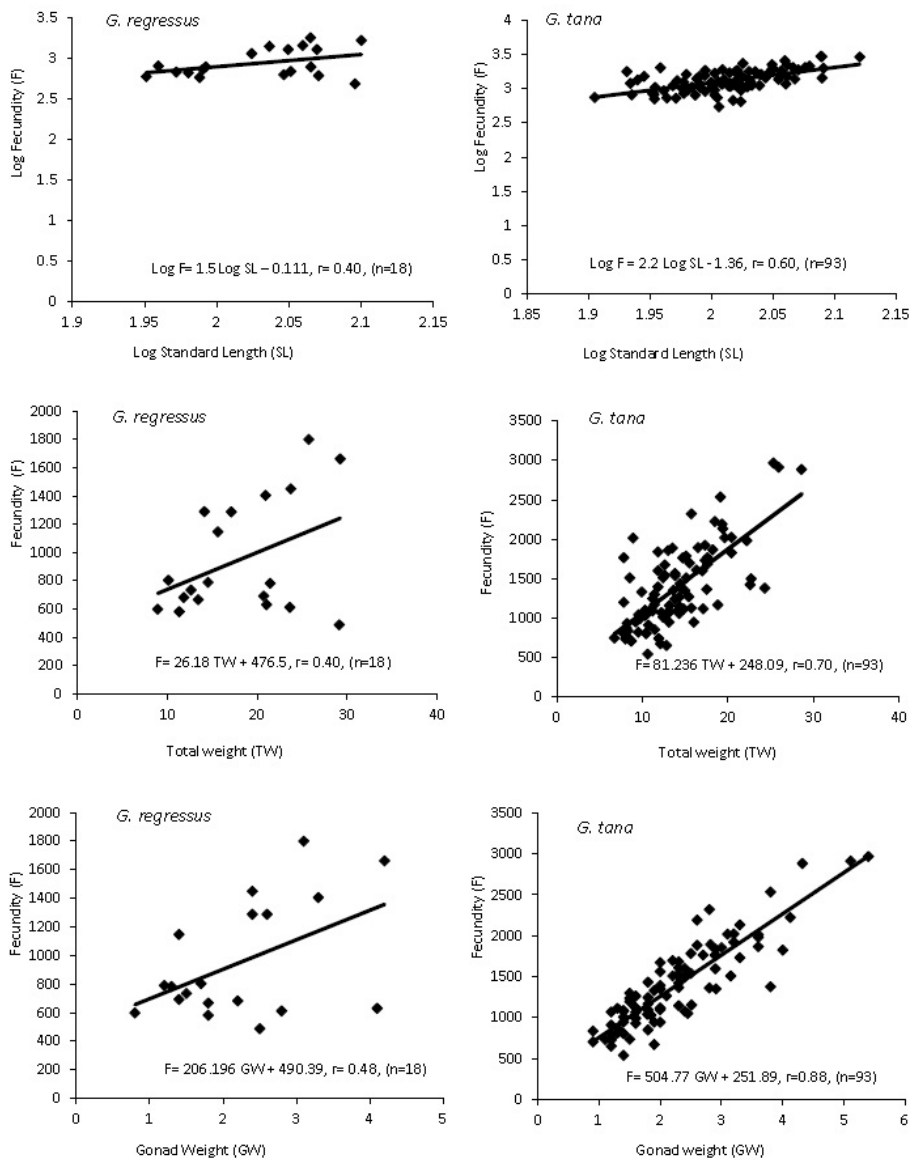


Figure 6. Absolute fecundity (F) as a function of Standard length (SL), Total Weight (TW), and Gonad Weight (GW) for *Garra regressus* and *G. tana* from Lake Tana.

frequency distribution of eggs showed bimodality and ovaries contained two kinds of oocytes (Fig. 7a): eggs with yolk (vitellogenic oocytes) and eggs without yolk. Only the vitellogenic eggs were counted to determine fecundity because only these oocytes would be spawned in subsequent peak. The size frequency distribution of eggs showed unimodality in *G. tana* (Fig. 7b).

From a total of 149 *G. regressus* examined, the sex ratio did not significantly differ from 1:1 during any period, except that in the total catch the ratio was female-biased (chi-square= 4.2, df= 1, P= 0.0404) (Table 3). From a total of 745 individuals of *G. tana* examined in 12 sampling occasions, a significant deviation from a 1:1 (female: male) ratio occurred only during March and

April, when females were more numerous, and during August when males were numerous (Table 3).

DISCUSSION

The results of GSI and frequency of females with ripe gonads, suggest that the two *Garra* species in Lake Tana has an intensive breeding activity coinciding with the major rainy season of the area. Therefore, biotic and abiotic factors that are associated with rainfall seem to be important cues to trigger breeding in the two species. Synchronization of sexual maturation and reproduction with rainy season is well documented in tropical

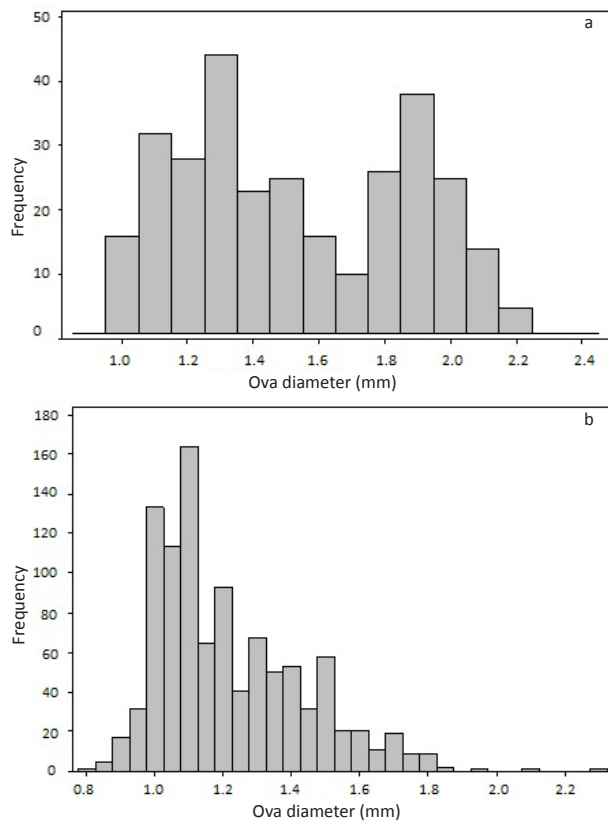


Figure 7. Ova diameter frequency histograms of (a) *Garra regressus* and (b) *G. tana* from Lake Tana

freshwater fish (Fryer & Illes 1972; Lowe-McConnell 1982; Wootton 1992). Temperature plays an important role in governing the reproductive activity of fish (Braum 1978; Sundararaj 1981). For instance, temperature

regime (i.e., the decrease in maximum and increase in the minimum air temperature (Fig. 2)) during the rainy season could possibly trigger reproductive activity, as observed in *Labeo horie* (Dadebo et al. 2003) and *G. rufa* (Bardakci et al. 2000). The relationship between intensive breeding activity and the rainy season has also been reported for other species of the genus *Garra*? (Jha et al. 2005).

The study also revealed considerable differences in reproductive periodicity of the two *Garra* species with a moderately long breeding season in *G. regressus* to an essentially continuous breeding in *G. tana*. Similar patterns of reproductive activity are known for other species of *Garra* in Iran (Abedi et al. 2011) and in characid fishes inhabiting a tropical stream in Panama (Kramer 1978). In this study no spatial differences in distribution patterns of *Garra* were observed between peak breeding periods and other periods.

The mean size at sexual maturity was similar for both species (and sexes) of *Garra*. Mean length at maturity (SL_m) is determined to predict size-assortative mating as well as management options by restricting mesh sizes to avoid over-exploitation, though exploitation is non-existent in this case. Size at maturity could vary among closely related species, among populations within species and among individuals within populations, suggesting that they can respond rapidly to natural selection (Stearns 1992 cited in Tsikliras & Antonoupoulou 2006). Length of maturity in many fish species depends on demographic condition, and is determined by both genes and the environment (Fryer

Table 3. Number of female and male fish, sex ratio (F:M) and the probability (P) of getting calculated chi-square value for *Garra regressus* and *G. tana* in the collected monthly samples from Lake Tana

Months	<i>G. regressus</i>				<i>G. tana</i>			
	F	M	P	Chi sqr	F	M	P	Chi sqr
Jan	2	6	0.1449	2.125	16	25	0.1573	2
Feb	6	1	0.054	3.714	14	21	0.2321	1.428
Mar	2	2	1	0	35	16	0.0077	7.098*
Apr	7	10	0.4432	0.588	21	10	0.0473	3.935*
May	2	2	1	0	105	81	0.0782	3.102
Jun	26	15	0.0846	2.975	53	41	0.2143	1.542
Jul	26	14	0.0569	3.625	82	62	0.0952	2.784
Aug	4	3	0.5934	0.285	13	33	0.0032	8.717*
Sep	3	3	1	0	25	25	1	0
Oct	5	2	0.2321	1.428	15	17	0.6929	0.156
Nov	0	2	0.1573	2	3	7	0.1923	1.7
Dec	4	2	0.3614	0.833	16	9	0.1573	2
Total	87	62	0.0404	4.201*	398	347	0.0617	3.492

& Illes 1972; Lowe-McConnell 1987; Wootton 1998); but can also be influenced by other factors such as long-term fishing pressure, which cannot be the case here.

The fecundity of the two *Garra* species increased in proportion to length, gonad weight and body weight. The positive correlation of absolute fecundity and body length has been reported for other *Garra* species (e.g., *G. ceylonensis* in Sri Lanka) (de Silva 1991). The parameter 'b' of the relationship of length with fecundity for *G. tana* ($b = 2.2$) is higher than that of *G. rufa* ($b = 1.8$) in Iranian waters (Abedi et al. 2011) and *G. ceylonensis* ($b = 1.22$) (de Silva 1991). But for *G. regressus*, this value ($b = 1.5$) is very close to the Asian species (*G. ceylonensis* and *G. rufa*). However, the 'b' value is generally comparable to previously reported values for other fish species (Bagenal 1978). In many fish species 'b' value is usually around three when fecundity is related to length, and about one when related to weight (Bagenal & Braum 1978). The relatively lower regression coefficient as well as weaker correlation between fecundity and standard length (Fig. 6) for both species in this study as well as for *G. rufa* (Abedi et al. 2011) and *G. ceylonensis* (de Silva 1991) indicates that fecundity could vary much within each size class. According to our findings, the coefficient of correlation for the relationship between fecundity and fish weight was 0.4 and 0.7, and for the relationship between fecundity and gonad weight 0.48 and 0.87 for *G. regressus* and *G. tana*, respectively. Thus, it is clear that the gonad weight has a better correlation with reproductive capacity than the body weight, as is the case in *G. rufa* (Abedi et al. 2011).

The high variability in absolute fecundity observed in these species could also be the result of either genetic differences among the females, or environmental conditions, or a combination of both (Wootton 1998; Rideout & Morgan 2007). The reproductive potential as reflected in the increase in absolute fecundity with length indicates that *Garra* allocates more energy to reproduction as it grows (Abedi et al. 2011).

The relative fecundity in *Garra* species of Lake Tana is low in comparison with other species of *Garra*; *G. rufa* from Iran (mean = 86.84 eggs/gram) (Esmaeili et al. 2005) and *G. ceylonensis* from Sri Lanka (mean = 866.7 eggs/gram) (de Silva 1991). However, *G. tana* has a comparatively higher relative fecundity (102 eggs/gram) than *G. regressus*. This might be a strategy for this abundant and highly predated prey species (Nagelkerke et al. 1995; de Graaf et al. 2000) in the deep off-shore part of the lake or possibly a difference in the reproductive investments or genetic variability (Rideout & Morgan 2007). The effect of predation on

one of the most abundant pelagic species in Lake Tana, *B. tanapelagius* was earlier reported in which the fish showed a larger relative fecundity at smaller length class (Dejen 2003). The piscivores in Lake Tana have their optimum prey size at 40–50 mm (de Graaf 2003), which is smaller than the modal length classes of *Garra* species (Geremew 2007). This might then indicate the lesser predation pressure on *Garra* species by the piscivores in the Lake. Thus, it is likely that less variability at different size classes in relative fecundity of *Garra* observed might be a different strategy to the one reported in *B. tanapelagius* (Dejen 2003).

The result of egg size frequency distribution showed that *G. tana* is a single spawner similar to *G. ceylonensis* (de Silva 1991; Sundrabanthy et al. 2005). However, *G. regressus* with an extended breeding season showed two distinct peaks in their ova size frequency distribution, suggesting multiple spawning. Multiple spawning helps to reduce competition for nursery and spawning sites by partitioning their use in time, helps in assuring the survival of some portion of the total spawn to recruitment, and provides more adaptability in highly variable environments (Rinchar & Kestemont 1996; Dejen 2003). Lower fecundity estimates in *G. regressus* reported here for a comparable size class may be related to the multiple spawning nature of this fish, or may be due to the larger mean size of its eggs as compared to *G. tana* (Table 3). It is known that smaller sized fishes with large sized eggs have smaller fecundities and produce multiple clutches of eggs in a season (Kramer 1978; Paugy 2002).

Female specimens predominated over males in *G. regressus*. However, *G. tana* showed no significant variation in the proportion of females over males from unity. It should be noted that this result does not necessarily indicate the sex ratio of the total population in the lake. This is because several factors may have an interactive effect as discussed in Admassu (1994) and Tadesse (1997). Sex based differences in activity associated with breeding behavior might be the reason for sex biased catches in some of the breeding months. For instance, *G. tana* showed preponderance of females in March and April which are periods at the start of peak breeding activity. On the other hand, males dominated in August, which is after the end of the peak breeding months. In *G. regressus*, although not statistically significant to support a deviation from theoretical one-to-one value, females were numerous than males in the catch during the peak breeding months of June and July. So without additional information on the spawning behavior of these species, it would be difficult

to eliminate this factor.

The present study provides vital information on the main spawning period and spawning area to inform conservation plans and fisheries management plans for *G. tana* and *G. regressus*. Thus, the proposed small-meshed fishery for small barbs in the lake should also consider *Garras*, especially in the pelagic habitat as this is shared with *B. tanapelagi* (Dejen et al. 2009). But prior to any management decision on small-meshed fisheries, studies on growth, mortality, biomass and potential yield of *Garra* stock is required.

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Amharic Abstract

በጣና ሐይቅ ብቻ የሚገኙ በሳይንሳዊ ስማቸው ጋራ ሪግረስስ እና ጋራ ጣና ተብለው የሚጠሩ ሁለት የአሳ ዝርያዎች ስላላቸው ስነ-ህይወታዊ የአረባብ ሁኔታ ለማጥናት በየወሩ (ከጥር-ታህሳስ 2006 እ.ኤ.አ) የአሳ ምዕሩ ከጣና ሐይቅ የደቡብ ባህር ወሽመጥ ለመሰብሰብ ተሞክሯል። በዚህ ጥናት መሰረት ጋራ ሪግረስስ የሚባለው ዝርያ የሚራባበት ወቅት ረዘም ያለ ሆኖ ከሚያዝያ እስከ ጥቅምት ወር ድረስ ብቻ ሲሆን፣ ጋራ ጣና የሚባለው ዝርያ ጣና አመቱን በሙሉ አንደሚራባ በረጋገጥም ከመጋቢት እስከ ሐምሌ ያሉት ወራት ለዚህ ዝርያ ዋጋኛ የርቢ ወቅቶች አንደሆኑ ለማረጋገጥ ተችሏል። የሁለቱም ዝርያዎች ለርቢ ዝግጁ የመሆኛ አማካይ የአካል መጠን (ስታንዳርድ ቁመት) በወንድ እና ሴት ጾታዎች መካከል መሰረታዊ ልዩነት አለመኖሩ ተረጋግጧል። ነገር ግን ሴት የጋራ ጣና ዝርያ ለርቢ ዝግጁ የምትሆንበት አማካይ የአካል መጠን ከሴት ጋራ ሪግረስስ በእጅጉ ያነሰ መሆኑ ለማረጋገጥ ተችሏል። ሴት የጋራ ሪግረስስ በርቢ ወቅት ከ580.8-1800 አንቀላሎችን የማምረት አቅም ሲኖራት የጋራ ጣና ዝርያ ጣና ከ538.9-2968 አንቀላሎችን ልታመርት ትችላለች። የሁለቱንም ዝርያዎች አንቀላል መጠን (ዳይሜትር) መሰረት በማድረግ በተሰራው ፐርሰንቴጅ ስርጭት ሴት ጋራ ሪግረስስ በአንድ የርቢ ወቅት ከአንድ ጊዜ በላይ አንቀላል የምትጥል ሲሆን ሴት ጋራ ጣና ጣና አንድ ጊዜ ብቻ የምትጥለው መሆኑን ለማረጋገጥ ተችሏል። በጠቅላላ የተሰበሰበውን የሁለቱን ዝርያዎች ምዕራ መሰረት በማድረግ የጾታ ፊሽኖ ሲሰራ ጋራ ሪግረስስ በብዛት የሴቶቹ ቁጥር አንድሚበዛ ሲያሳይ፣ ጋራ ጣና ዝርያ ጣና ከ 1:1 ፊሽኖ መሰረታዊ ልዩነት ያላሳየ መሆኑን በካይ ስኩዌር ዘዴ ለማረጋገጥ ተችሏል። በተጨማሪም ጋራ ጣና በሁለቱ ጾታዎች መካከል ለርቢ ዝግጁ የመሆኛ እድሜ ወይም የአካል መጠን መሰረታዊ ልዩነት አለመኖሩም ተረጋግጧል።

Author Details: KEWAKE GEREMEW is a lecturer in the department of Biology, Dilla University, Ethiopia. He served as an executive secretary of the Ethiopian Fisheries and Aquatic Sciences Association. His areas of specialization include fish biology and aquaculture. He has studied the taxonomy and biology of Lake Tana *Garra*. He is also currently working on tilapia nutrition. ABEBE GETAHUN is an Associate Professor and Chairman of the Department of Zoological Sciences, Addis Ababa University, Ethiopia. He is teaching undergraduate and post graduate courses; advising PhD, MSc. and undergraduate students and also involved in research projects. His research focus is on diversity, conservation and aquaculture of fishes. He has authored or co-authored over 30 articles in scientific journals and books. ESHETE DEJEN has been working for the past 15 years in fish biology, ecology and management of Lake Tana and its sub-basin. He served as Center Manager and Director for Livestock and Fisheries Department of ARARI, as Fisheries Officer at the FAO-Subregional Office for eastern Africa. He received National Green Award Programme-2009 from Individual Category for his contribution on Lake Tana’s Environmental Protection. Currently, he is working as Fisheries Technical Officer in IGAD.

