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Research Article

Reproductive biology and age determination of *Garra rufa* Heckel, 1843 (Actinopterygii: Cyprinidae) in central Iran

Masoud ABEDI[1], Amir Houshang SHIVA[2,*], Hamid MOHAMMADI[2], Rokhsareh MALEKPOUR[3]

[1]Islamic Azad University - Sepidan Branch, Department of Biology, Sepidan - IRAN

[2]Islamic Azad University - Jahrom Branch, Department of Biology, Jahrom - IRAN

[3]Islamic Azad University - Kazeroun Branch, Department of Biology, Kazeroun - IRAN

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Abstract: Some aspects of the reproductive biology of *Garra rufa* Heckel, 1843, a native cyprinid fish species from the Armand stream in Chaharmahal-o-Bakhtiari province, central Iran, were investigated by regular monthly collections throughout 1 year. A significant relationship between length and weight and the isometric growth pattern were observed in this fish. There were no significant differences in the total number of male and female specimens. The population of this cyprinid fish had a narrow age range of 0-4 years, and the maximum number of samples belonged to the age group of 2.01-3 years. Based on the patterns of gonadosomatic and Dobriyal indices, it was concluded that this fish population has a prolonged, active reproductive period, which is a type of adaptation by this population to environmental conditions. The average egg diameter was 0.67 mm; the highest diameters were seen in May and the lowest in November. The absolute and relative fecundity were 1179.6 and 109.4, respectively. There was a significant relationship between fecundity and fish size (total length and total weight), and also between absolute fecundity and gonad weight.

Key words: *Garra*, reproductive biology, age, fecundity, GSI, spawning, Iran

Introduction

There are about 140 fish species in the inland waters of Iran, which generally belong to three families: Cyprinidae, Balitoridae, and Cobitidae. The cyprinid species exhibit a wide range of geographical distribution, life histories, and reproductive styles (Winfield and Nelson, 1991). The family Cyprinidae, with about 220 genera and about 2420 species, is the largest family of freshwater fishes and, with the possible exception of Gobiidae, the largest family of vertebrates (Nelson, 2006). The members of the genus *Garra* Hamilton-Buchanan, 1822 are found throughout southwest Asia and from Africa to southeast Asia, and are predominantly adapted to life in swift-flowing waters, streams, and lakes (Krupp and Schneider, 1989).

The genus has about 73 species, and 4 are recognized from Iran: *Garra persica* Berg, 1913 in Hamun-e-Jaz Murian, Hormoz, and Sistan basins; *Garra rossica* Nikolsky, 1900 in Tedzhen River, Bedjestan, Sistan, Lut, Hamun-e-Jaz Murian, Mashkid, and Makran basins; *Garra variabilis* Heckel,

* E-mail: ah.shiva@gmail.com

1843 in the Tigris river basins; and *Garra rufa* Heckel, 1843 in the Tigris River, Kor River, Lake Maharlu, and the Gulf and Hormoz basins (Coad, 1995).

Knowledge of the reproductive cycle and the factors affecting it are important issues in fish and fisheries biology (Tomkiewicz et al., 2003). Studies of teleost reproduction often favor commercial or valuable native species (Matsuyama et al., 1988; Appleford et al., 1998; Fowler et al., 1999; Smith and Walker, 2004). Among Iranian inland fishes, *Garra rufa* is one of the important biological species that is native to the Tigris basin. It has a small size and no economic importance. Some of the common names of this fish used in Iran are Gel-cheragh, Gel-khorak, Mahi-e-sang lis, and Shirbot. *Garra rufa* has a wide dispersion, but there is little information on its biology in Iran. The purpose of this paper was to consider the age and different aspects of biology of this species, including the length-weight relationship, sex ratio, gonadosomatic index, fecundity, spawning season, and condition factors necessary for conservation measures. We believe that information on the reproductive biology of this native fish could be important for conserving its stock.

Materials and methods

The field study site was a rocky stream called Armand (31°40′N, 50°46′E). This stream is one of the branches of the Karun River and is located in the Armand plain, near the town of Lordegan, 120 km south of Shahr-e-Kord, in Chaharmahal-o-Bakhtiari province, central Iran. Fish were collected monthly from July 2006 to June 2007 using an electroshocker (200-350 V, 2-3 A). The specimens were immediately preserved in 10% formalin until they could be examined. The chi-square test was used to assess deviation from a 50:50 sex ratio (Robards et al., 1999). The total body weight (W) of all preserved fish was measured using an electronic balance to the nearest 0.001 g. The total length (TL) was also measured, to the nearest 0.05 mm, using a vernier caliper. The condition factor (K) was calculated monthly with the formula $K = (W/L^3) \times 100{,}000$, where W and L are the total weight and total length, respectively (Biswas, 1993). The length-weight relationship was calculated by the method of least squares using the parabolic equation suggested by Le Cren (1951), which is $W = a L^b$, and after logarithmic transformation has the form of $\log W = \log a + b \log L$, where W is the total weight in grams, L is the total length in millimeters, *a* is a coefficient related to body form, and *b* is an exponent indicating allometric growth when unequal to 3. The parameters *a* and *b* were estimated by linear regression of log-transformed weight and length (Bagenal and Braum, 1978; Elliott, 1995). The significance of the regression was assessed by analysis of variance (ANOVA). The significant difference of *b* from 3 was tested by using the equation given by Pauly (1984):

$$t = \frac{\mathrm{sd}\ln L}{\mathrm{sd}\ln W} \times \frac{|b-3|}{\sqrt{1-r}} \times \sqrt{n-2},$$

in which *n* is the number of specimens, *b* is the slope of the regression line, *sd* ln *L* and *sd* ln *W* are standard deviations of length and weight, respectively, and *r* is the regression coefficient.

The fish were dissected laterally and sex was ascertained macroscopically. To examine the monthly changes in the gonads as a means for estimating the spawning season of this cyprinid fish, the gonadosomatic index (GSI) and Dobriyal index (DI) were used, which were calculated by GSI = (weight of gonads/weight of fish) × 100 (Nikolsky, 1963), $DI = \sqrt[3]{GW}$ and by DI = (Dobriyal et al., 1999), where *GW* is the average gonad weight.

To determine the ovum diameter, the ovaries were fixed in a 10% formalin solution. Subsamples were taken from the anterior, middle, and posterior regions of the ovary. The diameters of 90 ova from all of the subsamples were measured, using a stereomicroscope that was fitted with an ocular micrometer. In this project, fecundity was defined as the number of ripe and maturing oocytes in the ovaries prior to spawning (Marshall, 1979; Payne and Collinson, 1983), and so the absolute fecundity (F) was measured in terms of the number of oocytes with a diameter greater than 0.7 mm (Leonardos and Sinis, 1998), using 69 female gonads. The relative fecundity (number of ova per unit of body weight) was also estimated using the method suggested by Bagenal (1967). The fecundity was then correlated with fish body size using $F = a L^b$, where F is the number of eggs per specimen; L is the total length (TL), total weight (W), or gonad weight (GW) of the fish; and *a* is a constant and *b* is the slope (Koutrakis and Tsikliras, 2003).

The age of all of the specimens was determined from the key sample scales taken from the left side of the body, between the lateral line and the beginning of the dorsal fin. The scales were cleaned mechanically using a fine brush and rinsed with distilled water. Cleaned scales were dried on filter paper. To avoid curling, the scales were mounted between 2 slides for 2-3 days. Observations were made using a stereomicroscope with reflected light. For each specimen, 4-5 scales were used and the number of annuli (growth lines) in each scale was counted.

Results

Sex ratio and age

During the present study, a total of 364 specimens of *G. rufa* were caught, ranging in total length from 29.11 to 151.27 mm. Of the total number of specimens, 191 were males and 173 were females. There was no significant difference in the total number of male and female specimens (chi-square = 0.617, P > 0.473).

Results revealed that the population of this cyprinid fish had a narrow age range of 0-4 years, and the maximum number of samples belonged to age group 2.01-3, followed by age groups 1.01-2, 3.01-4, and 0-1, respectively (Figure 1).

Length-weight relationship

There was a significant relationship with a high regression coefficient (r^3 0.991, P < 0.001) between the length and weight of the fish (Table 1), which can be described with the following equations. Total specimens: log W = -5.076 + 3.112 log TL, Males: log W = -5.092 + 3.134 log TL, Females: log W = -5.036

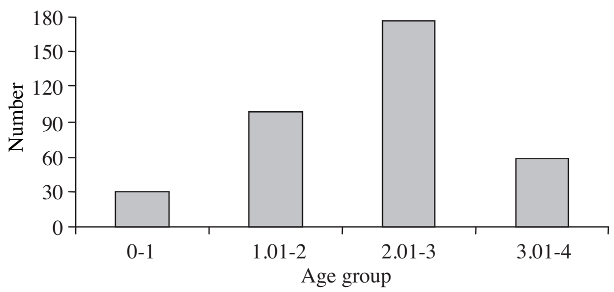

Figure 1. Number of specimens of *Garra rufa* in different age groups.

Table 1. Length-weight relationship of *Garra rufa*. n: number of specimens, r: correlation coefficient, r^2: coefficient of determination, *a* and *b*: constant of regression equation.

Specimens	n	R	r^2	log *a*	*b*	P
Female	173	0.991	0.981	-5.036	3.089	<0.001
Male	191	0.994	0.989	-5.092	3.134	<0.001
Total	364	0.993	0.984	-5.076	3.112	<0.001

+ 3.089 log TL. There was no significant difference in the parameters *b* and *a* between the sexes. However, the parameter *b* for male specimens was comparatively slightly higher than for females. As the *b* value was about 3, it could be concluded that the growth of this fish is isometric in the Armand rocky stream system.

Condition factor

For the pooled data, this factor ranged from 0.87 to 3.14 with a mean of 2.03 and fluctuated in different months. The ANOVA test showed that there was no significant difference between males and females (P = 0.428). The condition factor of females was the highest in May and reached the lowest value in November. From November to May, it showed an increasing pattern (Figure 2). In the case of males, the condition factor was the lowest in November and increased up until April. It showed almost decreasing trends from April to November (Figure 2).

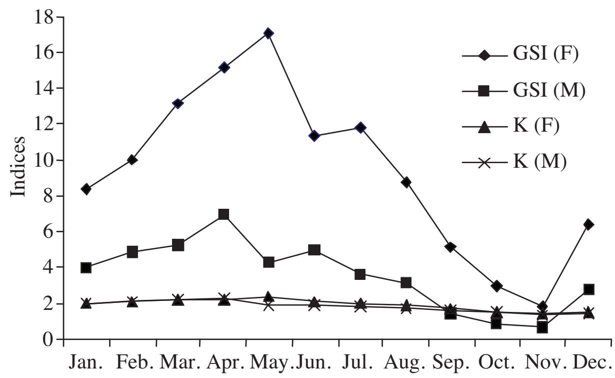

Figure 2. Variation of mean gonadosomatic index (GSI) and condition factor (k) of female (F) and male (M) *Garra rufa* in different months.

Gonadosomatic and Dobriyal indices

There was a significant difference in the male and female GSI in different months (ANOVA, $P < 0.001$). The female gonadosomatic index increased from November to May, peaking in the middle of spring and then decreasing slowly from the end of May to November (Figure 2), showing prolonged reproductive activity. In the case of males, the peak of the GSI plot was in April. This was due to an increase in gonad weight, indicating that the breeding season falls after April. In females, the DI was as high as 1.41 in May, with a clear fall during June. We observed a decreasing trend in the value from July to November, confirming a long period of spawning. In males, the DI showed an increasing trend from November to April, when it was as high as 1.17. There was a fall in the value during May. The DI decreased gradually from June to November (Figure 3).

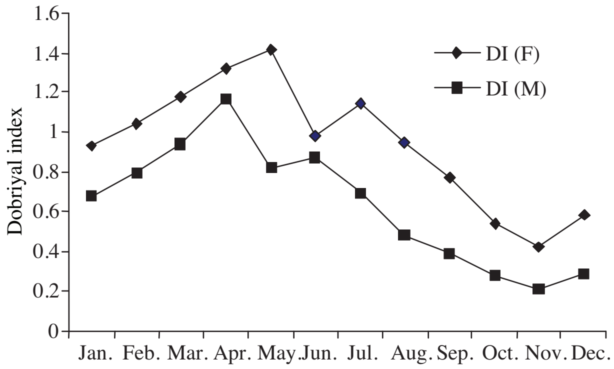

Figure 3. Variation of Dobriyal index (DI) of female (F) and male (M) *Garra rufa* in different months.

Fecundity and ova diameter

The absolute fecundity ranged from 283 to 3794 eggs per female, with an average of 1179.65. The mean relative fecundity was 109.43 per gram of body weight. We found a significant relationship between absolute fecundity and fish size (total length and weight), and also gonad weight (Table 2). The ovum diameters ranged from 0.028 to 1.98 mm, with a mean of 0.67 mm. The highest diameters were seen in May and the lowest in November (Figure 4).

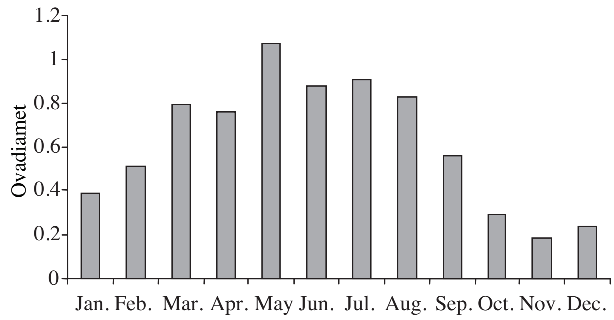

Figure 4. Mean ova diameter (mm) of *Garra rufa* in different months.

Table 2. Relationship between absolute fecundity (F) and total length (TL), weight (W), and gonad weight (GW) of *Garra rufa*.

Equation	N	r	P
$F = 0.412\ \mathbf{TL}^{1.875}$	69	0.596	<0.001
$F = 138.94\ \mathbf{W}^{0.627}$	69	0.634	<0.001
$F = 681.13\ \mathbf{GW}^{0.704}$	69	0.922	<0.001

Discussion

The study of reproductive biology is an effective method for recognizing the stocks and life cycle of fishes (Hosseinzadeh et al., 2001). A variety of useful concepts, centering on the body shape of individual fish, arise from the consideration of combined weight-length data. The equation suggested by Le Cren (1951) has proven to be a useful model for weight as a function of length (Murphy and Willis, 1996). The length-weight relationship in fishes is affected by a number of factors, including season, habitat, gonad maturity, sex, diet and stomach fullness, health, and preservation techniques (Tesch, 1968). Results of the present study indicated that the b value was about 3, so it could be concluded that the growth of this fish is isometric, meaning that the shape does not change as the fish grows. In general, the value of b lies between 2.5 and 4 and can be variable as a result of changes in fish shape, season, age, and food availability (Ricker, 1979). In the present study, the value of b remained within the expected range.

The monthly variations in the GSI were highly associated with the seasonality of the maturity stages assigned macroscopically, as found by Morato et al. (2003). An increase in the GSI of females was observed from November to April, which indicated the appearance of prespawning individuals. The GSI peaked in May, corresponding to the first observation of spawning individuals. A decrease in the GSI from May to November coincided with the long spawning season. In the case of males, the spawning season lasted from April to November, showing that perhaps male specimens start spawning earlier than females. A distinct fall in the GSI during June in females and May in males may be an indicator of heavy spawning of some fish in these months. As mentioned by De Silva (1991), it was concluded that the individuals of the *Garra rufa* population are asynchronous spawners. Not all females spawn at once, and the gonads of individuals include a range of developmental stages. All oocytes of a matured batch are generally spawned at once, but up to 20% may be retained for repeat spawning. In common carp, after spawning and absorption of residual oocytes, rematuration of the ovaries takes at least 3-4 months (Davies et al., 1986; Mills, 1991). The population of *G. rufa* in the Armand stream has a reproductive strategy in which, during the spawning period, different individuals release their eggs and sperms at different times. Such a lengthy breeding season is a type of adaptation by this population, which lives in an unstable habitat, to environmental conditions (Miller, 1979).

The fecundity is affected by many factors, such as the size and age of the female (Thorpe et al., 1984), the life history strategy (Morita and Takashima, 1998), food supply, and temperature (Fleming and Gross, 1990). The fecundity of this fish is low in comparison with the other cyprinid fishes; for example, the fecundity of *Labeo dero* is about 90,000 (Biswas et al., 1984). This shows that the population of *G. rufa* in the Armand stream is under stress. The Armand stream has a meandering and rocky bed, and boatmanship matches are held there, all of which can cause stress and consequently low fecundity. It is obvious that degeneration or malfunction of ovaries reduces the female fecundity and reproductive potential (Rideout and Burton, 2000). The fecundity of *Garra rufa* increases with fish size (total length and body weight). According to Jonsson and Jonsson (1999), fecundity increases with body size because the amount of energy available for egg production and the body cavity accommodating the eggs increases with fish size. According to our findings, the coefficient of correlation for the relationship between fecundity and fish weight was 0.634, and for the relationship between fecundity and gonad weight, it was 0.922 (Table 2). Thus, it is clear that the gonad weight has a better correlation with reproductive capacity than the body weight. Hence, the Dobriyal index could be used for determination of the spawning season, sexual maturity, and frequency of the spawning of fish. The DI involves only the data related to the sexual organs, which are easy for interpretation and calculation and provide a narrow range of index if the gonad weight is very low or very high (Esmaeili and Shiva, 2006). The ova diameter variation is probably one of the important pieces of evidence used in determination of fish reproductive strategy (Tomasini et al., 1996). The maximum diameter of ova was observed from March to September, showing the spawning season of this fish population.

The term condition was applied to analysis of the variation from the expected weight for length of individual fish or relevant groups of individuals as indications of fatness, general "well being," gonad development, and so on (Murphy and Willis, 1996). The condition factor of *G. rufa* was high at the beginning of the spawning season when more ripe eggs were present, and decreased slowly during the long spawning period. This shows the effect of gonad weight on the *k* value. In fact, releasing the oocytes in females and sperms in males decreased the weight of fish, so they became thin and this decreased the *k* amount. An increase or decrease in condition factor could be due to availability of food, spawning, stress, changes in temperature, pH or pollution of water, and so on (Türkmen et al., 1999; Erdoğan et al., 2002).

The study of fish scales is the best tool in fish biology, because fish biologists are unable to get large numbers of specimens for the studies due to the reduction in fish stocks. Numerous studies on the scale structure of fishes have been undertaken in the world, and the results have been successfully used for growth studies, calculation of minimum harvestable size, and determination of age in fishes (Tandon and

Johal, 1996; Rifflart et al., 2006). Our results revealed that the population of this cyprinid fish had a narrow age range of 0-4 years. This shows that *G. rufa* is a fish with populations mainly of young individuals.

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