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Research Article

Evaluation of some reproductive parameters and feeding habitat of the Caspian roach, *Rutilus caspicus* (Yakovlev, 1870), in the Caspian Sea (Golestan coastal waters)

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Abstract

A study on the feeding and reproductive habits of the Caspian roach (*Rutilus caspicus*) in Iranian waters was conducted from April 2016 to March 2017. The stomach contents of 330 specimens were analyzed and 104 specimens were chosen for detailed investigation. Of these, 47 had empty stomachs, and 57 contained food. The stomach emptiness index peaked in winter (73%) and was lowest in spring (11%). Gastropods were identified as the primary food source, alongside crabs, bivalves, and plant fragments. Reproductive analysis showed the largest mean ovum diameter (1.501±0.1 mm) at age 4 and the smallest (0.76±0.15 mm) at age 2. The gonadosomatic index (GSI) in females ranged from 0.05 to 0.10, with spawning beginning in March and lasting until mid-spring. The mean absolute fecundity was 19,469 eggs, with 50% of females reaching maturity at 138 mm and males at 125 mm. A significant linear relationship was observed between fork length, weight, and absolute fecundity ($p<0.05$). This research provides valuable insights into the seasonal diet, reproductive biology, and ecological behavior of *R. caspicus*. The findings are crucial for understanding the species' role in the Caspian Sea ecosystem and informing its management and conservation.

Introduction

The Caspian Sea, the world's largest enclosed inland body of water, presents a unique confluence of geographical, ecological, and geopolitical characteristics. Spanning approximately 371,000 square kilometers, it is bordered by five nations: Russia, Kazakhstan, Turkmenistan, Iran, and Azerbaijan. This vast expanse of saline water is often referred to as a sea due to its size and complexity, despite being classified as a lake due to its lack of direct access to the world's oceans. Ecologically, the Caspian Sea faces significant challenges, including pollution, overfishing, and the impacts of climate change. Industrial activities, particularly oil extraction, have led to ecological degradation, threatening both the unique marine species and the livelihoods of local communities. Coordinated efforts among the bordering nations are essential to manage these resources sustainably and prevent further harm to this invaluable ecosystem (Convention on the Legal Status of the Caspian Sea, 2018).

During the 1930s to 1950s, in addition to the decrease in the water level of the Caspian Sea, due to the use of river water to irrigate fields, the depletion of water in coastal wetlands, the entry of urban and rural sewage, and excessive sand harvesting from river mouths, The decrease in the amount of primary production and disruption in the food chain, as well as the destruction of the reproduction and spawning areas of bony fish, have caused a decrease in the reproduction rate and survival of young fish and a decrease in the stocks of these fish. The Caspian roach (*Rutilus caspicus*) is a freshwater fish native to the Caspian Sea and its adjacent river systems. This migratory anadromous fish is primarily distributed based on sea surface level, sea surface temperature, and freshwater discharge patterns (Fazli *et al*., 2022).

Its presence is most noticeable during the autumn and winter seasons, with higher abundance observed in the eastern regions of Iranian waters in the Caspian Sea (Fazli *et al*., 2021). Understanding the feeding habits of this species is crucial for both ecological studies and fisheries management. As a member of the cyprinid family, the Caspian roach exhibits omnivorous feeding behaviors, which significantly influence its role within the aquatic ecosystem. Caspian roach primarily consumes zooplankton, phytoplankton, and benthic invertebrates, demonstrating a preference for small organisms that inhabit the water column and substrate. This species employs a filter-feeding mechanism, utilizing its fine gill rakers to strain food particles from the water. During the warmer months, when productivity in the aquatic environment peaks, Caspian roach exhibit increased foraging activity, often forming schools to enhance their foraging efficiency. Their diet may also include detritus, indicating a secondary role in the decomposition processes within their habitat. The feeding habits of Caspian roach can exhibit variation based on environmental factors such as water temperature, availability of food sources, and competition with other fish species. Seasonal changes often lead to shifts in diet, prompting the roach to adapt its feeding strategies to optimize energy intake. In times of food scarcity, they may resort to more opportunistic feeding behaviors, consuming what is readily available. The feeding habits of the Caspian roach reflect its adaptability and ecological significance. By consuming a variety of food sources, it contributes to the cycling of nutrients in its habitat and supports the health of the aquatic ecosystem. Continued research is essential to further elucidate the dynamics of its feeding behavior, particularly in the context of environmental changes and human impacts (Naddafi *et al*., 2005).

The type of reproduction of this species is sexual, and its reproduction areas are mostly rivers related to the Caspian Sea, which have suitable vegetation. They prefer shallow bodies of water, where the temperature rises easily. In general, the best spawning conditions are provided for them in beds with suitable vegetation in early spring. Spawning takes place in one stage and during the life cycle 5-6 times (Kottelat and Freyhof, 2007).

Studies have been conducted on the feeding habits of Caspian roach, *R. caspicus*. Akbari Pasandi (2006), studied the biology of *R. caspicus*. The results showed that the average weight of word fish is 55.13 gr and its average length is 160 mm. Alaghi (1998) conducted a study on the age, growth, and production of *Rutilus caspicus* in the Gomishan Lagoon. Alaghi (1998) conducted a study on the age, growth, and production of *Rutilus caspicus* in the Gomishan Lagoon. The research involved analyzing 125 female and 100 male fish. Among the females, the most common length range was 180–190 mm, representing 10.39% of the samples. For the males, the most frequent length range was 170–180 mm, accounting for 16.39% of the specimens. In this study, the highest abundance was found in the length class of 200-210 mm for both male and female fish in two years. He also reported that the largest word of this region was obtained with a length of 330 mm and an age of 9 years. Pageh and Maqsoodlou (2000) investigated the age, growth, and reproduction of the fish in the Gomishan lagoon. The development of the gonads in the *R. caspicus* is proportional to age, and this proportion was observed in females in the Gomishan lagoon. Some biological characteristics of roach, *R. caspicus*, in Ghomishan Wetland and the Age, growth, and reproduction *R. caspicus* in the Anzali and the Gomishan wetlands, North Iran were studied by Naddafi *et al*., (2001, 2005), respectively. Naddafi *et al*. (2002) investigated and evaluated the biological features of the Turkmen *R. caspicus* in the Gomishan lagoon. Sex ratios based on the number in each age group showed a significant difference at the 0.05 level. There was also a significant correlation between total length, body weight, radius of scales, and scales. After performing background processing on the scales and calculating the length of the fish, the maximum growth was obtained at the age of one and two years. Also, Kashiri *et al*. (2019) conducted a study titled Population Structure of *R. caspicus* on the southern coasts of the Caspian Sea using Microsatellite Maker. Rahnama *et al*. (2017) conducted a study on the growth and mortality of *R. caspicus*, Yakovlev, 1870 in the southern waters of the Caspian Sea (Golestan Province). As well as Population structure comparison of the *R. caspicus* in Gorganrood and Bandar Anzali regions using microsatellite markers was done by Hosseinnia *et al*. (2014). As no study has been conducted on the feeding habits of this species, this research focuses on examining the feeding habits of the *R. caspicus*.

Materials and methods

In this study, 164 Caspian roach were collected from April 2016 until March 2017 in the waters of the southern Caspian Sea. This species was caught from the Golestan waters in the Southern Caspian Sea during the time that was not the fishing season, with beach seine nets and gill nets (Fig. 1). The fork (FL) and total length (TL) of fish were measured by biometry board (to the nearest 0.1 mm) and weighed to the nearest of 0.01 g.

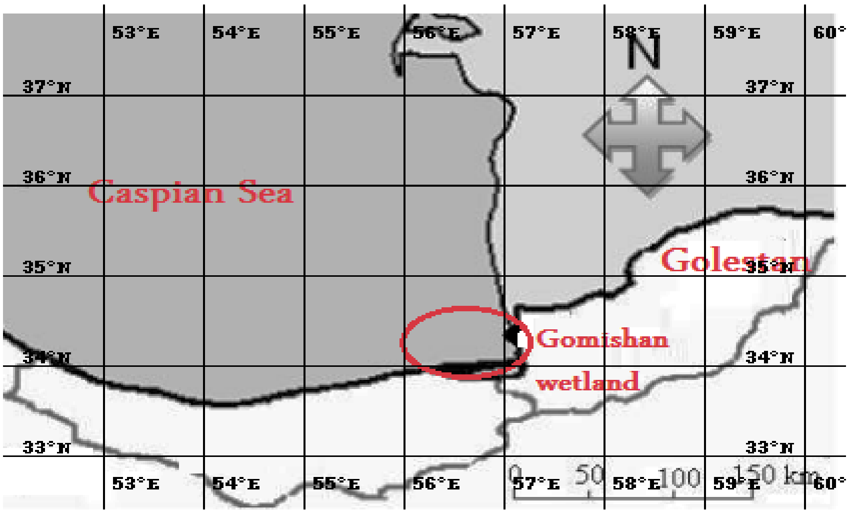

Figure 1: Sampling areas in the Southern Caspian Sea (Red Circle).

Assessing the nutrition of fish

The contents of the stomach were weighed to determine the diet, and then the contents of the gastrointestinal tract were diluted with water using scissors for cutting in Petri dishes. The samples from which their gastrointestinal were emptied and the contents of the digestive tract were examined using the numeric method (Biswas, 1993) and were assessed with the help of the invertebrate's atlas (Birstein *et al*., 2005).

The frequency of occurrence of the food item (Fi) in the sample was calculated as:

$$Fi= (Ni/N)\times 100$$

Where, Ni= the number of the stomachs that contain prey (i) and N = the number of the full stomachs that were examined (Hynes, 1950).

The percentage abundance (Ai) of prey type (i) can be described by the following equation:

$$Ai= (Si/St)\times 100$$

Where, (Si) is the stomach content (volume, weight, or number) composed by prey (i), and (St) is the total stomach content of all stomachs in the entire sample (Ammundsen *et al*. 1996). This method has been used to analyze the strategy of nutrition based on the frequency of occurrence (Fi) against the percentage abundance (Ai), and then each point according to the position of the inward chart is interpreted (Costello, 1990). The equation was used to calculate the vacuum index (Euzen, 1987):

VI= (Es/Ts)×100

Where, VI= stomach emptiness index, Es= empty stomachs, and Ts= total number of stomachs that were examined. The intensity of feeding, as indicated by the VI, is interpreted as:

- Edacious species 0 ≤VI < 20
- Relatively edacious species 20 ≤ VI < 40
- Moderate feeder 40 ≤ VI < 60
- Relatively abstemious 60 ≤ VI < 80
- Abstemious 80 ≤ VI <100

Index of preponderance (Ip) was used from the equation as:

Ip= (ViOi / ∑ (ViOi)×100

that (Vi) Percentage volume of food and (Oi) occurrence of particular food item (i) (Marshall and Elliott, 1997).

The length and weight of relationships were calculated using the equation (Froese and Pauly, 2018):

$W = aL^b$

In this equation, "W" is the weight, "a" is the width from the origin, "L" is the total length, and "b" is the line slope. By using the least squares method for the remainders, the optimal values for the coefficients ‘a’ and ‘b’ were obtained from the following equation (Haddon, 2011),

$$SSQ = \sum(Y - (a + bX)^2$$

The SSQ is the total sum of squared residuals. One of the criteria used to fit is the least squares method. The implication of this method is to look for a set of parameters that minimizes the disparity between observed data and model predictions and the values of particular parameters (Haddon, 2011).

In the momentary growth rate: analysis, the h1 and h2 equations were used to determine relative growth and equation g to determine the momentary growth rate (Ricker, 1979):

$$h_1 = \frac{l_i - l_{i-1}}{l_i} \times 100$$ L= Length (cm) h= Relative growth

$$h_2 = \frac{W_i - W_{i-1}}{W_i}$$ ،W= weight h= Relative growth

$$g = \ln \frac{W_i}{W_{i-1}}$$ ،W= weight g= momentary growth rate

Biology of reproduction

Determination of length at first puberty

Calculation of fish size at the time of first maturity based on total length was done using the following equation and least square method (King, 2006), in Excel software. In this method, fish whose ovaries are in mature stages (stages 4 to 5) were considered as adults:

$$P_l = \frac{1}{1 + e^{-r_m(l - lm50)}}$$

Where: Pl, the percentage of adult fish in the longitudinal group l; rm, slope of the curve lm50, fish length at sexual maturity (the length at which 50% of the fish are mature); l, average total length in length class (cm). The sex was determined macroscopically by splitting the abdomen of the fish and identifying the presence of ovaries (granular appearance) or testes (tube-shaped appearance) in it (King, 2006).

Sexual maturation cycle in male and female

Sexual stage I: It is when the ovaries and testicles of the fish are barely visible, which are located in the abdominal area as two thin threads. On both sides, there are swimming bags of fish adjacent to the sides.

At this stage, the sexual tissue of the fish cannot be seen and cannot be recognized.

Sexual stage II; at the same time as the fish grows, the ovaries and testicles grow and become a little bigger. At this stage, it is difficult to determine the species of the fish, but it can be recognized. Blood vessels can be seen on the ovaries. Which cannot be seen in the testicle, the egg cannot be seen with the naked eye. Stage III sex; Ovaries and testicles become bigger and grow so that they fill half of the body of the fish and the abdominal area of the fish. At this stage, the eggs are small and opaque. In the ovary, of course, by observing and without the need for an armed eye, the fish testicle becomes thicker at the beginning of the body and slightly thinner at the end of the body. Stage IV sex; In the middle of this stage, the growth of the genital organ has reached its maximum and fills about 2/3 of the abdominal area. Most of the internal volume of the fish is filled by ovaries and testicles. At this stage, the eggs are completely large and transparent. The testicle is completely white and full of liquid sperm. Sexual stage V; It is the spawning stage of the fish. The eggs inside the fish's belly have become liquid and are in a liquid state in the belly so that the egg comes out with the slightest pressure in the abdominal area of the fish. In the act of spawning, there is no pressure on the belly of the fish, but it is directing the eggs outwards. At this stage, the eggs are clear, large, and separate from the fish's stomach.

Fecundity

After the samples were transferred to the laboratory, they were subjected to biometry concerning the length of the fork using a digital caliper with an accuracy of 0.1 mm and the weight of the whole fish, the weight of the digestive system and the weight of the gonads using a digital scale with an accuracy of 0.01 Gram was measured. The sexual stages of the gonads were determined using the 6-stage method (King, 2006) and to determine the fecundity, the number of three samples weighing 0.35 to 0.53 grams from each ovary (in the 4th stage of sexual maturation) of the fish was measured using a digital scale with the accuracy of 0.01 gr was weighed, the number of eggs in each sample was counted, and fecundity was calculated using the following formula:

$$AF = \frac{c}{s} \times OW$$

Where AF is annual fecundity, C is the number of eggs counted in each sample, S is the weight of each sample (grams), and OW = ovary weight (grams) (Sivakumaran *et al*., 2003).

The sexual maturation index and GSI (gonadosomatic index) were calculated using the following formula (Billard *et al*., 1993):

GSI = (gonad weight/total weight)×100

Statistical analysis

The data analysis for this study was conducted using several statistical and modeling tools. The primary software used for statistical analysis was R (version 4.4.2), which facilitated various analyses, including descriptive statistics, correlation, and regression models. The SPSS software (version 22) was also employed for more detailed statistical testing, such as t-tests and ANOVA, to compare differences between groups. In addition, ArcGIS (version 10.8.2) was utilized for spatial analysis to examine the distribution of feeding habitats in the Golestan coastal waters. while Excel was used for preliminary data organization and cleaning.

Results

Nutrition (vacuity index)

A total of 47 specimens had empty digestive tracts, while 57 specimens had full digestive tracts. The vacuity index (VI) varied across different seasons, ranging from 11% to 73%. The highest VI was observed in winter, and the lowest in spring. Based on the VI, the species is considered to be highly feeding in spring and summer (0<VI<20), while in autumn and winter, it is relatively less active in feeding (60<VI<80) (Table 1).

Table 1*: The Seasonal survey of vacuity index (VI) of Rutilus caspicus*.

Season	Total sample	Filled intestines	Empty intestines	empty gastrointestinal index VI%
Spring	18	16	2	11.1
Summer	13	11	2	15.3
Autumn	27	18	19	70
Winter	46	12	34	73.9

Nutritional indices

Gastropods were a dominant food type. Crabs, Bivalvia, and remnants of plants were known as "generalized food for the Roach in Golestan province (Table 2).

Table 2*: The Importance of each prey in the digestive tract of Rutilus caspicus*.

Season	Prey	Importance of each prey in the digestive tract		
		Ip%	%A	%F
Spring (n = 18)	Crabs	25.29	24.5	12
	Plants Remnants	3.92	4.1	8
	Gastropods	53.15	44.9	10
	Aquatic insect	-	-	-
	Bivalvia	4.9	10.2	4
	Polychaeta	2.94	6.1	4
	Shrimps	-	-	-
	Fishes	9.80	10.2	8
Summer (n = 13)	Crabs	24.77	25.6	15
	Plants Remnants	3.59	3.3	10
	Gastropods	50.58	40.9	10
	Aquatic insect	0.26	0.7	18.6
	Bivalvia	8.55	12.7	3
	Polychaeta	1.33	5.5	3
	Shrimps	-	-	-
	Fishes	10.56	11.3	5

Table 2 (continued):

Season	Prey	Importance of each prey in the digestive tract		
		Ip%	%A	%F
Autumn (n = 27)	Crabs	2.85	3.5	25
	Plants Remnants	1.13	1.3	31.8
	Gastropods	95.5	92.2	45.6
	Aquatic insect	-	-	-
	Bivalvia	0.41	1.7	9.1
	Polychaeta	0.03	0.5	2.3
	Shrimps	0.02	0.3	3.3
	Fishes	0.06	0.5	4.5
winter (n = 46)	Crabs	2.17	4.7	12.5
	Plants Remnants	2.57	1.0	62.5
	Gastropoda	91	90	55.4
	Aquatic insect	-	-	-
	Bivalvia	1.85	1.9	31.3
	Polychaeta	0.06	0.4	6.3
	Shrimps	2.35	2	3.2
	Fishes	-	-	-

Costello model

According to Figure 2 (a), we can say that the dominant food was gastropods and shrimp, fish, and aquatic insects. Polychaeta was a rare prey item. Also, we can say that crabs, Bivalvia, and remnants of plants were known as generalized food for *R. caspicus* in Golestan province (Costello, 1990).

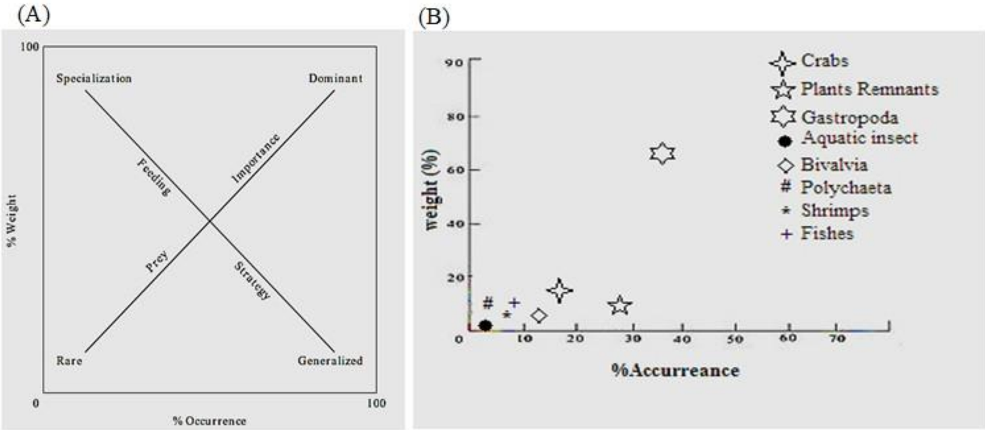

Figure 2: (A) Castello formula (1990) used to explain prey importance and feeding strategy. (B) The position of food prey in Golestan province on the Castello charts, which aids in interpreting the sampling method of the Castello- *Rutilus caspicus*.

Reproduction

Sex ratio

The sex ratios of male and female fish were examined across different months. In November, December, and February, a significant difference was observed in the ratio of male fish to the total population. However, no significant difference was found between the two sexes in the overall sample. An analysis of the sex ratios of male and female fish across different months revealed significant differences in the ratio of male fish to the total population during October, November, and December. In both October and December, males were dominant, while in January, females outnumbered males. Overall, the female sex was dominant in the total number of examined fish (Table 3).

Table 3: Sex ratio of *Rutilus caspicus*.

Month	N	Female	Male	Sig.
Sep	20	16	4	0.001
Oct	33	25	8	0.010
Nov	43	38	8	0.001
Dec	34	14	20	0.257
Jan	46	16	30	0.078
Feb	37	17	20	.739
Mar	32	15	17	0.738
Apr	32	17	15	0.088
May	37	14	13	0.501
Jun	36	12	14	0.751
Jul	28	12	16	0.811
Aug	23	10	13	0.701
Total	384	206	178	0.745

Examining the condition of the gonads

Monthly changes of GSI (Gonosomatic index)

From October 2014 to April 2015, the sexual maturation index (GSI) for the female species of *R. rutilus* in Golestan province varied from a minimum of 0.08 to a maximum of 0.17 and the decline or break of the curve started from the end of March and decreased during the April (Figs. 3 to 5).

Figure 3: Female (A) and male (B) sexual organs at the time of sexual maturation in the present study.

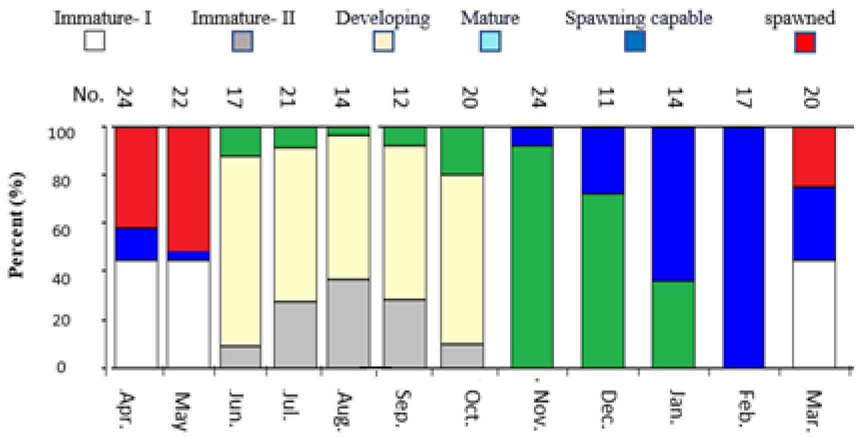

Figure 4: Monthly changes of different stages of sexual maturity in female *Rutilus caspicus* in the present study.

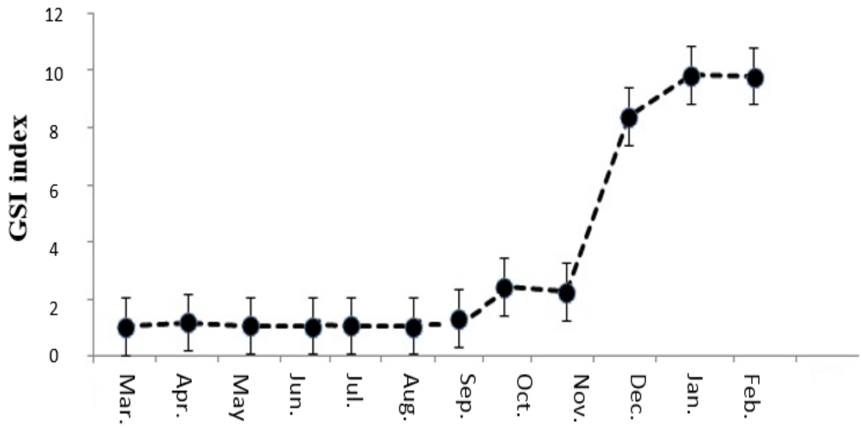

Figure 5: Monthly changes of GSI in *R. caspicus* in the present study.

Fecundity and its relationship with body weight and length

Fecundity was also investigated, and as shown in (Table 4), overall, with increasing age, the average number of eggs per millimeter, fork length, and absolute fecundity increased, while the average egg diameter decreased. The average number of eggs in one gram of ovary and the number of eggs in one gr of weight do not have any particular order.

Table 4: Fecundity status of Caspian roach (*Rutilus caspicus*) in the present study.

Age	Egg number		Egg diameter	Egg per 1 g	Relative Fec.	Absolute Fec.
2	45	Max	1.002	1951.8	258.1	20000.13
		Min	0.76	1274.8	103.4	7154.2
		Average±SD	0.88±0.13	1627.7	182.4	12000.3
3	80	Max	1.301	1863.6	201.1	23196.3
		Min	0.68	1182.5	103.8	7134.1
		Average±SD	0.85±0.15	1487.3	152.6	11824.2
4	20	Max	1.501	2250.1	215.1	19469.2
		Min	0.98	1357.1	130.1	11426.6
		Average±SD	1.057±0.1	1426.6	167.9	20000.13

In addition, a sample of one-year-old fish was also examined for fecundity, with the average egg diameter being 1.22 mm, the average number of eggs in one gr of ovary being 1412.8, the number of eggs in one gr fish weight 121.4, and the absolute fecundity 5891.6.

Relationship between length and weight with absolute fecundity

Figures 6 to 8 show that, the presence of linear relationships between fork length and total weight with fecundity, the results of the fecundity test with length and weight factors through Pearson's test showed that these relationships are significant (p<0.05).

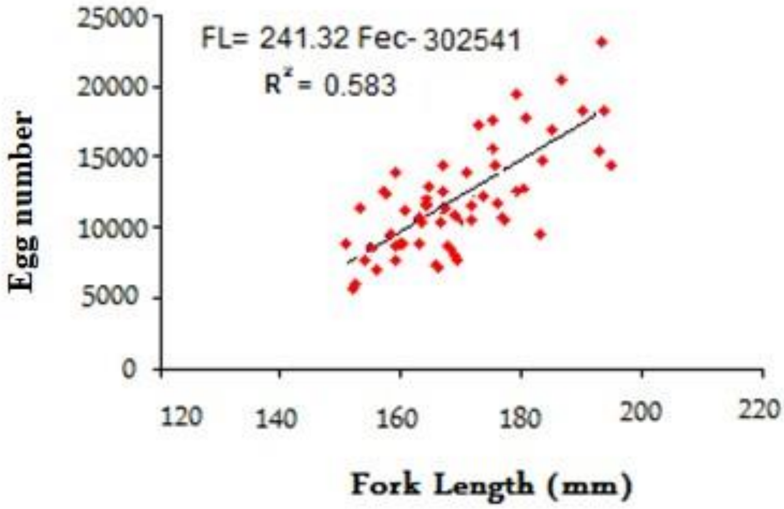

Figure 6: Relationship between fecundity and fish length of *R. caspicus* in the present study.

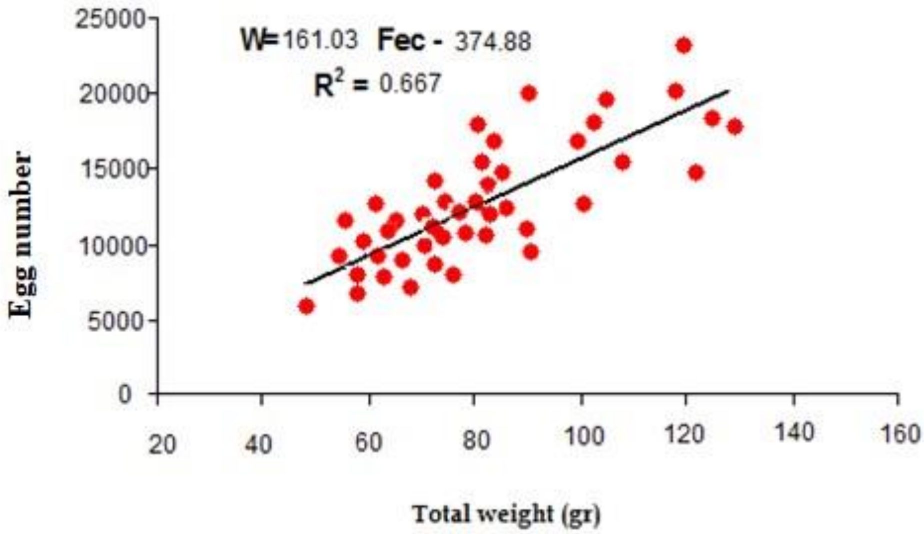

Figure 7: The relationship between fecundity and the weight of *R. caspicus* in the present study.

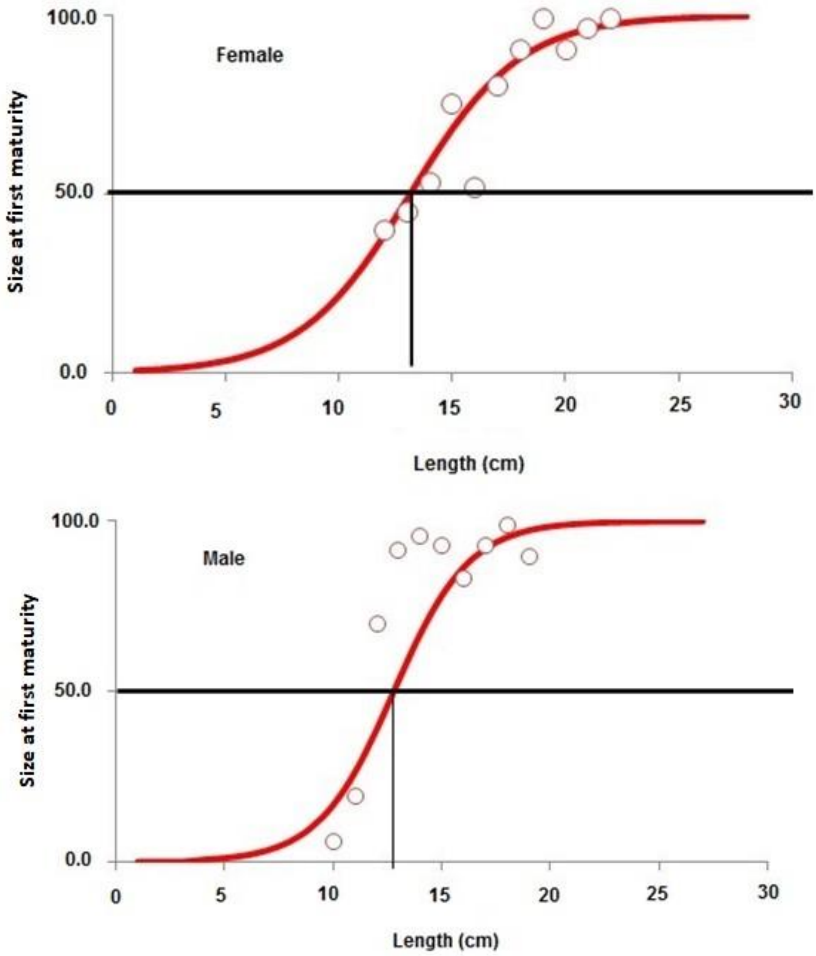

Figure 8: Size at first maturity (Lm50) estimated for females (F) and males (M) of *Rutilus caspicus* in the present study.

Discussion

According to Winfield and Winfield (1992), the roach feeds on infertility, zooplankton, grasses, and detritus, this is one of the most efficient substitutes for European roaches. Based on Jamet and Desmolles, (1994) on Aydat Lake, the main feeding was invertebrates (39.7%), sediment (29%), macrophytes (15.4%), and small zooplankton consumed by 2.4%. Horppila (1994) showed that the roach food items in both the coastal area and the Vesijarvi Lake in May and September-October were a combination of both. As the fish grew, the importance of zooplankton decreased, while the significance of benthos and plants increased. According to Kottelat and Freyhof 2007, fish commonly eat plant material and foul creatures. Young fish are basically "Plankton," while mature specimens are basically " Demersal fish." In examining the diet of the Caspian roach, the dominant and highest food was the gastropods (Gastropoda). Shrimp, fish, insects, zooplankton, and bivalves were some of the baits that were used in a smaller amount, and round crabs and plants were known as general food for Caspian roach.

The results of our investigation are consistent with the results of the mentioned studies, and because all the examined samples were more than one year old, which are considered adult fish, therefore, most of the detected baits were from communities of benthic organisms, which were mentioned in other studies.

Considering that the first age of maturity of this species in the province is one year old, the fish that are in the first age of maturity comprise more than 36% of the catch, which is important in terms of management and is considered a negative aspect of exploitation. Jan Henning *et al*. (1985) reported the first age of puberty for males and females of the *R. caspicus* in Norway to be 2 and 3 years, respectively. Zhukov (1965) suggests that Norway has a colder climate compared to northern Iran, which results in a slightly higher age of maturity for *R. caspicus* in Norway. The highest rate of weight gain compared to length increase in *R. caspicus* was related to the age of 2 to 3 years. According to Nikolsky (1969), the factors affecting growth include the quality of reproductives, reproduction time, reproduction environment temperature, abundance of food in the environment, growth of infants, main food sources, food competition, population density, temperature of the living environment and the conditions of the organisms in later ages. According to the environmental differences in this study, any of the mentioned factors or several factors can be effective in this field.

According to Jan Henning *et al*. (1985) in Arungen Lake, Norway, migration to the spawning areas starts at the beginning of April when the water level rises and the temperature is 6 to 10 degrees Celsius and continues until the beginning of June. Kestemont *et al*. (1999) in the Meuse River in Belgium showed that the migration of spawners of the *R. caspicus* is divided into two stages: the first stage, before spawning, which starts from the beginning of February and lasts until the end of March, and is related to the mass movement of *r. caspicus* to It is on the side of stagnant waters. The second stage of spawning migration is in April and May, returning from the previous period. According to Geraudie *et al.* (2009), natural conditions can effectively affect the sexual maturation cycle of *R. caspicus*, sexual maturation occurs at the end of winter to early spring, and the development of sex cells stops when the temperature drops below 6 degrees.

According to Alaghi (1998) and Naddafi *et al*. (2001) in the Gomishan wetland, the peak spawning of the *R. caspicus* occurs in March and April, which is consistent with the results of the present study. The peak spawning of the *R. caspicus* in Belgium and Norway was about a month later, which is probably due to the low average annual temperature of these two countries compared to Iran. In the study of Naddafi *et al*. (1999) 1: 1/1 was declared to be consistent with the sex ratio observed in Golestan province. In other words, at the peak of spawning, the male and female of the *R. caspicus* participate in natural reproduction. Zhukov (1965) reported the egg diameter of the *R. caspicus* to be about 1.5 to 1.5 mm, while the average size of the egg diameter of this fish in Gardno and Lebsko lakes is 1.295 and 1.374 mm, respectively, and the prevalence of this species the number of eggs varied from 700 to 77.000. Naddafi *et al.*(2002) reported the size of the fish eggs from 0.9 to 1.45 mm and the absolute number from 6.275 to 98.804. In the results of the present study, the egg diameter was 1.3 mm, and the absolute fecundity ranged from a minimum of 7,158 to a maximum of 22,185 eggs. This range is consistent with the findings of other studies. The lower maximum absolute fecundity observed in this study, in comparison to others, is likely attributed to the shorter lifespan of the specimens sampled. In our study, the maximum age of the examined individuals was 4 years, whereas in other studies, the oldest individuals reached 8 years.

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Confilicts of interest

The authors declare that there is no conflict of interest.

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