Genetic Sexuality Investigation of *Cobitis taenia* along with Biodiversity and Morphomeristic Characteristics

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Abstract: This work was conducted to investigate the diversity, distribution and sex determination in the population of the *Cobitis taenia* species in Siyahrud River of Qaemshahr city. Accordingly, to determine the population structure such as total length, fork length, head size, caudal fin and other parameters of biometry such as age, sexuality and other criteria, this research was carried out in three stations of Siyahrud River of Qaemshahr city in 2012. The selected stations were Karchang village located 15 kilometers from Qaemshahr city, Keshavarzi quarter in the middle of Qaemshahr and the station of Laheman village situated 15 kilometers from Qaemshahr on the way to Juybar, where sampling process was undertaken. Besides sampling, other parameters of water like temperature volume rate, pH, height, depth and the entry locations of pollutions were also analyzed. In regards to technical practices, a population of 246 fish was examined, using the above parameters with highly sensitive measuring equipment like digital caliper, digital scale, binocular and microscope. Sampling was performed by an electro shocker in which electricity was charged into water semi-paralyzing the fish, so that the fish moved to the surface of water. We investigated the parameters by catching the fish through a handy lace. Consequently, the average, standard deviation and coefficient of variation in Karchag village for body depth, fork length and head length parameters were respectively as: for average: 2.34, 12.1, 2, for standard deviation: 0.38, 0.46, 0.61, for coefficient of variation: 0.17, 0.086, 2, for Leheman village: for average: 3.10, 7, 1.47, for standard deviation: 0.44, 0.72, 0.32, for coefficient of variation: 0.4, 0.07, 0.19, for Keshavarzi Quarter: for average: 1.91, 9.45, 2, for standard deviation: 0.32, 0.40, 0.18, for coefficient of variation: 0.31, 3.8. We concluded: as we get farther from the sources and go to the lower directions, the size of the fish gets smaller and their life shorter.

Key words: Merphomeristic • Biodiversity • Sex Determining *Cobitis taenia*

INTRODUCTION

In most animal species, males and females behave differently, especially when it comes to sex and related social behaviors. These behaviors evolved to ensure successful reproduction and typically include some genetically preprogrammed displays. There are ~25,000 known species of fishes, by far the largest group of vertebrates and they express a remarkable variety of adaptive responses to aquatic habitats with associated ecological constraints [1]. Moreover, teleost's have evolved in a relatively short time (~200 My) [2] producing virtually every reproductive option open to vertebrates: they bear live young, brood offspring in their mouths or body cavities, lay eggs (sometimes even out of water) or simply release their gametes into the plankton. In addition, mating/brood-care systems range from monogamous bi-parental to polygamous non parental. In some species,
females can reproduce parthenogenetically, males may become pregnant, and fish of both genders can change sex [3, 4]. This brief summary of the range of fish reproductive behaviors highlights why they offer so much as reproductive systems for analysis. Some sex-determining mechanisms have been conserved over vast stretches of evolutionary time. For example, in birds and mammals, all extant species share a ZW system of female heterogamety (e.g., production of 2 kinds of gametes) and an XY system of male heterogamety. In both these cases, the different sexes are always represented by two different individuals (e.g., gonoehorism, [1]). In contrast, fish show a wide variety of sex determination systems, some via sex-determining chromosomes, other via autosomal genes and still others via environmental or social signals [3, 4]. Numerous studies have shown how morphological specialization and life-history differences between fish species translate directly into behavioral differentiation between the sexes. More subtle behavioral differences may arise as a consequence of the different reproduction roles taken by males and females. In gonochoristic fish species, all possible forms of genetic sex determination have been observed from male and female heterogamety with or without the influence of autosomal genes, to more complicated systems involving several loci but without sex chromosomes or with several pairs of sex chromosomes [5]. For example, in the striated spined loach (cobitis taenia) sex is determined through multiple sex chromosomes where females have \(x_1x_1x_2x_2\) while males have \(x_1x_2y\) sex chromosomes [6]. In many fish species temperature and/or pH of the hatching water determines sex. In a mouth brooding cichlid Oreochromism niloticus, Baroiller et al. [7] showed that housing mouth brooding females, in higher temperatures increased the male proportion in their brood from 33% to 81%. Phenotypic sex can also be fully reversed by hormone treatment in female Chinook salmon (Oncorhynchus tshawytscha) where a brief treatment with an aromatase (enzyme that converts T to E,) inhibitor during sex differentiation causes chromosomally female animals to develop as normal males [8]. Nonetheless, the core of the vertebrate sex determination differentiation cascade is conserved in fishes [1, 9, 10]. Dmrt1, named for a common DM domain, is considered to be involved in sex determination and/or sex differentiation and its expression is central to the development of the male tetrapod phenotype. This gene has a similar role in sex determination in both hermaphroditic and gonochoristic fish [11, 12]. In the medaka (Oryzias latipes) sex differentiation is chromosomal (male heterogamyy XX-XY) and recently, functional and expression analyses have shown that Dmrt1 is the master gene for male sex determination [13]. However, in many fish species, once sex has been determined, reproductive ability can be regulated through social cues [14]. Similarly, Iran has 23 native species, the most common of which is cyprinidae [15-17]. All of these fishes are typical of the inner waters in Iran and this has made them very important. In fact, because of being unique and special, these native fishes of Iran are capable of being placed next to aquarium fishes [18-20]. By studying the life and recognition of these fishes and also by scientific practices in cultivating them, we will be able to make more progresses and export these rare species. As one of these species, cobitis taenia has a special importance in luxurious fish families [16, 17, 21-23]. Thus, this study was done to investigate the sex determining biodiversity and morphomeristic characteristics of Cobitis taenia.

**MATERIALS AND METHODS**

This survey was carried out in 2012 with a samples of 246 fishes (122 male and 124 female). It was based in Quaemshahr city’s Siyahrud River, which flows from Alborz water sources down to Quaemshahr city and then enters Caspian Sea.

In this study, three stations of sampling were chosen, including Karchang village located 15 kilometers from the city; Keshavarzi quarter in the middle of the town; and Laheman village 15 kilometers after Quaemshahr city on the way to Juybar. In each station, using electroshocker device and electricity power, we semi-paralyzed the fish and collected them by handy lace. The fish went to the surface and were put in 10% diluted formalin. Then, they were taken to the laboratory.

In the laboratory, the specimens were examined by sensitive devices which considerably minimized the number of human faults. To do laboratorial operations, some technical facilities such as digital scale, digital caliper, biometrical board, microscope, minicalar, lam and scalp were used.

In the biometry analysis, some parameters such as standard length, total length, body depth, caudal fin, fin sizes, head length and some other factors like sex determination were also examined. These practices were performed by measuring fish and observing the inner parts as well as using recognition keys (color, sexual seeds and body length).
In determining the age, we estimated the age by observing scales under microscope. It is possible to determine the age by recognizing the concentric circles and counting the congestion of each group from these circles.

In sampling, we also checked the water specimens, the amounts of O₂ and pH in laboratory and water temperature of the place using a thermometer.

Figures are presented in a bar chart, making the comparative study of the data easier. Statistical data were inserted in Excel software and variance and average of data were calculated.

### RESULTS

Total, standard length and down head are represented in Figures 1, 2, 3 and 4. The distribution of sex groups are depicted in the tables. Other parameters are also illustrated in Tables 1, 2 and Figures 5, 6. Figure 1, 2 and 3 show that the differences of length parameters and weight in Lehman village is less than other stations, while the difference of biometric parameter with other stations are not significant. Figure 5 and 6 shows a high linear correlation was observed between weight and length.

#### Table 1: The number of fishes according to their sexuality in the three different regions.

<table>
<thead>
<tr>
<th>Region</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karchange</td>
<td>33</td>
<td>44</td>
<td>77</td>
</tr>
<tr>
<td>Laheman</td>
<td>37</td>
<td>38</td>
<td>75</td>
</tr>
<tr>
<td>Keshavarzi</td>
<td>52</td>
<td>40</td>
<td>94</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>122</strong></td>
<td><strong>124</strong></td>
<td><strong>246</strong></td>
</tr>
</tbody>
</table>

#### Table 2: Water parameters for in three different regions.

<table>
<thead>
<tr>
<th>Stations</th>
<th>Temperature</th>
<th>pH</th>
<th>O₂/mg/lit</th>
<th>CO₂/mg/lit</th>
<th>Length/m</th>
<th>Width/m</th>
<th>Depth/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karchange</td>
<td>21</td>
<td>5</td>
<td>5</td>
<td>20</td>
<td>4</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>Laheman</td>
<td>22</td>
<td>7.5</td>
<td>7.5</td>
<td>21</td>
<td>4.5</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Keshavarzi</td>
<td>21</td>
<td>7.5</td>
<td>7.5</td>
<td>22</td>
<td>5</td>
<td>3.5</td>
<td>15</td>
</tr>
</tbody>
</table>

#### Table 3: Comparisons between average, standard deviation and coefficient of variation in *cobitis taenia* in three different regions.

<table>
<thead>
<tr>
<th>Regions</th>
<th>Average</th>
<th>Variance</th>
<th>Standard Deviation</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karchange village</td>
<td>2.34</td>
<td>0.137</td>
<td>0.38</td>
<td>0.17</td>
</tr>
<tr>
<td>Body depth</td>
<td>12.1</td>
<td>0.9</td>
<td>0.46</td>
<td>0.086</td>
</tr>
<tr>
<td>Fork length</td>
<td>2</td>
<td>0.6</td>
<td>0.61</td>
<td>2</td>
</tr>
<tr>
<td>Head length</td>
<td>3</td>
<td>1</td>
<td>0.44</td>
<td>0.4</td>
</tr>
<tr>
<td>Laheman village</td>
<td>10.7</td>
<td>0.61</td>
<td>0.72</td>
<td>0.07</td>
</tr>
<tr>
<td>Body depth</td>
<td>1.47</td>
<td>0.09</td>
<td>0.32</td>
<td>0.19</td>
</tr>
<tr>
<td>Fork length</td>
<td>1.91</td>
<td>0.091</td>
<td>0.32</td>
<td>0.31</td>
</tr>
<tr>
<td>Keshavarzi Quarter</td>
<td>9.45</td>
<td>0.15</td>
<td>0.40</td>
<td>3</td>
</tr>
<tr>
<td>Body depth</td>
<td>2</td>
<td>0.019</td>
<td>0.18</td>
<td>8</td>
</tr>
</tbody>
</table>

Fig. 1: Chart of average total length, standard length and average size under the head in three different regions.
Fig. 2: Chart of dorsal size, the gap between two eyes and head length in three different regions.

Fig. 3: Chart of average weight in three different regions.

Fig. 4: Chart of depth, height, tail stem and caudal fin size in three different regions.

Fig. 5: Linear correlation of total length with total weight in Karchang village.
Fig 6: Linear correlation of total length with total weight in Laheman village.
Note: The above chart for Keshavarzi quarter was not remarkable.

DISCUSSION

Our finding shows that as we get farther from the sources and go to the lower directions, the size of the fish gets smaller and their life shorter. It means that the habitat of the fish because of the different PH, abundances of nutrients, photo synthesis, food cycle, water combinations and other physical and chemical parameters are so critical in fish morphology.

In lower parts of the river, due to the pollution of water, there is a decline in the extent of growth and a reduction in the age of the fish, meaning the population of the fish is taking a decreasing trajectory [21, 23].

These high levels of pollution are caused by different factors, including wastes from domestic and industrial factories, workshops, etc. which are located beside the path of this river. It might be possible to use this fish, like some of other animates, as a criterion for the pollution and cleanliness of water.

Water parameters obtained in this river are similar to life conditions in aquarium. For example, water temperature in the river is 21, that is approximately similar to aquarium conditions. This fish can adapt herself to the environment. That is, this animate has the tolerance of temperature pain in this range, but it should be noted that these temperature differences and alkali environments can cause a lot of stress for the fish and induce shocks to the fish [15,19].

This fish can complete the food cycle in its environment, plays a great role in the nourishment of bigger fishes and can transfer food from the lower levels of the cycle to the upper levels. As the name clearly indicates (cobitis taenia), the fish can clean its living environment of the aquarium and can refine its surroundings from pollution as well. In addition, by breeding this fish, we will be able to use it as living bait, or a living food, for bigger fishes in the aquarium. Fish express extremely diverse sex determination systems, mating systems, sensory systems and reproductive tactics that have evolved multiple times and account for their evolutionary success. The fine-tuning of each of these pathways is unlikely to be genetically determined but shaped by experience, ongoing behavior, the reproductive axis as well as neural and epigenetic changes.

Consequently, the inclusion of this species has resulted in an increase in interest in its conservation. As yet it is unknown if a number of endemic subspecies or species of spined loach have evolved in Iran due to the reproductive isolation of different populations. If this is the case, the entire complex of species will need to be conserved. Genetic research is currently in progress. Weed cutting and dredging will have short-term negative effects on this fish, but in the longer term, dredging may create more appropriate substrate conditions. Research into more detailed habitat requirements of this species in order to guide habitat management is underway.

REFERENCES