Identification of larval stages of fish in southeastern coastal waters of the Caspian Sea- Golestan Province

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Abstract
This study was carried out to develop a practical guide to identify species of fish larvae in the coastal waters of the Caspian Sea-Golestan Province. A total of 18 sample sites in the Caspian Sea and 6 sampling sites in the Gorgan Bay were sampled monthly from March to September 2015. The findings showed most of the fish larvae belonged to the postflexion and transitional stages. Some morphological and meristic characteristics of fish larvae were measured. Fish larvae were represented by 5 genera including: Atherina caspia (Eichwald, 1838), Rutilus caspicus (Jakowlew 1870), Rutilus kutum (Kamensky, 1901), Cyprinus carpio (Linnaeus, 1758), Chelon saliens (Risso, 1810), Chelon aurata (Risso, 1810) and Gambusia holbrooki (Girard, 1859). The results showed C. carpio, R. caspicus, and R. kutum were separated based on the pigment patterns on their body. While R. caspicus and R. kutum were discerned by the number of pre-anal myomers. Chelon sp. could be differentiated by a different pigment pattern, as well as some morphometric characters including eye diameter, body depth, and head length. Additionally, identifying characteristics for A. caspia and G. holbrooki are described. The results of this study revealed that in contrast to the current perception, the analysis of the morphometric variations of Chelon sp., Rutilus sp., and C. carpio fry could be used for their identification.

Keywords: Fish larvae, Morphometric and meristic, Coastal water, Caspian Sea

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Introduction
Larvae refer to the life stage prior to attaining the full complement of fin ray elements, the complete development of scales, and the loss of all larval characteristics (Neira et al., 1998). The developmental stages of larvae are defined, based on the development of notochord flexion during caudal fin development, as preflexion, flexion, and postflexion (Neira et al., 1998). During the larval stage fish undergo drastic changes in general shape and structural details (Loy et al., 2001). These developmental processes can be gradual to abrupt depending on the gene expression and environmental conditions (Gilbert and Bolker, 2003).

Larval stages are followed by a transformation stage during which individuals transform into juveniles, which morphologically resemble adults (Osse et al., 1997). Anatomical, physiological, and behavioral changes such as dentition, feeding behavior, and swimming capacity allow various larval stages to attain new capabilities for biological activities, which in turn enable them to occupy their preferred habitats and explore new environmental conditions (Moteki, 2002; Moteki et al., 2002; Pena and Dumas, 2009).

Understanding the biology of early life history stages of fish is essential for effective fisheries management (Hames Hichford, 2000; Mwaluma et al., 2014). For example, knowledge in fish development such as ontogenetic intervals and embryonic and larval development of fish species is a prerequisite to set the policies for their conservation and stock enhancement through larviculture (Rahman et al., 2009; Yanes-Roca et al., 2012; Amini et al., 2015; Andrade et al., 2016). However, the identification of eggs and newly hatched larvae has proven difficult due to having little or no resemblance to their adult stages, considerable overlap in the time and location of spawning among multiple fish species, similarity in morphology of eggs among species, their small size, and quick changes in shapes during the larval stage (Victor et al., 2009; Ko et al., 2013; Amini et al., 2015).

Several ichthyoplankton surveys have been carried out in the Persian Gulf and Oman Sea, located in the coastal waters of Khuzestan, Bushehr, Hormozgan and Sistan-Balochistan Provinces, Iran (Rabbaniha, 1998, 2002, 2007; 2011; Rabbaniha et al., 2003, 2013; Vosough et al., 2009, 2010; Koochaknejad et al., 2011; Rabbaniha and Owfi, 2011). They identified fish larvae, reported fish diversity, and developed density indices in the coastal area. A few studies were concerned about identifying early development characteristics of fish larvae. For example, in one study, Rastrelligar sp. and Scomberomorus sp. in the Khark and Kharo coralline islands were distinguished based on their morphological characters in the preflexion stage (Rabbaniha, 2011). In a survey of early life stages of Clupeiformes in the coastal waters of Bushehr Province, Sardinella sp., Dussumiera sp., Ilisha sp., Encrasicholina sp., and Thryssa sp. were differentiated based on the number of myomeres, dentate cover in the
mouth, and dorsal and anal fin locations (Rabbaniha, 2013). In another study, pigments on the tip of the lower jaw and snout were the discriminating characteristics between two species *Benthosema pterotum* and *B. fibulatum* (Rabbaniha and Saraji, 2014). However, there has not been sufficient information collected on the early developmental stages of the fishes in the Southern Caspian Sea and no identification key is available for the fish larvae in this region. This study is the first report on the early life stages of fish larvae collected from the natural habitat from the area. Therefore, the objective of this study was to develop a practical guide for identifying fish larvae in the Southeastern coastal waters of the Caspian Sea, Golestan Province. We also aimed to describe the larval characteristics of fish species based on morphometric and meristic variables and pigmentation patterns.

**Materials and methods**

**Study area**

Monthly sampling was conducted in the coastal waters of the Southeastern Caspian Sea, Golestan Province. A total of 24 sites were sampled along 6 transects near the Gomishan and Miakaleh Beaches within 36° 54´ 450´´ to 37° 13´ 355´´N and 53° 55´ 974´´ to 53º 47´ 080´´E, respectively, and 2 transects in the Gorgan Bay (Fig. 1) during March-September 2015, when the spawning of the majority of coastal fish species takes place in this region (Abdoli, 1999). The transects were perpendicular to the coast and placed at intervals of 2 km. The depth of the sampling locations ranged from 2 m to 7 m. Water depth, bottom type, bottom sediment size, discharge from rivers (Gorgan-Rud), and local surface currents (wind-induced type) were considered when choosing the sampling sites along the coastal line to represent the diversity of microhabitats in the region (Ghorbani et al., 2013).

![Figure 1: Location of sampling sites of fish larvae in the Southeastern coastal waters of the Caspian Sea.](image-url)
Data collection

Fish larvae were collected using a 46 cm bongo net with a mesh size of 330 µm during daylight hours. A flow meter was attached to the net to estimate the volume of filtered water. The net was swept obliquely from the bottom to the surface at a constant vessel speed of 0.3 knots to filter the water column (Sabates et al., 2007). The larvae samples were immediately fixed in 5% formalin in seawater buffered with borax (Lopez-Sanz et al., 2011), transferred to the fisheries laboratory, and identified to the lowest possible taxonomic level under a trinocular microscope (Nikon SMZ-745T) based on available identification keys (Auer, 1982; Moser, 1996; Ismail et al., 1998; Mousa, 2010; Makeyeva et al., 2011).

For each species, morphometric and meristic parameters were measured (Fig. 2), including body length (BL), head length (HL), snout length (SL), body depth (BD), eye diameter (ED), pre-dorsal length (PDL), pre-anal length (PAL), vent to anal fin length (VAFL), number of spines and fin rays of dorsal, anal, pectoral, abdominal (if exists) fins, and number of preanal and post-anal myomeres (Leis and Carson Ewart, 2000). Appearance characteristics such as the presence of spines, the shape of the eye, dentate cover in the mouth, and pigmentation (location and shape) were noted (Leis and Carson Ewart, 2000). Morphometric parameters were measured using Digimizer 4.1.1 (Digimizer Co. 2005-2011 MedCalc Software).

Statistical analysis

A preliminary analysis was performed using the analysis of covariance (ANCOVA) to check the relationship between the morphometric measurements and the total length. It showed no significant departure from the isometric relationship. To determine whether there are any statistically significant differences in each measurement among species, a one-way analysis of variance (ANOVA) and Duncan post hoc test were performed. To further elucidate the differences among the species, cross-validated discriminant analysis (DA) was used to determine the dissimilarity among the species and the ability of these characteristics to identify the specimens correctly. Data were analyzed in SPSS.
The morphological characteristics of fish larvae were summarized in Table 1. While *Atherina* sp., *Gambusia* sp., *Cyprinus* sp., and *Chelon* sp. were in the postflexion stage, *Rutilus* sp. was in both preflexion and postflexion stages. According to Table 1, *Chelon* sp. can be differentiated based on morphological characteristics especially based on pigmentation patterns.

### Table 1: List of fish larvae collected in the Southeastern coastal waters of the Caspian Sea.

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>English name</th>
<th>Morphological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherinidae</td>
<td><em>Atherina</em> sp.</td>
<td><em>Atherina caspia</em></td>
<td>Big scale sand smelt</td>
<td>Larvae have an elongate body and two dorsal fins. Abdominal part of the body is colorless and without pigment. Eyes are big in relation to the head length, and there is a distinct strip of black pigment along the dorsal, mid-ventral, and lateral line toward the caudal fin. The pigments are more abundant and larger on the head (Fig. 3A). In preflexion stage, larvae have curved body shape; the club-shaped yolk sac is about ( \frac{2}{3} ) of total length; eyes are big in relation to the head length; a few asterisk pigments are distributed on the neural cord, starting from the head and ending about ( \frac{1}{3} ) of total length; some pigment is also present on yolk sac.</td>
</tr>
<tr>
<td>Cyprinidae</td>
<td></td>
<td><em>Cyprinus aurata</em></td>
<td>Common carp</td>
<td>The larvae have an elongate body, similar to <em>R. caspicus</em>. Pigments are mostly on the head and the dorsal part of body in two rows along the body. Some single pigments are also present on the swim bladder, the tip of the snout, and caudal fin (Fig. 3C). Spherical eggs belonged to <em>Cyprinidae</em>, unfertilised, pale yellow, and diameter is about 1.5-1.8 mm.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Cyprinus carpio</em></td>
<td>Leaping mullet</td>
<td>Larvae have a very elongate body, small terminal mouth, shallow body depth, and long straight intestine. Pigments are distributed uniformly on the body except for the head and ventrum where no pigment occurs (Fig. 3D). Pigments are distributed uniformly on the body. The number of pigments is greater around the lateral line. They have two dorsal fins with the insertion of the second dorsal fin posterior to the insertion of the anal fin. Pigment density is lower compared with <em>Liza aurata</em>, and the depth of the caudal peduncle is shallower the width of the body in caudal peduncle is smaller in <em>Liza saliens</em> (Fig 3E). Pigments are distributed uniformly on the body. The number of pigments is greater around the lateral line, which appeared as a dark strip. They have two dorsal fins with the insertion of the second dorsal fin posterior to the insertion of the anal fin. Pigment density is greater compared with <em>Liza saliens</em>, and body appeared darker. The caudal peduncle has a greater width in <em>Liza aurata</em> (Fig. 3F). Larvae are characterized by superior mouth and small pelvic fin placed in first half of the body near pectoral fin. The location of the anal fin is prior of the dorsal fin. They have a round homocercal caudal fin. Pigmentation is minimal and randomly distributed on the body (Fig. 3G).</td>
</tr>
<tr>
<td>Mugilidae</td>
<td><em>Chelon</em> sp.</td>
<td><em>Chelon aurata</em></td>
<td>Golden grey mullet</td>
<td></td>
</tr>
<tr>
<td>Poeciliidea</td>
<td><em>Gambusia</em> holbrooki</td>
<td><em>Gambusia holbrooki</em></td>
<td>Eastern mosquito fish</td>
<td></td>
</tr>
</tbody>
</table>
Morphometric measurements of larvae in the postflexion stage are shown in Table 2. Except for the eye diameter, which was reported as relative to HL, all morphometric measurements were reported as relative to BL.

*C. aurata* had the maximum mean BL (23.17±7.87 mm), and *R. caspicus* had the minimum mean BL (9.9±0.83 mm). The analysis of variance (ANOVA) showed there were significant differences in HL, BD, and ED between *C. saliens* and *C. aurata* (*p*<0.0001). The maximum PAL, ED, BD, HL, and SL were measured in *A. caspia*, *C. saliens*, *R. caspicus*, *C. saliens*, and *C. saliens*, respectively. *A. caspia* had the minimum PDL, and *G. holbrooki* had the minimum PAL (*p*<0.0001).
Table 2: Morphological parameters (mm) of fish larva collected from the Southeastern Caspian Sea.

<table>
<thead>
<tr>
<th>Species</th>
<th>Parameter</th>
<th>BL</th>
<th>HL</th>
<th>SL</th>
<th>BD-3</th>
<th>ED</th>
<th>PDL</th>
<th>PAL</th>
<th>VAF-L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. caspia</strong></td>
<td>Min-Max</td>
<td>12.4-20.5</td>
<td>2.81±0.73</td>
<td>0.97±0.84</td>
<td>1.63±3.85</td>
<td>1.03±1.84</td>
<td>5.58±8.73</td>
<td>7.34±12.87</td>
<td>0.85±1.62</td>
</tr>
<tr>
<td></td>
<td>%Ratio</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>R. caspicus</strong></td>
<td>Min-Max</td>
<td>8.53-9.87</td>
<td>2.17±2.57</td>
<td>0.49±0.7</td>
<td>1.31±1.78</td>
<td>0.67±0.81</td>
<td>4.32±5.17</td>
<td>6.12±6.94</td>
<td>0.25±0.26</td>
</tr>
<tr>
<td></td>
<td>%Ratio</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>C. carpio</strong></td>
<td>Min-Max</td>
<td>8.34-10.68</td>
<td>1.83±2.24</td>
<td>0.25±0.33</td>
<td>0.9±1.3</td>
<td>0.63±0.84</td>
<td>4.69±5.44</td>
<td>5.63±7.60</td>
<td>0.11±0.18</td>
</tr>
<tr>
<td></td>
<td>%Ratio</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>C. aurata</strong></td>
<td>26.24±3.12</td>
<td>7.41±1</td>
<td>15.4±1.07</td>
<td>34.87±2.9</td>
<td>46.2±1.8</td>
<td>61.14±1.7</td>
<td>7.76±1.3</td>
<td>7.16±1.3</td>
<td>7.76±1.3</td>
</tr>
<tr>
<td><strong>C. saliens</strong></td>
<td>25.65±27</td>
<td>6.84±0.95</td>
<td>21.4±1.1</td>
<td>30.24±1.54</td>
<td>50.4±3.28</td>
<td>72.25±1.66</td>
<td>2.85±0.12</td>
<td>2.85±0.12</td>
<td>2.6±0.2</td>
</tr>
<tr>
<td><strong>C. holbrooki</strong></td>
<td>3.33±0.01</td>
<td>3.05±0.19</td>
<td>11.3±1.13</td>
<td>36.04±1.97</td>
<td>55.2±3.38</td>
<td>69.35±1.26</td>
<td>1.48±0.14</td>
<td>1.48±0.14</td>
<td>1.48±0.14</td>
</tr>
</tbody>
</table>

Means that do not share a letter are significantly different (α=0.05).

Meristic parameters in postflexion larvae were measured and summarized in Table 3. In Cyprinidae, the samples were too small, and the fins were not developed entirely. The total number of Myomers for A. caspia, R. caspicus, R. kutum, C. carpio, C. saliens, C. aurata, and G. holbrooki ranged from 36-39, 39-41, 43-45, 36-38, 20-22, 21, and 32-35, respectively. R. caspicus had a smaller number of myomers after anal in comparison to R. kutum.

Table 3: Meristic parameters of fish larva collected from the Southeastern Caspian Sea in 2015.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dorsal Fin1</th>
<th>Dorsal Fin2</th>
<th>Anal Fin</th>
<th>Pelvic Fin</th>
<th>Pectoral Fin</th>
<th>Myomer After Anal</th>
<th>Myomer Before Anal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. caspia</strong></td>
<td>(VI-VIII)</td>
<td>10-13(IV)</td>
<td>10-13 (II)</td>
<td>5(I)</td>
<td>12-13</td>
<td>24-25</td>
<td>12-14</td>
</tr>
<tr>
<td><strong>R. caspicus</strong></td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td>17</td>
</tr>
<tr>
<td><strong>R. kutum</strong></td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td>16-17</td>
</tr>
<tr>
<td><strong>C. carpio</strong></td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td>13-5</td>
</tr>
<tr>
<td><strong>C. saliens</strong></td>
<td>(IV)</td>
<td>9-10</td>
<td>8-10 (II)</td>
<td>5 (I)</td>
<td>14-15</td>
<td>11-12</td>
<td>9-10</td>
</tr>
<tr>
<td><strong>C. aurata</strong></td>
<td>(IV)</td>
<td>9(0-I)</td>
<td>8-10 (II)</td>
<td>5 (I)</td>
<td>12-15</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td><strong>G. holbrooki</strong></td>
<td>6-7</td>
<td>-----</td>
<td>9-10</td>
<td>5</td>
<td>9-11</td>
<td>13</td>
<td>19-22</td>
</tr>
</tbody>
</table>

The Roman numbers show the number of spines and the Arabic numbers represents the number of rays.

Because Cyprinidae larvae were similar in appearance, and detecting and counting myomers is challenging and needs some experiences of working with larva, discriminant analysis (DA) was used to differentiate the larvae based on morphological ratios, which are easier to measure. According to the results of DA, R. caspicus, R. kutum, and C. carpio were significantly discriminated (p<0.0001). The coefficients of the seven morphometric ratios associated with the first two discriminant functions are shown in Table 4. While the maximum standardized coefficients on the first discriminant function were on SL and PAL, the greatest coefficients for the second discriminant function were on HL, BD, and PDL. These discriminant
functions successfully identified the membership of one of the three species. The cross-validation results showed that all the observations were grouped into their original groups with 100% success rate. The first and second discriminant functions explained 79.4% and 20.6% of the total variance in the observations, respectively (Fig. 4).

Table 4. Coefficients for discriminant function on morphometric ratios.

<table>
<thead>
<tr>
<th>Canonical discriminant function Morphological Parameters</th>
<th>Function 1</th>
<th>Function 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL</td>
<td>-4.819 (-0.055)</td>
<td>63.173 (-0.722)</td>
</tr>
<tr>
<td>SL</td>
<td>127.051 (0.807)</td>
<td>-91.701 (-0.583)</td>
</tr>
<tr>
<td>BD</td>
<td>37.427 (0.394)</td>
<td>69.202 (0.728)</td>
</tr>
<tr>
<td>ED</td>
<td>-18.617 (-0.352)</td>
<td>12.22 (0.231)</td>
</tr>
<tr>
<td>PDL</td>
<td>-14.55 (-0.238)</td>
<td>-37.803 (-0.618)</td>
</tr>
<tr>
<td>PAL</td>
<td>-41.018 (-0.602)</td>
<td>17.623 (0.259)</td>
</tr>
<tr>
<td>VAFL</td>
<td>134.41 (0.463)</td>
<td>-5.462 (-0.019)</td>
</tr>
<tr>
<td>Constant</td>
<td>24.624</td>
<td>-16.572</td>
</tr>
</tbody>
</table>

1Standardized discriminant function coefficients were presented in parentheses.

DA results showed that some morphometric ratios could differentiate Chelon sp. significantly; these morphometric ratios include HL (p<0.05), BD (p<0.0001), and ED (p<0.0001). Canonical discriminant functions cover 100% of the variance (p<0.0001), and the observations were grouped into their original groups with a success rate of 97.1%. The unstandardized discriminant function coefficients for morphometric ratios were 6.88, 13.203, 102.851, 11.533, -4.349, -10.117, and -50.545 for HL, SL, BD, ED, PDL, PAL, and VAFL respectively. The constant term was -
The results of cross-validation are shown in Table 5.

Table 5: Classification of individuals into their original group by cross-validation.

<table>
<thead>
<tr>
<th>Species (group)</th>
<th>C. saliens</th>
<th>C. aurata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>C. saliens</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>C. aurata</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Cross-validated</td>
<td>4.2</td>
<td>95.8</td>
</tr>
</tbody>
</table>

97.1% of cross-validated grouped cases correctly classified

Discussion
Ichthyoplankton and macroinvertebrate assemblages were studied in the Gorgan Bay, previously. In the survey, six sites were sampled using bongo net, and no larvae had been collected (Mohammadkhani et al., 2010). Because turbidity was high in the sampling site (Gorgan Bay), the meshes were clogged quickly and could not filter the water column efficiently. The authors suggested decreasing sampling time to three minutes and changing sampling sites to increase the chances of catching larvae in the region (Mohammadkhani et al., 2010). In the present study, we modified the sampling sites in the Gorgan Bay and added some sampling locations in the Caspian Sea. This allowed us to collected larvae of seven fish species. We expected to sample other species such as Gobiidae, Clupeidae, Gasterosteidae, and Syngnathidae because our sampling period was between March and September which is the spawning season for a majority of coastal fish species in the region. A potential reason may be that some of these fish species release their eggs in aquatic plans, but a bongo net cannot be towed in very shallow waters with submerged macrophytes (Gregory and Powles, 1985). Light traps offer some advantages over more traditional methods that rely on towing nets. Light traps allow researchers to sample multiple locations simultaneously and to collect in areas where it is impractical to pull a net. They also allow collection of older larvae, which are often under-represented in towing nets due to net avoidance (Doherty, 1987; Choat et al., 1993). We suggest the use of light traps as an alternative for sampling fish larvae in the region in the future.

Makeyeva et al. (2011) described some features of A. boyeri (Eichwald) larvae. Their myomere formula, where the total number of myomeres equals the number anterior to the anal fin plus the number posterior to the anal fin (a+p), was 7+43 with the average length of 5.5 mm. The myomere formula for Labidesthes sicculus larvae (Atherinidae) was 12+25 (12-16mm) (Auer, 1982). The total number of myomeres in Atherinella eriarcha in postflexion and transformation stages were 38-39 (7-8+30-31) and 38 (13+25), respectively (White et al., 1984). For A. nepenthe, the number of myomeres was 39-40 (9-10+30-31) and 39-41 (14-15+25-26) for postflexion and transformation stages, respectively.
In the present study, the number of myomeres in *A. caspia* (Atherinidae) was 12-14+24-25 in larvae with an average length of 12-20 mm, which is a similar length of fish for which myomere counts were described in the previous studies.

Many species have unique pigmentation patterns. Pigment location, size, shape, and pattern, and their sequence of formation are very important taxonomic features (Mooig, 1989). Results of the present study showed that pigments in Cyprinidae were asterisk shaped, and pigmentation patterns were different among *R. caspicus*, *R. kutum*, and *C. carpio*. In *C. carpio*, the asterisk pigment were distributed uniformly on the whole body (Rainboth, 1996; Singanouvong et al., 1996; Baird and Phylavanh, 1999) while the asterisk pigments were more solid and round and distributed regularly in several rows along the body on the lateral line, dorsal, and abdominal part in *R. caspicus* (Mooig, 1989). A similar pattern was observed in *R. kutum* except that the pigments were organized in a heart shape near the heart in comparison to *R. caspicus*, and some asterisk pigments on *R. kutum* were on the urostyle (caudal fin) (Jafari et al., 2009; Makeyeva et al., 2011).

Although pigmentation pattern is usually invariable for a species (Mooig, 1989), pigment size and intensity show a wide variation affected by the light intensity when they are reserved or the environment they occupy. The pigments can expand in low light intensity (darkness) and contract in high light intensity (brightness). It is thought to be an adaptation to reduce visibility to predators (Mooig, 1989). Therefore, external pigmentation pattern alone is often not sufficient for discriminating species of larvae.

The post-anal myomeres were 17 in both *R. caspicus* and *R. kutum*, while the number of the pre-anal myomeres were greater (27-28) in *R. kutum* compared to *R. caspicus* (22-24). Jafari et al. (2009) enumerated the total number of myomeres in postfelexion larvae (mean length= 8.3 mm) of *R. kutum* as 10+19+14=43. Makeyeva et al. (2011) reported the myomere formula for *R. caspicus* (9.3 mm) and *R. kutum* (9mm) as 24+17 and 28+17, respectively. The number of myomeres postflexion larvae of *C. carpio* ranged from 39-40 (23-26 preanal myomeres) (Termvidchakorn and Hortle, 2013). In this study, the number of myomeres in *C. carpio* is consistent with the report of Makeyeva et al. (2011), who counted the total number of myomeres as 38 (23+15) in postflexion larvae. The HL, BD, ED, and SL ratios were 25-28%, 19-21%, 27-32%, and 18-30%, respectively, for postflexion larvae of *C. carpio* (Auer, 1982). Similar results were found in the present study (Table 1). Some previous studies suggested that identifying larva using the number of myomeres was time-consuming for use in ecological studies and the method is subject to large errors, especially when the specimens were damaged (Mooig, 1989). However, our results showed morphometric parameters reliably differentiated Cyprinidae.
A study by Ismail et al. (1998) reported the average HL, ED, BD, PDL, and PAL were 7.4, 1.9, 6.7, 16.8, and 12.5 mm, respectively, for *Liza Carinata* (22.8 mm); these values corresponded to 32.45%, 25.67%, 29.38%, 73.68%, and 45.82%, respectively. These ratios were 31.4%, 30.34%, 19.47%, 67.61%, and 52.1%, respectively for *C. aurata* and 33.35%, 24.92%, 17.23%, 66.6%, and 50.94%, respectively for *C. saliens*. This suggests that the morphometric parameters vary from species to species.

There are some major limitations in using morphometric characteristics to distinguish between species of larval fish because significant changes occur in the body proportion in these stages (Katselis et al., 2009). In this study, a similar range of body length was used in DA to overcome the limits. According to DA results, ED, BD, and HD can be used for discriminating between *C. saliens* and *C. aurata*. Katselis et al., (2009) reported that some morphometric characteristics such as BD, minimum body depth (FH), ED, PDL, and HL can be used for discriminating among four Mediterranean grey mullet species: *C. aurata*, *C. saliens*, *Mugil cephalus*, and *C. labrosus*.

In conclusion, the results of this study show that the analysis of the morphometric parameters could be used to accurately identify species of fish larvae in the southeastern coastal waters of the Caspian Sea. We present a practical guide to discriminate fish larvae in the region using meristic parameters such as number of myomeres and appearance characteristics such as pigmentation patterns. This guide is expected to help further studies on fish larvae and management of fisheries in the region.

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