Intergeneric Hybridization between Kutum, *Rutilus frisii kutum*, and Bream, *Abramis brama orientalis*, of the Caspian Sea

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Abstract

Artificial hybridization was performed between *Rutilus frisii kutum* and *Abramis brama orientalis* of the Caspian Sea. Synchronization of spawning of female broodstock of both species was induced by injection of carp pituitary extract. Reciprocal crossings between *R. frisii kutum* $\times A. brama orientalis$ (RA) and *A. brama orientalis* $\times R. frisii kutum$ (AR) produced viable hybrid larvae without any clear particular pre- or postzygotic isolation phenomena. RA and AR hybrid larvae were reared to fingerling stage with survival rates of 22.5 and 28% and average weight of 6.8 ± 0.17 g and 9.0 ± 0.79 g, respectively. A heterosis of 45% was calculated for weight at fingerling stage. RA and AR hybrid fingerlings were cultured in polyculture along with Chinese carps for 6–7 mo and reached an average weight of 190–195 g and 235–255 g, respectively. Karyotyping of these hybrids revealed a modal diploid number of $2n = 50$ for both groups, which is similar to those of the parental species. Discriminant function analysis on 28 morphometric and meristic characteristics of two parental species as well as their hybrids could separate these groups at highly significant level ($P < 0.001$). These results indicated an overall intermediate inheritance of the studied characters.

Interspecific hybridization of fishes has been used in aquaculture and fisheries contexts in order to improve performance traits such as growth rate, environmental tolerance, disease resistance, harvestability, flesh quality, and overall hardiness in culture condition. It has also been exploited to produce hybrids containing desirable traits of two original species, sterile fish stocks, and monosex populations (Bartley et al. 2001). Although a large number of hybridization trials have been attempted within a large variety of fish taxa so far, there are still numerous combinations to be examined for possible use by the aquaculture and/or fisheries industries. Because in hybridization of fish the outcome of each single combination of mating is inherently unpredictable (Tave 1993), each pair of reciprocal hybrids may potentially be heterotic and, therefore, of practical advantage for aquaculture and/or fisheries.

Kutum, *Rutilus frisii kutum*, is a cyprinid fish native to the Caspian Sea. This tasty fish is the most popular food fish in southern coastal region of the Caspian Sea in the north of Iran where it is locally known as Mahi-sefid (meaning white fish) because of its shiny scales. It has a great economic importance for the Iranian fishing industry in the southern Caspian Sea with more than 900 km of coastline. Despite its popularity and the fact that its maximum weight reaches as high as 5 kg in nature, kutum has not been cultured successfully to a marketable size in Iran. The only published work on this subject indicates mean weights of 147 and 155.3 g after 6 mo of monoculture and polyculture with Chinese carps in freshwater, respectively (Khosh-Asl 1997b). This may be partly
because of a lack of supply of proper natural food, that is, benthic organisms, possible salinity requirements for normal growth at grow-out stage, and poor genetic potential under culture conditions. However, its semi-artificial propagation and larval rearing in earthen ponds has been practiced for decades in Iran and currently some 180 million kutum fingerlings are annually released to the major rivers entering the Caspian Sea for conservation purposes.

On the other hand, bream, *Abramis brama orientalis*, which is another cyprinid fish of the Caspian Sea, is generally less selective for food compared with kutum. It appears to grow relatively faster than kutum under culture conditions and may reach an average weight of 200 and 250 g within 5 mo of monoculture and polyculture with Chinese carps, respectively (Khosh-Asl 1997a). This fish has been entered the Iranian fisheries list for conservation over past decades and currently more than 16 million fingerlings are released to rivers annually.

Artificial hybridization of kutum of the Caspian Sea has been previously attempted with roach, *Rutilus rutilus* (Hoseini 1993), and grass carp, *Ctenopharyngodon idella* (Hoseini 1996), whereas both natural (reviewed in Scribner et al. 2001) and artificial (Hoseini 1993) hybrids between bream and roach have already been reported. More recently, Mamcarz et al. (2006) hybridized bream with tench, *Tinca tinca*, successfully in reciprocal manner. However, to our best knowledge, neither natural nor artificial hybridization between kutum and bream of the Caspian Sea has been reported yet. In this study, the feasibility of establishing reciprocal matings between broodstock of these two species, larval and juvenile rearing, as well as grow-out performance of the hybrids in culture condition were studied. Moreover, karyological study of the hybrids as well as morphological comparisons among reciprocal hybrids and their parental species were performed.

**Materials and Methods**

**Broodstock**

Broodstock of kutum, as an anadromous fish living in sea and reproducing in rivers, were caught from rivers entering the Caspian Sea in Gilan province in the north of Iran (mainly Sefidrood and Havigh rivers) where they migrate for spawning during spring. Male and female spawners were then transferred to Shahid Ansari Fish Hatchery (Gilan, Iran) under oxygenated water condition for artificial propagation. Broodstock of bream were already maintained at this hatchery for conservation purposes.

**Artificial Propagation**

Two conspecific and two heterospecific types of mating were established using broodstock of two species as follows (indicated notations will be used throughout the rest of the article where appropriate):

- *R. frisii kutum* ♀ × *R. frisii kutum* ♂ (RR)
- *A. brama orientalis* ♀ × *A. brama orientalis* ♂ (AA)
- *R. frisii kutum* ♀ × *A. brama orientalis* ♂ (RA)
- *A. brama orientalis* ♀ × *R. frisii kutum* ♂ (AR)

For this purpose, in late April 2001, five pairs of each species were selected of which females were injected intramuscularly 2 and 4 mg/kg body weight (b.w.) carp pituitary extract for kutum (mean weight 1250 g) and bream (mean weight 450 g), respectively, in order to induce and synchronize ovulation. It is worth mentioning that the water temperature range at the time (18–21.5 °C) was relatively high for all broodstock. As a result, only a total of 65,000 eggs (240 g) approximately were obtained from three kutum females, whereas three bream females produced a total of circa 96,000 eggs (80 g).

In mid April 2002, 10 pairs of each species were chosen for artificial propagation. Female broodstock were injected carp pituitary extract at the dose mentioned above; however, only seven kutum (mean weight 1370 g) and six bream (mean weight 750 g) females were induced to spawn successfully. The water temperature range of the time was 12.5–15.5 °C, which was quite suitable for spawning of kutum while somewhat low for bream. A total of 185,000 (1500 g) and 490,000 (890 g) eggs were produced by kutum and bream females, respectively, which were not only higher in number but also of better quality than those of the year.
before because of more suitable environmental conditions.

Mean fertilization rate after 48 h (%), mean hatching time (h), and hatching rate (%) were determined for all fish groups in both years. The propagation criteria data are summarized in Table 1.

Fingerling Production

In yr 2001, the first feeding larvae were fed by cooked egg yolk and milk powder for 3 d. Then, 10,000 larvae resulting from each mating type were stocked in separate 500-m² earthen ponds (Table 2). These ponds were prepared based on the routine practices for carp fingerling production prior to introduction of larvae. The larvae were reared for 4 mo and fed with artificial SFK pellet food with an appropriate size (Chineh Co., Tehran, Iran) originally formulated for kutum fingerling. The ration size was adjusted to 10–6% of b.w. according to the manufacturer’s instructions. Developing larvae of all crosses in 2002 suffered from severe mortality during the rearing period as a result of fungal infection. These larvae were then discarded and no further data could be collected from them.

Grow Out

Grow-out performance of the reciprocal hybrids (i.e., RA and AR) was assessed through their polyculture with Chinese carps. For this purpose, two sets of four earthen ponds ranging from 2 to 4 ha were selected in two commercial fish farms in Talesh and Lakan areas of Gilan province. In each area, three ponds were stocked with 100 RA, 100 AR, or 50 RA plus 50 AR hybrid fingerling/ha along with the Chinese carps. The average weight of RA and AR hybrid fingerling at the stocking time were 6.8 and 9.0 g, respectively (n = 150 for each group). The fourth pond was stocked with the Chinese carps only and used as control. In the Talesh area, the stocking rate of the Chinese carps was 3000 fish/ha with composition of 1750 silver carp (average 200 g), 700 common carp (average 40 g), 300 grass carp (average 70 g), and 250 bighead carp (average 80 g), whereas in the Lakan area the stocking rate of the Chinese carp was 2000 fish/ha with composition of 1200 silver carp (average 500 g), 300 common carp (average 10 g), 300 grass carp (average 500 g), and bighead carp (average 500 g).

The culture practices were in accordance with the Chinese carp polyculture standards used routinely in carp production in Iran. Grow-out period was 6 mo in the Talesh area and 7 mo in the Lakan area. No attempt was made to culture the pure stocks of parental species because of facility limitations.

Karyology

Because the karyology of kutum and bream had already been reported by other workers (Noruz-Fashkhami and Khosroshahi 1995; Jankun et al. 1997; Nahavandi et al. 2001), only those of hybrids were studied. This was accomplished based on the methods by Kligerman and Bloom (1977) and Baksi and Means (1988) with some minor modifications. For this purpose, hybrid larvae were incubated in 0.07% colchicine bath for 3–4 h, hypotonized in 0.075 M KCl for 30 min, and fixed twice

Table 1. Characteristics of artificial propagation of kutum, Rutilus frisii kutum; bream, Abramis brama orientalis; and their reciprocal matings.

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>R. f. kutum ×</td>
<td>5/10</td>
<td>3/7</td>
<td>91/98</td>
<td>102/192</td>
<td>75/95</td>
</tr>
<tr>
<td>R. f. kutum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. b. orientalis ×</td>
<td>5/10</td>
<td>3/6</td>
<td>59/78</td>
<td>90/120</td>
<td>50/85</td>
</tr>
<tr>
<td>A. b. orientalis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. f. kutum ×</td>
<td>5/10</td>
<td>3/7</td>
<td>81/90</td>
<td>106/208</td>
<td>56/70</td>
</tr>
<tr>
<td>A. b. orientalis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. f. kutum ×</td>
<td>5/10</td>
<td>3/6</td>
<td>53/64</td>
<td>92/130</td>
<td>45/80</td>
</tr>
<tr>
<td>A. b. orientalis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
in freshly made Karnoy’s solution (3 methanol: 1 acetic acid) for 30 min. Chromosomal spreads were then made using tissue suspension splashed onto prewarmed slides to 45 C, followed by 20% Giemsa staining for 30 min. Arm lengths of chromosomes were measured under immersion microscope using Biocom 2000 computer software (BIOCOM) and their ratios were used to determine the types of chromosomes according to the nomenclature by Levan et al. (1964).

**Morphometrics and Meristics**

Twenty-eight morphometric and meristic criteria of pure species (i.e., RR and AA) as well as their reciprocal hybrids (i.e., RA and AR) were studied on 15 individuals from each group at the end of experimentation (Table 3). In addition, the pharyngeal teeth of 10 individuals of each hybrid group were studied and compared with those of parental species.

**Statistical Analyses**

Growth and morphometric data of fish groups were analyzed using ANOVA. Homogeneity of variances was tested prior to applying ANOVA. Tukey Honestly Significant Difference (HSD) and Games–Howell tests were used to detect significant differences among groups with homogenous and nonhomogenous variances, respectively. Discriminant function analysis was applied on analyzed morphometric and meristic data (Manly 1994; Sokal and Rohlf 1995). A level of \( P < 0.05 \) was adopted for significant differences for all analyses. Data processing and analysis was performed using Microsoft Excel 2003 (Microsoft Inc.) and SPSS 12.0 (SPSS Inc.) computer software.

**Results**

**Artificial Propagation**

The results of artificial propagation are summarized in Table 1. Both kutum and bream female broodstock responded well to carp pituitary extract injection for synchronized spawning (60–70%) in both years. It is worth mentioning that the optimal water temperature range for spawning of these two species differ considerably, being 13–14 C for kutum and 16–17 C for bream. Artificial fertilization was possible in reciprocal ways, and both RA and AR hybrid embryos were produced with relative ease. Mean fertilization rates in RR and AA pure species crosses were 91 and 59% in the first yr and 98 and 78% in the second yr, respectively. RA and AR hybrid crosses resulted in mean fertilization rates of 81 and 53% in the first yr and 90 and 64% in the second yr, respectively.

Mean hatching times for all crosses were much lower in the first yr than in the second yr mainly because of the higher water incubation temperature recorded in the late reproductive season of the first yr (Table 1). Mean hatching rates of pure parental crosses of RR and AA were 75 and 50% in the first yr and 95 and 85% in the second yr, respectively. Mean hatching rates of RA and AR hybrids were found to be 56 and 45% in the first yr and 70 and 80% in the second year, respectively.

**Fingerling Production**

Survival rates from larval to fingerling stages were 25 and 26% for RR and AA pure species, respectively. The average weight of RR fingerlings was 6.1 ± 0.15 g (\( n = 150 \)) and of AA fingerlings was 4.8 ± 0.38 g (\( n = 150 \)), which

<table>
<thead>
<tr>
<th>Fish stock</th>
<th>Pond area (m²)</th>
<th>Date of stocking</th>
<th>Date of harvesting</th>
<th>Days of culture</th>
<th>No. of larvae stocked</th>
<th>No. of fry harvested</th>
<th>Survival rate (%)</th>
<th>Mean weight at harvest (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td>500</td>
<td>12 May</td>
<td>11 September</td>
<td>122</td>
<td>10,000</td>
<td>2500</td>
<td>25.0</td>
<td>6.10</td>
</tr>
<tr>
<td>AA</td>
<td>500</td>
<td>12 May</td>
<td>11 September</td>
<td>122</td>
<td>10,000</td>
<td>2600</td>
<td>26.0</td>
<td>4.80</td>
</tr>
<tr>
<td>RA</td>
<td>500</td>
<td>12 May</td>
<td>11 September</td>
<td>122</td>
<td>10,000</td>
<td>2250</td>
<td>22.5</td>
<td>6.80</td>
</tr>
<tr>
<td>AR</td>
<td>500</td>
<td>12 May</td>
<td>11 September</td>
<td>122</td>
<td>10,000</td>
<td>2800</td>
<td>28.0</td>
<td>9.00</td>
</tr>
</tbody>
</table>

RA, *Rutilus frisii kutum* ♀ × *Abramis brama orientalis* ♂; AR, *A. brama orientalis* ♀ × *R. frisii kutum* ♂; RR, *R. frisii kutum* ♀ × *R. frisii kutum* ♂; AA, *A. brama orientalis* ♀ × *A. brama orientalis* ♂.
did not differ significantly from each other. During the same period, the survival of RA and AR hybrid larvae was 22.5 and 28%, respectively. RA hybrids reached an average weight of 6.8 ± 0.17 g (n = 150), which was higher than its paternal species (P < 0.05). On the other side, AR hybrids reached an average weight of 9.0 ± 0.79 g (n = 150), which was significantly higher than those of both parental species (P < 0.05), but not from RA hybrids. Midparent heterosis for this trait at fingerling stage was 45%.

**Grow Out**

In the Talesh area, RA hybrids reached an average weight of 190 ± 4.7 g after 6 mo of polyculture with Chinese carps. At the same time, AR hybrids weighted 235 ± 4.2 g, which was significantly higher than RA hybrids (P < 0.05). In the Lakan area, a similar pattern was achieved, with RA hybrids reaching a mean weight of 195 ± 3.4 g after 7 mo of polyculture, while AR hybrids showed an average weight of 255 ± 3.3 g, which was significantly higher than that of RA hybrids (P < 0.05). No comparisons were made between the results obtained from the two geographical regions because of their systematic differences. Moreover, no particular adverse effects of the presence of hybrid fish on the Chinese carp production were observed in any ponds.

**TABLE 3. Morphometric and meristic characteristics of kutum (RR), bream (AA), and their reciprocal hybrids (RA and AR).**

<table>
<thead>
<tr>
<th>Character</th>
<th>RR</th>
<th>AA</th>
<th>RA</th>
<th>AR</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of scales on lateral line</td>
<td>57.93a</td>
<td>53.07b</td>
<td>59.67a</td>
<td>55.27c</td>
<td>0.486</td>
</tr>
<tr>
<td>No. of soft rays in anal fin</td>
<td>10.20a</td>
<td>25.40b</td>
<td>17.20c</td>
<td>15.67d</td>
<td>0.249</td>
</tr>
<tr>
<td>No. of upper gill rakers</td>
<td>10.47a</td>
<td>25.93b</td>
<td>17.20c</td>
<td>17.67d</td>
<td>0.358</td>
</tr>
<tr>
<td>No. of lower gill rakers</td>
<td>11.13a</td>
<td>30.27a</td>
<td>18.87c</td>
<td>19.73d</td>
<td>0.313</td>
</tr>
<tr>
<td>Head length/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>24.48a</td>
<td>24.18a</td>
<td>25.30a</td>
<td>25.30a</td>
<td>0.546</td>
</tr>
<tr>
<td>Head height/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>18.36a</td>
<td>20.20a</td>
<td>20.09b</td>
<td>19.07c</td>
<td>0.180</td>
</tr>
<tr>
<td>Snout length/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>6.09ab</td>
<td>5.64a</td>
<td>6.53a</td>
<td>6.27b</td>
<td>0.122</td>
</tr>
<tr>
<td>Eye diameter/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>7.59a</td>
<td>9.19b</td>
<td>8.03ac</td>
<td>8.30c</td>
<td>0.133</td>
</tr>
<tr>
<td>Interorbital distance/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>7.81a</td>
<td>7.57a</td>
<td>7.68a</td>
<td>7.96a</td>
<td>0.181</td>
</tr>
<tr>
<td>Postorbital distance/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>10.46a</td>
<td>10.66a</td>
<td>10.95a</td>
<td>10.83a</td>
<td>0.167</td>
</tr>
<tr>
<td>Maximum body height/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>22.22a</td>
<td>26.11b</td>
<td>25.15bc</td>
<td>24.98c</td>
<td>0.261</td>
</tr>
<tr>
<td>Minimum body height/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>8.03a</td>
<td>8.89b</td>
<td>8.71b</td>
<td>8.89c</td>
<td>0.113</td>
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<tr>
<td>Caudal peduncle length/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>19.35a</td>
<td>14.08b</td>
<td>17.94c</td>
<td>17.86c</td>
<td>0.350</td>
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<tr>
<td>Caudal peduncle height/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>9.78a</td>
<td>11.80b</td>
<td>10.72c</td>
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<tr>
<td>Dorsal fin length/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>11.50ac</td>
<td>13.80b</td>
<td>12.10a</td>
<td>11.00b</td>
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<td>Dorsal fin height/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>18.32a</td>
<td>24.50b</td>
<td>19.91c</td>
<td>20.01c</td>
<td>0.326</td>
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<tr>
<td>Pectoral fin length/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>16.57a</td>
<td>19.04b</td>
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<td>Ventral fin length/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>13.73a</td>
<td>15.42b</td>
<td>14.64ab</td>
<td>13.71a</td>
<td>0.313</td>
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<tr>
<td>Pectoroventral distance/SL&lt;sup&gt;4&lt;/sup&gt;</td>
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<td>21.53b</td>
<td>23.94c</td>
<td>24.67ac</td>
<td>0.468</td>
</tr>
<tr>
<td>Ventroanal distance/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>21.72a</td>
<td>15.03b</td>
<td>20.87a</td>
<td>19.33c</td>
<td>0.408</td>
</tr>
<tr>
<td>Anal fin height/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>11.51a</td>
<td>17.83b</td>
<td>13.16c</td>
<td>13.02c</td>
<td>0.243</td>
</tr>
<tr>
<td>Anal fin length/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>9.17a</td>
<td>25.83b</td>
<td>16.64c</td>
<td>16.47c</td>
<td>0.316</td>
</tr>
<tr>
<td>Predorsal distance/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>52.09a</td>
<td>54.45b</td>
<td>52.76a</td>
<td>53.21ab</td>
<td>0.344</td>
</tr>
<tr>
<td>Postdorsal distance/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>37.05a</td>
<td>33.96b</td>
<td>37.48a</td>
<td>36.72a</td>
<td>0.359</td>
</tr>
<tr>
<td>Prevental distance/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>52.54a</td>
<td>48.75b</td>
<td>51.72a</td>
<td>52.46a</td>
<td>0.444</td>
</tr>
<tr>
<td>Preanal distance/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>73.53a</td>
<td>66.31b</td>
<td>70.21a</td>
<td>70.69a</td>
<td>0.806</td>
</tr>
<tr>
<td>Upper caudal fin length/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>20.03a</td>
<td>27.56b</td>
<td>22.12c</td>
<td>22.38c</td>
<td>0.283</td>
</tr>
<tr>
<td>Lower caudal fin length/SL&lt;sup&gt;4&lt;/sup&gt;</td>
<td>20.07a</td>
<td>29.84b</td>
<td>23.86c</td>
<td>23.77c</td>
<td>0.291</td>
</tr>
</tbody>
</table>

SL, standard length; RA, *Rutilus frisii* *kutum* ♀ × *Abramis brama* *orientalis* ♂; AR, *A. brama orientalis* ♀ × *R. frisii kutum* ♂; RR, *R. frisii kutum* ♀ × *R. frisii kutum* ♂; AA, *A. brama orientalis* ♀ × *A. brama orientalis* ♂.
1 All values except for pooled SEs are expressed as means.
2 All ratios are in percent.
3 Values in each row marked with different letters are significantly different (P < 0.05).
4 Ratio.
**Karyology**

Some chromosome pairs did not precisely match in terms of their length or arm ratio perhaps as a result of interspecific differences, mating type, and/or artifact; however, we tried our best to provide a typical karyotype for each hybrid group.

The chromosomal analysis of 45 metaphase plates obtained from RA hybrid larvae revealed a modal diploid number of $2n = 50$ with Fundamental Number (NF) = 188 as observed in 41 metaphase plates. In three metaphase plates, the chromosome number was 49 and in one plate it was 48. The chromosome constituent of this hybrid consisted of 10 metacentric pairs, 10 submetacentric pairs, two subtelocentric pairs, and three telocentric pairs. The karyotype of this hybrid is shown in Fig. 1.

The same analysis was carried out on the 40 metaphase plates obtained from AR hybrid larvae that revealed the same modal diploid number as the other hybrid group ($2n = 50$) but slightly different arm number (NF = 192) as observed in 37 metaphase plates. A lower number of chromosomes (48 and 49) was recorded in two of the plates. The chromosomal formula of hybrids was found to be 10 metacentric pairs, 10 submetacentric pairs, three subtelocentric pairs, as well as two telocentric pairs. The karyotype of this hybrid is reported in Fig. 2. Moreover, no polyploid chromosome counts were observed in either hybrid groups.

**Morphometrics and Meristics**

The morphometric and meristic characters analyzed in the parental species and hybrid groups are listed in Table 3. Discriminant function analysis showed that all four fish groups were significantly different from one another ($P < 0.05$). The two hybrid groups (i.e., RA and AR) not only produced distinctively separate clusters but these were separate from the two parental species too. Both clusters of hybrid groups lay between those of the pure species (Fig. 3).

The pharyngeal teeth formula of RA hybrids was found to be 5-5 in six individuals (similar to that of *A. brama orientalis*) and 6-5 in four individuals (similar to that of *R. frisii kutum*). However, all 10 studied AR hybrid specimens showed pharyngeal teeth formula of 5-5, which is the same as their maternal parent, that is, *A. brama orientalis*. The structure of pharyngeal teeth in both hybrids, particularly at the top edge, was found to be more similar to the corresponding maternal rather than paternal species.

**FIGURE 1.** Diploid metaphase plate ($2n = 50, \text{NF} = 188$) and its karyotype from a *Rutilus frisii kutum × Abramis brama orientalis* (RA) hybrid (*m* = metacentric, *sm* = submetacentric, *st* = subtelocentric, and *t* = telocentric).

**FIGURE 2.** Diploid metaphase plate ($2n = 50, \text{NF} = 192$) and its karyotype from *Abramis brama orientalis × Rutilus frisii kutum* (AR) hybrid (*m* = metacentric, *sm* = submetacentric, *st* = subtelocentric, and *t* = telocentric).
Hybridization is a nonbiotechnological breeding tool to develop new genetic stocks of fish for aquaculture and/or fisheries industries particularly when there is little additive genetic variation for desired traits of pure stocks to be exploited by selective breeding programs. Aquaculture has been found as the most prevalent anthropogenic factor involved in fish hybridization (Scribner et al. 2001). However, Bartley et al. (2001) pointed out that the utilization of interspecific hybrids in aquaculture and fisheries in many areas of the world has not been reported accordingly. In a review of more than 150 related articles, Scribner et al. (2001) found that among 19 freshwater fish families, Cyprinidae has been the most reported with the attempted hybridization of 68 species and the production of 56 interspecific hybrids. Most artificial hybridization trials among cyprinid fishes have been devoted to important cultured species such as common carp, Chinese carps, Indian carps, crucian carp, goldfish, and tench (Krasznai and Marian 1982; Bakos and Gorda 1995; Basavaraju et al. 1995; Hulata 1995; Bartley et al. 2001; Mamcarz et al. 2006). In contrast, little attention has been paid to numerous other possible combinations among cyprinid fishes including those native to the Caspian Sea such as bream and kutum. These species are of economical importance to the Iranian fishing industry in the southern coastal region of the Caspian Sea and of scientific significance to the Iranian fisheries scientists.

Generally, hybrids have been reported among fish species more than any other groups of vertebrates (Campton 1987; Epifanio and Nielson 2001; Scribner et al. 2001), and among fish families, Cyprinidae is the family with the most recorded natural hybridization in Europe and North America (Purdom 1993). Nevertheless, there is no record available on natural hybridization between kutum and bream although these fishes are sympatric species in the Caspian Sea. This may indicate the presence of one or several spatial, temporal, and behavioral isolating mechanisms. On the other hand, feasibility of artificial reciprocal mating and production of viable F₁ hybrids may not always be guaranteed in fish, particularly at an intergeneric level of hybridization, mainly because of karyological incompatibilities and/or different reproductively physiological prerequisites such as photoperiod, temperature, and so forth (Tave 1993). In this study, the reciprocal crossings between bream and kutum of the Caspian Sea, although belonging to two different genera, have proved feasible, and no particular pre- or postzygotic isolation mechanisms were shown to operate causing developmental incompatibility in either RA or AR hybrids.

Female broodstock of both species responded well to injection of carp pituitary extract in terms of synchronization and spawning. Although fecundity was somewhat low in the first year of study because of inappropriate thermal conditions of the late breeding season, in the second yr both species produced a larger number of eggs. In a study on A. brama from two rivers in the UK, Adámek et al. (2004) found mean relative stripping fecundities (RSFs) of 93,642 ± 20,896 and 151,179 ± 25,123 eggs/kg female biomass following carp pituitary extract injections. In the current study, the average of this parameter was found to be 71,111 and 108,889 eggs/kg female biomass in 2 successive yr, respectively. The relatively lower RSF of these fish could be attributed mainly to the smaller sizes of female broodstock used in this study compared to those used by

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Adámek et al. (2004). The observed variation of RSF between the 2 yr of the study is most probably because of different thermal regimes received by the corresponding broodstock. On the other hand, Azari-Takami (1979) reported an average relative fecundity of 53,480 (ranging 32,000–85,000) eggs/kg female biomass in *R. frisii kutum* caught from Havigh River, Gilan, Iran. In the current study, the average RSFs were 17,333 and 19,291 eggs/kg female biomass for the 2 successive yr of study, respectively. This remarkable lower relative fecundity may be attributed to the smaller size of female specimens and possibly to overall ecological changes occurred in the region over the past decades.

Although fertilization rates for hybrid crosses were generally lower than those of pure species, it does not seem to be a barrier for hybridization. Similarly, mean hatching rates for hybrids (45–80%) were lower than those of the parental species (50–95%). It seems that mean hatching rates in hybrids is much because of the egg quality of the maternal species. The survival rates of hybrids (22.5% and 28.0) were more or less similar to those of the pure species (25.0% and 26.0).

Although heterosis is evident in many hybrid fish, cyprinid hybrids generally do not show hybrid vigor (Mann 1991) and just a few cyprinid hybrids have shown value for aquaculture (Hulata 1995). In this study, a heterosis of 45% was calculated for weight of hybrids at fingerling stage; however, it cannot be extrapolated to larger sizes of fish because of nonlinearity of growth and the possible influence of other generic and nongenetic factors. Although the growth rates of RA (ca. 190–195 g) and AR (ca. 235–255 g) hybrids in polyculture with Chinese carps were comparable to those previously observed in kutum (ca. 155 g) and bream (ca. 250 g) by Khosh-Asl (1997a, 1997b), a solid conclusion cannot be made on the presence or absence of hybrid vigor at this stage because of the lack of pure parental species culture in this study.

The modal diploid number of both RA and AR hybrids did not differ from those of parental species and no evidence of either triploidy or heteroploidy was observed suggesting possible fertility of these hybrids. These hybrids need to be grown to a mature size for further studies on their fertility.

Morphometrics and meristics have been employed more frequently than any other alternative methods to identify hybrid fish (Scribner et al. 2001). Discriminant function analysis of these morphological data can maximally separate two or more groups of fish individuals in multivariate space (Campton 1987; Schaefer 1991). In this study, RA and AR hybrids were quite distinctive morphologically, yet more similar in appearance to their respective maternal than paternal parents indicating possible existence of maternal effects. Discriminant function analysis of morphometric and meristic data showed that the two hybrids not only differed from each other but also from their pure parental species regarding the studied characteristics. Both clusters of RA and AR hybrids laid between the parental clusters with no single overlapping individual indicating possible pure intermediate inheritance for these characters.

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**Literature Cited**


