

## 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 myomeres. *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., *Dussumieria* 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 *Benthoosema 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 Miankaleh Beaches within  $36^{\circ} 54' 450''$  to  $37^{\circ} 13' 355''$  N and  $53^{\circ} 55' 974''$  to  $53^{\circ} 47' 080''$  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).

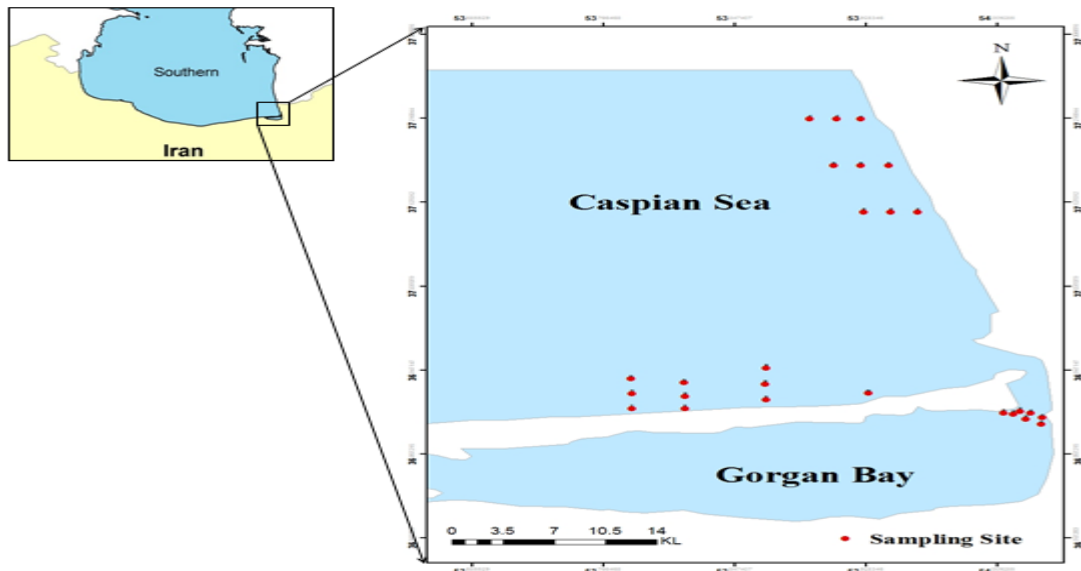


Figure 1: Location of sampling sites of fish larvae in the Southeastern coastal waters of the Caspian Sea.

### Data collection

Fish larvae were collected using a 46 cm bongo net with a mesh size of 330  $\mu\text{m}$  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).

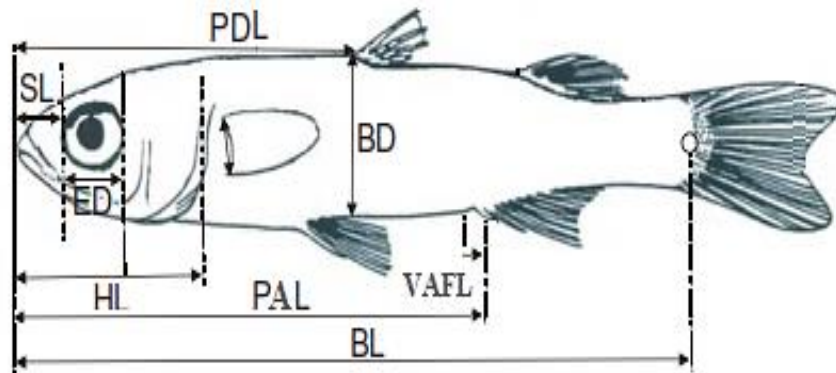


Figure 2: Morphometric parameters measured on the body of larvae.

### 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 (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY).

## Results

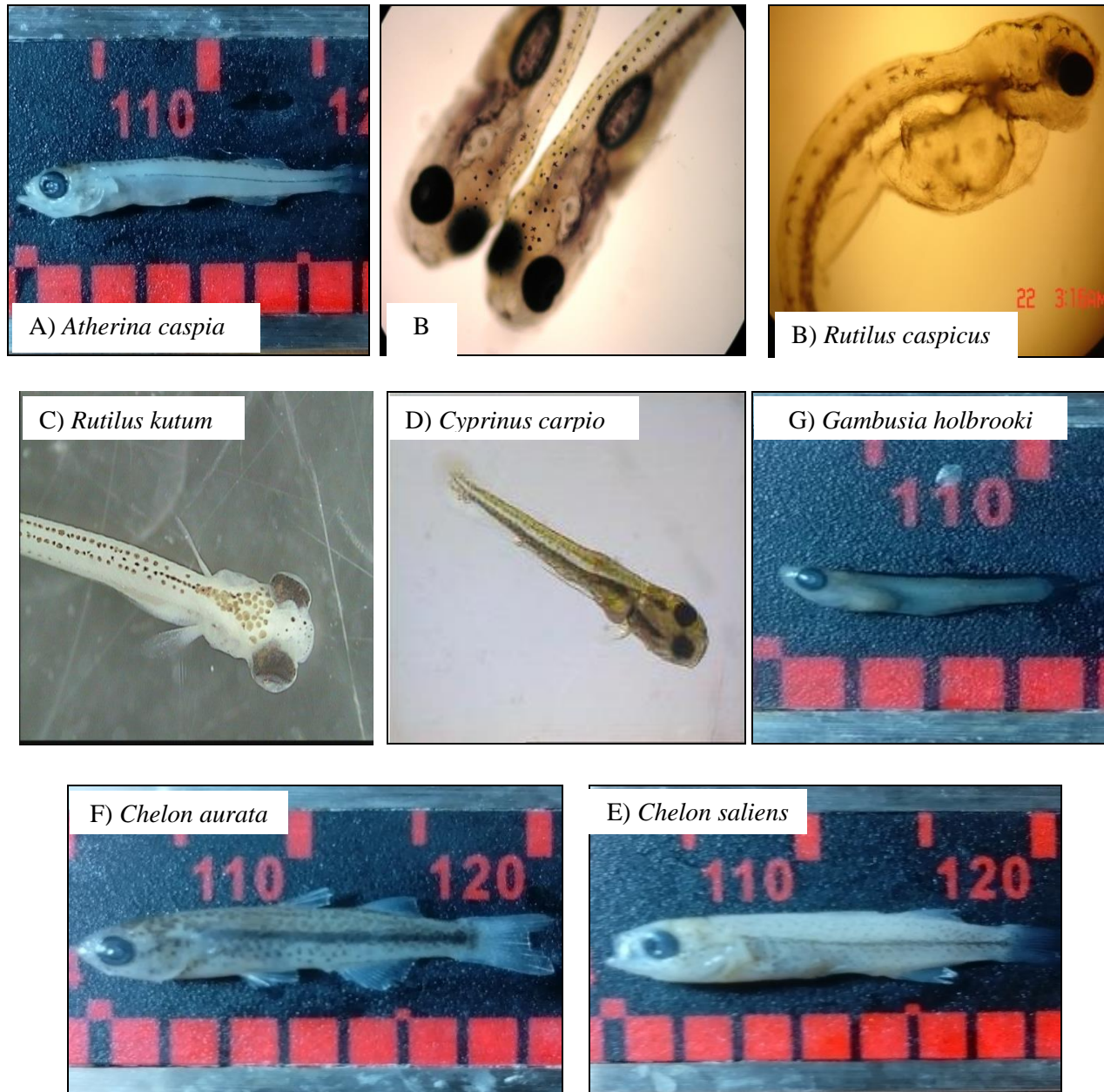
In this study, 196 larvae and 533 eggs were collected. They represented Atherinidae (one genus, one species), Cyprinidae (two genus, three species), Mugilidae (one genus, two species), and Poeciliidae (one genus, one species). The morphological characteristics of fish larvae were summarized in Table 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.**

Family	Genus	Species	English name	Morphological characteristics
Atherinidae	<i>Atherina</i> sp.	<i>Atherina caspia</i>	Big scale sand smelt	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. 3.A). 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
		<i>Rutilus caspicus</i>	Caspian roach	length; some pigment is also present on yolk sac.
	<i>Rutilus</i> sp.			In postflexion stage, the swim bladder is completely developed, and the asterisk pigments are mostly on swim bladder, head, dorsal, and mid-ventral part of the body (Fig. 3B).
Cyprinidae		<i>Rutilus kutum</i>	Caspian kutum	The larvae have an elongate body, similar to <i>R. caspicus</i> . 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 Cyprinidae, unfertilised, pale yellow, and diameter is about 1.5-1.8 mm.
	<i>Cyprinus</i> sp.	<i>Cyprinus carpio</i>	Common carp	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 <i>Liza aurata</i> , and the depth of the caudal peduncle is shallower the width of the body in caudal peduncle is smaller in <i>Liza saliens</i> (Fig 3E).
Mugilidae	<i>Chelon</i> sp.	<i>Chelon saliens</i>	Leaping mullet	
		<i>Chelon aurata</i>	Golden mullet grey	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 <i>Liza saliens</i> , and body appeared darker. The caudal peduncle has a greater width in <i>Liza aurata</i> (Fig. 3F).
Poeciliidae	<i>Gambusia</i> sp.	<i>Gambusia holbrooki</i>	Eastern mosquito fish	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).





**Figure 3: Fish larvae collected from the southeastern coastal waters of the Caspian Sea in 2015.**

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 ( $23.17 \pm 7.87$  mm), and *R. caspicus* had the minimum mean BL ( $9.9 \pm 0.83$  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. Means that do not share a letter are significantly different ( $\alpha=0.05$ ).**

Species	Parameter	BL	HL	SL	BD	ED	PDL	PAL	VAFL
<i>A. caspia</i>	Min-Max	12.4-20.5	2.81-5.73	0.97-1.84	1.63-3.85	1.03-1.84	5.58-8.73	7.34-12.87	0.85-1.62
	%Ratio	-	26.24±3.12 <sup>b</sup>	7.41±1 <sup>c</sup>	15.4±1.07 <sup>b</sup>	34.87±2.9 <sup>c</sup>	46.2±3.18 <sup>a</sup>	61.14±1.71 <sup>b</sup>	7.7±1.3 <sup>d</sup>
<i>R. caspicus</i>	Min-Max	8.53-9.87	2.17-2.57	0.49-0.7	1.31-1.78	0.67-0.81	4.32-5.17	6.12-6.94	0.25-0.26
	%Ratio	-	25.65±0.27 <sup>b</sup>	6.84±0.95 <sup>b</sup>	21.4±1.1 <sup>f</sup>	30.24±1.54 <sup>b</sup>	50.4±3.28 <sup>b</sup>	72.25±1.66 <sup>c</sup>	2.85±0.12 <sup>bc</sup>
<i>R. kutum</i>	Min-Max	8.34-10.68	1.83-2.24	0.25-0.33	0.9-1.3	0.63-0.84	4.69-5.44	5.63-7.60	0.11-0.18
	%Ratio	-	21.48±1.33 <sup>a</sup>	3.05±0.19 <sup>a</sup>	11.3±1.13 <sup>a</sup>	36.04±1.97 <sup>c</sup>	55.2±3.38 <sup>c</sup>	69.35±1.26 <sup>c</sup>	1.48±0.14 <sup>a</sup>
<i>C. carpio</i>	Min-Max	8.1-15.35	2.05-3.80	0.89-1.7	1.6-3.08	0.65-1.15	2.35-6.15	5.2-9.78	0.15-0.54
	%Ratio	-	25.42±1.35 <sup>b</sup>	11±1.97 <sup>c</sup>	20.6±1.11 <sup>c</sup>	30±0.95 <sup>b</sup>	55.3±3.29 <sup>c</sup>	64.23±1.25 <sup>b</sup>	3.16±0.24 <sup>bc</sup>
<i>C. saliens</i>	Min-Max	13.3-23.46	4.53-7.48	1.03-2.23	1.54-4.26	1.25-1.89	6.84-11.09	14.75-8.81	0.4-0.81
	%Ratio	-	33.35±1.46 <sup>d</sup>	9.21±1.39 <sup>d</sup>	15.6±1.07 <sup>b</sup>	24.92±1.8 <sup>a</sup>	50.2±3.19 <sup>b</sup>	66.6±3.46 <sup>c</sup>	3.43±0.42 <sup>c</sup>
<i>C. aurata</i>	Min-Max	14.4-40.46	4.04-13.46	1.06-3.15	2.7-7.89	1.35-3.56	7.37-21.35	9.05-29.23	0.41-1.4
	%Ratio	-	31.34±2.68 <sup>c</sup>	8.89±1.56 <sup>b</sup>	19.4±1.18 <sup>d</sup>	30.34±4.78 <sup>b</sup>	52.4±3.53 <sup>b</sup>	67.61±5.45 <sup>c</sup>	2.7±0.49 <sup>bc</sup>
<i>G. holbrooki</i>	Min-Max	7.46-17.88	2.44-5.22	0.53-1.35	1.45-3.15	0.9-1.64	4.61-11.52	3.87-10.21	0.17-0.65
	%Ratio	-	32.23±1.66 <sup>c</sup>	7.48±0.88 <sup>c</sup>	18±1.113 <sup>c</sup>	34.65±2.21 <sup>c</sup>	61.3±3.37 <sup>d</sup>	57.3±5.83 <sup>a</sup>	2.92±1.95 <sup>b</sup>

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 Myomeres 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 myomeres after anal in comparison to *R. kutum*.

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

Species	Dorsal Fin1	Dorsal Fin2	Anal Fin	Pelvic Fin	Pectoral Fin	Myomer After Anal	Myomer Before Anal
<i>A. caspia</i>	(VI- VIII)	10-13(IV) <sup>1</sup>	10-13 (II)	5(I)	12-13	24-25	12-14
<i>R. caspicus</i>	CC <sup>2</sup>	CC	CC	CC	CC	17	22-24
<i>R. kutum</i>	CC	CC	CC	CC	CC	16-17	27-28
<i>C. carpio</i>	CC	CC	CC	CC	CC	13-5	23-25
<i>C. saliens</i>	(IV)	9-10	8-10 (II)	5 (I)	14-15	11-12	9-10
<i>C. aurata</i>	(IV)	9(0- I)	8-10 (II)	5 (I)	12-15	12	9
<i>G. holbrooki</i>	6-7	-----	9-10	5	9-11	13	19-22

<sup>1</sup>The Roman numbers show the number of spines and the Arabic numbers represents the number of rays.

<sup>2</sup>CC = could not be counted.

Because Cyprinidae larvae were similar in appearance, and detecting and counting myomeres 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.

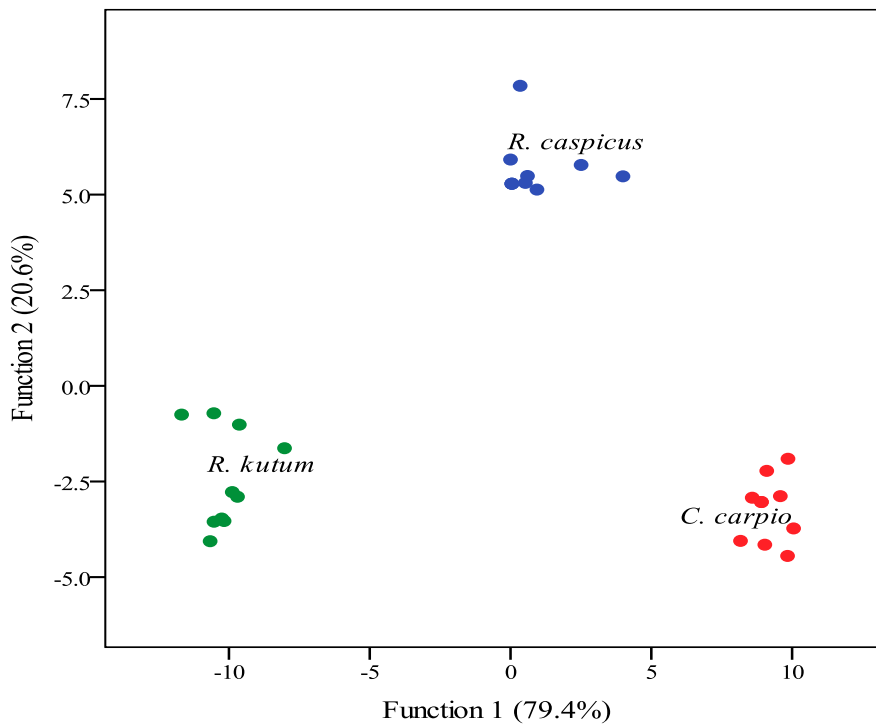
The cross-validation results showed that all the observations were grouped into their

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.**

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

<sup>1</sup>Standardized discriminant function coefficients were presented in parentheses.



**Figure 3: Observation scores on two canonical discriminant functions.**



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 ( $p$ -value  $< 0.0001$ ). 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 -15.148. The results of cross-validation are shown in Table 5.

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

	Species (group)	<i>C. saliens</i>	<i>C. aurata</i>
Original	<i>C. saliens</i>	0	100
	<i>C. aurata</i>	100	0
Cross-validated	<i>C. saliens</i>	100	0
	<i>C. aurata</i>	4.2	95.8

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 collect 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 plants, 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 (Moser, 1996). 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 moyomeres in postflexion 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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### References

- Abdoli, A., 1999.** Inland fishes of Iran, Natural Museum and Wild Life of Iran, 377 P (In Persian).
- Amini, M., Ghorbani, R., Shabani, A., Rabbaniha, M., Noorinejad, M. and Naddafi, R., 2015.** Composition of fish larvae in the central creeks of Bushehr Province, Persian Gulf in 2013-2014. *Journal of Applied Ichthyological Research*, 3(3), 1-18 (In Persian).
- Andrade, F.F., Lima, A.F., Assumpcao, L., Makrakis, S., Kasai, R.I.D. and Makrakis, M.C., 2016.** Characterization of the early development of *Pseudoplatystoma reticulatum* Eigenmann & Eigenmann, 1889 (Siluriformes: Pimelodidae) from the Paraguay River Basin. *Neotropical Ichthyology*, 14(2), 150032.
- Auer, N.A., 1982.** Identification of larval fishes of the Great Lake basin with emphasis on the Lake Michigan drainage. University of Michigan-Great Lake Research Division, 776 P.
- Baird, I.G. and Phylavanh, B., 1999.** Fishes and forests: fish foods and importance of seasonally flooded riverine habitats for Mekong River fish. Technical Report, Environmental Protection, and Community

- Development in the Siphandone Wetland, Champasak Province, Lao PDR Funded by the European Union, implemented by CESVI, 46 P.
- Choat, J.H., Doherty, P.J., Kerrigan, B. and Leis, J.M., 1993.** Larvae and pelagic young of coral reef fishes: comparison of three 44 towed nets, a purse seine and two light-aggregation devices. *Fishery Bulletin*, 91(2), 195-209.
- Doherty, P.J., 1987.** Light-traps: selective but useful devices for quantifying the distributions and abundances of larval fishes. *Bulletin of Marine Science*, 41, 423-431.
- Gregory, R.S. and Powles, P.M., 1985.** Chronology, distribution, and sizes of larval fish sampled by light traps in macrophytic Chemung Lake. *Canadian Journal of Zoology*, 63(11), 2569-2577.
- Ghorbani, R., Baghfalaki, M. and Shaloei, F., 2013.** Caspian Sea environment. Gorgan University of Agricultural Science and Natural Science, Iran, 336 P (In Persian).
- Gilbert, S.F. and Bolker, J.A., 2003.** Ecological developmental biology: preface to the symposium. *Evolution and Development*, 5, 3-8.
- Hames Hickford, M.J., 2000.** Patterns of distribution and abundance of larval fish in the southern temperate region. Ph.D. Thesis, University of Canterbury, Christchurch, New Zealand, 175 P.
- Ismail, W.A., Al-Abdul Elah, K. and Al-Yamani, F., 1998.** Larval development of the back keeled mullet *Liza carinata*. *Hydrobiologia*, 385, 87-105.
- Jafari, M., Kamarudin, M.S., Saad, C.R., Arshad, A., Oryan, S. and Bahmani, M., 2009.** Development of morphology in hatchery-reared *Rutilus frisii kutum* larvae. *European Journal of Scientific Research*, 38(2), 296-305.
- Katselis, G., Hotos, G., Minos, G. and Vidalis, K., 2009.** Phenotypic affinities on fry of four Mediterranean grey mullet species. *Turkish Journal of Fisheries and Aquatic Sciences*, 6, 49-55.
- Ko, K.L., Wang, Y.T., Chiu, T.S., Lee, M.A., Leu, M.Y., Chang, K.Z., Chen, W.Y. and Shao, K.T., 2013.** Evaluating the accuracy of morphological identification of larval fishes by applying DNA barcoding. *Plos One*, 8(1), 1-7(e5341).
- Koochaknejad, E., Savari, A., Dehghan Madiseh, S., Eskandari, G.H. and Sakhaiee, N., 2011.** Fish larvae assemblage in the northwestern coast of the Persian Gulf: Khure Mussa Channel. *Journal of the Persian Gulf*, 2(3), 25-29.
- Leis J.M. and Carson-Ewart B.M., 2000.** The larvae of Indo-Pacific coastal fishes (An identification guide to marine fish larvae, Brill) Fauna Malesiana Handbook. Brill, Netherlands, 850 P.
- Lopez-Sanz, A., Stelzenmuller, V., Maynou, F. and Sabates, A., 2011.** The influence of environmental characteristics on fish larvae spatial patterns related to a marine protected area: The Medes Islands (NW Mediterranean). *Estuarine, Coastal and Shellfish Science*, 92, 521-533.
- Loy, A., Bertelletti, M., Costa, C., Ferlin, L. and Cataudella, S., 2001.** Shape changes and growth trajectories in the early stages of three species of the genus *Diplodus* (Perciformes,

- Sparidae). *Journal of Morphology*, 250, 24-33.
- Makeyeva, A.P., Pavlov, D.S. and Pavlov, D.A., 2011.** Atlas of larvae and juveniles of freshwater fishes of Russia. Moscow: KMK Scientific Press. 383 P (in Russian).
- Mohammadkhani, H., Owfi, F., Poursedghi, T., Hami Tabari, A., Mansouri, B., Iyri, Y., Aghili, K., Jorjani, M., Lashgar Bloki, S. and Najafpour, S.H., 2010.** Comprehensive study on rivers and wetland of Caspian Sea basin (phase 2): Gorgan Bay. Iranian Fisheries Research Organization, Iran Shrimp Research Center, Inland Waters Aquatic Stocks Research Center, Gorgan, Iran, 160 P (In Persian).
- Mooig, W.M., 1989.** A key to the identification of larval bream, *Abramis brama*, white bream, *Beicca bjoerkna*, and roach, *Rutilus rutilus*. *Journal of Fish Biology*, 34, 111-118.
- Moser, H.G., 1996.** The early stages of fishes in the California Current region. California Cooperative Oceanic Fisheries Investigations, 1505 P.
- Moteki, M., 2002.** Morphological aspects of feeding and improvement in feeding ability in the early larval stages of red sea bream *Pagrus major*. *Fisheries Science*, 68, 996-1003
- Moteki, M., Ishikawa, T. and Fushimi, H., 2002.** Changes in feeding function inferred from osteological development in the early stage larvae of the Japanese flounder, *Paralichthys olivaceus*, reared in the laboratory. *Aquaculture Science*, 50, 285-294.
- Mousa, M.A., 2010.** Induced spawning and embryonic development of *Liza ramada* reared in freshwater ponds. *Animal Reproduction Science*, 119, 115-122.
- Mwaluma, J.M., Kaunda-Arara, B. and Strydom, N.A., 2014.** A guide to commonly occurring larval stages of fishes in Kenyan coastal waters. WIOMSA Book Series. No. 15, 73 P.
- Neira, F.J., Miskiewicz, A.G. and Trnski, T., 1998.** Larvae of temperate Australian fishes. laboratory guide for larval fish identification. University of Western Australia Press, Netherlands, 474 P.
- Osse, J., Van Den Boogaart, J., Van Snik, G. and Van Der Sluys, L., 1997.** Priorities during early growth of fish larvae. *Aquaculture*, 155, 249-258.
- Pena, R. and Dumas, S., 2009.** Development and allometric growth patterns during early larval stages of the spotted sand bass *Paralabrax maculatofasciatus* (Percoidei: Serranidae). *Scientia Marina*, 73, 183-189.
- Rabbaniha M., 1998.** Frequency and diversity of ichthyoplankton in Nayband Bay (Phase 1). Iranian Fisheries Research Organization, Iran Shrimp Research Center, 101 P (In Persian).
- Rabbaniha M., 2002.** Frequency and diversity of ichthyoplankton in coastal waters-B (Phase 4). Iranian Fisheries Research Organization, Iran Shrimp Research Center, 99 P (In Persian).
- Rabbaniha, M., Seifabadi, J., Sharifpour, I. and Owfi, F., 2003.** Frequency and diversity of fish larval in Farakeh Estuary- Bushehr Province. *Journal of Marine Science and Technology*, 2(4), 39 -47 (In Persian).

- Rabbania, M., 2007.** Identification, diversity, and distribution pattern of fish larvae in coral reef ecosystem of Kharg and Kharkoo-Persian Gulf using Geographical Information System (GIS). PhD Thesis, Science, and Research Branch Islamic Azad University. 150 P (In Persian).
- Rabbania, M., 2011.** Survey of distribution and early life history identify of Carangidae base on Iranian Waters larva of Persian Gulf. *Journal of Fisheries, Islamic Azad University, Azadshahr Branch*, 5(4), 1-11 (In Persian).
- Rabbania, M. and Owfi, F., 2011.** Changes in fish larvae indicators in Khark and Kharko coralline islands. *Journal of the Persian Gulf*, 2(4), 33-40.
- Rabbania, M., 2013.** Early life stages of Clupeiformes and its distribution in Persian Gulf. *Journal of Animal Environment*, 5(1), 61-70 (In Persian).
- Rabbania, M., Ghasemzadeh, J. and Owfi, F., 2013.** Spatial and temporal patterns of fish larvae assemblage in the northern coastal waters of Persian Gulf along Bushehr Province shoreline. *Journal of Fisheries Sciences*, 7(2), 141-151.
- Rabbania, M., 2014.** Introduction of Scomberid larvae in early life history from Iranian coastal waters of the Persian Gulf- Bushehr Waters. *Journal of Oceanography*, 5(17), 49-53 (In Persian).
- Rabbania, M. and Saraji, F., 2014.** Identification of larval stages of lantern fish in Iranian waters of Oman Sea. *Iranian Scientific Fisheries Journal*, 23(1), 41 -51(In Persian).
- Rahman, M.M., Miah, M.I., Taher, M.A. and Hasan, M.M., 2009.** Embryonic and larval development of guchibaim, *Mastacembelus pancalus* (Hamilton). *Journal of the Bangladesh Agricultural University*, 7 (1), 193–204.
- Rainboth, W.J., 1996.** Fishes of the Cambodian Mekong. FAO: Rome, Italy, 256 P.
- Sabatés, A., Olivar, M.P., Salat, J., Palomera, I. and Alemany, F., 2007.** Physical and biological processes controlling the distribution of fish larvae in the NW Mediterranean. *Progress in Oceanography*, 74, 355-376.
- Singanouvong, D., Soulignavong, C., Vonghachak, K., Saadsy, B. and Warren, T.J., 1996.** The main wet-season migration through Hoo Som Yai, a steep-gradient channel at the great fault line on the Mekong River, Champasak Province, Southern Lao PDR. IDRC Fisheries Ecology Technical Report. 4, 115.
- Termvidchakorn, A. and Hortle, K.G., 2013.** A guide to larvae and juveniles of some common fish species from the Mekong River Basin. MRC Technical Paper No. 38. Mekong River Commission, Phnom Penh, 234 P.
- Victor, B.C., Hanner, R., Shivji, M., Hyde, J. and Caldwell, C., 2009.** Identification of the larval and juvenile stages of the cubera snapper, *Lutjanus cyanopterus*, using DNA barcoding. *Zootaxa*, 2215, 24-36.
- Vosough, G.H., Fatemi, M.R., Jamili, S., Nikoyan, A.R. and Rabbania, M., 2009.** The fluctuation of coralline fish larvae of Khark and Kharko (Persian

- Gulf). *Journal of Fisheries and Aquatic Science*, 4(3), 136-142.
- Vosough, G.H., Fatemi, M.R., Rabbaniha, M., Jamili, S., Gharra, K. and Noorinejad, M., 2010.** Distribution pattern of coral & noncoralline fish larvae in Khark & Kharko (Persian Gulf). *Iranian Journal of Fisheries Sciences*, 9(1), 173-184.
- White, B.N., Lavenberg, R.J and McGowen, G.E., 1984.** Atheriniformes: development and relationships. In H.G. Moser., W.J. Richards., D.M. Cohen., M.P. Fahay., A.W. Kendall and S.L., Richardson, eds. *Ontogeny and systematics of fishes. American Society of Ichthyology and Herpetology Specialist Publication*, 1, 355-362.
- Yanes-Roca, C., Rhody, N.R., Nystrom, M., Wittenrich, M.L. and Main, K.L., 2012.** Embryonic and early larval development in hatchery-reared Common Snook. *North American Journal of Aquaculture*, 74, 499-511.