

## USE OF MELANOPHORE PATTERNS ON THE VENTRAL SIDE OF THE HEAD TO IDENTIFY FRY OF GREY MULLET (TELEOSTEI: MUGILIDAE)

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### Abstract

Examination of fry of five species of grey mullet revealed that the shape of the lower jaw and the melanophore patterns along the edge of the lower jaw and the ventral side of the head are species-specific. *Mugil cephalus* is characterized by an acute angle of the dentary symphysis and lightly pigmented ventro-opercular and gular regions of the head. In *Liza aurata*, the mandibular region has spots of pigment at the corners of the mouth, the gular region is lightly pigmented with a single row of melanophores, the ventro-opercular region of the head has two rows that form a distinct arch and, in specimens over 20 mm TL, at least one of these rows extends to the posterior ventro-opercular region. In *Liza ramada*, the pigmentation is darkest in the mandibular and gular regions of the head, the melanophores in the mandibular region in specimens of 30-40 mm TL are concentrated around the anterior tip of the jaw and the corners of the mouth, and the anterior ventro-opercular region usually has a single row of melanophores that may extend into the posterior ventro-opercular region. In *Chelon labrosus*, there are two rows of melanophores in the ventro-opercular region and one or two rows in the gular region which usually extend beyond the eye into the posterior sector. In *Liza saliens*, there are two rows of melanophores in the ventro-opercular region and two in the gular region which rarely extend beyond the eye to the posterior sector. These observations were used to create a key which may be useful for identifying fry of grey mullet species grown in aquaculture in the Mediterranean region.

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### Introduction

Grey mullets are important food fishes. The euryhalinity, simple diet and rapid growth of some species have made them the object of aquaculture in many parts of the world, including the Mediterranean (Anderson, 1982; Ardizzone et al., 1988; Harrison and Senou, 1999). In 1999, 15,821 tons of grey mullet were produced by aquaculture in marine and brackish waters in the Mediterranean and Black Sea regions (78% in Egypt) and 32,367 tons were produced by aquaculture in inland waters of countries bordering the Mediterranean and Black Seas (FAO, 2002).

Wild-caught grey mullet fry are often used to stock aquaculture ponds (Liao, 1981; Rogdakis et al., 1997) and inland waters (Rogdakis and Minos, 1998). Consequently, attempts have been made to find ways to enable biologists and commercial fishermen to quickly identify numerous small specimens in the laboratory or in the field, with minimal manipulation (Reay and Cornell, 1988; Serventi et al., 1996). The arrangement of the pyloric ceca and the morphology of the pharyngobranchial organ are useful for identifying grey mullet (Serventi et al., 1996) but require manipulation or sacrifice of the specimens. Identification using readily visible external features is preferable, however, difficult because features typically used to identify adults are undeveloped in fry (van der Elst and Wallace, 1976; Zismann, 1981; Reay and Cornell, 1988; Serventi et al., 1996).

Pigmentation patterns constitute an alternate set of characters for fry identification. Some authors have described differences in pigmentation on the flanks of fry (Perlmutter et al., 1957; Farrugio, 1977; Cambrony, 1984; Serventi et al., 1996) but the differences are not distinct for fry of all stages. Various authors have commented on the diagnostic value of melanophore patterns on the ventral side of the head (for example, Perlmutter et al., 1957; Farrugio, 1977; Zismann, 1981; Reay and Cornell, 1988; Serventi et al., 1996), however, none provided sufficient information for a range of body sizes for all of the most common species of grey mullet in the Mediterranean.

The purpose of this study, therefore, was to further describe melanophore patterns in the gular region of fry of five species of mullets common in the coastal waters of western Greece — *Mugil cephalus* (Linnaeus, 1758), *Liza ramada* (Risso, 1826), *L. aurata* (Risso, 1810), *Chelon labrosus* (Risso, 1826) and *L. saliens* (Risso, 1810) — and construct a key for identifying fry, especially newly recruited fry (10-30 mm), in the field or laboratory.

### Materials and Methods

Grey mullet fry were collected from western Greece (38°18'N, 21°32'E) between January 1992 and June 1993 using a fine-meshed beach-seine (Liao, 1981; Katselis et al., 1994; Katselis, 1996; Minos, 1996). The season during which fry of each species first appear along the coasts of western Greece (Katselis et al., 1994; Katselis, 1996; Minos, 1996) helped to predict which species would be found in the samples. The biotope and behavioral patterns of the fry also aided in preliminary identification of the fry during collection (Bograd, 1961; Katavic, 1980; Katselis 1996; Minos, 1996).

Mullet fry of 10-30 mm total length (TL) tend to form schools of a single species. Therefore, each haul of the beach seine aimed at collecting a single school, to increase the probability that the catch would not include a mix of species. Ten to 20 individuals from each haul were selected and lightly anesthetized. Using a hand lens, pigmentation patterns on the head and flanks of the specimens were observed and fry were preliminarily identified. The specimens were then transferred to an aquarium and kept alive for several months in the laboratory. The remaining fish of each haul were immediately killed with an overdose of the anesthetic MS-222 and preserved in 4% formalin. The melanophore patterns on these fish were examined after they had been preserved in formalin for 10 days. The shape of the lower jaw in ventral view and the angle of the dentary symphysis were observed. Identifications of these fish were checked by examining the number and arrangement of the pyloric ceca

(Zambriborch, 1951; Bograd, 1955; Morovic, 1957; Perlmutter et al., 1957; El Zarka and Kamel, 1965; Farrugio, 1977; Cambrony, 1984), and the pattern of the melanophores on the flanks and the lateral and dorsal sides of the head (Perlmutter et al., 1957; Farrugio, 1977; Cambrony, 1984; Serventi et al., 1996).

Every 10 to 20 days, several of the live individuals were killed with an overdose of MS-222, examined, identified and preserved in formalin. The pigmentation patterns and shape of the lower jaw in ventral view were observed to confirm the preliminary identifications. This process was continued until the fish reached the juvenile stage, after two to three months. By then, the fish had characteristics which made them easier to identify and they were used to check the accuracy of the earlier identifications.

Melanophore patterns on the ventral side of the head of the preserved specimens were examined using a WILD M5 stereoscope, following the techniques of earlier authors (Perlmutter et al., 1957; Farrugio, 1977; Zismann, 1981; Reay and Cornell, 1988; Minos et al., 1995). At least 30 fish of each species within the 15-30 mm TL range were studied.

To establish a standard reference for the arrangement of the melanophores, the ventral surface of the head was divided into two main sectors, an 'anterior sector' and a 'posterior sector' (Fig. 1). The anterior sector comprises the mandibular (M), anterior ventro-opercular (AV) and anterior gular (AG) regions. The posterior sector comprises the posterior ventro-opercular (PV), posterior gular (PG), suborbital and opercular regions. Rows of melanophores are identified according to the regions in which they are positioned. Hence, for example, mandibular rows on the lower lip are labeled M, the first and second rows in the anterior ventro-opercular region are labeled AV1 and AV2, respectively, and the extension of these rows into the posterior ventro-opercular regions are labeled PV1 and PV2, respectively.

The presence or absence of melanophores in the different regions of the ventral surface were recorded for each species. The presence of a row is expressed quantitatively as the

number of specimens in which the row is present relative to the total number of specimens observed at that length. Thus, for *M. cephalus* of 30-35 mm TL (Table 1), where 17 of 19 specimens had one melanophore row in the posterior ventro-opercular region (PV1), the presence row (pr) is 0.895 with a standard deviation of 0.07 ( $SD = \frac{pr[1-pr]}{[n-1]^{0.5}}$ , where n is the number of specimens in the TL size class; Zar, 1984).

### Results and Discussion

*Mugil cephalus*. The mandibular region in specimens of 20-30 mm TL has a single row of melanophores (Fig. 2). The ventro-opercular and gular regions are, generally, lightly pigmented. There is a single row of melanophores in the anterior ventro-opercular region, which extends into the posterior ventro-opercular region in some specimens. A single row of melanophores extends along the ventral midline from the anterior to the posterior sectors of the gular region, beyond the posterior margin of the eye. The row bifurcates at the level of the middle of the eye. The angle of the dentary symphysis is acute (less than 90°), giving the lower jaw a narrow V-shape in ventral view. In all other Mediterranean mullets, the angle of the dentary symphysis is more obtuse.

The characteristic acute angle of the dentary symphysis in *M. cephalus* has also been noted for fry from various parts of the Mediterranean (Perlmutter et al., 1957, for  $Pl_3$ , their Fig. D; Zismann, 1981; Serventi et al., 1996, their Fig. 2) and from British waters (Reay, 1992).

*Liza aurata*. The mandibular region of the head bears a single row of melanophores, with pigment spots concentrated at the corners of the mouth (Fig. 2). Two more or less regular rows of melanophores are present in the anterior ventro-opercular region (Table 2); these rows extend into the posterior ventro-opercular region in specimens over 20 mm TL, although they may be reduced to a single row in fish of 30-40 mm TL. The rows in the anterior ventro-opercular region form a distinct arch, outlining the lower jaw. There may be a few scattered melanophores forming a

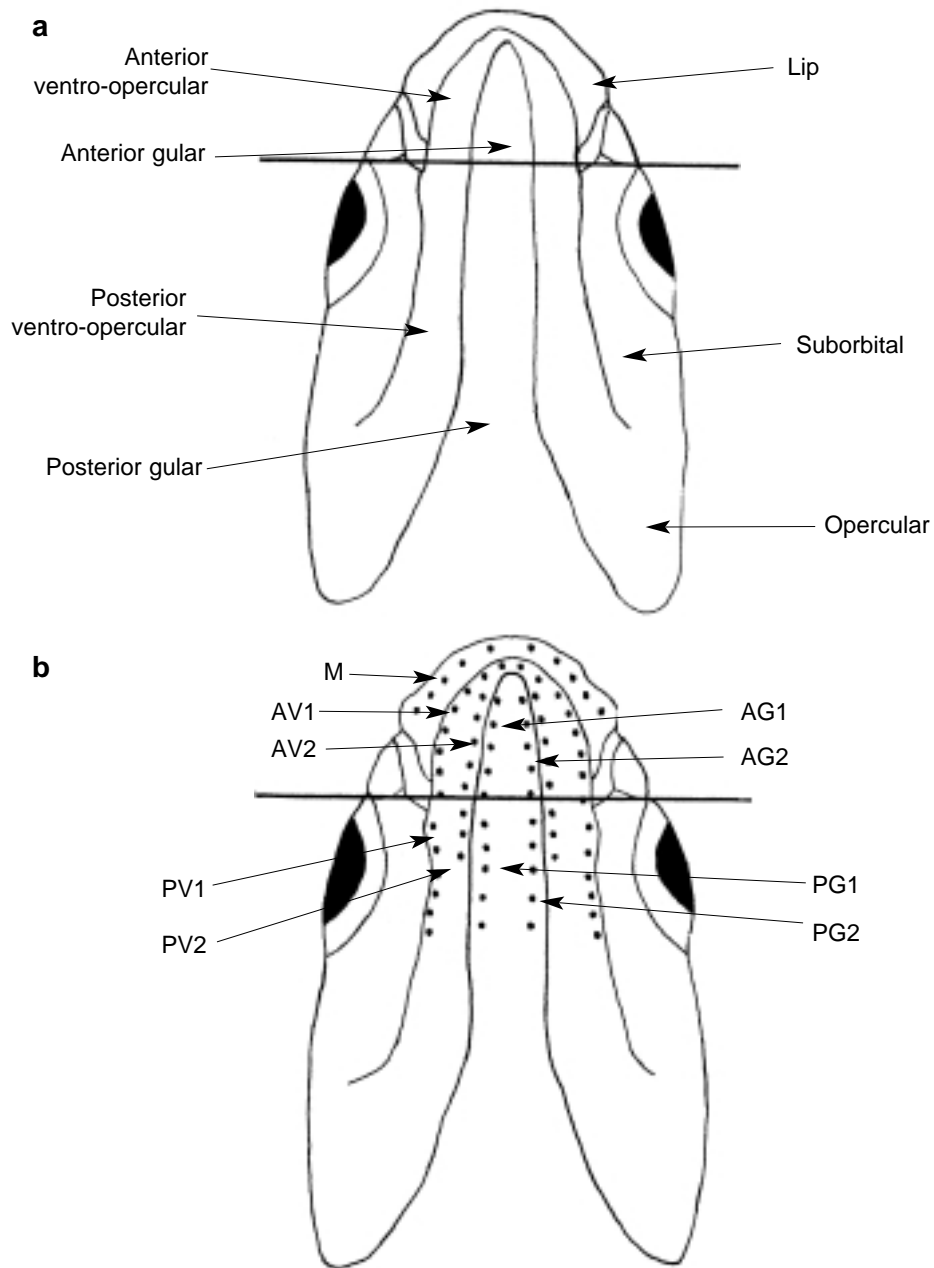


Fig. 1. (a) Regions on the ventral side of the head (terminology based on Reay and Cornell, 1988). Heavy horizontal line delimits the anterior and posterior sectors. (b) Terminology for rows of melanophores on ventral side of head. M = mandibular row (on lip); AV1 and AV2 = first and second anterior ventro-opercular rows, respectively; PV1 and PV2 = first and second posterior ventro-opercular rows, respectively; AG1 and AG2 = first and second anterior gular rows, respectively; PG1 and PG2 = first and second posterior gular rows, respectively.

Table 1. Presence of melanophore rows on the ventral surface of the head in *Mugil cephalus*.

TL	n	Anterior sector										Posterior sector										
		M		AV1		AV2		AG1		AG2		PV1		PV2		PG1		PG2				
		pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD			
20-25	7	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0		
25-30	13	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0		
30-35	19	1	0	1	0	0	0	1	0	0	0	0	0	0	0.89	0.07	0	0	1	0	0.84	0.09
35-40	23	1	0	1	0	0	0	1	0	0	0	0	0	0.52	0.11	0	0	1	0	0	0.87	0.07
40-45	4	1	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0.75	0.25

M = mandibular row (on lip); AV1 and AV2 = first and second anterior ventro-opercular rows; PV1 and PV2 = first and second posterior ventro-opercular rows; AG1 and AG2 = first and second anterior gular rows; PG1 and PG2 = first and second posterior gular rows.

TL = Total length range in mm; n = number of individuals; pr = presence of the row = number of specimens in which row is present/total number of specimens observed; SD = standard deviation

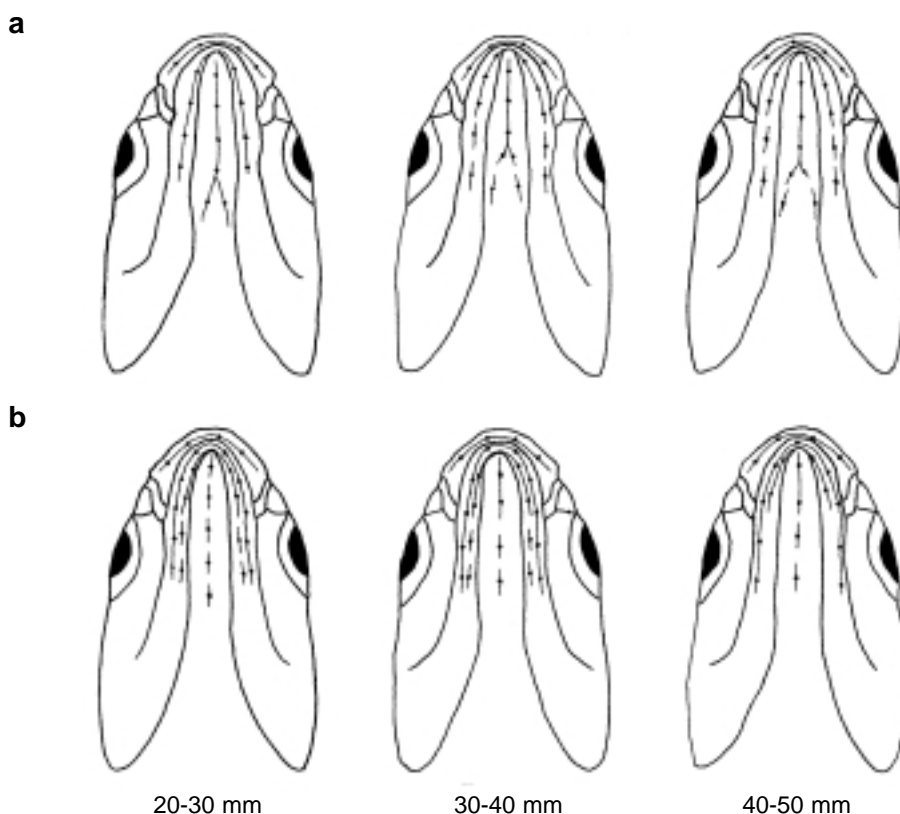


Fig. 2. Development of melanophore rows on ventral side of head for (a) *Mugil cephalus* and (b) *Liza aurata*. Unbroken line with dots (—•—•—) indicates the row of melanophores is always present; long broken line with dots (—•—•—) indicates the row of melanophores is frequently present; short broken line with dots (—•—•—) indicates the row of melanophores is occasionally present.

single line from the anterior to the posterior sectors of the gular region.

Reay and Cornell (1988, their Fig. 1) illustrate similar patterns of melanophores in the ventro-opercular and gular regions of the head in British specimens between 25 and 45 mm TL. Zismann (1981, his Fig. 2.4) illustrates melanophores concentrated at the corners of the mouth in fry from the Mediterranean coast of Israel.

*Liza ramada*. There is a single distinct line of melanophores across the mandibular region (Fig. 3) which becomes more distinct in large specimens (approximately 30-40 mm

TL). In these specimens, there is also a concentration of melanophores near the anterior tip of the jaw and at the corners of the mouth. The ventro-opercular region is lightly pigmented in 25-35 mm TL specimens, readily differentiating them from *C. labrosus* (also see Perlmutter et al., 1957). In most specimens, there is a single row of melanophores in the anterior sector of the ventro-opercular region which may extend, though less distinctly, into the posterior sector (Table 3). A second row of melanophores is present in the anterior ventro-opercular region of some specimens, but this is uncommon and the

Table 2. Presence of melanophore rows on the ventral surface of the head in *Liza aurata*\*.

TL	M			Anterior sector						Posterior sector							
	n	pr	SD	AV1	AV2	AG1		AG2		PV1	PV2	PG1		PG2			
						pr	SD	pr	SD			pr	SD	pr	SD		
20-25	2	1	0	1	0	1	0	0	0	1	0	1	0	1	0	0	0
25-30	23	1	0	1	0	0.96	0.04	0	0	1	0	0.70	0.10	0.96	0.04	0	0
30-35	35	1	0	1	0	1	0	0	0	1	0	0.57	0.08	0.86	0.06	0	0
35-40	15	1	0	1	0	1	0	0	0	1	0	0.07	0.07	0.80	0.11	0	0
40-45	5	1	0	1	0	1	0	0	0	1	0	0	0	0.60	0.24	0	0

\* See notes to Table 1.

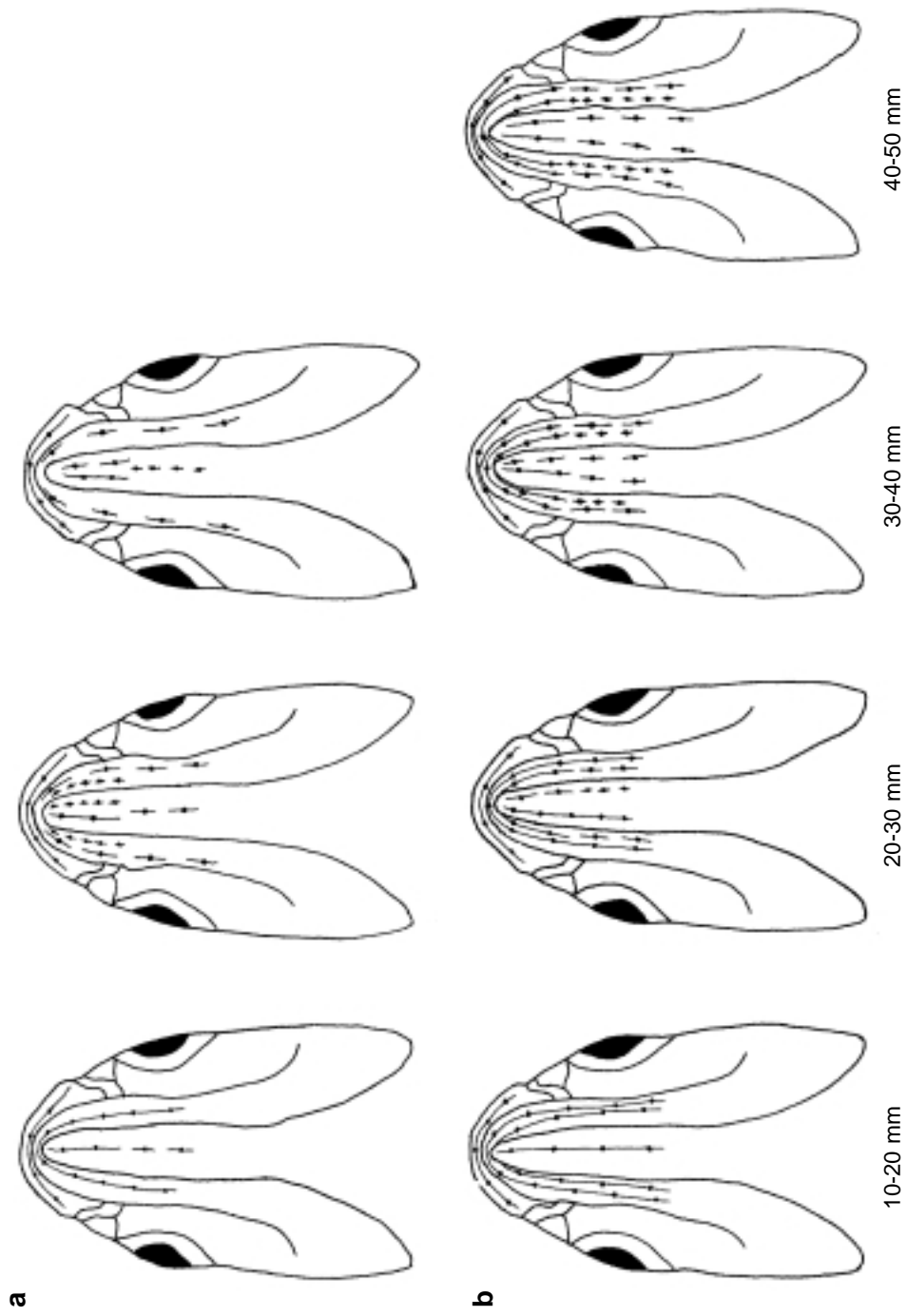


Fig. 3. Development of melanophore rows on ventral side of head for (a) *Liza ramada* and (b) *Chelon labrosus*. See Fig. 2 for explanation of lines.



Table 3. Presence of melanophore rows on the ventral surface of the head in *Liza ramada* \*.

TL	n	Anterior sector												Posterior sector								
		M		AV1		AV2		AG1		AG2		PV1		PV2		PG1		PG2				
		pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD			
15-20	5	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.80	0.20	0	0	
20-25	13	1	0	1	0	0.23	0.12	1	0	0.08	0.08	1	0	0	0	0	0	0	0.85	0.10	0	0
25-30	9	1	0	0.89	0.11	0.11	0.11	1	0	0.44	0.18	0.89	0.11	0	0	0	0	0.67	0.17	0	0	
30-35	17	1	0	0.88	0.08	0	0	1	0	0.47	0.12	0.94	0.06	0	0	0	0	0.24	0.11	0	0	
35-40	5	1	0	1	0	0	0	1	0	0.40	0.24	1	0	0	0	0	0	0.40	0.24	0	0	

\* See notes to Table 1.

row is never well developed. The anterior gular region has a row of melanophores running along the ventral midline which extends just into the posterior gular region. The row becomes more distinct in large specimens and a second row may be present in the anterior gular region (more commonly in specimens of 25-45 mm TL). Thus, in our specimens, the darkest regions on the ventral side of the head tended to be the mandibular and gular regions. A similar pigmentation pattern was illustrated for British specimens by Reay and Cornell (1988, their Fig. 1).

In fry of 19.1-32.7 mm TL collected from the Mediterranean coast of Israel, the mandibular region of the head is apparently more sparsely pigmented (Perlmutter et al., 1957, for Pl<sub>5</sub>) than we observed in our Greek specimens. However, according to Zismann (1981), specimens from Israel had a concentration of melanophores near the anterior tip of the jaw and the corners of the mouth, which we also note for Greek specimens.

*Chelon labrosus*. There is a single row of relatively large melanophores in the mandibular region of fry that are less than 20 mm TL; these melanophores become smaller and more diffuse in specimens over 25 mm (Fig. 3). There are two rows of melanophores in the anterior sector of the ventro-opercular region; the rows are sometimes irregular and usually extend beyond the level of the eye although this is less common in specimens over 30 mm TL. The anterior gular region has a single row of melanophores or, in specimens over 25 mm TL, there may be two irregular rows which may extend beyond the level of the anterior margin of the eye into the posterior gular region (Table 4). Our results generally agree with those of previous authors who indicated that fry of *C. labrosus* are more heavily pigmented on the ventral side of the head than other Mediterranean species (Perlmutter et al., 1957, for Pl<sub>4</sub>; Zismann, 1981; Reay and Cornell, 1988, their Fig. 1).

*Liza saliens*. The mandibular region is lightly pigmented compared to *L. aurata* and *L. ramada*. The ventral side of the head has some large discrete melanophores and the pigmentation may be concentrated at the anterior tip of

the lower jaw. There are two rows of melanophores in the anterior sector of the ventro-opercular region that rarely extend beyond the anterior margin of the eye (Fig. 4). The anterior gular region has two irregular rows of melanophores which rarely extend beyond the level of the anterior margin of the eye (extension of these rows into the posterior sector occurs more frequently in specimens of 16 mm TL or less; Table 5). The anterior gular region appears to be more heavily pigmented in *L. saliens* than in *L. aurata* or *L. ramada* (Fig. 5). However, the ventro-opercular region in *L. saliens* is not as densely pigmented as in *C. labrosus* (also see Perlmutter et al., 1957, for Pl<sub>4</sub> and Pl<sub>6</sub>; Serventi et al., 1996, their Fig. 2). The suborbital pigmentation of *L. saliens* is also less dense than that of *C. labrosus* and *L. aurata*, but more or less comparable to that of *L. ramada* (see Perlmutter et al., 1957, for Pl<sub>4-7</sub>).

Our observations of the mandibular pigmentation contradict those of Perlmutter et al. (1957, for Pl<sub>6</sub>), who indicated that the mandibular region of the head was relatively heavily pigmented in *L. saliens* (17.7-22.1 mm TL) from coastal waters of Israel. However, our results agree with those of Serventi et al. (1996, their Fig. 2b), who indicate that the mandibular region is lightly pigmented in fry of 20 mm SL from the Ligurian Sea. Our observations of the ventro-opercular and gular regions agree with those of Perlmutter et al. (1957) and Serventi et al. (1996) for specimens from the coasts of Israel and Italy, respectively. Zismann (1981) also commented on the presence of a few large melanophores along the midline of the gular region in fry from Israel.

*Key for identification*. The above descriptions indicate that there are species-specific differences in the pigmentation of the ventral side of the head in five species of grey mullets. Following is a key for distinguishing specimens in the range of 20-30 mm TL (Table 6), collected from coastal waters of Greece.

- 1a. Usually a single row of melanophores in the anterior ventro-opercular region; a second row is uncommon and, if present, never well developed...2

Table 4. Presence of melanophore rows on the ventral surface of the head in *Chelodon labrosus*\*

TL	n	Anterior sector												Posterior sector						
		M		AV1		AV2		AG1		AG2		PV1		PV2		PG1		PG2		
		pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	
15-20	9	1	0	1	0	1	0	1	0	0	0	1	0	0	1	0	1	0	0	0
20-25	13	1	0	1	0	1	0	1	0	0.15	0.10	1	0	0.85	0.10	1	0	0.08	0.08	
25-30	10	1	0	1	0	1	0	1	0	0.60	0.16	1	0	0.40	0.16	1	0	0.20	0.13	
30-35	11	1	0	1	0	1	0	1	0	1	0	1	0	0.27	0.14	1	0	0.73	0.14	
35-40	24	1	0	1	0	1	0	1	0	0.96	0.04	0.58	0.10	0.25	0.09	0.67	0.10	0.54	0.10	
40-45	14	1	0	1	0	1	0	1	0	1	0	0.57	0.14	0.29	0.13	0.64	0.13	0.50	0.14	
45-50	4	1	0	1	0	1	0	1	0	1	0	0.50	0.29	0.25	0.25	0.50	0.29	0.50	0.29	
50-55	1	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	

\* See notes to Table 1.

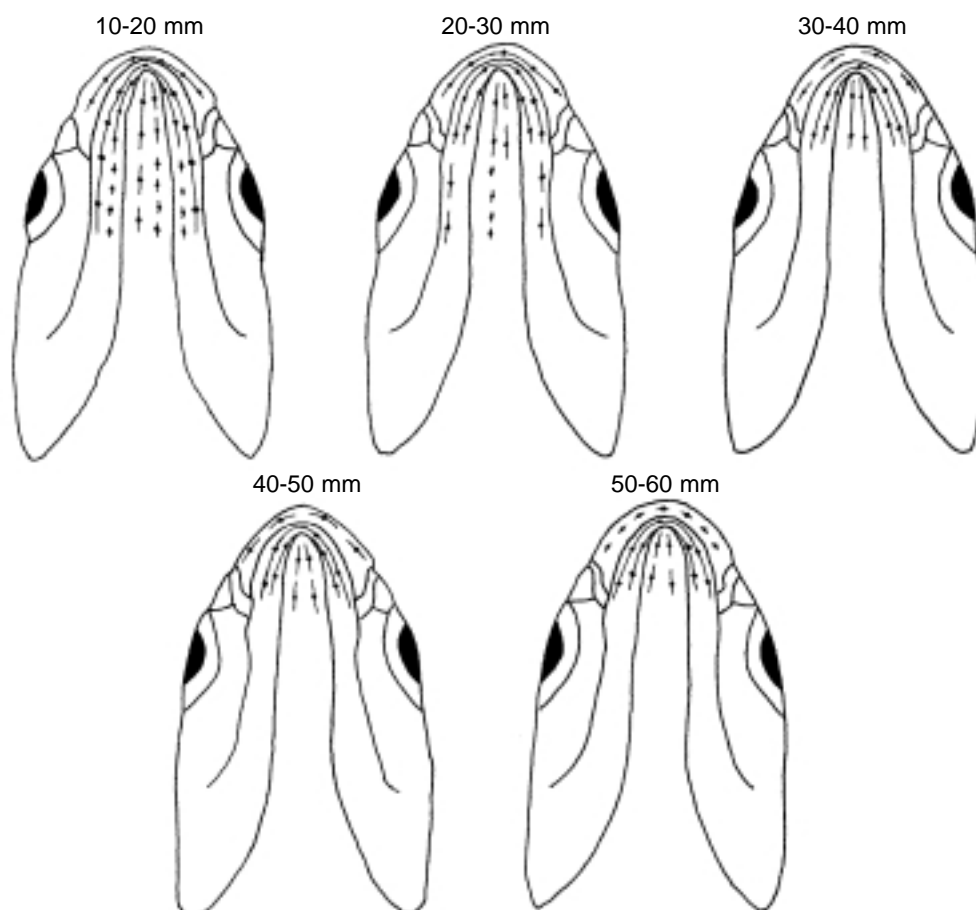


Fig. 4. Development of melanophore rows on ventral side of head for *Liza saliens*. See Fig. 2 for explanation of lines.

- 1b. Always a double row of melanophores in the anterior ventro-opercular region...3
- 2a. Single row of melanophores in the gular region that bifurcates at the level of the middle of the eye and extends beyond its posterior margin; acute angle of dentary symphysis...*Mugil cephalus*
- 2b. Usually a single row of melanophores in the gular region, that does not bifurcate; angle of the dentary symphysis obtuse...*Liza ramada*
- 3a. Sparse melanophores in the gular region, forming only a single row...*Liza aurata*
- 3b. Several melanophores in the gular region, forming one or two irregular rows...4
- 4a. The melanophores of the gular region usually do not extend beyond the level of the anterior margin of the eye (exception in specimens less than 20 mm TL)...*Liza saliens*
- 4b. The melanophores of the gular region usually extend beyond the level of the anterior margin of the eye...*Chelon labrosus*

This key, when accompanied by information regarding the season in which fry of the species appear, can be a significant tool for species identification for aquaculturists and ichthyologists in the field or in the laboratory.

Table 5. Presence of melanophore rows on the ventral surface of the head in *Liza saliens*\*.

TL	Anterior sector												Posterior sector								
	M		AV1		AV2		AG1		AG2		PV1		PV2		PG1		PG2				
	n	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD	pr	SD		
10-15	10	1	0	1	0	1	0	1	0	0	0	1	0	0	0	0	0	0.60	0.16	0.70	0.15
15-20	18	1	0	1	0	1	0	1	0	0.28	0.11	0.94	0.06	0.06	0.06	0.17	0.09	0.22	0.10	0.10	0.10
20-25	20	1	0	1	0	1	0	1	0	0.90	0.07	0.55	0.11	0	0	0.15	0.08	0	0	0	0
25-30	28	1	0	1	0	1	0	1	0	1	0	0.07	0.05	0	0	0.04	0.04	0	0	0	0
30-35	8	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
35-40	9	0.67	0.17	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
40-45	9	0.44	0.18	1	0	1	0	0.89	0.11	0.89	0.11	0.11	0.11	0	0	0	0	0	0	0	0
45-50	8	0.38	0.18	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
50-55	11	0.18	0.12	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
55-60	7	0.43	0.20	1	0	1	0	0.86	0.14	0.57	0.20	0	0	0	0	0	0	0	0	0	0

\* Seenotes to Table 1.

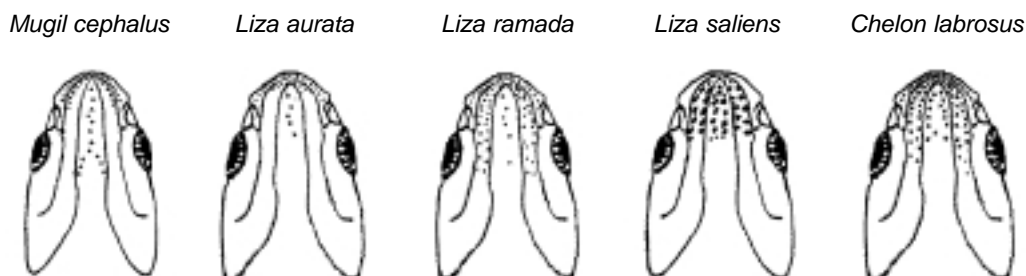


Fig. 5. Melanophore pattern on the ventral side of the head in five species of grey mullet (length range 20-30 mm). Interspecific differences in head shape are not represented.

Table 6. Melanophore rows\* on the ventral surface of the head in fry (20-30 mm TL) of five species of Mugilidae.

	<i>Mugil cephalus</i>	<i>Liza aurata</i>	<i>Liza ramada</i>	<i>Chelon labrosus</i>	<i>Liza saliens</i>
M	+	+	+	+	+
AV1	+	+	+	+	+
AV2	-	+	-	+	+
AG1	+	+	+	+	+
AG2	-	-	-(+)	-(+)	+
PV1	+	+	+	+	-(+)
PV2	-	+(-)	-	+(-)	-
PG1	+	+	+(-)	+	-
PG2	+	-	-	-(+)	-

\* See notes to Table 1.

+ = present (pr>0.9); - = absent (pr<0.8); +(-) = usually present (pr>0.5); -(+) = usually absence (pr<0.5)

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