Reproductive biology of *Merlangius merlangus* L. (Osteichthyes, Gadidae) in the northern Adriatic Sea

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The present paper investigates the reproductive biology of Merlangius merlangus. The reproductive period ranged November-May, based on the gonado-somatic index and gonad development in histological and anatomical terms. The length of first maturity was determined when over 50% of the sample was mature and was about 25 cm for females and 24 cm for males. No significant differences were found in sex ratio between specimens in different maturity stages.

Key words: maturity, spawning, Merlangius merlangus, northern Adriatic Sea

INTRODUCTION

The considerable growth in the fishing industry in the Mediterranean over the last decades and the subsequent depletion of natural resources (FAO, 1997; RELINI *et al.*, 1999) have made the study of the reproductive biology of commercial fish all the more urgent (VALLISNERI *et al.*, 2001). Taxonomy has been a subject of debate (TORTONESE, 1970) as well as the possible ecological and geographic separation between *Merlangius merlangus merlangus* of the Adriatic Sea and *Merlangius merlangus euxinus* of the Black Sea (UNGARO *et al.*, 1995). Information about the reproductive biology of this fish is scarce (GERRITSEN *et al.*, 2003), particularly in the Adriatic Sea (GIOVANARDI & RIZZOLI, 1983; VALLISNERI *et al.*, 2004). This preliminary study contributes information on the reproductive biology of *M. merlangus* in the Adriatic, including the spawning season and length at maturity in both sexes as indicated by gonado-somatic index, sexual maturity stage, and histological analysis.

MATERIALS AND METHODS

The study was conducted on 359 specimens from monthly catches from July 2001 to June 2002 between Cesenatico and Goro in the northern Adriatic Sea (Fig.1). Catches by commercial vessels were made with a tartantype trawl net with a cup-shaped 4-cm mesh. Measurements were made on specimens thawed in the laboratory and included total length (TL, mm), body weight (W, g), sex, gonad weight (gw, g), and stage of gonad maturity based on the modified empirical scale of HOLDEN & RAITT (1974). This scale has four stages: 1 = immature, 2 = maturing, 3 = ovipositing, and 4 = postoviposition, and seems more discriminate and less subject to interpretative error than other scales. The sex ratio (SR) was expressed as a percentage and determined by the formula SR = (no. males/total no. fish) x 100. The sex ratio was tested by the χ^2 test for significance. The gonado-somatic index (GSI) was determined as $GSI = (gw/W-gw) \times 100$. Gonad samples at various stages of development were fixed in Bouin's fluid, paraffin embedded, sectioned at 7 µm, and stained with Mayer Haemallum and Eosin B (Sigma) for histologic examination. The length at first maturity was determined as the mean total length of the specimens when at least 50% were mature, calculated for each size group only during the spawning period.

RESULTS

Total length

Table 1 shows total length (mm) for females and males by month.



Fig. 1. Capture sites of Merlangius merlangus specimens trawled in the Adriatic Sea

Gonado-somatic index

The GSI indicated that gonads began to mature in September-October for females and in November for males, with a peak for both sexes in December (Table 2). Likewise, the percentage of specimens in each maturity stage revealed that females began maturing in October-November and males in November, followed by a period of rapid growth for both sexes in December and a slow decline to May for females and to June for males (Table 3).

		Females			Males	
	n	TL (mm) range TL (mm) mean <u>+</u> SE	n	TL (mm) range T	<u>'L (mm) mean + SE</u>
July	18	137-277	225 <u>+</u> 11,3	11	208-242	223 <u>+</u> 3,6
September	21	157-272	216 <u>+</u> 5,9	13	192-247	224 <u>+</u> 5,5
October	16	169-331	231+11	15	150-253	190 <u>+</u> 7,5
November	26	178-283	225 <u>+</u> 4,9	2	219-240	230 <u>+</u> 10,6
December	14	201-265	232 <u>+</u> 4,5	17	185-267	220 <u>+</u> 5,3
January	18	184-302	236 <u>+</u> 7,3	22	155-215	191 <u>+</u> 3,4
February	14	206-304	239 <u>+</u> 7,2	24	174-258	221 <u>+</u> 5,1
March	15	202-285	240 <u>+</u> 6,3	19	172-239	210 <u>+</u> 4,4
April	17	194-302	241 <u>+</u> 6,4	18	187-238	205 <u>+</u> 3,1
May	5	243-302	267 <u>+</u> 9,8	30	204-282	235 <u>+</u> 1,8
June	13	164-269	208+11,1	11	164-254	228 <u>+</u> 7,8

Table 1. Total length (mm) of Merlangius merlangus females and males by month

		Females	5		Males	
	n	GSI	SD	n	GSI	SD
July	18	0,36	0,2	11	0,04	0,02
September	21	0,41	0,14	13	0,07	0,07
October	16	0,77	0,26	15	0,16	0,09
November	26	1,43	1,0	2	0,31	0,20
December	14	7,19	1,54	17	0,78	0,34
January	18	6,17	1,06	22	0,43	0,24
February	14	4,95	2,43	24	0,35	0,23
March	15	3,07	1,11	19	0,29	0,44
April	17	1,91	1,11	18	0,17	0,16
May	5	1,48	1,57	30	0,30	0,31
June	13	0,30	0,31	11	0,08	0,04

Table 2. Average gonado-somatic index (GSI) ± standard deviation (SD) for Merlangius merlangus females and males, by month

Table 3. Gonad maturity stage (%) of Merlangius merlangus *specimens: 1 = immature, 2 = maturing, 3 = ovipositing, 4 = post-oviposition (modified scale of HOLDEN & RAITT, 1974)*

	Females				Males				
	1	2	3	4	1	2	3	4	
July	22			78				100	
September	95	5			100				
October	62	38			100				
November	67	33				100			
December		21	79		11	24	65		
January		50	50		40	55	5		
February	7		93		29	33	38		
March		67	33		32	47	21		
April	11	24	65		39	39	22		
May		60	40		13	40	47		
June	77	15	8		36	55	9		

Length at first maturation

Gonad anatomy

The length at first maturity was estimated for both sexes as the total length at which 50% of the fish were mature (Table 4). In fact, over 50% of the individuals were in stage 3 in the sample taken during the reproductive period (November-May). Length at first maturity was 25 cm for females and 24 cm for males. In both sexes, some 19-cm specimens had already reached the ovipositing stage. Gonads originated as two folds in the peritoneal wall, attached to the vault of the abdominal cavity in a medial position with respect to the mesonephros, between the swim bladder and the intestine. In both sexes the gonads were double but partially jointed along the median line, with greater vascularization on the medial side. Ovaries were elongated, tubular in shape, with a more or less circular section and a yellowish

	F	emale	es			Males		
TL (mm)	1	2	3	4	1	2	3	4
180	100				80	20		
190	75		25		44	50	6	
200	42	29	29		29	57	14	
210	40	20	40		27	55	18	
220	33	33	34		13	50	37	
230	17	39	44		10	51	39	
240	10	42	48		6	28	66	
250	9	36	55				100	
260		43	57		17	17	66	
270		50	50				100	
280	17		83				100	
290								
300			100					

Table 4. Percent of specimens in each stage of gonad maturity during the reproductive period (November-May), according to total length (TL). For stages, see Table 3

orange color that became brighter with advancing stages of maturity (Fig. 2). Testes were elongated, bilobate, whitish, and linked to the dorsal wall of the body cavity by short mesorchiums.

Gonad histology

Histological analyses of the gonads were essentially in line with gonad weight and development patterns. During the non-reproductive period, there were oocytes in the previtellogenic stage (Fig. 3). During the reproductive period, there were oocytes at the end of the vitellogenic stage with differing amounts of yolk together with oocytes of different sizes in the previtellogenic stage. Such findings allow us to classify the species as partially ovipositing with asynchronous oocyte development. Histological analysis of the testes was also essentially in line with gonad weight and development patterns. During the non-reproductive period, there were seminiferous lobules in the earliest stages of spermatogenesis (Fig. 4). During the reproductive period, there were seminiferous lobules in various stages of spermatogenesis.

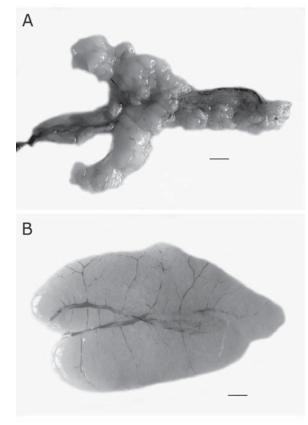


Fig. 2. Anatomy of maturing gonads in (A) males and (B) females; scale bar 5 mm

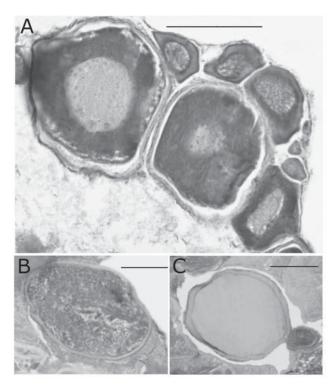


Fig. 3. Ovaries sectioned for histological analysis: (A) previtellogenic oocytes of different sizes (scale bar 100 μm), (B) vitellogenic oocyte (scale bar 50 μm), (C) hydrated oocyte (scale bar 100 μm)

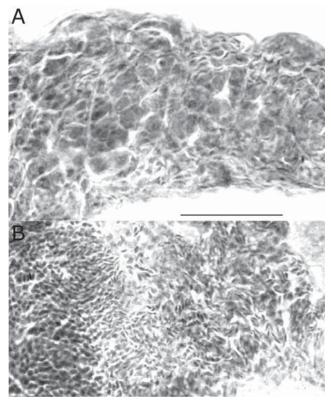


Fig. 4. Testes sectioned for histological analysis: (A) early spermatogenesis and (B) advanced spermatogenesis (scale bar 50 μm)

Parameters	n Males	n Females	Sex-ratio	Statistical results
Total number	182	177	51	$\chi^2=0,03; d.f.=1; P=ns$
Reproductive period	132	109	55	χ ² =1,10; d.f.=1; P=ns
Non-reproductive period	50	68	42	χ ² =1,37; d.f.=1; P=ns
Pre-maturity size	131	118	53	χ ² =0,34; d.f.=1; P=ns
Post-maturity size	51	59	46	χ ² =0,29; d.f.=1; P=ns

Table 5. Sex ratio in different stages of maturity

Sex ratio

No significant differences were found in sex ratio between reproductive periods (Table 5).

DISCUSSION

All three criteria used in this study, i.e., gonado-somatic index, sexual maturity stage, and histological analysis, yielded similar findings, i.e., that the reproductive period is November-May with a peak in December and that the size at sexual maturity is about 25 cm for females and 24 cm for males.

The reproductive period was within the range (winter-spring) already determined for the Adriatic Sea by GIOVANARDI & RIZZOLI (1982) but differs from that found in the Atlantic Ocean off the Scottish coast by COOPER (1983), i.e., March-

June, and from that found in the Irish Sea by GERRITSEN *et al.* (2003), i.e., February-June with a spawning peak in March and April depending on latitude and climatic conditions.

Length at first maturity was less than that of Atlantic Scottish whiting which was 27.5 cm for females and 25.5 cm for males (COOPER, 1983). The difference in length is most likely due to the difference in latitude (58°N in the Atlantic Sea study and 45°N in the Adriatic; VALLISNERI *et al.*, 2003). Males reached sexual maturity earlier than females, and hence at a smaller length, in both latitudes.

According to COOPER (1983) for the west coast of Scotland and GERRITSEN (2003) for the Irish Sea, the sex ratio of whiting generally does not significantly differ from 1:1 for the overall data or within each reproductive period.

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Reprodukcija pišmolja, *Merlangius merlangus* L. (Osteichthyes, Gadidae) u sjevernom dijelu Jadranskog mora

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U radu se iznose zapažanja o reprodukciji pišmolja *Merlangius merlangus*. Gonado-somatski indeks, te razvoj gonada u histološkom i anatomskom smislu pokazuju da je reproduktivno razdoblje trajalo je od studenog do svibnja. Duljina prve spolne zrelosti je određena u trenutku kada je više od 50% uzorka postiglo spolnu zrelost i iznosi oko 25 cm za ženke i 24 cm za mužjake. Nije utvrđena statistički značajna razlika u odnosu spolova između jedinki u različitim stadijima zrelosti.

Ključne riječi: zrelost, mriješćenje, Merlangius merlangus, sjeverni dio Jadranskog mora