

## Reproductive biology of the striped goby, *Gobius vittatus* (Gobiidae) in the northern Adriatic Sea

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**SUMMARY:** The striped goby, *Gobius vittatus* Vinciguerra, 1883 is a Mediterranean gobiid species considered to be rare and lacking in data on its reproduction. Sex ratio, length and age at first maturity, annual cycle of gonad development, spawning period and fecundity of *G. vittatus* were studied on 704 specimens collected by SCUBA diving in the northern Adriatic Sea (Kvarner area) from April 2001 to March 2002. The monthly sex ratio did not differ significantly, except during the spawning season, when it was strongly biased in favour of females. The estimated length at first maturity was 32.8 and 35.4 mm for females and males, respectively. In both sexes gonad development started in the first year of life. All specimens were mature by the third year. The spawning season was from April to July. Total fecundity ranged from 560 to 3045, with an average of  $1426 \pm 89$  ripe eggs/fish. The simultaneous presence of three clearly distinct sizes of oocytes in ripe ovaries indicate that this species is a multiple spawner.

**Keywords:** Gobiidae, sex ratio, maturity, spawning, fecundity.

**RESUMEN:** BIOLOGÍA REPRODUCTIVA DE *GOBIOUS VITTATUS* (GOBIIDAE) EN EL NORTE DEL MAR ADRIÁTICO. – El góbido listado, *Gobius vittatus* Vinciguerra, 1883 es una especie mediterránea considerada rara de la que se desconoce todo sobre su reproducción. En este estudio se han establecido la proporción sexos, la longitud y la edad de la primera madurez, el ciclo anual del desarrollo gonadal, el período de freza y la fecundidad de *G. vittatus* en base a 704 individuos, capturados mediante inmersión con escafandra autónoma en el norte del Adriático (región de Kvarner), desde abril 2001 hasta marzo 2002. La proporción entre sexos por mes no resulta significativamente distinta, excepto durante el período de freza, cuando se observaba una marcada desviación a favor de las hembras. La longitud estimada de la primera madurez es de 32.8 mm y 35.4 mm para machos y hembras respectivamente. El desarrollo de las gónadas se produce en el primer año de la vida en ambos sexos. Todos los individuos son maduros a los tres años. El período de freza se extiende de abril hasta julio y la fecundidad total va de 560 a 3045 huevos/hembra madura, con una media de  $1426 \pm 86$ . La presencia simultánea de tres tallas diferentes de oocitos en los ovarios maduros indica que la reproducción de esta especie es múltiple.

**Palabras clave:** Gobiidae, proporción de sexos, madurez, freza, fecundidad.

### INTRODUCTION

With 59 species, Gobiidae is the most specious fish family in the Mediterranean (Kovačić, 2005). Great ecological diversity seen in gobies indicates their value as research material in life-history evolution (Miller, 1984). However, most gobies are not easily collected and the conventional fishing gears are in most cases useless. Detailed studies of reproductive biology of gobiid species based on year-

round samples are therefore rare and currently there are no data on reproduction for more than half of the Mediterranean and northeastern Atlantic gobiid species (Kovačić, 2001). Among them, the striped goby, *Gobius vittatus* Vinciguerra, 1883 was considered to be rare (Tortonese, 1975) or very rare (Jardas, 1985) due to the lack of data. For a long time only morphology and habitat of the striped goby were known from the few papers published since the species description, based on one or two specimens

(Vinciguerra, 1883; Kolombatović, 1886; 1891; Fage, 1918; De Buen, 1923; Tortonese, 1975). Heymer and Zander (1978) described habitat, diet and morphology on 21 specimens from Banyuls-sur-Mer (France). Some biological aspects of *G. vittatus* were studied in detail in the northern Adriatic Sea, where an abundant population of this species was found in a characteristic ground using SCUBA diving (Kovačić, 2004, 2006, 2007). Goby *G. vittatus* is a Mediterranean endemic species present on coralline grounds from 20 to 42 m depth (Heymer and Zander, 1978) and on a mixed bottom of rock and sand, with a steep to medium slope from 5 to 34 m depth (Kovačić, 2004). It is a small-sized species, with a maximum recorded size of 58 mm (Heymer and Zander, 1978). It is a carnivore and generalist, feeding on a wide variety of prey items, particularly polychaetes, gastropods, copepods, ostracods and bivalves (Heymer and Zander, 1978; Kovačić, 2004, 2007). Maximum estimated age in the northern Adriatic Sea was 4 and 3 years for females and males, respectively (Kovačić, 2004, 2006). The aim of this paper is to provide the first data on reproduction of *G. vittatus*, including the sex ratio, length and age at first maturity, annual cycle of gonad development, spawning period, relationship of the reproductive cycle and liver storage, fecundity and recruitment of young.

## MATERIAL AND METHODS

Seven hundred and four specimens of *G. vittatus* were obtained at three locations (Stara voda, Oštro and Selce) in the Kvarner area, northern Adriatic Sea, from April 2001 to March 2002 (Fig. 1). All fish, including the first records of the young recruited to the demersal adult population, were collected between 8 and 20 m depth, using a hand net and the anaesthetic quinaldine during SCUBA dives. Each month twenty specimens were collected at each location. However, only four specimens were collected in January at Oštro due to bad weather and low temperature. During the SCUBA dives the water temperature was measured at the collecting depth. All specimens were killed by over-anaesthesia with quinaldine and fixed in 65% alcohol.

In the laboratory, the total length (TL) of each individual was measured to the nearest 0.1 mm (they were later grouped into 5 mm length classes) and wet mass was weighed to the nearest 0.001 g after blotting dry on absorbent paper. Sagittal otoliths

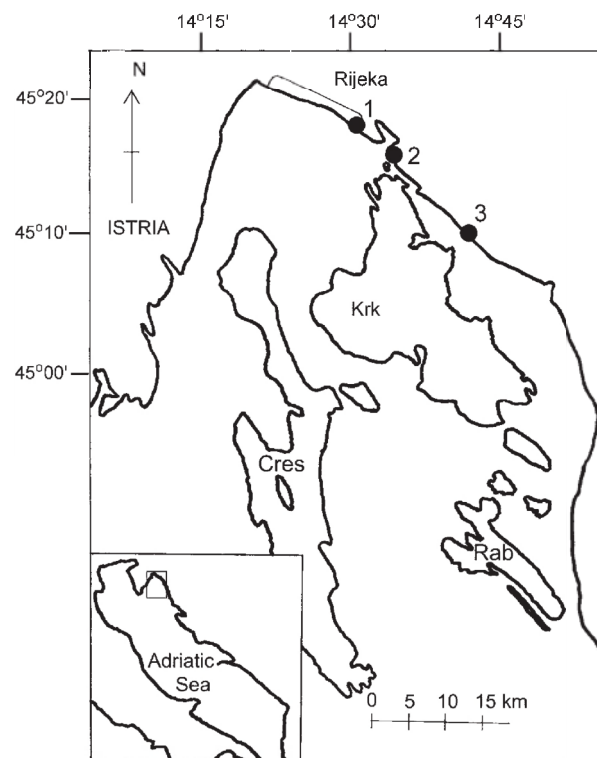


FIG. 1. – The Kvarner area, Croatia. Collecting sites: (1) Stara voda, (2) Oštro and (3) Selce.

were removed and used for ageing purposes, as described in detail in Kovačić (2006). The specimens were dissected under a stereomicroscope and gut, liver and gonads were removed. Sex was recorded and gonads were macroscopically staged according to the gonad development classification for gobies with a five-point scale of maturity: I - immature, II - developing virgin and recovering spent, III - ripening (for females IIIa - early ripening and IIIb - late ripening), IV - ripe, V - spent (Miller, 1961). The wet mass of gonads and liver was obtained to the nearest 0.1 mg after blotting dry on absorbent paper. In males all testicular apparatus (testes and seminal vesicles) was weighed as gonad mass. Furthermore, the seminal vesicles were weighed separately (Kovačić, 2001). The running ripe ovaries (stage IV) were used to calculate fecundity. A portion of ovaries was wet weighed and only the ripe oocytes were counted to estimate total fecundity ( $F = \text{wet mass of ovaries} \times \text{wet mass of counted portion}^{-1} \times \text{number of oocytes in portion}$ ). Oocyte size was measured as maximum diameter using a micrometric scale.

Sex ratio was calculated monthly and the significance of deviation from 1:1 null hypothesis was tested by  $\chi^2$  test (Sokal and Rohlf, 1995). The length and

age at first maturity were estimated by the proportion of mature specimens (i.e. in stages II-V, Miller, 1961). The length at first maturity, defined as the length at which 50% of fish are mature, was estimated by means of a logistic function fitted to the proportion of the mature specimens pooled in 5 mm length classes (L). The quasi-Newton algorithm for non-linear least square estimation of function parameters was applied to data according to the following equation:  $P_L = (1 + e^{-a(L-b)})^{-1}$ , where  $P_L$  is the proportion of mature fish at length L; a and b are estimated parameters, where a is the slope of the curve and b corresponds to a proportion of 0.5 mature fish (Morato *et al.*, 2003). The annual cycle of gonad development was investigated using maturity stages as in Miller (1961) and calculating gonadosomatic index (GSI= gonad wet mass x 100 x body wet mass<sup>-1</sup>) and seminal-vesical-somatic index (SVSI= seminal vesicle wet mass x 100 x body wet mass<sup>-1</sup>) (Patzner *et al.*, 1991) on monthly samples. The hepatosomatic index (HSI) was calculated as HSI= liver wet mass x 100 x body wet mass<sup>-1</sup> (Fouda *et al.*, 1993), as a measure proportional to energy reserves stored in this organ. All indices were compared across months using the non-parametric Kruskal-Wallis test, and the HSI was compared between sexes using the Mann-Whitney U-test, since the assumptions of normality and homogeneity were not met after arc-sine or logarithmic transformation (Sokal and Rohlf, 1995). Homogeneity of variance and normality were tested with Levene's test and the Kolmogorov-Smirnov test, respectively. The relationship between total fecundity and fish length was assessed by regression analysis of log-transformed data. Descriptive statistics are reported as mean  $\pm$  standard error. A non-linear estimate of logistic function parameters for length at first maturity was calculated using Statistica 5.1. Other data analyses were carried out using the Excel and SPSS software packages.

## RESULTS

### Sex ratio

All 704 specimens of *G. vittatus* were sexed successfully. The overall ratio of males to females was 1:1.33, diverging significantly from 1:1 ( $\chi^2 = 14.2$ , d.f. = 1,  $P < 0.001$ ). Monthly sex ratio however, did not differ significantly from 1:1, except for the period May to July 2001 (Table 1).

TABLE 1. – Monthly sex ratio of *G. vittatus* from the Kvarner area (April 2001 to March 2002) tested by  $\chi^2$  analysis. P, significance level; \*,  $P < 0.05$ .

Month	No. of males	No. of females	Sex ratio	P
Apr 2001	24	36	1:1.5	0.121
May 2001	18	42	1:2.33	0.002*
Jun 2001	10	50	1:5	<0.001*
Jul 2001	17	43	1:2.52	<0.001*
Aug 2001	24	36	1:1.5	0.121
Sep 2001	30	30	1:1	1
Oct 2001	27	33	1:1.22	0.439
Nov 2001	30	30	1:1	1
Dec 2001	25	35	1:1.4	0.197
Jan 2002	25	19	1:1.3	0.366
Feb 2002	37	23	1:0.62	0.071
Mar 2002	35	25	1:0.71	0.197

### Length and age at first maturity

The length at first maturity was estimated on all 402 females, ranging from 20.1 to 54.0 mm, and on all 302 males, ranging from 19.2 to 52.6 mm. Female length at maturity was estimated at 32.8 mm, and male length at maturity at 35.4 mm. The estimates of logistic function parameters for length at first maturity (a and b) for females and males are shown in Table 2. Out of 704 specimens examined (402 females and 302 males), 677 specimens (383 females and 294 males) were aged successfully (Kovačić, 2006). In both sexes gonad development started in the first age class (0+ fish: females 35.2%, males 31.3%). During the second year of life (1° and 1+), 88.9% of females and 82.4% of males were mature. In older age groups all specimens were mature.

### Annual cycle of gonad development and spawning period

The proportion of maturity stages for both sexes varied strongly with season (Table 3). Female gonadosomatic index (GSI) (Fig. 2a) varied significantly across months (H Kruskal-Wallis = 279.7,  $P < 0.001$ ),

TABLE 2. – The estimates of parameters (a and b) for length at first maturity of logistic function  $P_L = (1 + e^{-a(L-b)})^{-1}$  for females and males of *G. vittatus* from the Kvarner area ( $r^2$  coefficient of determination, SE standard error).

Parameter	Females		Males	
	Estimate	SE	Estimate	SE
a	0.394	0.046	0.301	0.029
b	32.79	0.32	35.43	0.33
$r^2$	0.994		0.995	

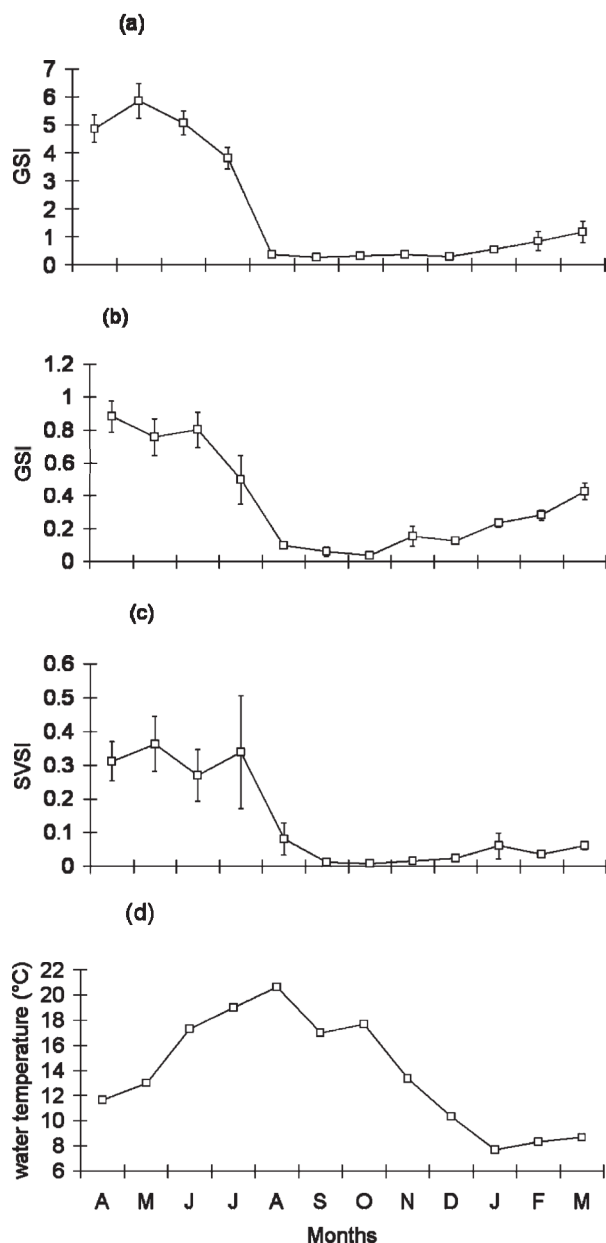


FIG. 2. – Mean ( $\pm$  standard error) monthly gonadosomatic index (GSI) of (a) female and (b) male and the seminal-vesicle-somatic index (SVSI) (c) of *G. vittatus* from the Kvarner area. Mean monthly sea temperature at the collecting depth (d) is also given.

as did male gonadosomatic index (GSI) (H Kruskal-Wallis = 199.6,  $P < 0.001$ ) and seminal-vesicle-somatic index (SVSI) (H Kruskal-Wallis = 86.7,  $P < 0.001$ ) (Figs 2b and 2c). In males all stages indicating gonad development occurred one or two months earlier in the annual cycle than in females. Females and males in stage II (i.e. developing virgin and recovering spent) were mostly found in winter. In this period, female GSI and male SVSI increased slowly, while male GSI showed a steady increase. Ripening males (stage III)

were the most numerous in early spring, while ripening females were numerous throughout the entire spring. By April male GSI ( $0.88 \pm 0.10$ ) and by May female GSI ( $5.86 \pm 0.62$ ) and male SVSI ( $0.36 \pm 0.08$ ) were at their maximum and remained high through to July. Ripe males (stage IV) were already present in March. However, the spawning season lasted from April to July, when ripening fish (stage IV) of both sexes were present and all gonad indices were high. In the post-spawning period (from August to November) GSI and SVSI reached their lowest value, and mostly spent (stage V) and immature (stage I) specimens were present. The late pre-spawning and spawning period occurred during an increase in the sea water temperature (Table 3 and Fig. 2).

### Hepatosomatic index

Hepatosomatic indices (HSI) of females ( $1.96 \pm 0.05$ ) and males ( $1.77 \pm 0.05$ ) were not significantly different (Mann-Whitney U-test,  $Z = 1.87$ ,  $P > 0.05$ ). HSI of both sexes showed a clear seasonal pattern, evidencing significant variation across months (females: H Kruskal-Wallis = 87.7,  $P < 0.001$ ; males: H Kruskal-Wallis = 54.7,  $P < 0.001$ ) (Figs 3a and 3b). Female HSI ( $2.51 \pm 0.14$ ) and male HSI ( $2.20 \pm 0.16$ ) peaked in April. The hepatosomatic index of both sexes decreased during the spawning season from April to July, when female HSI reached the

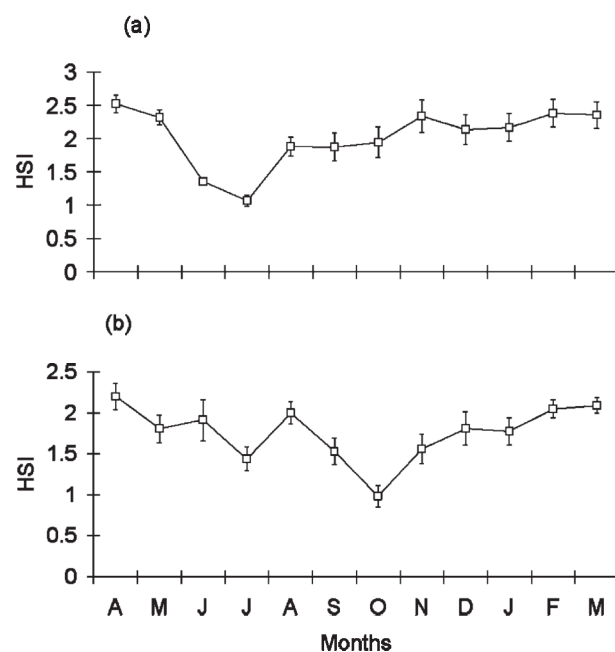


FIG. 3. – Mean ( $\pm$  standard error) monthly hepatosomatic index (HSI) of (a) female and (b) male of *G. vittatus* from the Kvarner area.

TABLE 3. – The number of females and males of *G. vittatus* from the Kvarner area in each maturity stages by month: I - immature, II - developing virgin and recovering spent, III – ripening (for females IIIa – early ripening and IIIb – late ripening), IV - ripe, V - spent. In parentheses: number of females and males of maturity stages II-V in proportion to total number of mature females and males for each month.

Females	I	II	Maturity stages IIIa	IIIb	IV	V
Apr 2001	-	2 (5.6%)	9 (25.0%)	15 (41.7%)	10 (27.8%)	-
May 2001	-	-	13 (31.0%)	18 (42.9%)	10 (23.8%)	1 (2.4%)
Jun 2001	-	5 (10.0%)	10 (20.0%)	21 (42.0%)	13 (26.0%)	1 (2.0%)
Jul 2001	6	3 (8.1%)	8 (21.6%)	14 (37.8%)	12 (32.4%)	-
Aug 2001	11	1 (4.0%)	-	1 (4.0%)	-	23 (92.0%)
Sep 2001	12	-	-	-	-	18 (100.0%)
Oct 2001	17	1 (6.3%)	-	-	-	15 (93.8%)
Nov 2001	13	-	-	-	-	17 (100.0%)
Dec 2001	17	8 (44.4%)	-	-	-	10 (55.6%)
Jan 2002	10	9 (100.0%)	-	-	-	-
Feb 2002	8	13 (86.7%)	2 (13.3%)	-	-	-
Mar 2002	3	11 (50.0%)	6 (27.3%)	5 (22.7%)	-	-
Males	I	II	III	IV	V	
Apr 2001	2	3 (13.6%)	12 (54.5%)	7 (31.8%)	-	
May 2001	1	5 (29.4%)	6 (35.3%)	6 (35.3%)	-	
Jun 2001	1	-	2 (22.2%)	7 (77.8%)	-	
Jul 2001	7	-	5 (50.0%)	2 (20.0%)	3 (30.0%)	
Aug 2001	9	1 (6.7%)	-	2 (13.3%)	12 (80.0%)	
Sep 2001	18	-	-	-	12 (100.0%)	
Oct 2001	19	2 (25.0%)	-	-	6 (75.0%)	
Nov 2001	18	4 (33.3%)	-	-	8 (66.7%)	
Dec 2001	10	12 (80.0%)	3 (20.0%)	-	-	
Jan 2002	12	7 (53.8%)	6 (46.2%)	-	-	
Feb 2002	22	8 (53.3%)	7 (46.7%)	-	-	
Mar 2002	9	16 (61.5%)	6 (23.1%)	4 (15.4%)	-	

lowest value, followed by a continuous recovery until the beginning of the next spawning season. On the other hand, male HSI showed a new decrease from August to October, when it reached the lowest value, followed by a steady increase until the next spawning season.

### Fecundity and egg size

Fecundity was estimated on 45 ripe females from 36.9 to 53.6 mm TL. The total fecundity ranged from 560 to 3045, with an average of  $1426 \pm 89$  ripe eggs/fish. There was a significant positive relationship between fecundity and fish length ( $F = 76.2$ ,  $P < 0.001$ ) (Fig. 4). The regression model between total fecundity and total length was:

$$\ln F = 3.172 \ln TL - 4.995$$

However, considerable variability in fecundity of females of similar length was present and the proportion of the variance of fecundity explained by length was not high (coefficient of determination  $r^2 = 0.64$ ).

The measurements of the oocyte diameters showed that a ripe ovary contained fully ripe

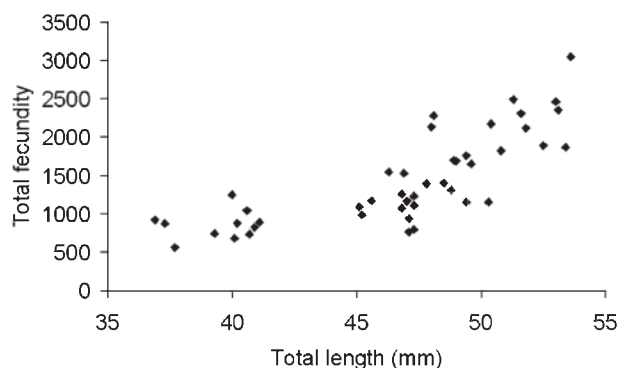


FIG. 4. – Plots of total fecundity against total length of *G. vittatus* from the Kvarner area.

oocytes (0.44-0.60 mm), early ripening oocytes (0.16-0.24 mm) and numerous, very small ones (<0.08 mm). The presence of three clearly distinct sizes of oocytes in ripe ovaries indicated that each female could spawn at least twice during the breeding season.

### Recruitment of young

The young of the striped goby recruited to the demersal adult population were first found in August



2001. Total length of eleven specimens ranged from 19.2 to 24.8 mm, with an average of  $21.6 \pm 0.6$  mm.

## DISCUSSION

The reproductive biology of *G. vittatus* has never been investigated before the present study. The present data can be compared with those of closely related *Gobius* species. The sex ratio was strongly biased in favour of females for the period May to July 2001, which coincides with the observed spawning season. The prevalence of females observed during the spawning period was probably due to a decrease in the availability of males. Indeed, during the spawning period males of most gobiid species guard nests, so they are hardly caught at all. The predominantly female sex ratio during the spawning season, as a probable result of the cryptobenthic behaviour of males, was also found in *Gobius niger* (Nash, 1984; Silva and Gordo, 1997). Nash (1984), as well as the present study, found no significant differences in the number of males and females of *G. niger* out of the spawning period, whereas Silva and Gordo (1997) noted a significant dominance of males for the same species. Hence, data which do not refer to the spawning period provide a more realistic evaluation of sex ratio in populations of most gobiid species featuring nest guarding behaviour.

Similarly to *G. niger* (Fabi and Frogia, 1984; Vesey and Langford, 1985; Joyeux *et al.*, 1991), *G. vittatus* manifests early maturation, starting gonad development in the first year of life. In other *Gobius* species, such as *G. paganellus* and *G. roulei*, gonad development starts in the second year of life (Miller, 1961; Kovačić, 2001). However, all these species show late maturation in comparison with short lifespan gobiid species such as *Pomatoschistus* spp., whose populations are fully mature during the first year of life (Miller, 1961; Gibson and Ezzi, 1981; Bouchereau *et al.*, 1989, 1993).

The spawning period similar to that of *G. vittatus*, from April to August, has also been observed in *G. roulei* from the Kvarner area and *G. niger* from the central Adriatic Sea (Fabi and Frogia, 1984; Kovačić, 2001). All *Gobius* species of the northeast Atlantic and the Mediterranean breed during spring and summer, with a breeding season of two to six months (Miller, 1961; Gibson, 1970; Vaas *et al.*, 1975; Dunne, 1978; Nash, 1984; Vesey and Langford, 1985; Joyeux *et al.*, 1991, 1992; Arruda *et*

*al.*, 1993; Silva and Gordo, 1997; Azevedo and Simas, 2000). Nevertheless, the spawning season of *Gobius* species in the northeast Atlantic usually begins earlier and is shorter than in the Mediterranean. At higher latitudes, limitation of productivity to short periods of the year cannot support a long season of repeat-spawning of species originated under warmer conditions (Miller, 1979).

Closely linked with reproduction is phenology of storage (Miller, 1979). Annual variations in liver mass could reflect the process of storage and transfer of energy from periods of intensive feeding to periods of restricted feeding but high energy demands. The data on the relationship of the liver storage and the reproductive cycle of gobies are rare (Miller, 1984; Joyeux *et al.*, 1992; Fouda *et al.*, 1993). These data, as well as the present study, show that liver plays an important role in storage during the annual cycle of gonad development. A marked reduction in liver mass was noticed at the beginning of or during the breeding season (Joyeux *et al.*, 1992; Fouda *et al.*, 1993).

The size frequency of oocytes within mature ovary of *Gobius* species shows that they are repeat spawners, like the majority of gobies (Miller, 1984). The total fecundity estimated on ripe oocytes therefore represents the batch fecundity and not the annual fecundity. The fecundity of the striped goby is generally lower than in other *Gobius* species such as *G. cobitis*, *G. paganellus* and *G. roulei* (Miller, 1961; Gibson, 1970; Dunne, 1978; Kovačić, 2001). The ripe oocytes of *G. vittatus* were similar in size to those of *G. roulei* (Kovačić, 2001), but smaller than those of other, larger *Gobius* species, which measure more than 1 mm in diameter (Miller, 1961; Gibson, 1970). Among *Gobius* species, fecundity and egg size generally depended on species size. In addition, the reduction of egg size in smaller species slows down the decrease of fecundity linked to a decrease in body and ovarian mass. The estimation of number of egg batches laid by *Gobius* species during the spawning season is only known for *G. paganellus*, which spawned twice per season (Miller, 1984). With *G. vittatus* the number of oocyte classes found in the ripe ovaries does not necessarily reflect the number of batches produced during one season, because of possible recruitment from numerous immature oocytes. Multiple spawning and the parental care may increase the reproductive success in the striped goby and compensate for the relatively low fecundity.

The young of *G. paganellus* recruited to the demersal adult population were first found four months after the beginning of the spawning season (Miller, 1961) and four months after the GSI reached maximum values (Azevedo and Simas, 2000), similarly to the present results on the striped goby.

The striped goby is the smallest *Gobius* species. Considering the data already published, it shares characteristics of reproductive biology with congeners. Small size, compared to other *Gobius* species, resulted in a relatively early maturation, low fecundity and small egg size.

## ACKNOWLEDGEMENTS

I am grateful to M. La Mesa for criticism and comments on this paper and to S. Padavić for the improvement of language and style. Diving assistance was provided by M. Arko-Pijevac.

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Scient. ed.: A. Garcia-Rubies.

Received June 29, 2006. November 27, 2006.

Published online February 26, 2007.

