THE REPRODUCTIVE CYCLE OF *OPHICHTHUS RUFUS* (ANGUILLIFORMES) IN THE NORTHWEST MEDITERRANEAN

by

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ABSTRACT.– The reproductive biology of the Mediterranean snake eel, *Ophichthus rufus* (Ophichthidae), was studied on the basis of microscopic and macroscopic analysis. Results show that *O. rufus* is an ovuliparous species with a group synchronous ovary type. The maturation process of the gonads starts in December and progress untill August, when ovulation and spawning take place. It is a fractional spawner with buoyant eggs. Potential annual fecundity estimates ranged from 1426 to 23,605 oocytes. Except for small sizes, a clear predominance of females was observed.

RÉSUMÉ.– Cycle de la reproduction d'*Ophichthus rufus* (Anguilliformes) en Méditerranée nord-ouest.

La biologie reproductive d'*Ophichthus rufus* (Ophichthidae) a été étudiée sous ses aspects macroscopiques et microscopiques. *O. rufus* est ovipare, avec un ovaire du type "groupe synchronique". Le processus de maturation des gonades commence en décembre et se poursuit jusqu'en août, lorsque l'ovulation et la ponte ont lieu. Cette ponte est fractionnée et les oeufs sont pélagiques. La fécondité annuelle potentielle est estimée entre 1426 et 23605 ovocytes. Une grande prédominance de femelles a été observée, sauf chez les individus de petite taille.

Key words.– Ophichthidae - *Ophichthus rufus* - MED - Reproduction - Ovogenesis - Gonadosomatic index - Fecundity.

Ophichthus rufus (Rafinesque, 1810), the Rufus snake eel, is a benthic species distributed exclusively in the mud bottoms of the Mediterranean shelf (Bauchot, 1986). Along the north-eastern coast of Spain it is usually found in sand or mud bottoms at depths between 50 and 150 m (Matallanas, 1979). It is a nocturnally-active species. During the day it buries itself in the mud, using its tail, and probably emerges to feed only at the night (Matallanas, 1979).

Few studies have been carried out on this species. Casadevall *et al*. (1994) have described its feeding habits, but little attention has been paid to its size and distribution (Tortonese, 1960). Apart from when the breeding period occurs (Spartà, 1937), no further information is available about its reproduction.

This paper presents a description of the reproductive biology of *O. rufus*. On the basis of histological observations, oocyte development stages are identified and measured to describe the previtellogenesis and vitellogenesis phases. The reproductive style and annual cycle are evaluated using the gonadosomatic index (GSI), macroscopic observations of the gonads, sex ratio, and fecundity.

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MATERIALS AND METHODS

The sample for this study was obtained by professional trawling in a region of sandy-mud bottom called 'Els Capets', located near Blanes (Costa-Brava, Catalan sea, north-west Mediterranean). A total of 679 individuals, collected monthly between 1985 and 1987, were used to calculate the sex ratio (SR) and the gonado-somatic index (GSI).

$$SR = \frac{\text{no males}}{\text{no females}} \qquad GSI = \frac{\text{gonad weight (g)}}{\text{body eviscerated weight (g)}} \times 100$$

The gonads of two adult females collected in February, with body total lengths (TL) of 360 and 375 mm, were used for the description of oocyte stages. These were medium-sized females (maximum total length in the sample was 605 mm), with ovaries in the vitellogenic phase, and oocytes in all stages of development. Because of the structure of the ovaries, it was impossible to obtain good histological slides for larger and more mature females.

For the histological study, gonads were embedded in glycol methacrylate, sectioned at 3-5 μm and stained by methylene blue-basic fuchsin and by toluidine blue. Differences in stain affinities, nuclear evolution and cytoplasm modifications were used to classify six stages of oocyte development.

Oocytes were measured using the eye-piece micrometer. Oocyte size was obtained by taking the mean of the maximum and minimum diameter of only those oocytes that had been sectioned through the nucleus. The NPR (nucleo-plasmatic ratio) was calculated as:

$$NPR = \frac{V_n}{V_c - V_n}$$

where Vn is the nucleus volume and Vc is the cytoplasm volume.

The ovaries of 26 females (320 to 540 mm TL) caught in July were used to estimate fecundity. Measurement of total fecundity must be effected before the first spawning. Ovulation takes place in August, and eggs were released from the body cavity of most females when dissected. These eggs are free, making it impossible to weigh the whole gonad and to separate a representative subsample. Furthermore, spawning may have begun at this time, and some advanced oocytes may be lost. July samples were selected for this reason, it being the month when ovaries are still outlined and covered by the *tunica albuginea*. Following the classification of Hunter *et al.* (1992), July females are mature non-spawning females: they showed no evidence of recent or imminent spawning, but were capable of spawning in the near future.

Ovaries were removed from the fish, weighed, and then two subsamples with an individual weight of approximately 10% of the total weight of every ovary were taken (one from the top and the other from the back of the gonad). These subsamples were immersed in Gilson's fixative (modified by Simpson, 1951) and shaken frequently to facilitate membrane breakdown and subsequent oocyte release. When the oocytes had been completely released, they were separated by suspending each sample in water and with the latter poured through a series of sieves with a mesh size of between 0.1 and 2 mm.

Absolute fecundity was calculated as:

$$F_{abs} = 1 \Big/ N \sum_{i=1}^{N} \frac{w_i}{b_i} n_i$$

where w = ovary weight, N = number of subsamples, b = weight of subsample and n = number of oocytes.

For each ovary sample, both the absolute fecundity (i.e., the number of oocytes, advanced yolked oocytes, destined for spawning, per female and year) (Aboussouan and Lahaye, 1979) and the relative fecundity, or the fecundity relative to the corporal weight (eggs/g body eviscerated), were determined.

Considering the stage of maturity of ovaries in July, the absolute fecundity obtained must be considered equivalent to the potential annual fecundity described by Hunter *et al*. (1992), i.e., the number of total advanced yolked oocytes matured per year, uncorrected for atretic losses. Atresia was not observed in February ovaries, but atretic losses could not be measured in more advanced stages of maturation, so there is likely to be an overestimation of fecundity.

RESULTS

Stages of oocyte development

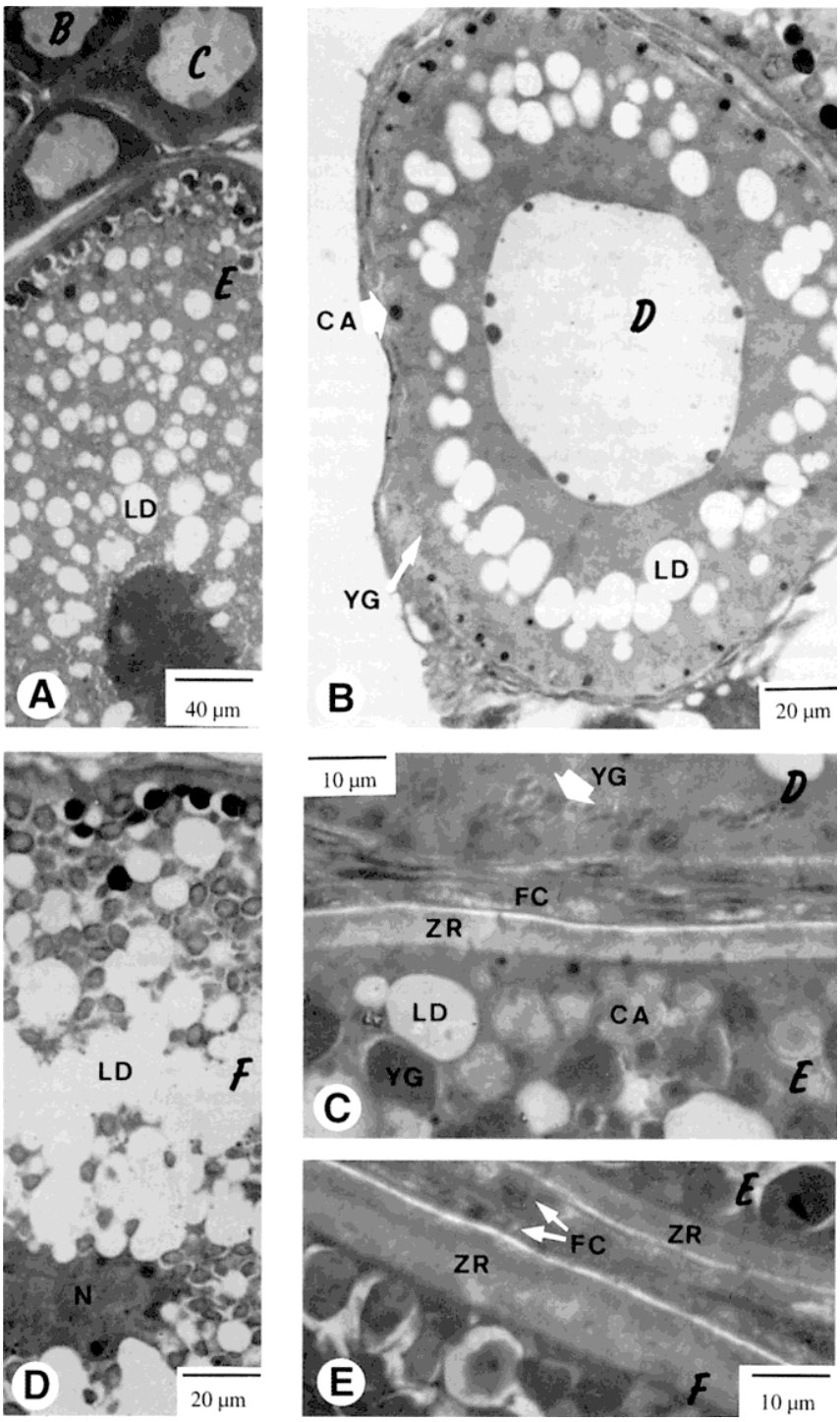
The ovaries of *Ophichthus rufus* are paired, flat (with semicircular section), and lamellar, and are spread along the length of the body cavity.

Following Takashima and Hibiya (1995), all the teleost ovaries are of the cystovarian type; however, in *O. rufus* there is no oviduct and eggs are released directly into the body cavity.

The ovarian stroma, a well-irrigated muscular-connective tissue raphe, runs longitudinally through the ventral margin of the gonad. Each ovarian follicle projects laterally from it and consists of a musculo-connective axis with a central arteriole and germinal tissue formed by germinal cells. Each ovarian follicle is covered by a thin single layer of epithelium. The gonad is covered by a thin *tunica albuginea*. In the ovarian follicles of the vitellogenic female, oogonia and oocytes may coexist in the previtellogenic phase as well as oocytes undergoing vitellogenesis. As far as the characteristics of the different stages of oocyte development are concerned, the following A, B, and C stages correspond to the previtellogenesis phase, and D, E and F stages to the vitellogenic phase. (See table 1 for details of mean diameters and nucleo-plasmatic ratio of oocytes at the different stages.)

Table 1. Measurements of oocytes at the different stages of development. M.D.: Mean diameter; NPR: Nucleo-plasmatic ratio; SE: Standard error.

Stages	M.D. (μm)	SE	Number	NPR
Oogonia	22.7	1.46	9	0.260
A	59.0	3.70	21	0.108
B	105.4	3.94	18	0.106
C	150.2	3.61	18	0.080
D	166.0	9.73	12	0.080
E	281.0	8.58	17	0.030
F	422.8	6.07	17	0.007


B
C
E
LD
A
40 µm
CA
D
YG
LD
B
20 µm
LD
F
N
D
20 µm
10 µm
YG
D
FC
ZR
LD
CA
YG
E
C
E
FC
ZR
ZR
E
F
10 µm

The oogonia can be recognised by the large, lightly stained nucleus and the fibroreticular cytoplasm, stained deeply with basic fuchsin. As many as six nucleoli (per 3□μm section) are observed, among which one or two are usually larger than the others (Fig.□1).

In stage A, a change of the stain affinities in the cytoplasm is observed: there is a gradual loss of basic fuchsin stain affinity and it begins to stain with methylene blue. The nucleus maintains its volume at this stage, and a clear increase in the nucleolus number is not observed. There is, however, one group nucleolus with a larger diameter than the others. Moreover, an initial migratory movement of nucleolus towards the nuclear envelope is observed at this stage.

All the cytoplasm in stage B is now stained deeply with toluidine blue (Fig.□1A). A small increase in the nucleolar number (as many as ten per 3□μm section) is observed, all of them located peripherally in the vicinity of the nuclear envelope. The presence of very variable nucleolar diameters indicates that nucleolar excision has not yet finished at this stage.

At the stage C, cytoplasm loses affinity for toluidine blue and is much paler in appearance (Fig.□1A). The nucleolar number stabilizes around 22 (3□μm section), and all of them are of similar diameter and located peripherally.

A series of events takes place during the vitellogenesis phase, including the formation of the yolk vesicles and yolk globules, and the progressive volumetric increase of the oocytes, considerably greater than during the previtellogenic phase.

At stage D (cortical alveoli stage), yolk vesicles (or cortical alveoli) line the cortical part of the ooplasm; they are organized in two or three layers, and are stained deeply by toluidine blue (Fig.□1B, C). Lipid droplets, bigger and less numerous than the cortical alveoli at this stage, appear in a more central position, and without a clear stain affinity. At the same time, some groups of small, but numerous, granules become visible in the vicinity of cortical alveoli, strongly stained by basic fuchsin. Nucleoli maintain their perinuclear position.

At stage E (yolk granules stage), the cellular volume of oocytes increases suddenly (Fig.□1A, C). Cortical alveoli remain in the same position, whereas lipid droplets, much more numerous, increasingly fill the entire ooplasm. At this stage the nucleus stains deeply and shows a more irregular outline. The yolk granules are now scattered throughout the ooplasm and have increased in size, and stain deeply with basic fuchsin.

Stage F (advanced yolked) oocytes are the last ones that are observed in the samples (Fig.□1D, E). Lipid droplets start to fuse with each other, initially near the nuclear envelope, extending subsequently to the rest of the ooplasm. Cortical alveoli are still differentiated in the peripheral zone. At this stage, the vitelline envelope, or *zona radiata*, has developed to its maximum thickness (10□μm) and the follicle cells are clearly differentiated.

No ripe egg stage has been observed, as neither have pre-ovulatory *corpora atretica* or post-ovulatory *corpora lutea*.

Fig.□1.□□Oocyte stages of development. CA: cortical alveoli; FC: follicle cells; LD: lipid droplets; N: nucleus; ZR: *zona radiata*; YG: yolk granules. **A**: Stages B, C and E (toluidine blue), scale bar□=□40□μm; **B**: Stage D or cortical alveoli stage (toluidine blue), scale bar□=□20□μm; **C**: stages D and E, detail of the ooplasm peripheral zone (methylene blue-basic fuchsin), scale bar□=□10□μm; **D**: Stage F, lipid droplets start to fuse with each other (toluidine blue), scale bar□=□20□μm; **E**: Stages E and F, detail of the oocyte envelope (methylene blue-basic fuchsin), scale bar□=□10□μm.

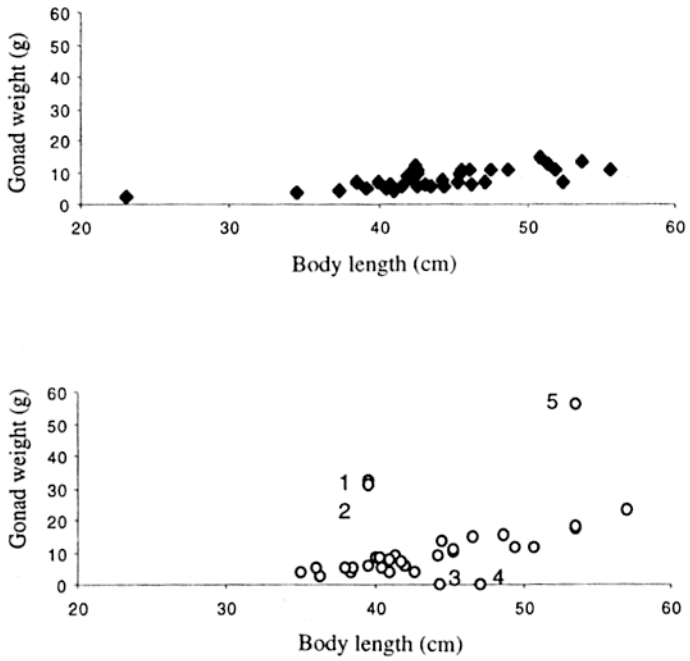


Fig. 2. - Relationship between gonad weight (g) and body length (mm), in July and August. Points 1, 2 and 5 correspond to absolutely full ovaries; points 3 and 4 correspond to spent ovaries; all the other points correspond to ovaries that had already started to spawn.

Table II. - Monthly distribution of specimens of *O. rufus* in the sample (1985-1987). GSI: Mean gonadosomatic index; SR: Sex-ratio.

Month	Males	GSI	Females	GSI	SR
January	8	0.199	67	2.679	0.12
February	14	0.280	82	3.817	0.17
March	45	0.351	218	4.236	0.20
April	7	0.396	49	5.542	0.14
May	6	0.807	21	6.441	0.28
June	-	-	-	-	-
July	0	-	36	15.652	0.00
August	5	2.898	33	20.754	0.15
September	1	1.780	21	1.440	0.05
October	0	-	41	1.358	0.00
November	1	0.420	12	1.147	0.08
December	3	0.150	11	1.770	0.27
Total	88		501		0.15

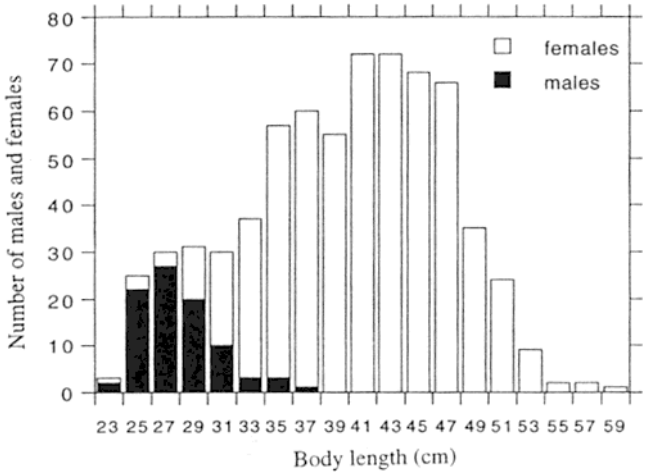


Fig. 3. Male and female size distribution in the sample.

Gonadosomatic index and sex ratio

Gonadosomatic indexes for males show highly significant differences throughout the year (χ^2 = 39.35, df = 37, p < 0.0001), with a tendency to increase in May, achieving maximum values in August, and with a clear decrease in September (Table II).

The monthly variations of the GSI for females also show highly significant differences (χ^2 = 53.57, df = 30, p < 0.0001), with a tendency to increase from January to a maximum in August, with a sudden decrease in September.

When the evolution in ovary weight is analysed in July, a regular increase in all sizes is observed, but in August (Fig. 4) some points (1, 2, 3, 4, and 5) are totally differentiated from the major group. Points 1, 2, and 5 correspond to very heavy ovaries as compared to the others, while points 3 and 4 are spent ovaries. The rest of the points are in the middle, which means that a part of the ovulated clutch has already been spawned.

In relation to the sex ratio, females predominate; 88 of 679 individuals were males, so the SR is generally 0.15 (χ^2 = 296.95, df = 1, p < 0.0001). In a monthly analysis of the SR, very similar results were found, as can be seen in table II.

On the other hand, if the sex ratio is analysed for different sizes (Fig. 3), the number of males is seen to be superior in small sizes whereas males disappear in larger sizes. The smaller individuals in the sample (a female of 230 mm TL and a male 233 mm TL) were already mature so it was impossible to determine the size of the first sexual maturation. The largest male observed in the sample reached 359 mm. However, the possibility of being a protandrous hermaphrodite was disregarded after examination of the gonads.

Oocyte size-frequency distribution, potential annual fecundity and relative fecundity

In August, advancing sexual maturity in females is accompanied by bulky abdomen and is detectable when the abdomen is pressed, easyly releasing sexual products in males and females. In fact, this clearly places spawning in August, so that pre-spawning July samples are the most useful for estimating fecundity.

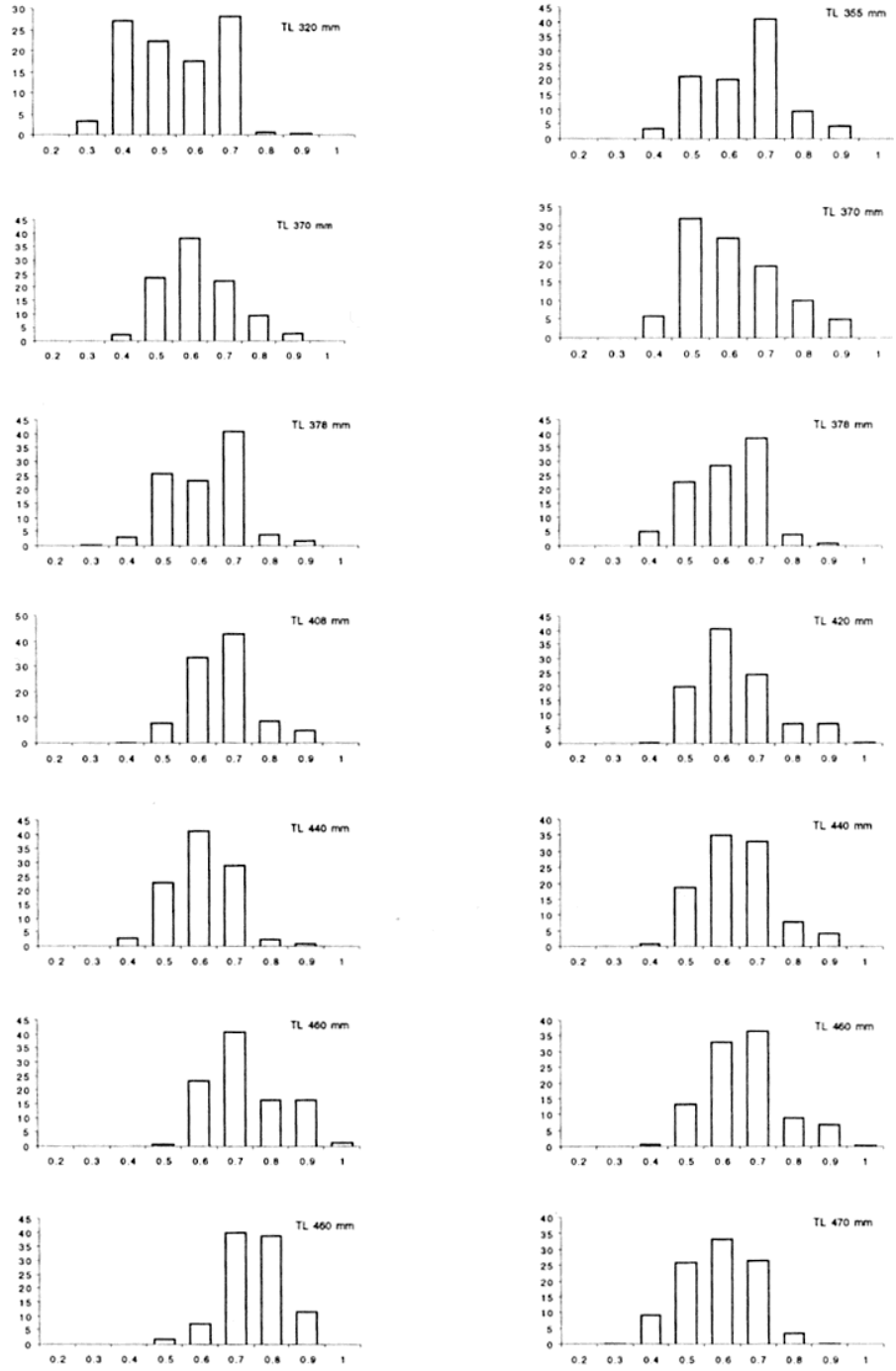


Fig. 4. Oocytes size-frequency distribution of July ovaries.

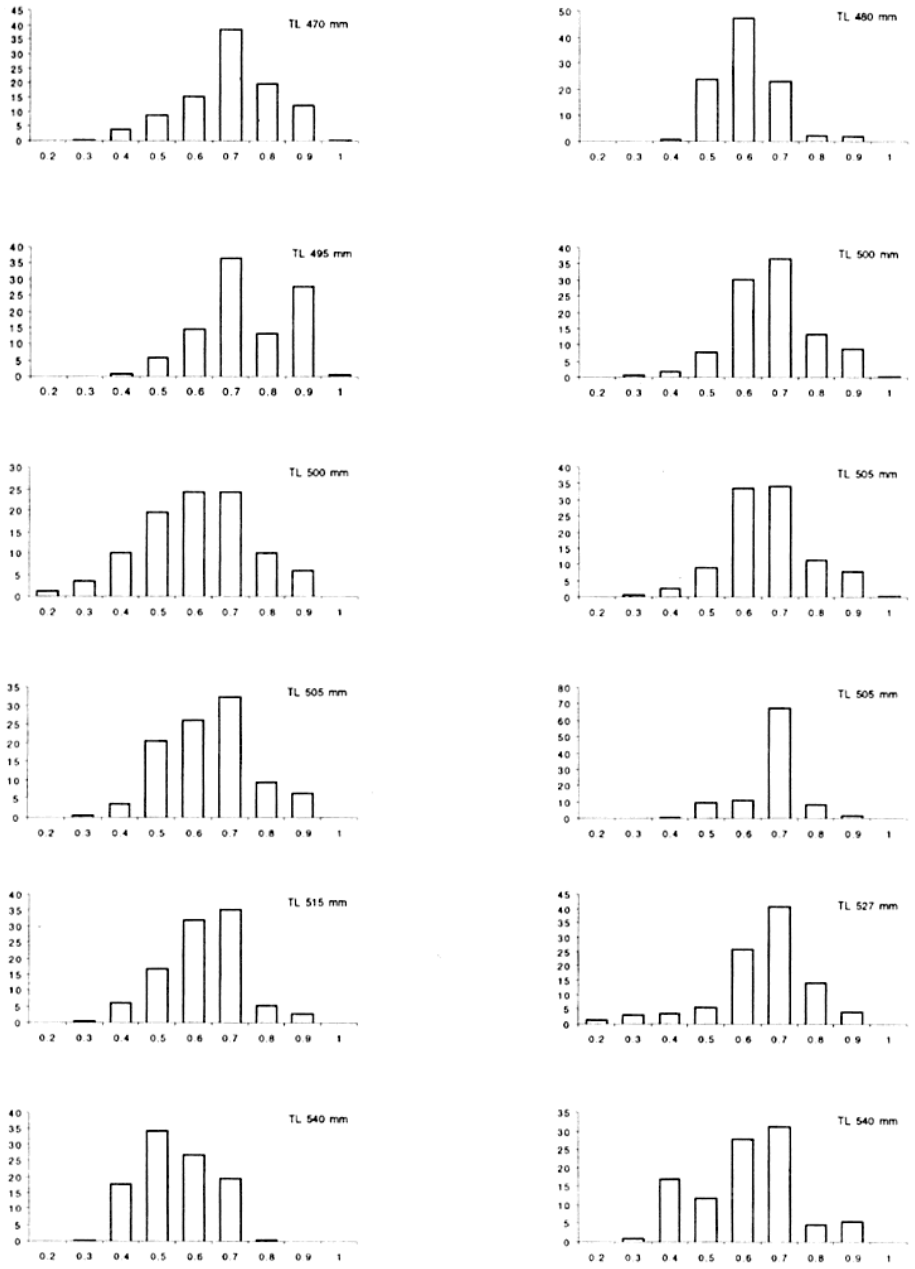


Fig. 4. Oocytes size-frequency distribution of July ovaries (continued).

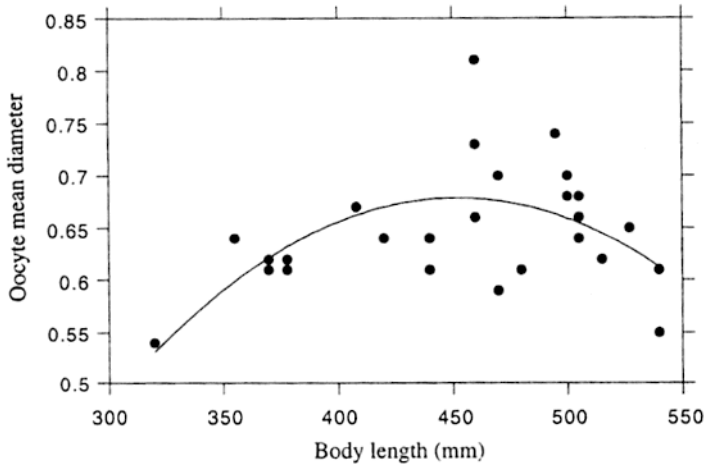


Fig. 5. Relationship between oocytes mean diameter and specimens size (body length in mm). The line shows the general tendency in the mean diameters.

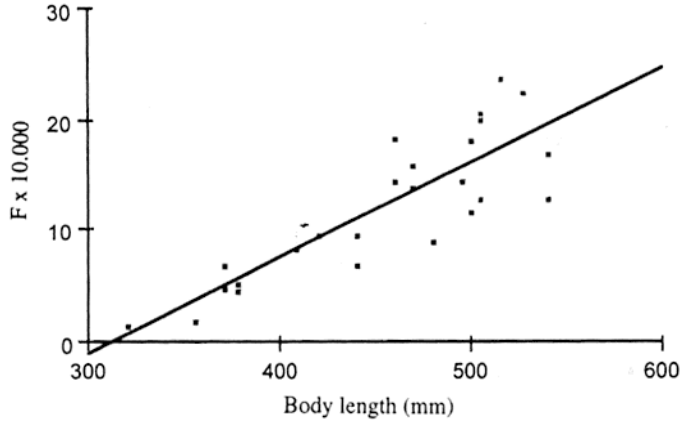


Fig. 6. Relationship between potential annual fecundity (F) and body length (mm), determined by linear regression analysis: log F = -3.07999 + 4.27167 log TL (r^2 = 0.86912).

As a point of reference for the recruitment, using histological data it was decided to use oocytes with a minimum diameter of 0.2 mm, since this size corresponds to the vitellogenic oocytes observed microscopically.

In the histological sections of gonads collected in February all stages of oocytes development are observed. However, in July ovaries, a large mass of vitellogenic oocytes is found in a reduced range of diameters (0.5-0.7 mm), as can be seen in the oocyte size frequency distribution (Fig. 4). This means the large group, or clutch, is very homogeneous. Among them, a small number of previtellogenic oocytes are observed microscopically. Ovulated eggs from gonads in August have a mean diameter of 1 mm. The mean diameter of oocytes increases with size, except in the largest females, where a gentle decrease is observed (Fig. 5).

In the case of *Ophichthus rufus*, the total potential annual fecundity 'F' was considered as the number of ripe oocytes present in the ovary immediately before spawning, according to Bagenal (1973), as there is a single spawning batch per year.

Potential annual fecundity estimates ranged from 1426 oocytes in a fish of 320 mm TL, to 23,605 oocytes in a fish of 515 mm TL.

The relationships of fecundity (F) to body length and body eviscerated weight (We) were examined by correlation analysis. Logarithmic transformation gave the rectilinear relations:

$\log F = -3.07999 + 4.27167 \log TL \quad (r^2 = 0.86912)$

$\log F = 1.95094 + 1.26378 \log We \quad (r^2 = 0.87996)$

There is a good correlation in both cases. Results indicate that fecundity increases with weight at a very similar rate, but with respect to body length, fecundity increases regularly and at a higher rate (Fig. 6).

Relative fecundity (RF) varies between 103 and 489, with a mean of 264.11 ± 17.65. There is not a good relationship between relative fecundity and body length ($r^2 = 0.39$); the best correlation is found when RF is compared with gonad weight:

$\log RF = 3.36386 + 0.81672 \log Wg \quad (r^2 = 0.94951)$

In this case, there is a gradually decreasing allometry probably related to the progressive increase in volume and weight of the individual oocytes.

DISCUSSION

Blüm (1986) observed that segmented ovaries in the shape of lamellae occur in primitive teleosts, and the absence of oviducts is not rare in Anguilliformes. Actually, the loss of gonoducts in males and females was described in family Anguillidae (Goodrich, 1930), and we have observed the same pattern in *Gnathophis mystax* (Congridae) (Casadevall, 1991).

Considering the three major categories of oviparity, namely ovuliparity, zygoparity and embrioparity (Blackburn *et al.*, 1985; Wourms *et al.*, 1988), *O. rufus* is an ovuliparous species, like all fishes that undergo external fertilization.

According to Spartà (1937), *Ophichthus rufus* has freely floating pelagic eggs due to the presence of between 6 and 22 lipid droplets. Many more lipid droplets were observed in histological sections, but they fuse together in more advanced stages of development.

During the spawning season the ovary contained a large clutch of maturing oocytes, homogeneous in diameter, and a gap develops between these and the less advanced oocytes. In addition, early vitellogenic oocytes (0.2 to 0.3 mm in diameter) are nearly absent.

For this reason, according to the classification of Marza (1938) and of Wallace and Selman (1981), the ovary of *O. rufus* is a 'group synchronous ovary'. Holden and Raitt (1974) point out that fish with group synchronous development shed the whole clutch of developed oocytes over a short period of a week or so.

On the basis of our observations, both ovulation and spawning take place in August. Following De Vlaming (1983), the term fractional spawning must be used for species that spawn part of an ovulated clutch. The results obtained on the evolution of ovary weight in August demonstrated that *O. rufus* is a fractional spawner, because at this time absolutely full ovaries, spent ovaries and a large group in intermediate stages were

observed simultaneously.

The gonadosomatic index is useful in this case to validate anatomical and histological observations. Indeed, the GSI shows a gentle and progressive increase from December to August, only to fall drastically in September. Then, *O. rufus* maintains a resting condition throughout September, October and November, and starts a new maturation process from December onwards.

A previous study on the feeding habits of the same population sample (Casadevall *et al.*, 1994), showed a maximum feeding activity of *O. rufus* in summer and autumm, coinciding with the spawning and post-spawning periods. That contrasts with the observations of Cau and Manconi (1984) for *Conger conger*, and of Cau (1981) for *Nemichthys scolopaceus*, showing that these species ceased to feed when they were about to deposit their gametic products.

According to Fisher and Pearcy (1983), high gonad index values for ripe fish may indicate that maturation of more than one complement of oocytes is unlikely, which seems to be the case for *O. rufus*.

With reference to the distribution of males and females in the sample, the general trend for *O. rufus* is, for males, to dominate in the first size-class, after which a dramatic inversion in the intermediate sizes occurs, and then they disappear at larger sizes, where only females occur. According to Kartas and Quignard (1984), this may be caused by several factors: (a) males maturing earlier; (b) a tendency for slow growth in males; (c) a higher mortality rate in males. Another possible reason could be a spatial displacement of sexes, which was already observed for *Conger conger* (Cau and Manconi, 1983).

The reason for the apparent abundance of larger fish (i.e., females) in the total sample, probably lies in the selectivity of the fishing net size. The spatial displacement of sexes is unlikely; Matallanas (1979) prospected the same fishing area from 0 to 700 m, and found that *O. rufus* only occurred between 90 and 130 m. In this study, neither an earlier maturity in males, which is rather common in teleosts, seems to be the more probable cause.

With regards to fecundity, estimations taken early in the spawning period may be biased if only the more mature yolked oocytes are considered, because all oocytes may not have been recruited into the advanced stock of yolked oocytes. In *O. rufus* there is no doubt when looking at the shape of the oocyte size-frequency distribution, that there is an advanced stock of yolked oocytes (0.5-0.7 mm in diameter) destined to be spawned. The gap existing in diameters of 0.2 and 0.3 mm oocytes makes it clear that recruitment of oocytes into this advanced stock had finished for the season in these pre-spawning females.

Ophichthus rufus seems to be a moderately fecund species, when we consider that it has freely floating eggs, which are dispersed by the sea from the spawning ground. Kartas and Quignard (1984) observed that, in general, species with high population densities were less fecund than others. *Ophichthus rufus* is a rather common species in the area studied.

Regarding the relationships of fecundity to body length and body eviscerated weight, results show that the increase in length causes a much more pronounced increase in fecundity than changes in weight. In fact, *O. rufus* has a typical serpentiform body (long and narrow), whereby an increase in length does not imply a large increase in weight, there being an increase in space available for the elongation of the gonads, which are also very long structures.

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