PRELIMINARY RESULTS ON FECUNDITY OF ATLANTIC BONITO (*SARDA SARDA*) CAUGHT IN SOUTH WESTERN MEDITERRANEAN TRAP

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SUMMARY

The Atlantic bonito (Sarda sarda) is one of the most abundant small tuna species in the Mediterranean Sea. Although this species is commercially exploited off the Spanish coast by traditional fisheries the biological and reproductive information about this species is scarce. In order to improve our knowledge about the reproductive parameters, specifically, the fecundity of this population, gonads were collect along June (2003) in a Spanish Mediterranean trap (la Azohia, Murcia). The fish collected range from 410 to 460 mm in fork length. All these fishes were mature and their reproductive characteristics indicate a spawning area near to the trap location. Batch fecundity was estimated using uniquely the pre-spawning and spawning females without postovulatory follicles in their ovaries. The average fecundity was 79,432 oocytes by spawning batch. The relative batch fecundity, a parameter particularly useful to compare female of different size class, ranged from 111 to 44 oocytes g^{-1}, with an average value of 65 oocytes g^{-1}. Histological and stereological methods allow the obtaining of adequate estimates of fecundity in this species. These and other biological aspects (size distribution, size/weight relations, etc) will be discussed in this paper.

RÉSUMÉ

La bonite à dos rayé (Sarda sarda) est l'une des espèces thonières de petite dimension qui abondent le plus en Méditerranée. Bien que cette espèce soit commercialement exploitée au large du littoral espagnol par des pêcheries traditionnelles, il existe peu d'informations sur sa biologie et sa reproduction. Afin d'améliorer nos connaissances quant aux paramètres reproducteurs, notamment la fécondité de cette population, des gonades ont été prélevées au mois de juin 2003 dans une madrague espagnole en Méditerranée (la Azohia, Murcie). Les poissons recueillis mesuraient entre 410 mm et 460 mm de longueur à la fourche. Tous ces poissons étaient en âge de maturité et leurs caractéristiques reproductives indiquent une zone de frai située à proximité de la madrague. La fécondité par fraction de ponte a été estimée en utilisant uniquement les femelles en état de préponte et de ponte sans follicule post-ovulatoire dans leurs ovaires. La fécondité moyenne était de 79.432 ovocytes par fraction de ponte. La fécondité par fraction de ponte relative est un paramètre particulièrement utile pour comparer des femelles de différentes classes d'âge, s'inscrivant dans une fourchette de 111 à 44 ovocytes g^{-1}, avec une valeur moyenne de 65 ovocytes g^{-1}. Les méthodes histologiques et stéréologiques permettent d'obtenir des estimations adéquates de la fécondité de cette espèce. Ces aspects biologiques et d'autres (distribution par taille, rapports taille/poids, etc.) seront examinés dans le présent document.

RESUMEN

El bonito (Sarda sarda) es una de las especies de pequeños túnidos más abundante en el mar Mediterráneo. Aunque esta especie es explotada comercialmente en las aguas de la costa española por pesquerías tradicionales, la información reproductiva y biológica de esta especie es escasa. Con el fin de mejorar nuestro conocimiento de los parámetros reproductivos, concretamente la fecundidad de esta población, se recogieron gónadas durante el mes de junio (2003) en una almadraba española del Mediterráneo (La Azohía, Murcia). La talla de los peces recogidos osciló entre 410 y 460 mm de longitud a la horquilla. Todos estos peces habían alcanzado la madurez y sus características reproductivas indicaban la existencia de una zona de desove cercana a la almadraba. Se estimó la fecundidad por lotes utilizando únicamente hembras reproductoras y pre-reproductoras con folículos postovulatorios en sus ovarios. La

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fecundidad media era 79.432 oocitos por lote de puesta. La fecundidad relativa por lote, un parámetro especialmente útil a la hora de comparar hembras de diferentes clases de talla, oscilaba entre 111 y 44 oocitos g^{-1}, con un promedio de 65 oocitos g^{-1}. Los métodos histológicos y estereológicos permiten obtener estimaciones adecuadas de la fecundidad de esta especie. En este documento se discuten estos y otros aspectos biológicos (distribución por tallas, relaciones talla-peso, etc.).

KEYWORDS

Sarda sarda, Atlantic Bonito, Spawning, Fecundity, South Western Mediterranean

1. Introduction

The Atlantic Bonito (*Sarda sarda*) inhabit temperate and tropical areas of both hemispheres of Atlantic Ocean, Gulf of Mexico, the Black Sea and Mediterranean. It forms large mixed schools with other tuna species near the surface (Collete and Nauen 1983; Anon ICCAT 2003). The Atlantic bonito (*Sarda sarda*) is one of the most abundant small tuna species in the Mediterranean Sea where has been commercially exploited since ancient times (Demir 1963). In Spain, the species have been caught traditionally by seasonal coastal fisheries. Several fishing gears have been used to catch it: Traps and other minor passive gears, purse-seine and *curricán* (Rey *et al*. 1984). In spite of the importance to local economy of this species, little is currently known about the biology of this small tuna.

The first maturity size has been stated in 38 cm (FL) when the fish is one year old (Rey *et al*. 1984; Anon Iccat, 2003). Several authors have observed that the species spawn in the Mediterranean although its spawning areas are better known in the eastern part of the Mediterranean and Black Sea (Mayorova and Tkacheva 1959; Demir 1963). Very little is known about reproductive areas of Atlantic bonito in the Western Mediterranean, although have been noted a scarce number of larvae and eggs around the Balearic Islands and Algerian coast (Duclerc *et al*. 1974; Piccinetti *et al*. 1996; Ehrenbaum 1924 and Piccinetti and Piccinetti Manfrin 1994). The spawning period extends from May to July (Sanzo 1932; Rodriguez-Roda 1966). The species is a multiple spawner with asynchronous oocyte development that carried out 3 or 4 spawning batches by reproductive season (Majorova y Tkacheva 1959; Rey *et al*. 1984). The information available about the fecundity of this species is scarce and fragmentary. Most of these authors calculate the absolute fecundity using the more advance oocyte class in pre-spawning female (Postel 1955; Rey *et al*. 1983).

The aim of this paper is to describe and discuss the results obtained about reproductive characteristics and fecundity rates of the Atlantic bonito in the study area.

2. Material and methods

2.1 Specimen collection

A total of 183 specimens were measured and weighed during a scientific survey carried out during 2003 in the "La Azohía" trap (Murcia) in the southwestern Mediterranean. 40 specimens caught from 2 June 2003 to 10 June 2003 were dissected to obtain gonad, liver and muscular tissue for reproductive studies and hard structures for age determination like spines, vertebrae and otoliths. For each specimen fork length (FL) to the nearest milimeter, weight and sex were determined. In addition the gonad weight to the nearest g was obtained with the purpose of calculate the gonad-somatic index (Kume and Joseph 1969) indicating the levels of sexual maturity of the bonito specimens. Gonads were collected from each fish.

Each pair of gonads (ovaries, testis) were removed from the gut cavity and identified as male or female. Then a section of 2 cm width from the central part of only one single gonad was removed, preserved in 10 % buffered formalin and stored. A portion of each preserved section was washed in buffer solution, dehydrated in ethanol and n-butanol series and embedded in paraffin. The samples were sectioned at approximately 10 μm with a microtome, mounted on slides and stained with Mallory´s Trichrome Stain for a general assessment of the histology of the gonad. Finally, the slides were examined and photographed in a Leyca photo-microscope.

2.2 Staging and measurement of oocytes

Five well-established developmental stages of oocytes (perinucleolar, previtelogenic, partially yolked, totally yolked and hydrated oocytes) were defined histologically following several authors (Yamamoto, 1956, Forberg, 1982 & Hunter *et al*. 1992). Developmental stage and measurement of whole oocytes were determined from 7 females in different developmental stages. Oocyte sizes were obtained by measuring of two diameters of 100 oocytes of each stage using an analysing system of only those oocytes that had been sectioned through the nucleus. Finally, the histological analysis and oocyte diameter distribution in each female was used to assess the spawning sequence (West 1990).

2.3 Histologic classification

To estimate reproductive condition of Atlantic Bonito, two different histological classification systems were used: one for estimating the sexual maturity and the other for assessing the activity stage of mature females. Each ovary was histologically classified according to both systems (Hunter and Goldberg 1980; Hunter and Macewicz 1980, 1985a, b; Hunter, Macevicz and Sibert 1986).

2.3.1 Sexual maturity

It is considered that a female is a mature one when has the capability to reproduce in a determinate spawning season. The presence in the ovary of yolked oocytes, hydrated oocytes or postovulatory follicles is a histological sign of maturity. The immature females have not reached the sexual maturity and are unable to reproduce in a determinate season.

2.3.2 Sexual activity

2.3.2.1 Five different stages of activity have been taken into consideration in the Atlantic bonito female:

a) Inactive females: the histological analysis indicates that the ovary contains no yolked oocytes and no atresic structures.
b) Active females: females were classified as active when the ovary contained yolked oocytes and there was no atresia or only minor atresia can be found. Active females were further classified into other stages according to additional criteria.
c) Ripening females (Maturating): Those females showing signs of sexual maturity (Yolked oocytes) but not signs of imminent spawn or signs of past spawns batches.
d) Pre-spawning females (Ripe): Those females showing signs of an imminent spawning like hydrated or nuclear migration phase oocytes but not postovulatory follicles or extended atresia. High density of oocytes in the ovary can be seen.
e) Spawning females: Those female whose ovaries present postovulatory follicles or imminent spawning signs like hydrated or migratory-nucleus oocytes. The histological analysis shows signs of past spawning (postovulatory follicles) and enough vitellogenic oocytes to complete more spawning.
f) Post-spawning females: Those females showing signs of past spawning (postovulatory follicles) but have not enough vitellogenic oocytes to complete more spawning. Extended atresia in vitellogenic oocytes. Low oocytes density in the ovary.

The gonadosomatic index was calculated according to Kume and Joseph (1969)

2.4 Fecundity estimates

Once the maturity stage was identified, size, number and area occupied by each class of oocytes were measured in each female in order to estimate the batch and relative fecundity.

For estimation of the number of each oocyte type contained in the gonads, the stereological method of Weibel & Gómez (1962) was applied. This technique has been previously used for counting fish oocytes with considerable success (Emerson *et al*. 1990; Greer Walker *et al*. 1994; Coward & Bromage 1998. Medina *et al*. 2002).

From micrographs of histological sections, the numerical density (N_V), was calculated for each oocyte developmental stage according to the formula developed by Weibel & Gómez (1962) as further modified by Weibel *et al*. (1966).

$$N_v = K/\beta . N_a^{3/2}/V_i^{1/2}$$

N_v = Number of oocytes by volume unit.
N_a = Number of trans-sections by area unit.
V_i = Volume fraction occupied by each type of oocyte.
β = Shape coefficient
K = Size distribution of oocytes coefficient.

The used specimens, data on maturity and other biological characteristics of the sample can be found summarised in the **Table 1**.

3. Results

3.1 Maturity

3.1.1 Ripening females

Three females (16.6%) were classified like maturating. These females displayed ovary profile characterised by the presence of perinucleolar, previtellogenic and yolked oocytes in the ovary. No atretic follicles and post-ovulatoy follicles can be found in the ovary. 82% of ovary follicles were not yolked ones. No signs of imminent spawning activities can be found in the ovary.

3.1.2 Ripe females

Seven females (38.8%) were classified like totally mature. These females displayed ovary profile characterised by the presence of perinucleolar, previtellogenic, partially yolked and post-vitellogenic oocytes (nuclear migration or hydrated oocytes) in the ovary. Minor atretic follicles can be found in the ovary (0.9%) and no recent post-ovulatoy follicles were presented in the ovary. Six of these females were used to estimate the fecundity.

3.1.3 Spawning females

Seven spawning females (38.8%) were caught in the sample. These females showed some differential characteristics. All these females showed perinucleolar, previtellogenic, yolked oocytes, atretic structures and recent postovulatory follicles. But only some of these females presented hydrated oocytes. The presence of post-ovullatory follicles in all females indicates recent spawning.

3.1.4 Post-spawning females

Only one female was post-spawning one (5.5%). This female showed perinuclear and previtellogenic oocytes and large extended atresia of yolked and hydrated oocytes

3.2 Fecundity estimates

3.2.1 Shape coefficient

For each oocyte type, β was determined approximately as a function of the axial ratio (calculated previously from multiple sections of the samples) using the function provided by Weibel (1969) for cylinder (perinucleolar stage) or ellipsoidal particles. The estimated values of β were summarised in the **Table 2**.

3.2.2 Size distribution coefficient (K)

K is defined as:

$$K=(M3/M1)^{3/2}$$

Where M1 and M3 are the first and third moment of the size distribution (Weibel 1969; Williams 1977). *K* values can be found summarised in the **Table 3**.

3.2.3 Number of transsections by area unit

N_a is the number of transsections of oocytes per unit of section area. This parameter was calculated as the number of oocyte profiles lying within the stereological test system divided by the test area (transsections that are cut by the right and lower margins are counted, whereas those cut by the left and upper margins are rejected). The results obtained are showed in the **Table 4**.

3.2.4 Volume density

V_i is the fraction of volume occupied by oocytes of a given category (volume density). The volume density was estimated counting the number of points (cross-points of the lines) that overlaid the profiles of the oocyte type considered and dividing by the total number of points of the lattice (Weibel & Gómez 1962; Weibel *et al*. 1966; Weibel 1969; Williams 1977). The results obtained have been summarised in the **Table 5**.

3.2.5 Fecundity

The total number of oocytes of each category contained in the gonad of every individual was then obtained multiplying V_i by the entire ovarian volume. The ovary volumes are showed in the **Table 6** and the fecundity estimates in the **Table 7**.

Batch fecundity was estimated using the number of hydrated oocytes of those specimens showing hydrated oocytes but not postovulatory follicles. The female BON03-30 does not show hydrated oocytes so it cannot be used in the batch fecundity estimates. BON03-40 showed some postovullatory follicles. This fact indicates that the spawning was initiated when the female was caught. The batch fecundity estimates were significantly lower in this female (3550 oocytes) than those obtained in the other ones (from 56.440 to 136.702). In order to avoid bias in the average batch fecundity this female was excluded of the calculation. Finally the females used for fecundity estimates were: BON 03-21, BON03-25 BON 03-27 and BON 03-30. The average batch fecundity was 79.432 oocytes.

From this estimate, the number of oocytes g^{-1} of body mass (Relative fecundity) was calculated. This parameter is particularly useful for comparison among related females that have a markedly different size. The results have been shown in the **Table 8**. The relative fecundity ranges from 44 to 111 oocytes x g^{-1}. The average value was 65 oocytes x g^{-1}.

4. Discussion

The reproductive activity pattern in the studied area was deduced from the histological analysis of Atlantic Bonito ovaries. The catches of pre-spawning, spawning and recent post-spawning mature female in the study area during June highly support the hypothesis of a reproductive area in the South-western Spanish Mediterranean. The most important spawning areas in the Mediterranean had been located in the eastern Mediterranean (Mayorova and Tkacheva 1959; Yoshida 1980).

The results of this study also indicate that Bonito is a multiple spawner with asynchronous oocyte development. We have shown that there are no gaps between distributions of dominating stages of oocyte development (Macías *et al.* 2004). We also found several modal values in yolked oocytes. These characteristics suggest that Atlantic Bonito is a species with indeterminate fecundity. Indeterminate fecundity pattern depends on estimates of batch fecundity and spawning frequency to determinate potential annual fecundity (Hunter and Macewitz 1985 a; Hunter *et al*. 1992).

Previous works had used volumetric methods to estimate the potential fecundity (Postel 1955; Rey *et al*. 1984). These authors assume a determinate minimum oocyte size to calculate the annual fecundity, as Bonito shows an indeterminate pattern, this assumption is inadequate. The use of histological and stereological approach avoids the bias due to the inclusion of atretic and yolked follicles in the batch of hydrated oocytes.

The estimates of annual fecundity for this species in the western Mediterranean obtained by Rey *et al*. (1984) ranged from 220.000 to 1.500.000 oocytes. Using the same criteria, our results (obtained by the sum of hydrated, yolked and atretic follicles) ranged from 304.000 to 1.150.000. These results agree with those of Rey *et al* (1984). The average batch fecundity obtained in this study was 79.432 oocytes. If we assume 3 or 4 spawning batches, according to Mayorova and Tkacheva (1959), the annual fecundity of this species could range from

170.000 to 550.000 oocytes, much lower than current estimates. These differences suggest the possibility of more spawning batches.

The relative batch fecundity obtained (65 oocytes x g^{-1}) result surprisingly higher than the relative total fecundity obtained by Rey *et al*. (1984) (24-62 oocytes x g^{-1}). If we consider that the specimens used in those studies were larger than those used in the present paper (specimens used by Rey *et al*. (1984) ranged from 47 to 70 cm in FL and those used in the present study ranged from 40 to 44 cm) this results are even more unexpected and suggest that fecundity could remains stable with the age/size. Further studies should be done in order to clarify this question.

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Table 1. Biological characteristics of Atlantic Bonito used in this study.

CODE	*Length(cm)*	*Weight (g)*	*Dressed Weight*	*Gonad W*	*GSI*	*Sexo*	*Maturity*
BON1	44,2	1154	1060	50,5	5,85	F	Ripening
BON10	43,5	1112	1014	35,5	4,31	M	mature
BON11	43,2	1082	1008	24	2,98	M	mature
BON12	41,3	970	896	28	3,97	M	mature
BON13	41,8	1054	976	26	3,56	F	Spawning
BON14	42,8	1182	1066	37,5	4,78	M	mature
BON15	42,4	1044	964	40	5,25	M	mature
BON16	43,4	1146	1052	43	5,26	M	mature
BON17	42	1078	998	38	5,13	M	mature
BON18	43,5	1006	934	22,5	2,73	M	mature
BON19	44,2	1214	1134	39,5	4,57	M	mature
BON2	41,5	1008	928	31	4,34	M	mature
BON20	42,2	1076	988	41	5,46	M	mature
BON21	43	1156	1028	71	8,93	F	Ripe
BON22	43	1028	940	42	5,28	F	Ripe
BON23	44	1102	1036	20,5	2,41	M	mature
BON24	42,5	1122	1024	43,5	5,67	F	Spawning
BON25	46	1280	1150	63	6,47	F	Ripe
BON26	42,5	1182	1118	12	1,56	M	mature
BON27	44	1186	1050	81	9,51	F	Ripe
BON28	41,5	1066	970	50,5	7,07	M	mature
BON29	44	1164	1058	47	5,52	F	Spawning
BON3	42,2	1036	962	24	3,19	F	Spawning
BON30	45	1146	1038	49	5,38	F	Ripe
BON31	44,5	1228	1078	89	10,10	F	Ripe
BON32	44	1102	1000	46	5,40	F	Ripening
BON33	47	1384	1264	68,5	6,60	M	mature
BON34	43,5	1160	1016	60	7,29	F	Spawning
BON35	43	1132	1044	40	5,03	M	mature
BON36	43	1096	1008	30,5	3,84	F	Spawning
BON37	44,5	1118	1050	11	1,25	F	Post-spawning
BON38	42,5	1050	960	21,5	2,80	M	mature
BON39	42,5	1048	974	25	3,26	M	mature
BON4	41,6	968	882	30	4,17	M	mature
BON40	43,5	1098	976	69	8,38	F	Ripe
BON5	42,2	1070	972	47	6,25	M	mature
BON6	41	946	872	33	4,79	M	mature
BON7	45	1240	1128	52	5,71	F	Ripening
BON8	41,5	1052	972	26	3,64	F	Spawning
BON9	43	1110	1018	36,5	4,59	M	mature

Table 2. Estimated β coefficient for each oocyte type.

	Perinucleolar	*Previtellogenic*	*Partially yolked*	*Totally yolked*	*Hydrated*	*Atretic*
E	0,800	0,857	0,861	0,868	0,879	0,719
b cylinder	1,70	1,66	1,66	1,66	1,65	1,75
b elipsoide	1,45	1,42	1,42	1,42	1,40	1,54

Table 3. K coefficient results for each Atlantic Bonito female studied.

BON 21	*Perinucleolar*	*Previtellogenic*	*Partially yolked*	*Totally yolked*	*Hydrated*
M1	96,6	202,5	297,0	466,5	732,4
M3	101,7	212,6	301,5	472,8	737,6
K	1,08	1,08	1,02	1,02	1,01
M3/M1	1,1	1,0	1,0	1,0	1,0
BON 25	*Perinucleolar*	*Previtellogenic*	*Partially yolked*	*Totally yolked*	*Hydrated*
M1	62,88	175,2	269,4	479,7	716,1
M3	67,0	183,3	271,0	489,3	734,1
K	1,10	1,07	1,01	1,03	1,04
M3/M1	1,1	1,0	1,0	1,0	1,0
BON 27	*Perinucleolar*	*Previtellogenic*	*Partially yolked*	*Totally yolked*	*Hydrated*
M1	65,36	151,5	261,1	476,7	626,6
M3	68,7	158,8	263,5	491,7	638,6
K	1,08	1,07	1,01	1,05	1,03
M3/M1	1,1	1,0	1,0	1,0	1,0
BON 30	*Perinucleolar*	*Previtellogenic*	*Partially yolked*	*Totally yolked*	
M1	55,5	182,9	332,5	537,8	
M3	60,4	204,0	337,6	546,9	
K	1,14	1,18	1,02	1,03	
M3/M1	1,1	1,1	1,0	1,0	
BON 31	*Perinucleolar*	*Previtellogenic*	*Partially yolked*	*Totally yolked*	*Hydrated*
M1	74,5	195,6	287,6	481,2	695,3
M3	79,0	201,0	289,4	494,4	707,3
K	1,09	1,04	1,01	1,04	1,03
M3/M1	1,1	1,0	1,0	1,0	1,0
BON 40	*Perinucleolar*	*Previtellogenic*	*Partially yolked*	*Totally yolked*	*Hydrated*
M1	63,48	167,8	274,9	472,5	575,7
M3	65,5	175,8	277,0	482,0	577,7
K	1,05	1,07	1,01	1,03	1,01
M3/M1	1,0	1,0	1,0	1,0	1,0

Table 4. Number of transections of oocytes per unit section area.

Nº Ovocitos/mm2	*BON 03-21*	*BON 03-25*	*BON 03-27*	*BON 03-30*	*BON 03-31*	*BON 03-40*
Perinucleolar	6,5	8,6	8,7	9,3	6,1	7,1
Previtellogenic	1,0	1,7	1,3	1,0	0,9	1,3
Partially yolked	0,3	1,0	0,7	0,2	0,6	0,6
Totally yolked	1,6	1,8	2,9	3,0	1,6	3,1
Hydrated	0,7	0,8	0,6	0,0	1,2	0,046
Atretic	0,0	0,1	0,0	0,1	0,0	0,1
Postovulatory	0,0	0,1	0,0	0,1	0,0	0,0

Table 5. Volume density of each type of oocytes of the different female studied.

	BON 03-21	*BON 03-25*	*BON 03-27*	*BON 03-30*	*BON 03-31*	*BON 03-40*
Perinucleolar	0,065	0,057	0,071	0,096	0,062	0,057
Previtellogenic	0,045	0,063	0,046	0,045	0,030	0,045
Partially yolked	0,026	0,088	0,058	0,021	0,039	0,045
Totally yolked	0,276	0,318	0,505	0,526	0,277	0,571
Hydrated	0,282	0,328	0,167	0,000	0,388	0,002
Atretic	0,003	0,006	0,004	0,004	0,025	0,005
Postovulatory	0,000	0,004	0,000	0,002	0,000	0,000

Table 6. Gonads volume of Atlantic Bonito caught along 2003.

	Gonad Volume (mm3)	*Gonad Weight (gr)*	*Gonad density (gr/cm3)*
BON 03-21	70937	71	1,00
BON 03-25	61511	63	0,98
BON 03-27	80667	81	1,00
BON 03-30	47749	49	0,97
BON 03-31	88046	89	0,99
BON 03-40	77798	69	1,13

Table 7. Number of oocytes by volume unit.

BON 03-21	*K*	*β*	*K/β*	*Na*	*Na 3/2*	*Vi*	*Vi1/2*	*Nv*
Perinucleolar	1,08	1,7	0,64	6,5	16,6	0,065	0,25	41,29
Previtellogenic	1,08	1,42	0,76	1	1,0	0,045	0,21	3,59
Partially yolked	1,02	1,42	0,72	0,3	0,2	0,026	0,16	0,73
Totally yolked	1,02	1,42	0,72	1,6	2,0	0,276	0,53	2,77
Hydrated	1,01	1,4	0,72	0,7	0,6	0,282	0,53	0,80
Atretic		1,54	0,00	0	0,0	0,003	0,05	0,00
BON 03-25	*K*	*β*	*K/β*	*Na*	*Na 3/2*	*Vi*	*Vi1/2*	*Nv*
Perinucleolar	1,1	1,7	0,65	8,6	25,2	0,057	0,24	68,35
Previtellogenic	1,07	1,42	0,75	1,7	2,2	0,063	0,25	6,65
Partially yolked	1,01	1,42	0,71	1	1,0	0,088	0,30	2,40
Totally yolked	1,03	1,42	0,73	1,8	2,4	0,318	0,56	3,11
Hydrated	1,04	1,4	0,74	0,8	0,7	0,328	0,57	0,93
Atretic		1,54	0,00	0,1	0,0	0,006	0,08	0,00
BON 03-27	*K*	*β*	*K/β*	*Na*	*Na 3/2*	*Vi*	*Vi1/2*	*Nv*
Perinucleolar	1,08	1,7	0,64	8,7	25,7	0,071	0,27	61,18
Previtellogenic	1,07	1,42	0,75	1,3	1,5	0,046	0,21	5,21
Partially yolked	1,01	1,42	0,71	0,7	0,6	0,058	0,24	1,73
Totally yolked	1,05	1,42	0,74	2,9	4,9	0,505	0,71	5,14
Hydrated	1,03	1,4	0,74	0,6	0,5	0,167	0,41	0,84
Atretic		1,54	0,00	0	0,0	0,004	0,06	0,00
BON 03-30	*K*	*β*	*K/β*	*Na*	*Na 3/2*	*Vi*	*Vi1/2*	*Nv*
Perinucleolar	1,14	1,7	0,67	9,3	28,4	0,096	0,31	61,38
Previtellogenic	1,18	1,42	0,83	1	1,0	0,045	0,21	3,92
Partially yolked	1,02	1,42	0,72	0,2	0,1	0,021	0,14	0,44
Totally yolked	1,03	1,42	0,73	3	5,2	0,526	0,73	5,20
Hydrated	0	1,4	0,00	0	0,0	0	0,00	0,00
Atretic	0	1,54	0,00	0,1	0,0	0,004	0,06	0,00
BON 03-31	*K*	*β*	*K/β*	*Na*	*Na 3/2*	*Vi*	*Vi1/2*	*Nv*
Perinucleolar	1,09	1,7	0,64	6,1	15,1	0,062	0,25	38,80
Previtellogenic	1,04	1,42	0,73	0,9	0,9	0,03	0,17	3,61
Partially yolked	1,01	1,42	0,71	0,6	0,5	0,039	0,20	1,67
Totally yolked	1,04	1,42	0,73	1,6	2,0	0,277	0,53	2,82
Hydrated	1,03	1,4	0,74	1,2	1,3	0,388	0,62	1,55
Atretic		1,54	0,00	0	0,0	0,025	0,16	
BON 03-40	*K*	*β*	*K/β*	*Na*	*Na 3/2*	*Vi*	*Vi1/2*	*Nv*
Perinucleolar	1,05	1,7	0,62	7,1	18,9	0,057	0,24	48,94
Previtellogenic	1,07	1,42	0,75	1,3	1,5	0,045	0,21	5,27
Partially yolked	1,01	1,42	0,71	0,6	0,5	0,045	0,21	1,56
Totally yolked	1,03	1,42	0,73	3,1	5,5	0,571	0,76	5,24
Hydrated	1,01	1,4	0,72	0	0,02	0,002	0,04	0,05
Atretic		1,54	0,00	0,1	0,0	0,005	0,07	0,00

Table 8. Fecundity estimates.

BON 03	*BON 03-21*	*BON 03-25*	*BON 03-27*	*BON 03-30*	*BON 03-31*	*BON 03-40*	*Average fecundity*
Perinucleolar	2929281	4204427	4935378	2930954	3415750	3807667	3703909
Previtellogenic	254333	409309	420076	187047	317876	409612	333042
Partially yolked	51925	147484	139528	21170	147380	121233	104787
Totally yolked	196295	191072	414523	248143	247968	407607	284268
Hydrated	56440	57090	67495	0	136702	3550	79432

Relative fecundity (Nº oocytes/gr)							*Average RF*
Perinucleolar	2534	3285	4161	2558	2782	3468	3131
Previtellogenic	220	320	354	163	259	373	282
Partially yolked	45	115	118	19	120	110	88
Totally yolked	170	149	350	217	202	371	243
Hydrated	49	45	57	0	111	3	65