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Some reproductive patterns of the sardine, *Sardina pilchardus* (Walb, 1792), in Boka Kotorska Bay (Montenegro, southern Adriatic Sea)

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*The annual alteration of gonad morphology in sardine (*Sardina pilchardus*, Walbaum 1792) caught in the period from November 2006 to October 2007 using beach seines in the region of Boka Kotorska Bay (Montenegro, southern Adriatic) was studied. Samples were taken in the middle of each month. Total length of individuals ranged from 8.7 to 14.7 cm and weight ranged from 4.67 to 22.61 g. Their gonads were extracted and weighed, and a piece of gonad tissue was sampled for histological analysis. The length–weight relationship of all sardine specimens was described by the equation:* $W=0.0059\ L_T^{3.0891}$; $(r^2 = 0.963)$. *The lowest gonadosomatic index (GSI) values, below 1, were found in June, July and August, corresponding to the state of gonad rest. The GSI increased gradually from September and October and reached its highest value in February, at 5.22 for females and 6.58 for males. After February the GSI started to decrease throughout March (4.5), April (2.4) and May (2.4). In June all gonads had a GSI bellow 1. Although primary oocytes (stage I) were present during all months, their percentage increased from May and was the highest during the summer months (June-September 100%). Mature stage IV oocytes were recorded from November to April, with the highest percentage recorded during January (26.7%). An increase in the percentage of oocytes in the yolk vesicle (II) and yolk (III) stages occurred in October, remaining almost unchanged until May. During the summer months (May – September), only stage I spermatogonia were present in the testicles. In October, spermatocytes (stage II) started to appear, while from November the spermatids (stage III) appeared as well. Spermatozoa (stage IV) appeared in December and reached their maximum level in January and February.*

Key words: sardine, *Sardina pilchardus*, oogenesis, reproduction, length structure, length-weight relationship, condition factor, Boka Kotorska Bay

INTRODUCTION

The sardine, *Sardina pilchardus,* is one of the most abundant, and commercially most important, fish species in the Adriatic Sea (SINOVČIĆ, 1991, 2000, 2003). In recent decades there have been large inter-annual natural fluctuations of sardine biomass and catches in the entire Adriatic Sea (CINGOLANI *et al*., 2003). A severe collapse occurred in 1991 and continued until 1997. Since then, sardine stock assessment has indicated a recent slight increase in biomass throughout the entire Adriatic Sea (CINGOLANI *et al*., 2003). The collapse was attributed to poor recruitment during that period, but the reasons for recruitment failure still remain unknown.

Montenegrin industrial fishing of sardine and anchovy is still undeveloped. They are mainly caught through small-scale fishery, mostly using beach seines of small mesh size (5-6 mm) in Boka Kotorska Bay, targeting small individuals. As this practice is traditional, though neither organized nor based on modern fishery management practice, there is a need to regulate it. However, so far there has been no biological data on the sardine in Boka Kotorska Bay and Montenegrin Sea in spite of its necessity for fishery management.

The sardine, *Sardina pilchardus*, like most clupeids, is a batch spawner (ROY *et al*., 1989). The reproductive features of sardine in the Adriatic Sea have been studied using various methods. MUŽINIĆ (1954) studied macroscopic properties of gonad maturity stages during the year, while NEJEDLI *et al*. (2004) investigated oogenesis and the spawning period by histological methods. KARLOVAC (1964) and REGNER *et al*. (1981, 1983) investigated the duration of the sardine spawning season by means of egg and larval surveys whereas SINOVČIĆ (1983, 1983–84) investigated sardine fecundity. These investigations showed that the sardine in the Adriatic spawns mainly from October to April.

The study of the gonad cycle is of crucial interest for determining the time and duration of egg maturation. This was probably the reason why the majority of researchers studying fish reproduction have focused on females and why little attention has been given to the reproductive cycle of males. This paper is the first one to report on the results of histological investigations of spermatogenesis in sardines from the Adriatic Sea and histological investigations of oogenesis in sardines from Boka Kotorska Bay and the eastern side of the South Adriatic.

MATERIAL AND METHODS

Sardines were collected monthly by beach seine in Boka Kotorska Bay (Fig. 1). Boka Kotorska Bay is a closed marine bay with many freshwater springs and runoffs, and is prone to rather large temperature and salinity variations. It is one of the most productive areas of the Montenegrin coast and it seems to be a nursery ground for sardine and other small pelagic fish species.

The beach seine samples were collected monthly from November 2006 to October 2007. Total length (LT) of 1257 individuals was measured to the nearest 0.1 cm by digital calliper and weighed (W) to the nearest 0.01 g by digital balance. After that, 382 individuals were dissected and the gonads from both sexes were extracted (159 males and 223 females).

The length–weight relationship was determined according to the logarithmic form of the original exponential equation (RICKER, 1975):

$$\log W = \log a + b \log L_T,$$

where a is the proportionality constant, b the allometry coefficient, W is fish weight in grams, and L_T is total length in centimetres. The allometric condition index was determined according to the formula: $Ka = W/aLt^b$ (LE CREN, 1951) where a and b are the coefficient and power of the length–weight relationship, respectively. Only fish less than 10.5 cm in length were used in the analysis, for the purpose of avoiding a length/size bias. Sex ratio (R) was calculated using the expression R= F/(F+M), where F is the number of females and M that of males. The gonadosomatic index (GSI) was determined on the basis of their body mass and their ovary mass by applying the formula: GSI = 100 X G/W, in which G is gonad weight and W is body weight.

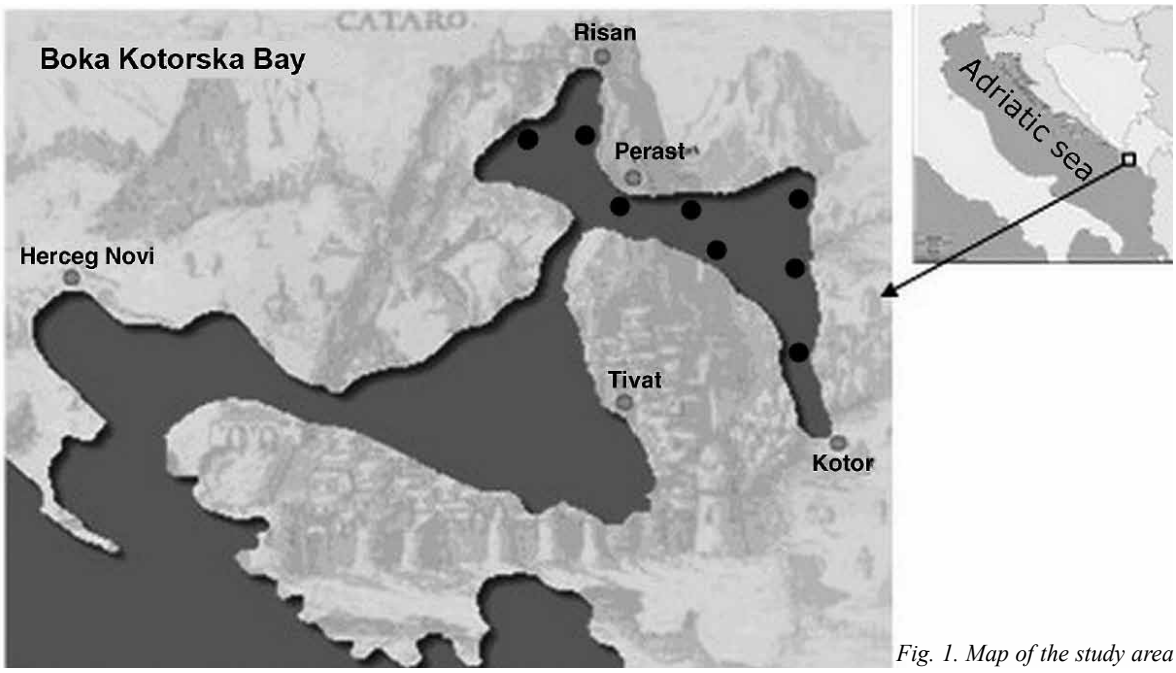

Fig. 1. Map of the study area

Gonads were weighed and preserved in 4% formaldehyde solution. Subsequently, a piece of tissue from each of the anterior, median and posterior parts of the gonad was subjected to histological analysis. Tissue samples were dehydrated, clarified in xylol, and embedded in paraffin. Sections (4–6 μm) were cut and stained with Mayer's hematoxylin and eosin Y (CLARK, 1981). The oocytes were counted and measured under a binocular microscope.

In the histological slices the oocytes were divided into four groups according to their morphologic characteristics, their frequency and mutual relationship being presented graphically. The stages of oogenesis were described according to ZIMMERMANN (1997). These stages are: Stage I – Peri-nucleolus stage (Primary oocytes), Stage II - Yolk vesicle stage, Stage III - Yolk stage, Stage IV - Mature stage (Migratory nucleus). The process of spermatogenesis is roughly divided into four phases as follows (SANTOS *et al*., 2006): Stage I - Spermatogonia, Stage II – Spermatocytes, Stage III – Spermatids, Stage IV – Spermatozoa.

RESULTS

Sample structure

The total length of the sardines ranged from 8.7 cm to 14.7 cm, with a mean of 11.3 ± 1.32 cm (SE 0.06) and W ranged from 4.67 to 23.79 g with a mean of 11.22 ± 4.14 g (SE 0.21). The total length of females ranged from 8.7 to 14.4 cm, with a mean of 11.5 ± 1.26 cm (SE 0.08), while weight ranged from 4.67 to 21.75 g with a mean of 11.55 ± 4.0 g (SE 0.26). The length of males ranged from 8.9 to 14.7 cm, with a mean of 11.1 ± 1.38 cm (SE 0.1), and weight ranged from 5.04 to 22.61 g with a mean of 10.76 ± 4.3 g (SE 0.34).

Sex ratio

Of all analyzed sardines, 58.40% of them were females and 41.60% were males. Females were predominant in all length classes above 10.5 cm, while males were predominant in the length classes below this value.

Length–weight relationship

The sardine length–weight relationship was calculated for females and males. The relationship for the whole sardine sample was: W=0.0059 $L_T^{3.0891}$; r^2 =0.963. Regression coef-

ficients for both sexes (males: b=3.1132, r^2 =0.965; females: b = 3.0774, r^2 =0.961) indicated that deviations of allometric coefficients from the value of 3 were not statistically significant (males, t=2.4298, females t=1.8465, df=1, $p<0.05$).

Condition

The average value of the allometric condition coefficient (*Ka*) of sardine specimens during the investigated period was *K*a=1.0408 ± 0.061. The condition index decreased rapidly during successive months, from December (*Ka*=1.0658 ± 0.161) and January (*K*a=0.9966 ± 0.128) to February (*K*a=0.9916 ± 0.111). After that, it increased again in April (*K*a=1.1381 ± 0.151)

GSI

The changes of mean monthly values of GSI showed similar patterns for both females and males. The mean GSI started to increase from September and October and reached its highest values of 5.22 for females and 6.58 for males in February. After February, the GSI started to decrease during March (4.5), April (2.4) and May (2.4). In June all gonads had a GSI of less than 1 which corresponded to spent gonads. Such low GSI values remained throughout both July and August (Fig. 2).

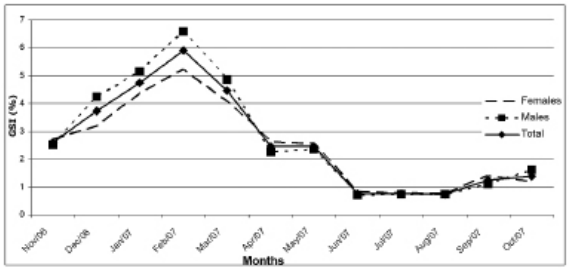

Fig. 2 Annual cycle of the gonadosomatic index in females, males and both sexes together of Sardina pilchardus*, over the period November 2006 to October 2007*

Reproductive cycle

During most of the investigated period it was noted that all four developmental phases of oocytes were present in the ovaries, ranging from the oogonia to mature egg cells, except during the June–September period when only stage I was present. (Fig. 3).

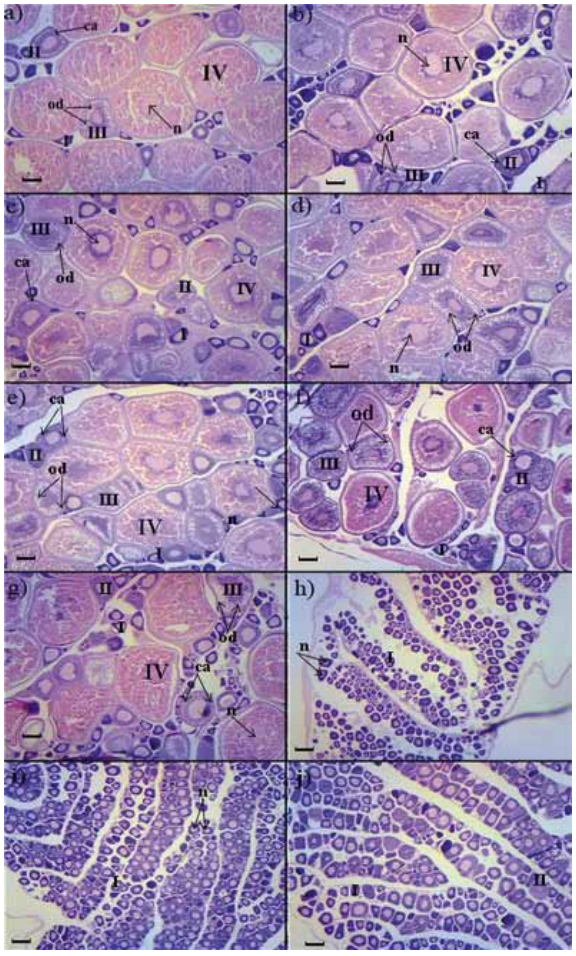

Fig. 3. Consecutive stages of oocyte development in Sardina pilchardus *(40 X magnification) – a) November; b) December; c) January; d) February; e) March; f) April; g) May; h) June; i) September; j) October. I – Peri-nucleolus stage; II – Yolk vesicle stage; III – Yolk stage; IV – Mature stage; n: nucleus; ca: cortical alveoli; od: oil droplets*

During the March–September period, the testicles of sardines were mainly composed of spermatogonia. From October, spermatocytes started to appear and from November spermatids also appeared. Spermatozoa appeared in December and in the January–March period the testicles were full of spermatids and spermatozoa (Fig. 4).

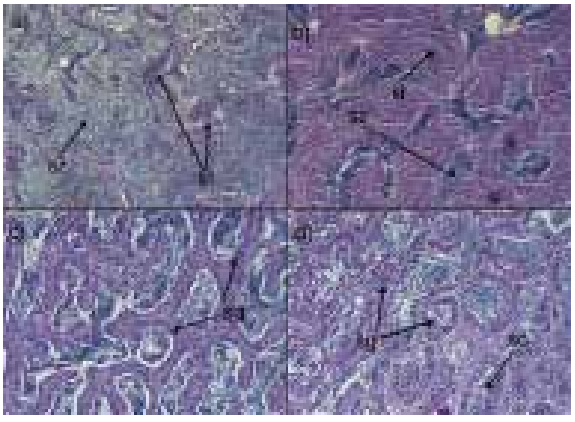

Fig. 4. Consecutive stages of testicular development in Sardina pilchardus *(100 X magnification) – a) January; b) February; c) May; d) October. Sg – spermatogonia, Sc – spermatocytes, St – spermatids, Sz – spermatozoa*

During the June–September period, the ovaries were composed of primary oocytes alone, 50-70 μm in diameter. The second, yolk vesicle stage of oocytes, which were 100-200 μm in diameter, appeared in October and represented 5% of the total number of oocytes. Starting from November, all four developmental stages were present in ovaries. Stage II, the yolk vesicle stage, was present by approximately 20% in the November–April period, reaching its lowest value in May (7%). Stage III, the yolk stage of approximately 300 μm in diameter, also appeared in November, comprising 10-16% of the total number of oocytes in ovaries. Mature cells, with an average diameter of 450 μm, represented over 20% of the total oocyte number in the November–April period, whereas in May their number was 14.7%. From June, only primary oocytes were present in ovaries (Fig. 5).

Average oocyte size (Fig. 6) was greatest in

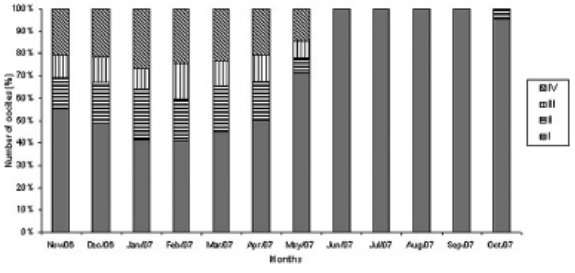

Fig. 5. Annual oocyte composition in the ovaries of Sardina pilchardus*. I – Peri-nucleolus stage; II – Yolk vesicle stage; III – Yolk stage; IV – Mature stage*

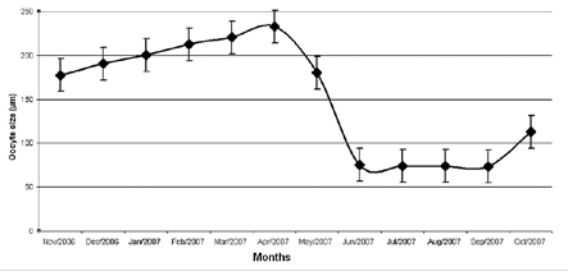

Fig. 6. Annual change of average oocyte size (mean ±SE) in ovaries of Sardina pilchardus

April (232.6 μm ± 16.33). The size of oocytes was smallest in the June-September period with an average of 74.26 μm ± 0.87. In October, the average size of oocytes in ovaries started to increase up to 113 μm ± 3.29, through November (177.5 μm ± 10.23), December (190.7 μm ± 9.79), January (200.3 μm ± 9.49), February (212.6 μm ± 10.45) and March (220.36 μm ± 9.61). After its greatest value in April, the average oocyte size began to decrease in May (180 μm ± 10.46) which coincided with an increase in the number of primary oocytes in the ovaries.

DISCUSSION

Boka Kotorska Bay is an important spawning and nursery ground for small pelagic fish species in the south-eastern Adriatic. On the other hand, small pelagic species in this area are the target of traditional small-scale local fishery. As this fishery resource is important for the local economy, there is a definite need to compare the status of this part of the sardine stock with others in the Adriatic and prepare recommendations for sustainable management.

Small sardine, of LT less than 9 cm, spawn for the first time in Boka Kotorska Bay and then depart to the open sea. This is consistent with the findings of SINOVČIĆ (1991) who reported that sardine spawn for the first time at the end of the first year of life, as well as of SINOVČIĆ *et al*. (2008) who reported that fifty percent of the sardine population in the middle Adriatic Sea are sexually mature at 7.9 cm total length.

The sex ratio of sardines in this study was skewed towards females in almost all length classes. This is an important characteristic of the

Boka Kotorska Bay sardine population. WOOTTON (1982) pointed out that a greater number of females is advantageous since populations with a sex ratio biased towards females have a greater rate of reproduction. The sardine is a species characterized by fast growth, though with a short life span, although SINOVČIĆ (1984) reported that they can live up to the age of 9 years.

The mean changes of the allometric condition factor (*K*a) showed that a decrease begins in the November-December period which coincides with the beginning of sardine spawning, which generally extends from October to April, with the highest gonad activity in the November-January period (MUŽINIĆ, 1954). A decrease in the condition factor could be explained by reserves being used for gonadic maturation and spawning instead of for maintenance and growth of the individuals for which they are normally used. These results are in agreement with previous observations (TOMASINI *et al*., 1989; PEREZ *et al*., 1992; ABAD & GIRALDEZ, 1993; ZWOLINSKI *et al*., 2001). SINOVČIĆ *et al*. (2008) reported lower values of the condition factor for sardine from the middle Adriatic Sea (for the period 2002/03 average *K*a=0.998, 2003/04 average *K*a = 1.001 and for 2004/05 average *K*a=1.010) which may be explained as a consequence of anthropogenic and natural eutrophication which causes high primary production and food availability in Boka Kotorska Bay (REGNER *et al*., 2005, 2008).

This investigation of the reproductive cycle in sardines revealed that oocytes in stage I (perinucleolus stage) were present in ovaries during the entire year (Fig. 3). All oocytes in ovaries (100%) were in that stage during the summer months (June to September) while in October, which could be considered the start of the reproductive cycle, there were 5% of oocytes in stage II (Fig. 5). After October, a decrease in the proportion of oocytes in stage I was noted with their lowest occurrence in January (41.54%), when the number of oocytes in stage IV of oogenesis was the greatest (26.7%). NEJEDLI *et al.* (2004) reported for the northern Adriatic Sea that the number of oocytes in stage IV is greater from October to April with the greatest values found in November (36.3%).

The increase in the number of oocytes in phases II and III of oogenesis noticed in October, which remained up to May, was a result of intensive vitellogenesis in the liver that represents the initial phase of growth and maturation of oocytes, ending with the deposition of yolk vesicles during the process of maturation. The increase in the number of oocytes in phases II and III of oogenesis accompanied with the increase in their diameter (Fig. 6) is a prominent characteristic of oogenesis in fish (FUJITA *et al*., 1997; KOYA *et al*., 1998).

The increase in both number and size of mature oocytes that preceded the spawning of the sardine was noticed in November and reached the greatest values in January. A decrease of oocytes in stage IV of oogenesis occurred in May and after that they were completely absent from the ovaries. In the northern Adriatic Sea all four stages of oogenesis are present in the ovaries during the entire year with the lowest number of oocytes in stage IV recorded in August (NEJEDLI *et al*., 2004).

The sardine's testicles passed through successive stages of maturation during their annual cycle (Fig. 4). Four stages commonly recognized for testicular development (SANTOS *et al.*, 2006) were clearly distinguished histologically. The reproductive cycle of males coincided very well with the oogenesis in females during the year. The occurrence of spermatogonia (stage I) during the summer and appearance of spermatocytes (stage II) in October corresponded to the occurrence of oogonia and appearance of oocytes in stage II in ovaries during the same period. Likewise, the appearance and differentiation of mature spermatozoa in testicles that preceded spawning during the December–February period corresponded to the domination in the number of mature oocytes (stage IV) in the ovaries of females.

Investigation of the GSI revealed that this parameter in the sardine under investigation was also a useful indicator of the progress in gametogenesis (FUJITA *et al*., 1997; ZIMMERMANN, 1997; KOYA *et al.*, 1998). The lowest value of the GSI within this investigation was found from June to August when gonad mass accounted for less

than 1% of body mass (Fig. 2). In this period the ovaries were very small, with 100% of their composition being oocytes in the initial stage I of oogenesis. The start of oocyte development that occurred in October coincided well with the increase in ovary mass, from slightly greater than 1% of total mass to a maximum of almost 6% of total mass recorded in February. Thus, the oscillation of GSI during the year corresponded well to the increase or decrease in the number of mature oocytes in sardine ovaries. There was also a good correlation between changes in GSI and histological events in the gonads of males since the low GSI value of less than 1 in the summer months corresponded to the domination of spermatogonia (stage I), and an increase of GSI to values greater than 1, to the maximal value of 6.5 from October to February, was due to the differentiation of spermatocytes (stage II), spermatids (stage III) and spermatozoa (mature cells).

Long spawning periods in female and male sardine suggest that this species is a multiple spawner. In our investigation the description of oogenesis and spermatogenesis confirms previous reports that the spawning of the sardine population in the investigated region of the southern Adriatic Sea begins in October and extends until May (MUŽINIĆ, 1954; KARLOVAC, 1964; REGNER *et al*., 1981, 1983; GAMULIN & HURE, 1983; SINOVČIĆ, 1983–84; NEJEDLI *et al*., 2004).

The results of this study indicate that some important management decisions need to be taken. The most important should be a ban on sardine fishing for a two-month period (January–February) and a ban on fishing in the area of spawning

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Reproduktivne značajke srdele, *Sardina pilchardus* (Walb, 1792), u Boki Kotorskoj (Crna Gora, južni Jadran)

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SAŽETAK

Istraživana je godišnja promjena morfologije gonada kod srdele (*Sardina pilchardus* Walbaum 1792) ulovljene obalnom mrežom potegačom u razdoblju od studenog 2006. do listopada 2007. na području Bokokotorskog zaljeva (Crna Gora, južni Jadran). Ukupna dužina primjeraka kretala se od 8.7 do 14.7 cm, a težina od 4.67 do 22.61 g. Obavljena je histološka analiza gonada. Dužinsko–težinski odnos za sve jedinke može se opisati jednadžbom: $W=0.0059\ L_T^{3.0891}$ ($r^2 = 0.963$). Najniže vrijednosti gonadosomatskog indeksa (GSI) utvrđene su u lipnju, srpnju i kolovozu, što odgovara stadiju mirovanja gonada. GSI vrijednosti su se postupno povećavale u rujnu i listopadu te dosegle najviše vrijednosti u veljači 5.22 za ženke, odnosno 6.58 za mužjake. Nakon veljače, GSI vrijednosti su opadale, kroz ožujak (4.5), travanj (2.4) i svibanj (2.4). U lipnju su GSI vrijednosti bile manje od 1. Iako su primarne oocite bile prisutne u svim mjesecima, njihova zastupljenost je počela rasti u svibnju i dosegla najvišu vrijednost u ljetnim mjesecima (100% u razdoblju od srpnja do rujna). Zrele oocite u stadiju IV zabilježene su od studenog do travnja, sa najvišom zastupljenošću u siječnju (26.7%). Povećanje postotka oocita u stadijima II i III uočeno je u listopadu i ostaje gotovo nepromijenjeno do svibnja. Za ljetnjih mjeseci (svibanj–rujan), u testisima su bili prisutni samo spermatogoniji stadija I. U listopadu se započinju pojavljivati spermatociti (stadij II), a od studenog i spermatidi (stadij III). Spermatozoi (stadij IV) se javljaju u prosincu, a najvišu razinu dostižu u tijekom siječnja i veljače.

Ključne riječi: srdela, *Sardina pilchardus*, oogeneza, duljina, dužinsko-maseni odnos, razmnožavanje, hidrografski uvjeti, zaljev Boka Kotorska