

Annual oscillation of ovarian morphology in European pilchard (*Sardina pilchardus* Walbaum) in the northern Adriatic Sea

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ABSTRACT

Annual oscillation of ovarian morphology in European pilchards (*Sardina pilchardus* Walbaum) caught in the period July 2001 to June 2002 in the region of the Northern Adriatic (Croatia) was studied. Fifty females were taken in the middle of each month and determining body mass they were dissected, ovaries extracted and weighed, and the centre of each ovary was sampled for histological analysis. Samples were fixed in Bouin's solution, mounted in paraffin and cut into 6 µm-thick slices which were stained with hematoxylin and eosin (HE) and using the Periodic-acid Schiff method (PAS). During oogenesis the oocytes passed through four developmental phases: peri-nucleolus stage (phase I), yolk vesicle stage (phase II), yolk stage (phase III) and mature stage (phase IV). Oocytes in phase I were present during all months, forming more than 40% of all oocytes. Their percentage increased from May to August ($P<0.05$) and was the highest in August (84%). Percentage of oocytes in phase IV increased from October to April ($P<0.05$) and was the highest in November (36.3%). An increase in percentage in phases II and III of oogenesis was noticed in September and remained at an

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almost equal level until May. The developmental cycle of oocytes began in September and October, coinciding with an increase in the ovarian mass, which achieved its maximum level in October, remaining at an almost equal level until April. During this period the mass of ovaries reached more than 4% of body mass, and the GSI (gonadosomatic index) was significantly different ($P < 0.05$) from other months.

Key words: *Sardina pilchardus* Walbaum, ovaries, oocyte stages, Adriatic Sea, Croatia

Introduction

The existence of a fish population depends on many factors. Besides the environment, regular reproduction as well production of high quality egg cells is particularly important. The course of a reproductive cycle is related to a certain period of the year, to the quantity of available aliments, temperature, etc.

The reproductive cycle must ensure a sufficient quantity of mature egg cells, which is possible only within the regular process of oogenesis. Oogenesis is a very dynamic process in ovaries, in which the oocyte passes through various phases of development that are very similar in different fish species. Generally speaking, the oogenetic process may be synchronic, group synchronic and asynchronic (SELMAN and WALLACE, 1989). In the case of synchronic oogenesis, all oocytes develop at the same time, ovulation also being simultaneous. In the case of the synchronic oogenesis group the maturation of oocytes is accomplished in two groups so that some immature stages are always present within the ovaries. In the case of asynchronic ovulation, different development stages of oocyte maturation and ovulation in groups may be found within the ovaries.

The study of the oogenesis during the reproductive cycle is of great interest in order to determine the exact ovulation time when the number of the mature egg cells is maximal. The importance of such investigations has been indicated by certain authors: (RAMSAY and WITTHAMES, 1996; FUJITA et al., 1997; ZIMMERMANN, 1997; BROOKS et al., 1997; KOYA et al., 1998) who were able to determine the exact spawning time of several fish species by studying the histological changes within the ovaries. As far as is known from the available literature, investigations of such type were not performed on the fish species in the Adriatic Sea.

The sardine is a typical pelagic fish species. It lives in rather large shoals and is important from the economic point of view because in the

Adriatic Sea it is fished throughout the whole year. Therefore, it may be of interest to determine the annual progress of the oogenesis as well as the exact spawning time of sardines by means of the histological methods.

Materials and methods

In the investigations we used sardines caught in the period July 2001 to June 2002 in the region of the Northern Adriatic. Fifty female fish were taken in the middle of each month and, after determining the body mass of the fish, they were dissected and their ovaries extracted. Ovaries were weighed and a sample was taken for histological analysis from the middle of each ovary. Gonadosomatic index was determined on the basis of their body mass and their ovary mass by applying the formula: $GSI = 100 \times GW/BW$, in which GSI = gonadosomatic index, GW = gonadal weight, BW = body weight.

Average GSI value for each month is presented in a linear graph.

Samples for histological investigations were fixed in the Bouin's solution, mounted in paraffin and cut into 6 μm -thick slices. The slices were stained with hematoxylin and eosin (HE) and by the Periodic-acid Schiff method (PAS) (ROMEIS, 1968). In these histological slices the oocytes were divided into four groups according to their morphologic characteristics, their participation and mutual relationship being presented graphically.

Approximate age of the sardines used in our investigations was determined on the basis of their body mass (SINOVIĆ, 1986; SINOVIĆ, 1991).

Diameters of oocytes were measured with a microscale on histological slides.

Statistical analyses of results were made by using Student's t-test.

Results

During oogenesis it was noticed that the oocytes passed through several development phases, ranging from the oogonium to mature egg cells (Fig. 1). The process of oogenesis can be roughly divided into four phases as follows.

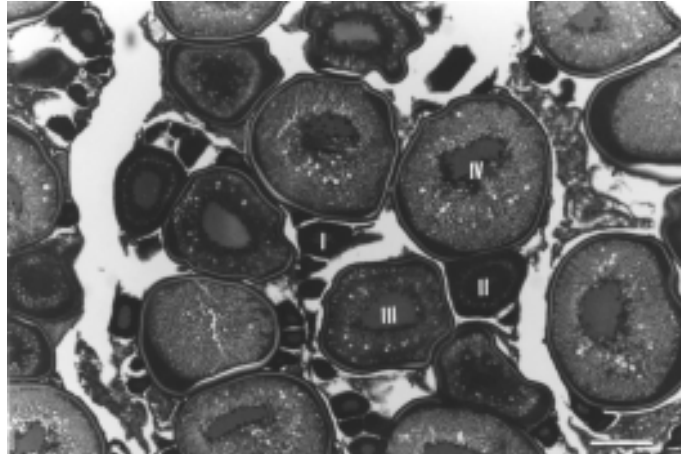


Fig. 1. Ovarian histology of *Sardina pilchardus* Walbaum. I - peri-nucleolus stage, II - yolk vesicle stage, III - yolk stage, IV - mature stage. Scale bar = 100 μm .

Phase I (peri-nucleolus stage). Oocytes are present within the ovaries throughout the whole year. In this phase a characteristic basophilia appears in the oocyte cytoplasm, which gradually decreases with the growth of oocytes. A large number of nucleoles appear, situated in the periphery of nucleolus beneath the nuclear membrane. Average oocyte diameter is 49 μm .

Phase II (yolk vesicle stage). Within the cytoplasm a number of yolk granules appear around the oocyte nucleus. The yolk granules are stained by the PAS-method and their number and size increase with the growth of the oocyte. A layer of the follicular cells may be seen around the oocyte. Average diameter of the oocyte is 96 μm .

Phase III (yolk stage). This phase of oogenesis is accompanied by a series of changes within the oocyte which is characterized by the appearance of yolk granules and oil droplets. Initially, the yolk granules and oil droplets are few in number, being generally distributed in the peripheral region of the oocyte. The yolk granules later appear, forming many layers in the oocyte periphery, with the oil droplets forming a ring around the nucleus. A yolk capsule surrounding the oocyte appears at the end of this phase. Average diameter of oocytes is 201 μm .

S. Nejedli et al.: Annual oscillation of ovarian morphology in European pilchard (*Sardina pilchardus* Walbaum) in the northern Adriatic Sea

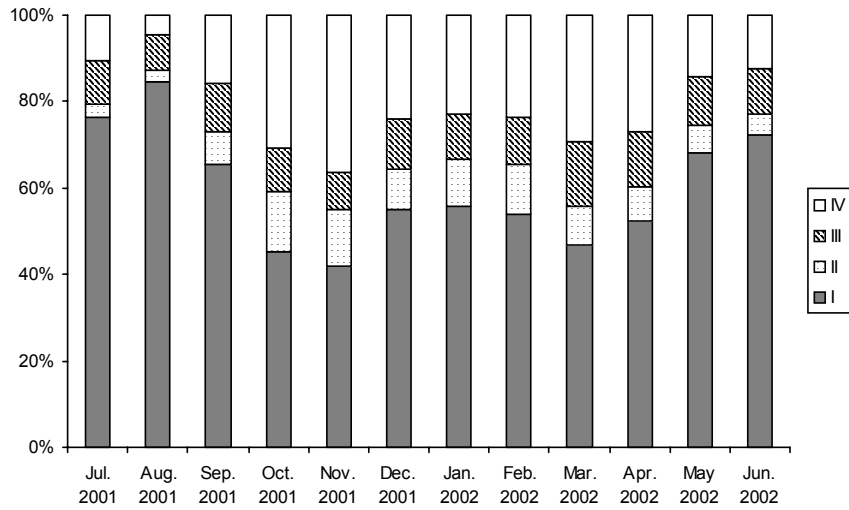


Fig. 2. Annual change of oocyte composition in ovaries of *Sardina pilchardus* Walbaum. I - peri-nucleolus stage; II - yolk vesicle stage; III - yolk stage; IV - mature stage

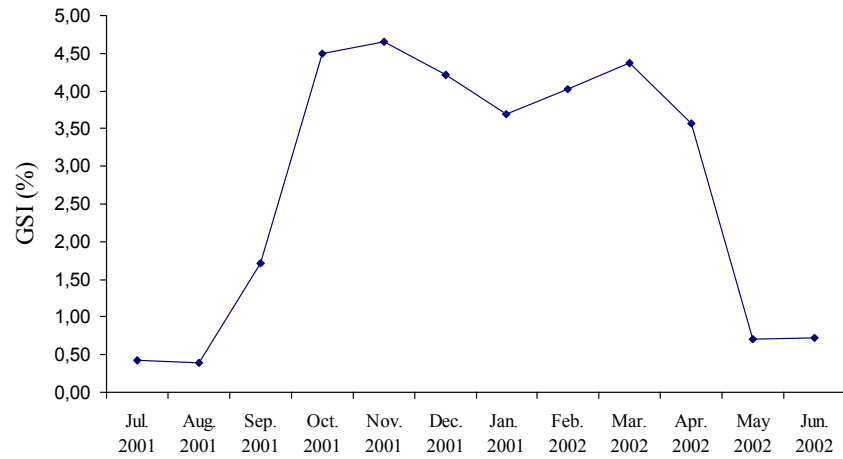


Fig. 3. Annual changes in female GSI

Phase IV (mature stage). In this phase of oogenesis the whole egg cell is filled with a large number of manifestly PAS positive yolk granules. The nucleus moves from the central part of the egg cell toward its periphery. This phase precedes ovulation. Average diameter of oocytes is 286 μm .

In each development phase oocytes were counted by microscope, their percentage ratio being determined within the ovary for each month of the year, as presented in Fig. 2.

The gonadosomatic index show the participation ratio of the ovary mass, expressed as a percentage within the total body mass and is precisely linked with the growth and maturation of oocytes.

Values of the gonadosomatic index in each month are shown in Fig. 3.

Through determination of their body mass, age of investigated fish was found to be 2-4 years old.

Discussion

Oogenesis in sardines is manifested by a series of changes in oocytes, which makes possible their division into four basic groups, which in the literature are known as: peri-nucleolus stage, yolk vesicle stage, yolk stage, and mature stage (ZIMMERMANN, 1997). The oocytes of each group show a characteristic aspect corresponding to the view of oocytes within other teleostean fish (FORBERG, 1982; SELMAN and WALLACE, 1986; BEGOVAC and WALLACE, 1987; DOWN and LEATHERLAND, 1989; FUJITA et al., 1997; ZIMMERMANN, 1997).

The reproductive cycle of the sardines do not coincide with phases of the calendar year. According to our investigations, all four stages of oogenesis were present within the ovaries. Ovaries were present in phase I of oogenesis during all months, forming more than 40% of all oocytes. During the investigation period the number of oocytes in phase I is greater from May to September ($P < 0.05$), and most numerous in August (84%). The number of oocytes in phase IV is greater from October to April ($P < 0.05$) and most numerous in November (36.3%). The increase of oocytes in phases II and III of oogenesis noticed in September, and remaining at an almost equal value up to May, was a result of an intensive vitellogenesis presenting an initial phase of growth and maturation of the oocytes. Vitellogenesis

began under the influence of estrogens, with synthesis of vitellogenin within the liver. Through the circulation it occurs as a precursor of the yolk appearing in the gonads (HAMAZAKI et al., 1987; HYLLNER et al., 1991). The increase in the number of oocytes in phases II and III of oogenesis was also accompanied by an increase in their diameter, which was characteristic of the oogenesis of fish, as described in other fish (FUJITA et al., 1997; KOYA et al., 1998). An increased number of oocytes appeared simultaneously in phase IV. The appearance of yolk vesicles within oocytes was an indication of the process of maturation.

Investigation concerning the ratio of ovary mass in proportion to body mass (GSI) is also one of the indicators of the progress of oogenesis (FUJITA et al., 1997; ZIMMERMANN, 1997; KOYA et al., 1998). The lowest value of the gonadosomatic index within the investigated sardines was found from May to August, when ovary mass was less than 1% of body mass. In this period, the ovaries were very small, with about 70% of their composition being oocytes in the initial phase (I) of oogenesis. The start of development of oocytes occurred in September and October and coincided with an increase in ovary mass, attaining its maximum level in October, and remaining at an almost equal value up until April. During this period, the mass of ovaries was more than 4% of body mass, and the GSI was significantly different ($P < 0.05$) from other months. Oscillation of GSI during the year corresponds to the increase or decrease in the number of the mature oocytes. These findings correspond to those of FUJITA et al. (1997).

Sardines attain sexual maturity in the second year of their life (SINOVIĆ, 1986; SINOVIĆ, 1991). In our investigations, fish were 2-4 years old and all were sexual mature, which corresponds with the findings of SINOVIĆ (1986; 1991).

According to the manner of their oogenesis and ovulation, sardines belong to the group of multiple spawning fish. Such a description of oogenesis indicates that the spawning of the sardine population in the investigated region of the Adriatic Sea begins in August-September and extends to May. The obtained findings correspond to those of GAMULIN and HURE (1983), who quote the same annual periods as the spawning time of sardines in the Northern Adriatic. Our findings, as well as those by GAMULIN and HURE (1983), are also supported by the findings of REGNER et

al. (1987), describing an increase in the quantity of phytoplankton in those Adriatic regions considered to be the locations of the most intense spawning.

Conclusion

Oogenesis in sardines is manifested in a series of changes in oocytes, which makes possible their division into four basic groups: peri-nucleolus stage (phase I), yolk vesicle stage (phase II), yolk stage (phase III), and mature stage (phase IV).

According to their changes in number during the year it is possible to determine the onset of oogenesis and the spawning time of fish. Using these methods, we concluded that the reproductive cycle of the sardine population in the investigated region of the Adriatic Sea commences from August-September and extends to May.

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S. Nejedli et al.: Annual oscillation of ovarian morphology in European pilchard (*Sardina pilchardus* Walbaum) in the northern Adriatic Sea

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SAŽETAK

Istraživane su godišnje promjene u građi jajnika srdela (*Sardina pilchardus* Walbaum) ulovljenih u periodu od srpnja 2001. do lipnja 2002. na području sjevernog Jadrana (Hrvatska). Sredinom svakog mjeseca uzimano je po 50 riba ženskog spola. Nakon određivanja mase tijela ribe su secirane i odvojena su im oba jajnika. Jajnici su izvagani i od sredine svakog jajnika uzet je uzorak za histološku analizu. Uzorci su fiksirani u Bouin's otopini, uklopljeni u parafin i izrezani na 6 µm debele rezove. Rezovi su obojeni hematoksilinom i eozinom (HE) te perijodnom kiselinom- Schiff metodom (PAS). Za vrijeme nastanka oocite prolaze četiri razvojne faze: faza I (faza izvan jezgrice), faza II (faza mjehurića), faza III (žumanjčana faza) i faza IV (faza zrenja). Oocite u fazi I prisutne su tijekom svih mjeseci i čine više od 40% svih oocita, a njihov broj povećava se od svibnja do rujna ($P < 0,05$) te je najveći u kolovozu (84%). Udio oocita u fazi IV povećava se od listopada do travnja ($P < 0,05$), a najveći je u studenom (36,3%). Povećanje broja oocita u fazi II i III oogeneze zapaža se u rujnu i ostaje gotovo na istoj razini do svibnja. Razvojni ciklus oocita počinje tijekom rujna i listopada te se

S. Nejedli et al.: Annual oscillation of ovarian morphology in European pilchard (*Sardina pilchardus* Walbaum) in the northern Adriatic Sea

podudara s povećanjem mase jajnika koja dostiže svoj vrhunac u listopadu i ostaje na gotovo istoj razini do travnja. U tom razdoblju težina jajnika dostiže više od 4% tjelesne mase, a gonadosomatski indeks (GSI) je značajno različit ($P < 0,05$) od drugih mjeseci.

Ključne riječi: *Sardina pilchardus* Walbaum, jajnici, faze oocita, reprodukcijski ciklus, Hrvatska
