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Short Communication

Oogenesis and spermatogenesis in round sardinella (*Sardinella aurita* Valenciennes, 1847) from the eastern part of the Adriatic Sea

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Abstract

Background and purpose: *Small pelagic fish species round sardinella (*Sardinella aurita*), a thermophilic species, has expanded its habitat during the last few decades towards colder parts of the Mediterranean Sea. In the Adriatic Sea, this investigation is the first attempt to study its gonad development histologically.*

Materials and methods: S. aurita *specimens were sampled monthly from commercial landings of purse seine catches from the eastern part of the Adriatic Sea from November 2007 to January 2009. After biometric parameters were determined, gonads were removed from the fish and weighed. Sex and gonad development were determined macroscopically by the shape and the structure of gonads and afterwards gonads were analysed histologically.*

Results: *The phases of gonadal development of both sexes of* S. aurita *were: immature, developing, spawning capable, regressing, and regenerating. An annual reproduction cycle based on gamete development revealed four stages of oocyte development: chromatin nucleolar and perinucleolar stage (primary growth stage), yolk vesicle (cortical alveoli) formation, vitellogenic (yolk) stage, and ripe (mature) stage. Spermatogenesis was described through three stages: spermatocytogenesis (formation of primary spermatocytes from spermatogonia), meiosis (formation of secondary spermatocytes and spermatids) and spermiogenesis.*

Conclusions: *Gonadal histology analyses of both sex of* S. aurita *indicated summer spawning manner of this species, with a peak in June-August period.*

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INTRODUCTION

Round sardinella (*Sardinella aurita* Valenciennes, 1847) is a small pelagic fish species widespread in the Atlantic, the Mediterranean and the Black Sea (*1*). In recent decades, this thermophilic species has expanded its habitat into the northern Mediterranean (*2*, *3*).

S. aurita is an iteroparous gonochorist. It spawns during the whole year in Venezuela, while in the Mediterranean region only during the summer months (*3*, *4*). In general, histological studies on ovarian development of some small pelagic fish species: sardine and anchovy have been reported (*5*, *6*), while those of *S. aurita* have been scarce or insufficient, especially for Mediterranean region. On the other hand, Fontana

(*7*) studied ovarian development in *S. aurita* and *S. eba* from the Point-noire region of the Congo. The oocytes development was also studied in Venezuela (*4*).

The ovarian developing cycle is crucial for determining the duration and stages of egg maturation, which are the basic parameters in the stock size assessments (*8*, *9*). Although only histological analyses provide a resolution of assessing fish maturity and had been widely applied in studies of fecundity, daily egg production method (DEPM) and in estimation of spawning stock biomass (SSB), it is an expensive and time-consuming method (*10*, *11*).

Data on the *S. aurita* reproduction cycle in the Adriatic Sea is very scarce. Recently, Mustać and Sinovčić (*12*) found that this species spawns from May to September, peaking in June-August period. In the mentioned study, the *S. aurita* reproduction cycle was investigated by analysing gonad maturity stages macroscopically, as well as fecundity, gonad mass, and gonado-somatic index (I_G) fluctuations over the year. So, the aim of this study was to analyse developing phases of this species' oogenesis and spermatogenesis, to compare microscopically gonad maturation with previously mentioned macroscopically analyses (*12*), in order to extend current knowledge on *S. aurita* reproduction.

Reproductive strategies in marine fishes are extremely diverse - they may vary greatly for a given species within its geographical range, especially within colder seas (*10*, *13*). Therefore, the objective of this study is also to fill that gap in *S. aurita* reproduction knowledge, since reproductive performance can affect recruitment and thus population growth, the essential factors in the integration of ecosystem-based fisheries management.

MATERIALS AND METHODS

A total of 2,004 *S. aurita* specimens was analysed from November 2007 to January 2009 (Table 1). A random sample of 150-200 specimens was taken monthly from purse seine catches (mesh size: 8 mm/bar length) in the eastern Adriatic Sea (43°30 N; 15°30 E; and 44°30 N; 15°00 E).

Each fish was weighed to the nearest milligram and measured to the nearest millimeter. Gonads were removed from the fish and weighed. Sex and gonad development of *S. aurita* were determined macroscopically, and gonadal development was determined using the phase scale according to the study of Brown-Peterson *et al.* (*14*). Hence, in this study the term "phase" is used to indicate gonadal development and the term "stage" to define processes during gamete development. The phases of gonadal development of both sexes were divided into five groups: immature, developing, spawning capable, regressing, and regenerating.

Classification of oocyte development was done in accordance with the histological staging of West (*15*), while spermatogenesis was described based on Selman and Wallace's (*16*) division into three principal stages.

For histological analysis subsamples of both ovaries and testis of 15-20 specimens (with total length range from 19.2 to 29.5 cm, body masses from 54.1 to 149.6 g and gonad masses from 0.05 to 4.56 g per month were fixed in formaldehyde (4%). A tissue sample was taken from middle (central) part of gonad. A tissue sample was dehydrated, cleared in xylol and embedded in paraffin. Sections (4-6 µm) were cut with microtome and stained with hematoxylin and eosin. During the histological examination observed under the microscope, photos of gonads were taken and those which most clearly showed gonad developing phases and gamete developing stages for males and females were presented.

RESULTS

Gonad developing phases

Ovaries and testis of cca.15-20 *S. aurita* specimens per month were analysed for gonad developing phases and stages.

1. The immature phase

The immature phase is defined by gonadal differentiation and gamete growth, for male: primary spermatogonia and female: oogonia and primary growth oocytes (P, Sg1 and Sg2 in Figures 1 and 4). In *S. aurita* from the Adriatic Sea, the immature phase was observed only in the smallest specimens. The absence of lumen in lobules was observed in immature testes of *S. aurita*.

2. The developing phase

The developing phase of *S. aurita* occurred from October to May. At the beginning of this phase, besides early cortical alveolar oocytes, primary growth oocytes were also present in ovaries, while primary spermatocytes were present in testes (Ca, P and Sc in Figures 1 and 5). In the end of this phase, however, early vitellogenic stage oocytes were present in females (Vtg1 and Vtg 2 in Figure 2). In males, spermatocysts, spermatocytes, and smaller spermatids were present at the end of the developing phase (Sc and Sd in Figure 5). A lumen of lobules was formed as well.

3. The spawning capable phase

Within advance gamete development fish became spawning capable. In observed *S. aurita* specimens, besides mature, late vitellogenic stage oocytes, other -early stage oocytes- were also present in ovaries (B, E and P in Figure 3). Gonadal histology of testes sampled from June to August revealed domination of mature spermatozoa in cysts and in sperm ducts (Figure 6).

4. The regressing phase

During this phase, in September, post-spawning ovaries and testes were still large, but watery and mostly empty with few remaining primary growth oocytes or some residual sperms and some mature atretic egg cells, postovulatory follicle complexes with bloody periphery tissue.

5. The regenerating phase.

During the regenerating phase (September - October) *S. aurita* samples were reproductively inactive but their gonads were preparing for the next reproductive cycle. Oogonia and primary growth oocytes occur in females, with some late-stage atresia and primary spermatogonia usually with residual spermatozoa in sperm ducts and the lumen of lobules in males.

Oocytes developing stages

1. The chromatin nucleolar and the perinucleolar stage (primary growth stage)

In this stage during October, *S. aurita* oocytes were extremely small. The oocyte had a proportionally large nucleus (germinal vesicle) mostly located in the centre of oocyte with thin layer of cytoplasm around it (P in Figure 1). It was surrounded by squamous follicle cells. One large nucleolus was in each nucleus. Homogenous and basophilic cytoplasm decreased as oocyte grew.

2. Yolk vesicle (cortical alveoli) formation

During the yolk vesicle (cortical alveoli) formation stage, small spherical vesicles started to appear in the cytoplasm (Figure 1). In the early developing phase, CA ocytes were mostly hexagonal. This oocyte stage dominated in *S. aurita* ovaries from October to May. During this stage cortical alveoli increased in number and size and formed several peripheral rows and later they released their content into the perivitelline space inside the egg membranes during fertilization (Figure 2). Oil droplets also began to accumulate in cytoplasm during this stage and they became involved in the formation of lipid globule in matured egg.

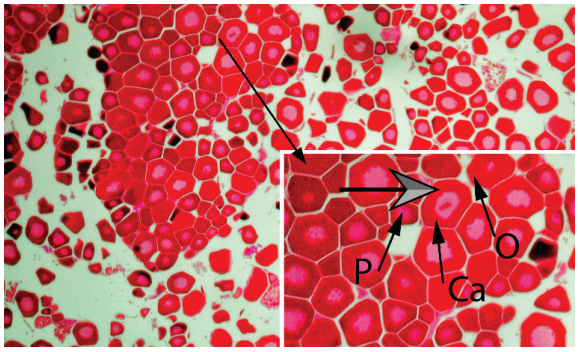

Figure 1. *Yolk vesicle (cortical alveoli) formation stage in ovaries of S. aurita from the middle eastern Adriatic Sea, female sample from May 2008 (L=29.5cm; M=147.82g; Mgonad=1.90g; early developing phase) (x 400); Ca: early cortical alveoli oocytes, P: primary growth oocytes, O: oil droplets; tiny clear inclusions around cytoplasm (arrow)*

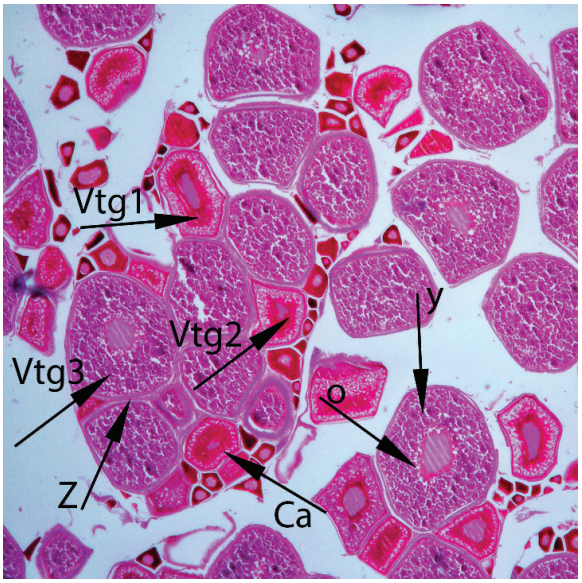

Figure 2. *Vitellogenic (yolk) stage in ovaries of S. aurita from the middle eastern Adriatic Sea, (a) female sample from July 2008 (L=27.2cm; M=149.60g; Mgonad=2.67g; late developing phase) (x 200); Ca: late cortical alveoli oocytes, Z: zona radiata, Y: yolk granule; O: oil droplets; Vtg1 and Vtg2: earlier vitellogenic stage, Vtg3: late vitellogenic stage*

3. The vitellogenic (yolk) stage

The vitellogenic (yolk) stage, which arised in June was characterized by the appearance of yolk proteins in granule of yolk stage oocytes. The ovary of *S. aurita* was filled with vitellogenic oocytes in different stages of yolk depo-

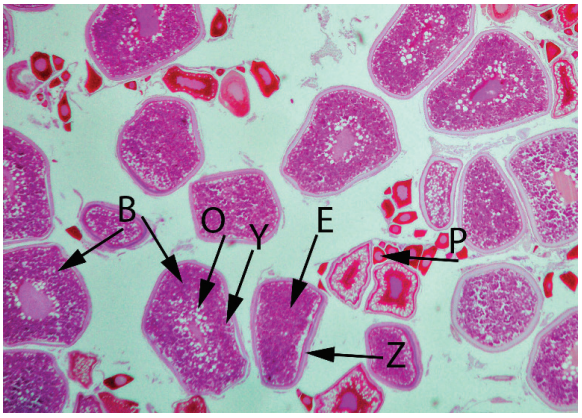

Figure 3. *Ripe (mature) stage of oocyte development in mature ovaries of S. aurita from the middle eastern Adriatic Sea, female sample from July 2008 (L=27.2cm; M=149.60g; Mgonad=2.67; spawning capable phase) (x 100); oocyte in the beginning (B) and the end (E) of spawning capable phase; P: primary growth oocyte, Y: yolk granule, O: oil droplets, Z: zona radiate*

sition (Figure 2). Hence, in the beginning of this stage, yolk granules were very small and rare (Vtg1) and afterwards amount of cytoplasm filled with yolk grew (Vtg2 and Vtg3). At the end of this stage oil droplets were more distributed around the nucleus (Figures 2 and 3). A layer of zona radiata (z) was also formed during this stage.

4. Ripe (mature) stage

This stage dominated in the ovaries from June to September. The fully grown vitellogenic oocyte (egg cell) was much larger than oocytes from earlier developing phases (Figure 3), which were also present in the mature, spawning capable ovaries, indicating group synchronic type of oogenesis. The whole egg cell was filled with a great number of yolk granules, and the nucleus moved from the central toward the animal pole.

Sperm developing stages

Spermatocytogenesis

In October, testes of *S. aurita* specimens contained chromatin nucleolar spermatogonias, which dominated on the periphery of cysts and near interlobular connective tissues. Larger primary and smaller secondary spermatogonia were observed in cysts of lobules without a lumen (Figure 4).

Meiosis

During further development, which occurred from November to May, primary spermatocytes become half size smaller (secondary spermatocytes). A number of spermatocytes were growing and developing during this stage. Later testes developed spermatids that resulted from the second meiotic division of secondary spermatocytes. These spermatids were smaller than spermatocytes, which were scattered in the periphery of lobules (Figure 5).

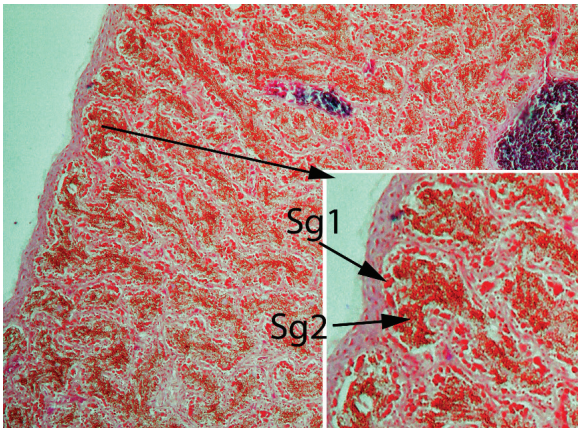

Figure 4. *Spermatocytogenesis in immature testes S. aurita from the middle eastern Adriatic Sea; male sample from October 2008 (L=24.5cm; M=113.31g; Mgonad=0.40g; testes development phase - immature) (x 400); Sg1: primary spermatogonia, Sg2: secundary spermatogonia*

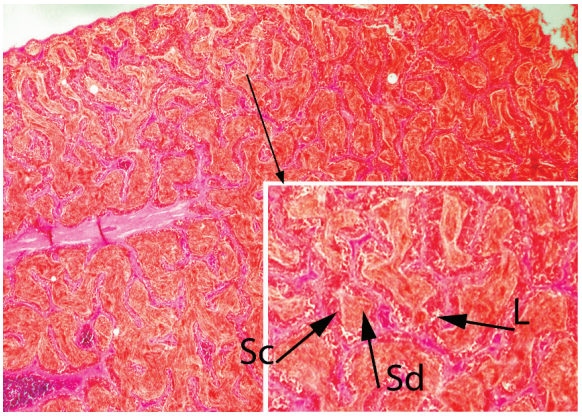

Figure 5. *Meiosis stage in developing testes of S. aurita from the middle Eastern Adriatic Sea, male sample from May, 2008 (L=20.5cm; M=67.11g; Mgonad=1.43g; developing phase) (x 400); Sc: spermatocytes, Sd: spermatids, L: lumen of lobules*

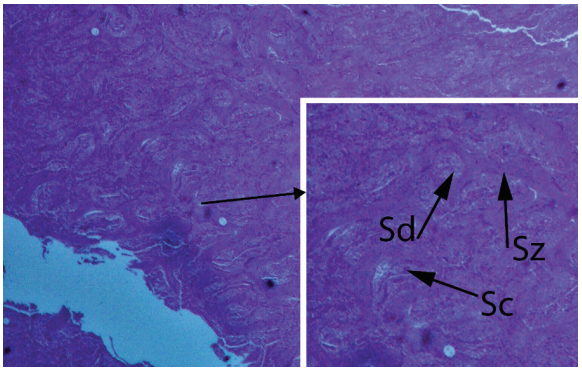

Figure 6. *Spermiogenesis in mature testes of S. aurita from the middle eastern Adriatic Sea, male sample from July 2008 (L=23.0cm; M=85.68g; Mgonad=4.56g; spawning capable phase) (x 200); Sc: spermatocytes, Sd: spermatids; Sz: spermatozoa*

Spermiogenesis

Just prior to spawning, during May – June period, mature spermatozoa were developing. They were present in lumen of lobules. The matured sperm had a uniformly dense nucleus and was found in cysts adjacent to the efferent ducts. In June, when spawning of *S. aurita* occurred, most males were mature, with spermatozoa in sperm ducts. During the active reproductive months (June – August) all stages of spermatocytes were present in lobules, with spermatozoa prevalent (Figure 6).

DISCUSSION

In the study, an annual reproduction cycle of *S. aurita*, based on gamete development, revealed four stages for oocyte development in females and three stages of spermatogenesis in males. For the same species Fontana (*7*) described six maturity phases for oocyte development in female samples from Congo, while in Venezuela, Guzmán

et al. (*8*) found also four groups of oocyte development. They have found mature female fish during all year, while in this study mature fish were only observed in summer months. The spawning period of *S. aurita* seems to be determined by the regional conditions, mainly temperature; its spawning time is longer in the seas with higher temperatures than in those of colder ones such as the Adriatic Sea and Ionian Sea (*3*, *12*, *17*).

The annual reproductive cycle of *S. aurita* from the Adriatic Sea revealed that from October to May, only primary growth oocytes (i.e., previtellogenic oocytes) were present; then from May to June cortical alveoli (CA) oocytes are recruited, as a beginning of secondary growth of the oocyte (i.e., vitellogenic oocytes), which lasted until September, indicating a short annual period of secondary oocyte growth. In *S. aurita* females GSI ranged from 0.45% (October) to 3.15% (July) which corresponded with oocyte maturing in this study (*12*).

Spermatogenesis development was in accordance with the oogenesis process. The male sample from October was also immature. During the spawning capable phase of testes, spermatids and mature spermatozoas were most abundant, while after spawning, spent testes were large, but flaccid, sperm ducts were empty, or with some residual sperm indicating regressing phase. Monthly variations of GSI and gonad weight of *S. aurita* males matched spermatogenesis development presented in this paper (*12*).

According to this study, gonadal histology analyses have confirmed summer spawning regime of *S. aurita* in the eastern part of the Adriatic Sea, peaking in June-August period (Figures 3 and 6). Mustać and Sinovčić (*12*) have found the greatest values of the gonado-somatic index (I_G) in males and females from June to September, which clearly corresponded with the oogenesis and spermatogenesis documented in the present research. They have found highest monthly average values of GSI in July (3.38%). Hence, an overlap in *S. aurita* microscopically and macroscopically gonad analyses was confirmed.

At the end of *S. aurita* oogenesis, when mature egg cells were mostly present (Figure 3) oocytes with other development stages (primary growth oocytes) were also present. The spawning oocyte batch started to separate in size from the adjacent smaller oocytes when the secondary vitellogenesis started (Figures 2 and 3). Hence, *S. aurita* in the eastern part of the Adriatic Sea has revealed a group synchronic type of the oogenesis process in which at least two groups of differently mature oocytes are present at the same time: a batch of larger oocytes and heterogeneous batches of smaller oocytes, suggesting that this species is multiple spawner (*18*).

In multiple, batch-spawning fish species with indeterminate fecundity, the hydrated oocytes that present the spawning batch are separated from the diverse batches of remaining oocytes (*12*). Consequently, *S. aurita* spawns during the whole year in warm waters, but in the Adriatic Sea and the Ionian Sea this species spawns only over summer months (*3*, *4*, *12*). Blaxter and Hunter (*19*) reported that the presence of more than one group of yolked eggs has become an accepted criterion of the presence of more than a single spawning event.

The oocyte batch in which the period of oocyte growth is shorter than the spawning period is characterized as indeterminate fecundity (*20*). In the multiple spawners with indeterminate fecundity, previtellogenic oocytes continue to recruit into vitellogenic stock during the spawning season. The different developmental stages observed in mature gonads and the absence of a gap between immature and mature oocytes implies that *S. aurita* in warm waters has an indeterminate fecundity pattern (*4*). Furthermore, the presence of more determinate spawners in cold water has been already reported, as has the more indeterminate spawners in warm-water stocks (*20*).

Various developmental oocyte stages observed in spawning capable gonads and the absence of a gap between immature and mature oocytes found during this study implied that *S. aurita* in the Adriatic Sea might have an indeterminate fecundity. To confirm that, more frequent sampling during spawning period with more detailed research on precise oocyte growth and diameter are needed.

REFERENCES

1. WHITEHEAD PJP 1985 Clupeoid fishes of the world (suborder Clupeoidei). FAO Fisheries synopsis, Rome, p. 125
2. SINOVČIĆ G, FRANIČEVIĆ M, ČIKEŠ KEĆ V 2004 Unusual occurrence and some aspects of biology of juvenile gilt sardine (*Sardinella aurita* Valenciennes, 1847) in the Zrmanja River estuary (eastern Adriatic). *J Appl Ichthyol* 20: 53–57. https://doi.org/10.1111/j.1439-0426.2004.00482.x
3. TSIKLIRAS AC, ANTONOPOULOU E 2006 Reproductive biology of round sardinella (*Sardinella aurita*) in the north-eastern Mediterranean. *Sci Mar* 70: 281–290. https://doi.org/10.3989/scimar.2006.70n2281
4. GUZMÁN R, AROCHA F, GÓMEZ G 2001 Tipo de desove de la sardina (*Sardinella aurita,* Valenciennes, 1847) en el nororiente de Venezuela: Resultados preliminares. *Zoot Trop* 19: 173–183.
5. GANIAS K, SOMARAKIS S, MACHIAS A, THEODOROU A 2004 Pattern of oocyte development and batch fecundity in the Mediterranean Sardine. *Fish Res* 67: 13–24. https://doi.org/10.1016/j.fishres.2003.08.008
6. EL OUIZGANI H, SNAIKI Y, CHOUHAM S 2009 Histological examination of ovarian development of anchovy (*Engraulis encrasicolus*) from the Agadir Bay (Morocco Atlantic Coast). In: Wyanski DM, Brown-Peterson NJ (eds) Proceeding of the 4th Workshop on gonadal histology of fishes, El Puerto de Santa María, Cadiz, Spain, pp 168–172. https://doi.org/10261/24937
7. FONTANA A 1969 Etude de la maturité sexuelle des sardinelles *Sardinella eba* (Val.) et *Sardinella aurita* (C et V) de la région de Pointe-Noire. Cah O.R.S.T.O.M. Série Océanogr 7: 101–114.
8. REGNER S, PICCINETTI C, SPECCHI M, SINOVČIĆ G 1981 Preliminary statistical analysis of sardine stock estimation from data obtained by egg surveys. *FAO Fish Rep* 253: 143–154.

9. PEŠIĆ A, ĐUROVIĆ M, JOKSIMOVIĆ A, REGNER S, SIMONOVIĆ P, GLAMUZINA B 2010 Some reproductive patterns of the sardine, *Sardina pilchardus* (Walb, 1792), in Boka Kotorska Bay (Montenegro, southern Adriatic Sea). *Acta Adriat* 51: 85–92.
10. LOWERRE-BARBIERI S K, BROWN-PETERSON N J, MURUA H, TOMKIEWICZ J, WYANSKI D M, SABORIDO-REY F 2011 Emerging Issues and Methodological Advances in Fisheries Reproductive Biology. *Mar Coast Fish* 3: 32–51. https://doi.org/10.1080/19425120.2011.555725
11. RIDEOUT RM, ROSE GA, BURTON MPM 2005 Skipped spawning in female iteroparous fishes. *Fish and Fisheries* 6: 50–72. https://doi.org/10.1111/j.1467-2679.2005.00174.x
12. MUSTAĆ B, SINOVČIĆ G 2012 Reproductive cycle of gilt sardine, *Sardinella aurita*, in the eastern middle Adriatic Sea. *J Appl Ichthyol* 28: 46–50. https://doi.org/10.1111/j.1439-0426.2011.01908.x
13. HUNTER JR, LO NCH, LEONG HJ 1985 Batch fecundity in multiple spawning fishes. In: Lasker R (ed) An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, *Engraulis mordax* NOAA Tech Rep No. NMFS 36, p 67–77.
14. BROWN-PETERSON NJ, WYANSKI DM, SABORIDO-REY F, MACEWICZ BJ, LOWERRE-BARBIERI SK 2011 A standardized terminology for describing reproductive development in fishes. *Mar Coast Fish Dynam Manag Ecosys Sci* 3: 52–70. https://doi.org/10.1080/19425120.2011.555724
15. WEST G 1990 Methods of assessing ovarian development in fishes-a review. *Aust J Marine Freshwater Res* 41: 199–222. https://doi.org/10.1071/MF9900199
16. SELMAN K, WALLACE R A 1986 Gametogenesis in *Fundulus heteroclitus*. *Amer Zool* 26: 173–192. https://doi.org/10.1093/icb/26.1.173
17. QUAATEY SNK, MARAVELIAS CD 1999 Maturity and spawning pattern of *Sardinella aurita* in relation to water temperature and zooplankton abundance off Ghana, West Africa. *J Appl Ichthyol* 15: 63–69. https://doi.org/10.1046/j.1439-0426.1999.00111.x
18. SELMAN K, WALLACE RA 1989 Cellular aspects of oocyte growth in Teleosts. *Zool Sci* 6: 211–231.
19. BLAXTER JHS, HUNTER JR 1982 The biology of the clupeoid fishes. Adv Mar Biol 20: 1-223. https://doi.org/10.1016/S0065-2881(08)60140-6
20. GANIAS K, LOWERRE-BARBIERIB SK, COOPER W 2015 Understanding the determinate-indeterminate fecundity dichotomy in fish populations using a temperature dependent oocyte growth model. *J Sea Res* 96: 1–10. https://doi.org/10.1016/j.seares.2014.10.018