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Short communication

Gonadal development and spawning season of white mullet *Mugil curema* (Mugilidae) in a tropical bay

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Introduction

The white mullet *Mugil curema* Valenciennes, 1836 is a widespread coastal fish found in the western Atlantic from Massachusetts to southern Brazil. It is considered to be catadromous, and, as such, the juvenile fish are recruited to lagoons and estuaries following a period of offshore spawning (Ibáñez-Aguirre, 1993; Ditty and Shaw, 1996). The white mullet is an important economic resource supporting many small communities through both fishing and aquaculture (Alvarez-Lajonchere, 1982; Gómez and Cervigón, 1987).

The life cycle of *M. curema* has been well documented in temperate areas (Jacot, 1920; Anderson, 1957; Moore, 1974) and even in some subtropical and tropical countries, such as Venezuela (Angell, 1973; Alvarez-Lajonchere, 1976; Marin E et al., 2003), Mexico (Yáñez-Arancibia, 1976; Ibáñez-Aguirre, 1993), Cuba (Garcia and Bustamente, 1981; Alvarez-Lajonchere, 1982) and Trinidad and Tobago (Solomon and Ramnarine, 2007). In Brazil, such investigations are rare and superficial. Couto and Nascimento (1980) described their histological observations of the ovary from aquaculture specimens, and Benetti and Fagundes Netto (1991) studied growth rates under experimental conditions. To date, there have not been any histological observations of the seasonal variations in the gonadal cell development to determine the spawning season of *M. curema* from the Brazilian coast.

The aim of this study was to examine some key parameters related to reproduction in *M. curema* from a coastal bay in southeastern Brazil, focusing on gonadal development and the spawning season. In addition, an attempt was made to correlate the spawning season exhibited by this species with the hepatosomatic index and condition factor.

Materials and methods

Fresh specimens of *Mugil curema* (males: n = 36, 239–355 mm total length; females: n = 136, 230–445 mm total length) were collected from artisanal commercial catches from July 2006 to June 2007 in Sepetiba Bay (22°54′–23°04′S; 43°34′–44°10′W), an area of ca. 450 km^2 in Rio de Janeiro State, Brazil. The nets were 1500 m long, 3 m in depth and had three panels of different mesh sizes (35, 40 and 45 mm stretched mesh).

Individuals were collected each month. All fishes were packed in ice and transported to the laboratory. Total length (TL) to the nearest mm was measured. Total (TW) and eviscerated (EW) weight were measured to the nearest gram (g), and liver weight (LW) and gonad weight (GW) were determined to a precision of 0.01 g.

The gonadosomatic index ($GSI = GW \times EW^{-1} \times 100$) and frequency distribution of gonad maturation stages determined the gonadal cycle. Fish condition was established through the hepatosomatic index ($HSI = LW \times EW^{-1} \times 100$) and condition factor [$K = (EW \times TL^{-1})^3 \times 1000$].

The GSI, HSI and K were tested for eventual correlation by using the Spearman test (r_{sp}). Analysis of variance (one-way ANOVA) was used to determine whether seasonal changes in the means of GSI, HSI and K were significantly different ($P < 0.05$). Eviscerated weight was used in all calculations of indexes to avoid any influence of the contents of the gonad and stomach on the weights.

Gonads were preserved in 70% alcohol after 4 h in Bouin's solution. The stages of ovaries macroscopic maturity followed Diaz-Pardo and Hernandez-Vazquez (1980) and Ibáñez Aguirre and Gallardo-Cabello (2004). Small pieces of the gonads were taken from preserved samples and dehydrated by a graded ethanol series, imbibed in paraffin wax, cut in 5 μm sections and then stained with haematoxylin-eosin. The terminology used to describe the development of oocytes and testicular cells in histological sections followed West (1990) and Kanak and Tachihara (2008), with slight modification. By examining cross-sections of different parts of the gonads we determined that there were no regional differences in the course of gametogenesis. Measurements of cells of ovarian development were taken from the histological slides for approximately 300 cells of each type. For males, only gonadal microscopic development was examined in detail, due to the low number of males caught during the study.

Results

Ovarian tissue

Immature ovaries contained germ cells (oogonia) that were undergoing profound changes in their nuclear structure, cytoplasm and membranes. The oocyte development was classified into two stages: previtellogenic stage (young germ cells and peri-nucleolus oocytes from reserve stock) and vitellogenic stage (oocytes with lipid vitellogenesis, oocytes with lipid and protein vitellogenesis and post-vitellogenic stage).

Oogonia (Fig. 1a). Very small, < 10 μm in diameter, and spherical to slightly oval in shape. Cytoplasm is lightly dyed. There is a large and basophilic nucleus, usually with a single

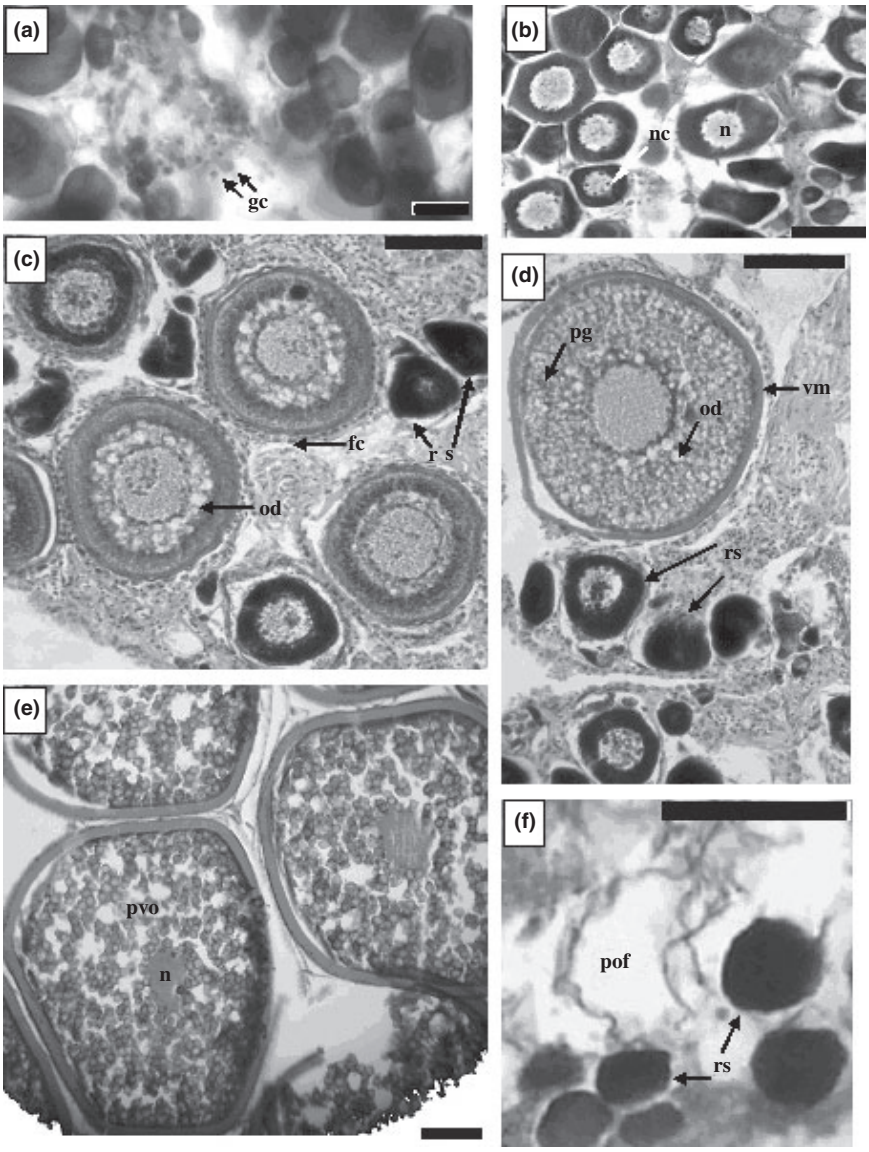

Fig. 1. Photographs of histologically observed ovary sections and different maturity stages of *Mugil curema* from Sepetiba Bay. (a) Immature ovary (I) containing germ cells, (b) Resting ovary (II) containing peri-nucleolus oocytes, (c) Developing ovary (III) containing lipid vitellogenesis oocytes, (d) Maturing ovary (IV) containing lipid and protein vitellogenesis, (e) Ripe / running ripe ovary (V) containing post-vitellogenic oocytes and (f) Recovering ovary (VI) containing post-ovulatory follicle. gc, germ cells; pn, peri-nucleolus stage; fc, follicular cells; n, nucleus; yg, yolk globule; nc, nucleolus; rs, reserve stock oocytes; od, oil droplet; pg, protein granules; vm, vitelline membrane; pvo, pos-vitellogenic oocytes; pof, post-ovulatory follicle. Scale bars, (a) = 10 μm, (b, c, d and e) = 50 μm, (f) = 100 μm

nucleolus. These cells are sited on the periphery of the ovarian lamellae, isolated or forming cysts and visible in immature (I) and recovering / resting (II) ovaries. Present throughout the reproductive cycle.

Peri-nucleolus stage (Fig. 1b,f). Spherical to multifaceted due pressure of the cells, and ranging from 10 to 120 μm (58 $\pm$ 37 μm; mean $\pm$ standard deviation) in diameter. Cytoplasm with strong affinity for haematoxylin. Nucleus more evident, ranging from less than 10 to 70 μm (36 $\pm$ 19.4 μm; mean $\pm$ standard deviation), with multiple nucleoli (5–15 nucleoli depending on cell size; nucleoli number seems to increase with cell size), generally peripheral and next to the nuclear membrane. Follicular layer present but difficult to observe. Oogonia and the peri-nucleolus stage are present in the ovaries throughout the entire annual cycle and represent the reserve stock for futures maturations.

Lipid vitellogenesis (Fig. 1c). Accumulation of lipid inclusions in the cytoplasm has begun. Yolk vesicles (oil droplets) appear in the cytoplasm beneath the cell membrane and surrounding the nucleus. Vesicles have increased progressively in both number and size. There is progressive loss of affinity for haematoxylin by the cytoplasm. Follicular layer and vitelline membrane are visible. The nucleus, ranging from 40 to 80 μm (56.4 $\pm$ 12.8 μm), becomes irregular and is still centrally located with nucleolus next to the membrane. Present in the ovary in developing (III) stages, ranging from 80 to 240 μm (162 $\pm$ 58 μm) in diameter.

Lipid and protein vitellogenesis (Fig. 1d). Yolk granules (acidophilic protein granules) are present. Oil droplet formed by fusion of lipid inclusions. Follicular layer and vitelline membrane are perfectly visible. The latter is stained with eosin and consists of two layers: an internal light-pink layer and an external darker layer. The nucleus is similar, in the last stage ranging from 50 to 90 μm (69.4 $\pm$ 13.4 μm). Present in ovary in maturing (IV) stages and ranging from 130 to 350 μm (248 $\pm$ 75 μm) in diameter.

Post-vitellogenic stage (Fig. 1e). Oocytes greatly increased in diameter, ranging from 340 to 700 μm (520 $\pm$ 127 μm). The nucleus is still present, ranging from 70 to 100 μm (83.4 $\pm$ 9.7 μm), and the fusion of yolk granules (protein)

and lipid droplets occurs to varying degrees; however, no single large complete yolk mass can be observed. The thickness of the vitelline membrane is greatest at this stage, ranging from 20 to 25 μm. Oocytes appear to be arrested at this stage and are present in ripe / running ripe (V) stages.

Testicular tissue

The testes are involved with the tunica albuginea, which also involved the seminiferous tubules. Internal to the seminiferous tubules are Sertoli cells that surround the cysts formed by spermatogenesis cells, which are all smaller than 10 μm (spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa). Functionally, the testes are divided into three stages according to cellular type: immature, maturing and functional maturation.

Immature (Fig. 2a). Appearance of spermatogonia, the largest cells of the spermatogenic lineage, generally associated with the tunica albuginea, with voluminous nucleus containing scattered chromatin and several nucleoli.

Maturation (Fig. 2b,c). All stages of cell development are present after mitotic divisions from spermatogonia. Spermatocytes are smaller than spermatogonia. Primary spermatocytes can form cysts by mitotic division, and the nucleus is strongly stained with haematoxylin. The cytoplasm has little affinity for dyes. Secondary spermatocytes are somewhat smaller, with a nucleus that stains weakly. Spermatids are even smaller than secondary spermatocytes, and their nuclei have denser chromatin.

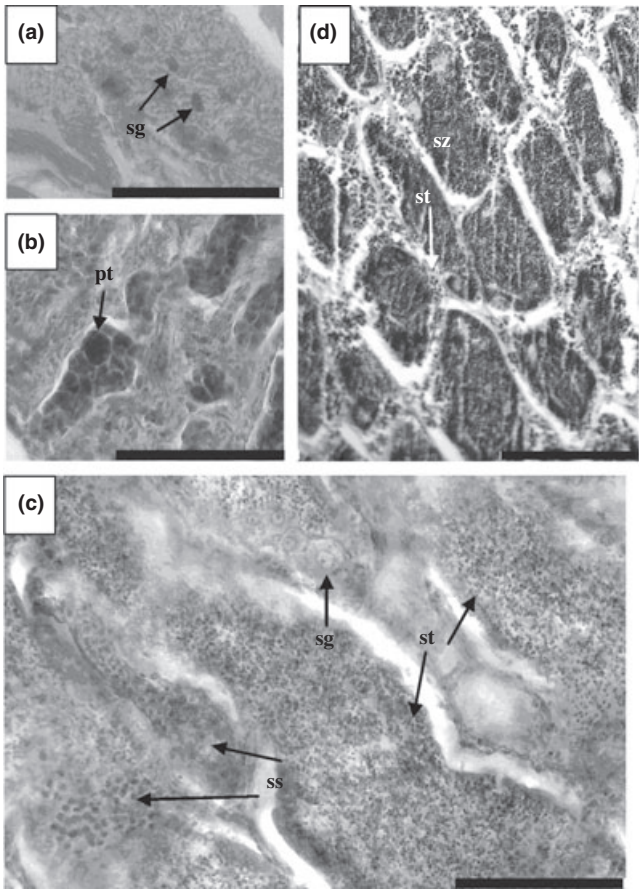

Fig. 2. Photographs of histologically observed testes sections and different maturity stages of *Mugil curema* from Sepetiba Bay. (a) Immature testis containing spermatogonia, (b) arrangement of primary spermatocytes (maturation stage), (c) arrangement of different testicular cells (maturation stage), and (d) spreading pattern of spermatozoa in a functional maturity stage; spermatids also present in small quantities. sg, spermatogonia; ps, primary spermatocytes; ss, secondary spermatocytes; st, spermatids; and sz, spermatozoa. Scale bars, (a, b and c) = 25 μm and (d) = 50 μm

Functional maturity (Fig. 2d). Tubules full of spermatozoa which begins to accumulate in deferent ducts. Spermatids are more visible next to the wall of the tubules, although all cellular types are present.

Ripe ovaries (V) were observed between August and January. The only sign of a spent ovary (VI) was recorded in November. Immature (I) and recovering / resting (II) ovaries with peri-nucleolus stage oocytes were recorded between December and January and from November to June, respectively. Developing ovaries (III) with yolk vesicle oocytes were found throughout the study period; the highest percentages were observed in April and May. Maturing ovaries (IV) with lipid and protein vitellogenesis were observed between July and January, with the highest percentages in September and October (Fig. 3).

The mean GSI from examined females showed seasonal differences during the study period ($F = 8.56$; $P < 0.01$; Fig. 4). The lowest GSI were recorded between February and June; these values then increased in July and August, peaked in October and then dropped sharply in November, December and January. The mean HSI and K from examined females also showed seasonal differences during the study ($F = 11.63$; $P < 0.01$; $F = 3.73$; $P < 0.01$, respectively) (Fig. 4). From May onwards, the HSI gradually increased until reaching a peak in August, then decreased from September to February, when the lowest value was found. The mean K did not show a well defined seasonal pattern of variation, shifting throughout the study period. A trend was, however, observed: values were low between July and December; they increased to a peak in March and then decreased in the following months. A significant positive relationship was detected between GSI and HSI ($r_{sp} = 0.71$; $P < 0.05$), but no significant correlation was found between GSI and K ($r_{sp} = -0.13$; $P > 0.05$) or HSI and K ($r_{sp} = 0.02$; $P > 0.05$).

Discussion

Histological analyses indicated that the white mullet exhibits synchronous group oocyte development, in that at least two populations (clutches) of oocytes can be distinguished in the

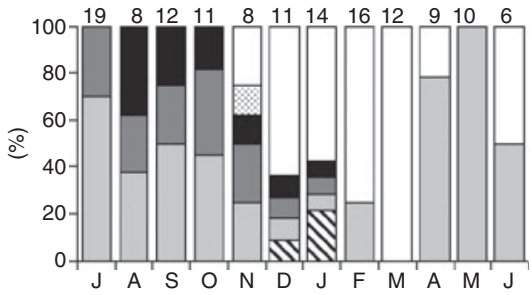

Fig. 3. Monthly changes in the percent of maturity stages of female *Mugil curema* from Sepetiba Bay. Samples sizes are given above bars. I (▨); II (□); III (▢); IV (▩); V (■); VI

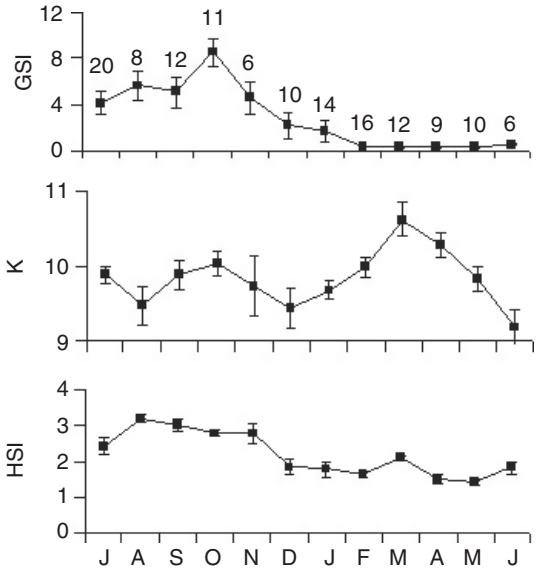

Fig. 4. Temporal variation (mean ± standard error) in gonadosomatic index (GSI), condition factor (K) and hepatosomatic index (HSI) of female *Mugil curema* from Sepetiba Bay. Sample sizes are given above means

ovary at the same time during the reproductive cycle (Wallace and Selman, 1981). In *M. curema*, two clutches of oocytes were present in the ripe ovary: peri-nucleolus stage oocytes and post-vitellogenic oocytes, suggesting total spawning. These patterns may not agree with the findings of Solomon and Ramnarine (2007), who reported that three clutches of oocytes were present in the *M. curema* ovary prior to spawning: primary oocytes, yolk vesicle oocytes and vitellogenic or post-vitellogenic oocytes. Differences in ovary development in *M. curema* of the Caribbean (Solomon and Ramnarine, 2007) and southwestern Atlantic (this study) could be explained by the fact that this species is present in these two areas in completely isolated populations. Furthermore, the sampled area (inner zone of Sepetiba Bay) may be non-spawning grounds, where only non-spawning fish occur.

This work showed a long reproductive period for *M. curema* of approx. 6 months (August–January) with a clear peak in October, based on GSI, macroscopic observation and histological analyses. Reproductive periodicity in white mullet varies with geographic distribution. In temperate areas, several works have shown that *M. curema* has a wide reproductive period, ranging basically from April to August, with peaks in spring (Jacot, 1920; Anderson, 1957; Moore, 1974). Ibáñez-Aguirre (1993), studying the coexistence of *M. cephalus* (Linnaeus, 1758) and *M. curema* in the Golf of Mexico, established that white mullet spawning occurs from January to June. In tropical areas, there is no agreement on the reproductive season, with spawning time varying according to the study area. One wide, reproductive period has been reported from November to July by Solomon and Ramnarine (2007) and from March to August by Maia and Vilela do Nascimento (1980) for northeastern Brazil. On the other hand, there is a short spawning season from July and September (Angell, 1973) in Venezuelan waters. Spawning seasons have been reported from Cuba for the white mullet, June to August, November to January (Alvarez-Lajonchere, 1976) and June / July and September / August (Alvarez-Lajonchere, 1980). Despite the range of reproductive periods in different geographic regions, the white mullet spawns mainly from late winter to summer, peaking in spring, as in this study.

HSI and K have been used to assess fish condition and to relate this condition to reproduction. Several authors have used these parameters, coupled with GSI, to assess the reproductive period (Ibáñez-Aguirre and Gallardo-Cabello, 2004; Kanak and Tachihara, 2008), possibly because vitellogenesis and gametogenesis mobilize hepatic energy and body fat (Kanak and Tachihara, 2008). In this study, K was not closely associated with GSI or HSI. This result could suggest that reproduction does not influence fish condition (muscular condition), which here was calculated according to the eviscerated weight of the individual fish.

The hepatosomatic index was found to be positively associated with GSI, indicating that the liver increases in mass during the reproductive season. Increasing hepatocyte number and size is linked to vitellogenesis, as the provisioning of eggs with yolk takes place in the ovaries but the precursors of the yolk are synthesized in the liver (Wootton, 1990). Fish in the pre-spawning period (June and July) have a high concentration of visceral fat (not measured). This phenomenon suggests that visceral fat bodies are likely to be mobilized in early winter for the purpose of reproduction. Kanak and Tachihara (2008) reported that decreasing visceral fat is associated with vitellogenesis in females of *Gerres oyena* (Forsskål, 1775). In conclusion, we suggest that the relationship between HSI and GSI shows that these two indexes may be correlated to predict the reproductive period of *M. curema* in Sepetiba Bay. Also, a lipidosomatic index (LSI = Fat Weight $\times$ EW^{-1}) could describe the relationship between energy depletion and vitellogenesis better than the condition factor (K).

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