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A N I N T E R N A T I O N A L J O U R N A L

Validation of an Ovarian Biopsy Method for Monitoring Oocyte Development in the Fat Snook, *Centropomus parallelus* Poey, 1860 in Captivity

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ABSTRACT

The validation of an ovarian biopsy method for in vivo assessment of oocyte maturation in Centropomus parallelus was studied. Diameters of intra-ovarian oocytes siphoned with cannula were analyzed fresh and preserved with 1% formalin in 0.7% NaCl solution. Oocytes in different stages were present along the ovaries, up to the tertiary yolk globule stage, which had a unimodal diameter frequency distribution. The oocyte diameter means were not significantly different at four sites along the ovaries ($P > 0.05$). Samples obtained with cannula were representative of the ovary central portion, in vivo and in vitro samples of the seven females examined were not significantly different ($P > 0.05$). An estimate of the coefficient of variation corrected for bias ($P < 0.05$) for 8 repeated in vivo samples was 1.9 ± 0.6. The results demonstrated that for the species, the biopsy method was satisfactory, providing representative samples of the ovaries.

Key words: Ovarian Biopsy, Fat Snook, *Centropomus parallelus*

INTRODUCTION

Indo-Pacific and American species of the family Centropomidae, mainly the barramundi *Lates calcarifer* and the snook *Centropomus spp*. respectively, are valuable game and commercial fishes (Tucker, 1987; Barlow et al., 1993). The commercial culture of *L. calcarifer* is well established, with annual production based on reliable technologies for mass production of juveniles (National Institute of Coastal Aquaculture, 1986; Dhert et al., 1992). However, although *Centropomus spp*., shows good potential for culture (Tucker, 1987), production of juveniles is still at an experimental level (Edwards and Henderson, 1987; Tucker, 1987; Amador del Angel and Cabrera Rodriguez, 1994).

Spawning induction trials with the fat snook *Centropomus parallelus* Poey have been done in Santa Catarina (Brazil) (Lat. 27º 37.5'S and Long. 48º 27.0'W) by Cerqueira (1995) and Cerqueira et al. (1995). In order to assess sexual development in females, before spawning induction treatments, it is important to select individuals with the highest

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probabilities of positive results, and to determine correct hormonal dosages. In previous spawning experiments with fat snook, intra-ovarian oocytes were taken with a catheter to estimate their diameter, based on studies done on other species (Shehadeh et al., 1973; Garcia, 1989-a). The aim of the present study was to validate this biopsy method for the assessment of intra-ovarian oocyte development of fat snook spawners.

MATERIALS AND METHODS

The study was done in March 1998, during the natural breeding season for fat snook *C. parallelus* in Florianópolis, Santa Catarina (Brazil). Females were collected from a captive broodstock held in floating cages within an undrainable tide-pond containing brackish water (Lagoa da Conceição). The biopsies were taken during the morning (09.00-10.00 hours) from females anesthetized with benzocaine (50 ppm), using a 0.8-mm diameter polyethylene cannula inserted through the oviduct to approximately the central portion of one of the ovaries. The intra-ovarian oocyte samples were drawn by suctioning while the cannula was slowly withdrawn and then either measured immediately or preserved in 10-mL vials with a solution of 1% formalin in 0.9% NaCl (Shehadeh et al., 1973).

The oocytes were examined on a 60 mm-diameter Petri dish, under stereomicroscope and their diameter individually measured with an ocular micrometer to the smallest division, which measured 25 µm. Particular attention was given to those oocytes in the tertiary yolk globule stage with an opaque appearance. The sample size for the diameter measurements was estimated with the iterative procedure described by Sokal and Rolf (1981). The effect of fixation on the oocyte diameter was analyzed by measuring the diameter of 100 individual fresh oocytes, and further measuring them again 1, 2, 4, and 24h after they were placed in the preservative solution.

The external morphology of the ovaries of this species is peculiar, because the oviduct of a sexually mature female is located approximately at the posterior forth portion of the ovary length. Taking this into consideration, it was determined if there were differences in oocyte development across the length of the ovary. Seven females were sacrificed and from one ovary of each, oocyte samples were taken from four sites: (1) near the cloacal opening, (2) on the anterior end, (3) on the central portion, and (4) on the posterior end.

Paired samples were taken from the central portion of the ovary of seven females, one *in vivo* with the cannula and one *in vitro* from the central portion of one ovary after each female was sacrificed, to determine if the *in vivo* oocyte samples were representative of the ovaries.

The precision of the oocyte mean diameter estimates was calculated by the coefficient of variation corrected for bias with the confidence limits (Sokal and Rolf, 1981) of 8 *in vivo* samples obtained with a cannula from one female. Statistical differences between mean diameters were calculated by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test, and Student's t-test in the case of the paired *in vivo* and *in vitro* samples at $P = 0.05$, after the homogeneity of variances was demonstrated by Bartlett's test (Sokal and Rolf, 1981).

RESULTS

Females examined, 375 - 410 mm in total length and 547 - 771 g in total weight, had oocytes in different prematuration stages in the four ovary sites studied. Those in the tertiary yolk-globule stage had a unimodal frequency distribution of their diameter. A sample size of 50 oocytes was estimated by the iterative procedure for the mean diameter to be 90% certain of detecting a 5% difference between two means of the four sites of the ovaries at the 1% level of significance with a previous coefficient of variation estimate of 6 %. There were no detected effects of the preservative solution on the oocyte diameter ($P > 0.05$) between fresh and those preserved over the 24-h interval of the test (Table 1).

Mean oocyte diameters from four sites on the ovaries of six females were not significantly different ($P > 0.05$). However, in female number IV (Fig. 1) the mean oocyte diameter from the rear portion of the ovary (site 4) was smaller than from the other sites ($P < 0.05$). Comparisons of mean oocyte diameters between *in vivo* and *in vitro* paired samples from the central portion of the ovaries of seven females (Table 2) showed no

statistically significant differences (P > 0.05). The estimate of the coefficient of variation corrected for bias (V*) with its confidence limits for the 8 means of repeated *in vivo* samples from one female was:

$$V^* \pm t_{0.05[7]} S_{V^*} = 1.9 \pm 0.6.$$

Table 1 - Oocyte mean diameters (n=100) ± standard error of the mean (SEM) from fresh and fixed (1% formalin in 0.9% NaCl solution) samples, and Student *t*-test comparisons with 0 h (*P* = 0.05) of biopsied snook *Centropomus parallelus*.

Fixation period (h)	Mean diameter ± SEM (µm)	$t_{0.05}$	Statistics
0 (Fresh)	379 ± 5	-	-
1	381 ± 6	0.14	NS
2	385 ± 4	0.29	NS
4	384 ± 5	0.24	NS
24	382 ± 4	0.21	NS

Table 2 - Oocyte diameter-frequency distributions of *in vivo* (a) and *in vitro* (b) paired samples, mean ± standard error of the mean (SEM) of oocytes, and Student *t*-test comparisons of paired means (*P* = 0.05) from each of seven snook *Centropomus parallelus* females.

Sample number	Oocyte diameters (µm)									
	325	350	375	400	425	450	Mean ± SEM	N	$t_{0.05}$	Statistics
1a		14	40	27	4		379 ± 5	85	1.72	NS
1b	1	19	38	39	7	3	385 ± 5	107		
2a	1	8	19	14	7		384 ± 7	49	1.01	NS
2b		6	16	23	5		389 ± 6	50		
3a	1	2	12	20	14	1	398 ± 7	50	0.20	NS
3b	2	2	10	23	13	1	397 ± 7	51		
4a	6	6	18	18	6	1	382 ± 8	55	0.84	NS
4b	1	4	22	18	5		386 ± 6	50		
5a	1	1	15	20	11	1	396 ± 6	50	0.53	NS
5b	3	2	3	31	10	2	399 ± 7	51		
6a	4	2	7	19	16	2	399 ±7	50	0.02	NS
6b	1	5	10	17	14	3	398 ± 8	50		
7a	4	11	12	13	9	1	383 ± 6	50	0.26	NS
7b	3	5	25	11	6		381 ± 7	50		

DISCUSSION

The preservative solution did not affect the oocyte diameters within 24 h. The same was observed in other species (Shehadeh et al., 1973; Alvarez-Lajonchère et al., 1983, 2001; Tamaru et al., 1988), although Garcia (1989-a) reported an increase in *L. calcarifer* oocyte diameters after one hour in a buffered 5% formalin solution. Ovarian biopsy methods have been validated on synchronous as well as asynchronous oocyte-development species (Shehadeh et al., 1973, Markmann and Doroshov, 1983; Alvarez-Lajonchère et al., 1983, 2001; Rodriguez and Garzo, 1986).

The observation of multiple oocyte developmental stages in the present study was the characteristic of asynchronous oogenesis of batch spawners, in agreement with reports on other Centropomids (Alvarez-Lajonchère et al., 1982, 2001; Garcia, 1989-b). For successful induced spawning in batch spawners, hormonal treatments should stimulate final maturational changes in the most advanced oocytes, which must be at late vitellogenic or at postvitellogenic stages, still opaque and non-hydrated. In this study, hydrated oocytes were never observed. In fact, this stage has been observed in fat snook only with hormone induced females (Cerqueira, 1995; Cerqueira et al., 1995).

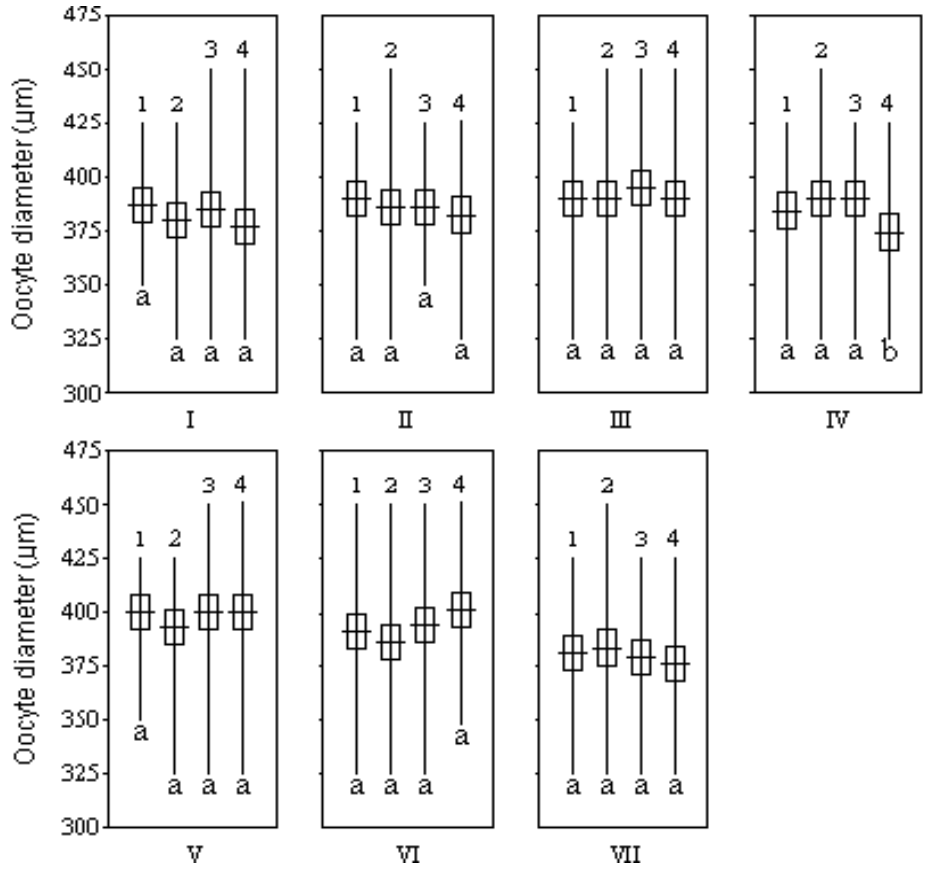

Figure 1 - Oocytes measurements (mean oocyte diameter ± standard error of 50 oocytes mean at $P = 0.05$, and data range) from four ovary sites (1 to 4) in seven snook *Centropomus parallelus* females (I to VII). Different letters (a and b) correspond to significant difference at $P < 0.05$.

Hormonal treatments for induced spawning of *C. parallelus* were applied to females with mean oocyte diameter of at least 390 µm (Cerqueira, 1995), which were within the 95% confidence intervals of estimated means of 67% of the females in the present study. Tucker and Campbell (1988) reported a mean diameter for sectioned yolked oocytes of *C. undecimalis* in the range of the vitellogenic or postvitellogenic opaque oocytes found in the present study. Wallace et al. (1993) reported that to induce final maturation in *C. undecimalis* fresh oocytes should have diameters larger than 0.5 mm.

Based on the present data, to further improve the established biopsy method and effectively select fat snook females for spawning induction treatments, complementary studies are recommended with an *in vitro* oocyte maturational competence test (Greeley et al., 1987; Patiño and Thomas, 1990; Wallace et al., 1993) to estimate the minimum oocyte diameter that would positively respond to an acute hormonal treatment.

The *in vivo* method for monitoring ovarian sexual development applied to *C. parallelus* in the present study fulfilled the conditions required in species with asynchronous oocyte development. Oocyte samples obtained with a polyethylene cannula from the central portion of the ovaries exhibited the same proportions between developmental stages, when compared with the different ovary sites, and

specially the same characteristics of the most advanced oocyte group (late vitellogenic and postvitellogenic).

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RESUMO

A validação de um método de biópsia ovariana para determinação *in vivo* da maturação ovocitária em *Centropomus parallelus* foi descrita. Os diâmetros de ovócitos, obtidos de amostras intra-ovarianas sifonadas por cânula, foram analisados a fresco e preservados com formalina (1%) em solução de NaCl (0,7%). Ovócitos em diferentes estádios de maturação estavam presentes ao longo dos ovários, até o estádio de vitelogênese completa, apresentando uma distribuição de freqüência de diâmetros unimodal. O diâmetro médio dos ovócitos não diferiu significativamente entre as quatro regiões dos ovários ($P > 0,05$). Amostras obtidas com a cânula são representativas da porção central do ovário, uma vez que as amostras *in vivo* e *in vitro* das sete fêmeas examinadas não foram significativamente diferentes ($P > 0,05$). Uma estimativa do coeficiente de variação corrigido para "bias" ($P < 0,05$) para oito amostras repetidas *in vivo* foi de $1,9 \pm 0,6$. Os resultados demonstraram que para esta espécie, este método de biópsia é satisfatório, provendo amostras representativas dos ovários.

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