Oocyte distribution within and between ovary lobes is largely homogeneous in *Lachnolaimus maximus* (Perciformes: Labridae)

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Abstract: In fish reproduction, previous information of ovary oocyte distribution is necessary, when oocytes quantitative estimates are required to estimate batch or annual fecundity. Heterogeneous oocyte distribution requires a standardized sampling protocol to prevent bias in estimates, whereas homogeneous distribution, allows sampling of any portion of gonads with no risk of bias. We studied gonad oocyte distribution patterns in the hogfish *Lachnolaimus maximus* population from Southern Gulf of Mexico. For this, 23 mature females were selected from a total of 47 individuals exhibiting visible oocytes in the ovaries. These females were classified by reproductive phase and sub-phase (early developing-ED, developing-D, spawning capable-SC and actively spawning-AS). Six histological sections were taken from the anterior, middle and posterior regions of the left and right ovary lobes of each individual. Digital image processing (AxioVision and Image ProPlus programs) was used to estimate oocyte density per unit area, and for different development stages. Contingency tables were used to analyze oocyte distribution frequencies between the regions of each lobe, and between the lobes of each ovary. This was supported with a Pearson's χ^2 test for goodness-of-fit and a replicated G test to confirm distribution heterogeneity. Oocyte stage distribution was homogeneous in almost all 23 females regardless of the ovary lobe. In the left ovary lobe, oocyte distribution was uniform in all three regions sampled regardless the female phase or sub-phase. In the right ovary lobe, oocyte frequencies were similarly uniform for the ED, D and AS phase and sub-phases; nevertheless, during SC phase, some heterogeneity was observed in tertiary vitellogenesis-Vtg3 oocytes, especially in samples from middle and posterior regions of this lobe. Females in AS sub-phase are normally used to estimate batch fecundity in fish such as *L. maximus*, which has shown to have asynchronous oocyte development and batch spawning. Given the homogeneous oocyte distribution pattern within and between the ovary lobes in females in AS sub-phase, no systematization is required of the gonad histological sampling protocol to estimate species batch fecundity. Nevertheless, due to the heterogeneous Vtg3 oocytes distribution in the right ovary lobe of females in the SC phase, it is best to systematically take sections of any region in the left ovary lobe when conducting a study encompassing all of a species' reproductive aspects. Rev. Biol. Trop. 65 (1): 293-303. Epub 2017 March 01.

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Reproduction has direct implications in the exploitation, management and cultivation of fishes (Wootton & Smith, 2015). Fish reproduction studies involving microscopic observation of gonads require preliminary analyses of oocyte distribution homogeneity in females at different ovary development stages. During maturation, fish ovaries undergo voluminous development. For practical reasons, histological observations of large ovaries are normally done using randomly sampled areas within a section of one of the ovary's two lobes. Quantitative

analysis of the structure of any biological tissue using histological sections requires basic data on cell type distribution in the studied organ. If distribution of the different oocytes stages is homogeneous within the gonad during a fish species' reproductive season, samples can be collected from any region of either lobes, and will be representative of the female´s degree of maturity (e.g. Ma, Kjesbu, & Jirgensen, 1998; Witthames et al., 2009; McElroy, Wuenschel, Press, Towle, & McBride, 2013). On the other hand, if distribution among the different regions of a lobe or between the two lobes is heterogeneous, a systematic protocol is required for collecting samples for histological analysis (Witthames & Greer Walker, 1995; Nichol & Acuna, 2001; Kennedy, Witthames, & Nash, 2007; Hainfellner, de Souza, Nascimento, Freitas, & Batlouni, 2011). Generally, a lack of a standardized ovary sample collection method, may create significant bias in data for fecundity estimations (Murua et al., 2003).

A number of fish reproduction studies have addressed oocyte distribution pattern in the ovary. Most of these studies have focused on a species' reproductive biology, defining spawning season, and characterizing sexual differentiation and gonad development. To identify the homogeneity degree in ovary oogenesis, analyses have been commonly done along three regions (anterior, middle and posterior) of each ovary lobe (right and left). Generally, three parameters have been used in most of these studies: average oocyte diameter, oocyte density per unit of weight; and oocyte density per unit of ovary tissue surface. Studies have addressed females in different maturation phases and all oocyte stages present in the ovary (Tricas & Hiramoto, 1989; Lee, Liu, Su, & Wu, 2005; Weng, Liu, Lee, & Tsai, 2005; Hainfellner et al., 2011; Liao & Chang, 2011, Wu, Su, Liu, Weng, & Wu, 2012), as well as only vitellogenic or mature oocytes (Witthames & Greer Walker, 1995; Ma et al., 1998; Nichol & Acuna, 2001; McElroy et al., 2013). Some do not include exact data on female maturation phases and/or the oocyte stages analyzed (Matić-Skoko, Kraljević, & Dulčić,

2004; Kennedy et al., 2007; Reñones, Grau, Mas, Riera, & Saborido-Rey, 2010; Sequeira, Neves, Paiva, Vieira, & Gordo, 2012; Yang, Chen, & Hu, 2013).

Gonadal development studies have focused in standard histological methods, and very few studies have used digital image analysis, to calculate average oocyte diameter and estimate oocyte density (Nichol & Acuna, 2001; McElroy et al., 2013); although this type of analysis has been in use in the biological sciences for over a decade (Klibansky & Juanes, 2008). The application of digital image processing techniques may generate data on structure from images normally intended to improve image quality and in the search of data (Pertusa, 2003). They can also be used to count and measure cells or other small particles in an image, and to make precise measurements; besides, the use of these techniques has reduced the processing time, health risks and costs of standard histological methods.

Hogfish *Lachnolaimus maximus* (Walbaum, 1792) is a labrid fish species distributed from the mid-Atlantic seaboard of the United States (North Carolina) to the North coast of South America (Venezuela and Guyana), including the Bermudas, the Gulf of Mexico and the Caribbean Sea (Carpenter, 2002). It is important in commercial and recreational fisheries throughout its range, and presently, the IUCN (2016) has considered the species to be at high risk of extinction in the wild (Vulnerable A2bd). Growth and recruitment stage overfishing has been reported in a *L. maximus* population in South Florida (US) (McBride & Murphy, 2003; McBride & Richardson, 2007; McBride, Thurman, & Bullock, 2008). The *L. maximus* population in Southern Gulf of Mexico, on the continental shelf of the Yucatan Peninsula (known as Campeche Bank), is considered a high value alternative target fishing resource. To date, no studies have been done on the biology, exploitation level and conservation status of *L. maximus* population in Campeche Bank.

The reproductive biology of this species' has been studied in populations of Puerto Rico

(Colin, 1982), Cuba (Claro, García-Cagide, & Fernández de Alaiza, 1989), North Carolina (Parker, 2000), the Northeast Gulf of Mexico, and South Florida (Davis, 1976; McBride, Johnson, Bullock, & Stengard, 2001; Robinson & Prince, 2003; McBride & Johnson, 2007; McBride et al., 2008; Muñoz, Burton, Brennan, & Parker, 2010; Collins & McBride, 2015). Of these studies, six have included histological analysis of gonads to identify species sexuality (protogynous hermaphrodite) and reproductive cycle, and to estimate fecundity. Only two (Davis, 1976; McBride & Johnson, 2007) have addressed spatial development of oocytes in the ovaries. Neither of these reported studies have evidence of heterogeneous oocyte development between lobes or within sections of the same lobe. But Davis (1976) only analyzed two females, while McBride and Johnson (2007) made a cursory examination of a small number of slides (3 locations) from a limited number of females (n= 14) to ensure that no clear difference in oocyte distribution patterns occurred between locations (McBride, personal communication, May 19, 2016).

The objective of the present study was to characterize oocyte distribution patterns in different developmental stages and different sections of the ovary of *L. maximus,* by the use digital images taken from histological sections of female gonads, in different development stages. The resulting oocyte distribution patterns will determine the sampling protocol for histological sampling from gonads of this species, thus providing more accuracy to analyses of main reproductive parameters (i.e. maturity, reproductive cycle and fecundity) of *L. maximus* in Campeche Bank.

MATERIALS AND METHODS

Specimen collection: The data analyzed here were collected as part of a project studying *L. maximus* reproduction in Campeche Bank. Specimens were collected between May 2013 and January 2014 in three coastal marine zones, corresponding to areas reported to have substantial abundance of *L. maximus* along the coast of the state of Yucatán (Mexicano-Cíntora, Leonce-Valencia, Salas, & Vega-Cendejas, 2007): Celestún (west zone: 20°51'33'' N - 90°24'0'' W); Dzilam de Bravo (central zone: 21°23'33'' N - 88°53'29'' W); and Río Lagartos (East zone: 21°35'51'' N - 88°9'28'' W). Specimens were caught between six and 22 m depth by professional fishers using harpoons and compressor diving gears. Sex identification was done based on sexual dimorphism traits described by Davis (1976), Claro et al. (1989) and McBride and Johnson (2007). For each specimen we recorded fork length (FL; nearest 0.1 cm) with a graduated ictiometer and whole weight (WW; with an electric scale Sartorius model TE2101 with a 2 100 (± 0.1) g capacity). A total of 850 specimens were collected, of which 646 were female (size range: 13.9-39.4 cm FL; 135-1 132 g WW) and 204 were male (26.3-47.4 cm FL; 344-1 885 g WW). Based on previous data on its reproductive cycle of populations in Florida and Cuba (Davis, 1976; Claro et al., 1989; McBride et al., 2008; Collins & McBride, 2015), the nine-month collection period considered, included the probable maturation season of *L. maximus* in Campeche Bank.

Female selection and ovary histology: From the total sample of females, a preselection was made of those exhibiting visible oocytes in the ovaries (N = 47; 20.2-35.3 cm FL; 171-939 g WW), taking care to identify mature adult individuals with secondary growth oocytes. Six samples were extracted from each preselected ovary for histological analysis: one from the anterior, middle and posterior sections of the left and right ovarian lobes. These were fixed in Bouin's solution for four to five days, embedded in paraplast, sectioned at a 6 µm thickness (4 to five sections per sampled region), and stained with Gabe and Martoja's one-step trichrome stain (Gabe, 1968). Based on the histological analyses, the preselected females were classified by their gonad development phase into one of the following reproductive phases or sub-phases (Brown-Peterson, Wyansky, Saborido-Rey,

Macewicz, & Lowerre-Barbieri, 2011): early developing (ED), developing (D), spawning capable (SC) and actively spawning (AS).

The histological sections of only 23 females (20.3-34.0 cm FL; 191-814 g WW) were of sufficient quality (i.e. sections with no broken or incomplete oocyte and adequate color contrast) to allow digital image processing.

Image analysis: Digital images were taken from three microscopic fields (each field = 3.8 mm^2) of each ovary region section using a camera (Axiocam MRc) attached to a microscope (Axioscop; 25X). Each image was analyzed with the Image-Pro Plus 6.0 program (Media Cybernetics, Inc.).

Before beginning the oocyte count, all oocytes (that did not appear complete in the right and upper portions of each field) were excluded to prevent recounting of the same oocyte. Each image was processed with spectral analysis and a binary mask generated within which the selected oocytes were counted. Oocyte stages were identified based on the criteria developed by Wallace and Selman (1981), Brown-Peterson, Thomas and Arnold (1988) and Lowerre-Barbieri et al. (2009): primary growth (PG) oocyte; cortical alveolar (CA) oocyte; primary, secondary and tertiary vitellogenic (Vtg1, 2 and 3, respectively) oocytes; and oocyte maturation (OM). The oocyte maturation stage included: germinal vesicle migration, germinal vesicle breakdown, yolk coalescence, and hydration events.

Contingency tables (rows x columns) were applied to each group of females which had been classified into the four reproductive phases or sub-phases to analyze the frequency distributions of oocyte stage (columns) at two levels (rows): 1) anterior, middle and posterior regions of each ovary lobe, and 2) right and left lobes of the ovary. A Pearson's Chi-squared (χ^2) was used to determine if oocyte frequencies were uniform between rows and columns. When distributions were not uniform, a replicated goodness-of-fit test (heterogeneity G-test) (Sokal & Rohlf, 1995) was applied to identify which rows differed in terms of

oocyte frequency between columns. All statistical analyses ($\alpha = 0.05$) were run with the InfoStat program (Di Rienzo et al., 2013).

RESULTS

Following the degree of ovary development, the 23 selected females analyzed were classified in four reproductive phases or sub-phases: eight were in the ED sub-phase; four in the D phase; five in the SC phase; and six in the AS sub-phase (Fig. 1). The latter did not exhibit newly collapsed post-ovulatory follicles (POFs) in the ovary. The different oocyte stages characteristic of each female reproductive phases and sub-phases were observed in all the examined ovary sections and lobes (Table 1). In addition, the oocyte density typical of each phase and sub-phase was generally very similar between ovary regions and lobes; the one exception being Vtg3 oocytes in the right ovary of SC phase females.

The oocyte frequency analysis showed that the oocyte distribution was uniform in all three regions of the left lobe (χ^2, 0.1423 ≤ P ≤ 0.3858), no matter the phase or sub-phase (Table 2). In the right lobe, oocyte frequencies were also uniform in females at the ED sub-phase (χ^2, P = 0.2683), the D phase (χ^2, P = 0.4006) and the AS sub-phase (χ^2, P = 0.6852). However, females in the SC phase exhibited significant differences in oocyte frequencies in this lobe (χ^2, P = 0.0146). This discrepancy in frequencies in the SC phase females was confirmed by the G test (P = 0.0086; Table 3). Significant differences were present between any combination of the three regions in the right lobe of these females, the highest being between the middle and posterior lobes. This difference was probably caused by variability in Vtg3 oocyte frequency between the three regions of the right lobe (Table 1). The SC phase females analyzed here were likely just entering that phase. Nonetheless, if this oocyte category is excluded from the statistical analysis, distribution of all the other oocyte categories (PG, CA, Vtg1 and 2 oocytes) was homogeneous throughout the three regions
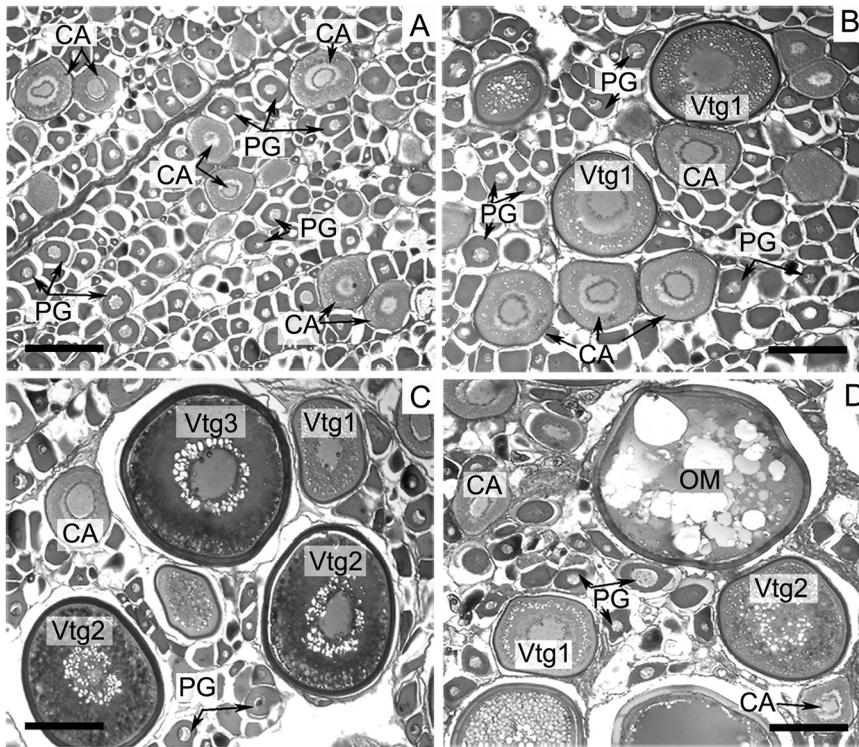
Fig. 1. Histological sections of ovaries from *Lachnolaimus maximus* collected on Campeche Bank (Gabe and Martoja's one-step trichrome stain; scale bar = 200 microns). **A)** female in early developing reproductive sub-phase (30.6 cm FL), collected 16 Oct. 2013; **B)** female in developing reproductive phase (31.2 cm FL), collected 16 Oct. 2013; **C)** female in spawning capable reproductive sub-phase (27.8 cm FL), collected 15 Oct. 2013; and **D)** female in actively spawning reproductive sub-phase (20.6 cm FL), collected 22 May 2013. CA = cortical alveolar oocyte; PG = primary growth oocyte; OM = oocyte maturation (germinal vesicle breakdown and yolk coalescence); Vtg1 = primary vitellogenic oocyte; Vtg2 = secondary vitellogenic oocyte; Vtg3 = tertiary vitellogenic oocyte.

TABLE 1

Oocyte density (number per unit area) frequencies observed in anterior, middle and posterior regions of left and right ovary lobes of *Lachnolaimus maximus* females in different reproductive phases and sub-phases

Female reproductive phase	Ovary		Total oocyte counts						Total area analyzed [b] (mm²)
	Lobe	Region	PG	CA	Vtg1	Vtg2	Vtg3	OM	
Early developing[a] (n = 8)	Left	A	1 833	321	-	-	-	-	91.2
		M	1 918	302	-	-	-	-	
		P	1 815	336	-	-	-	-	
	Right	A	1 913	316	-	-	-	-	
		M	1 833	283	-	-	-	-	
		P	1 774	316	-	-	-	-	
Developing (n = 4)	Left	A	695	142	81	-	-	-	45.6
		M	728	146	81	-	-	-	
		P	816	154	63	-	-	-	
	Right	A	766	129	94	-	-	-	
		M	729	150	92	-	-	-	
		P	788	127	96	-	-	-	

TABLE 1 (Continued)

| Female reproductive phase | Ovary | | Total oocyte counts | | | | | | Total area analyzed [b] (mm²) |
	Lobe	Region	PG	CA	Vtg1	Vtg2	Vtg3	OM	
Spawning capable (n = 5)	Left	A	900	131	61	27	9	-	57.0
		M	971	158	70	23	5	-	
		P	930	148	91	17	4	-	
	Right	A	894	124	84	32	12	-	
		M	942	138	81	16	5	-	
		P	970	131	92	33	1	-	
Actively spawning[a] (n = 6)	Left	A	627	112	66	48	30	49	68.4
		M	696	104	83	46	27	65	
		P	720	94	70	36	26	59	
	Right	A	638	93	69	40	35	56	
		M	653	104	64	45	40	49	
		P	654	107	65	41	25	66	

n = number of females analyzed; [a] = reproductive sub-phases; [b] = three 3.8 mm² microscopic fields x n; A = anterior, M = middle; P = posterior; PG = primary growth oocyte; CA = cortical alveolar oocyte; Vtg1 = primary vitellogenic oocyte; Vtg2 = secondary vitellogenic oocyte; Vtg3 = tertiary vitellogenic oocyte; OM = oocyte maturation.

TABLE 2

Chi-square goodness-of-fit test results for oocyte density frequencies in anterior, middle and posterior regions of left and right ovary lobes of *Lachnolaimus maximus* females in four reproductive phases and sub-phases

Female reproductive phase	n	Ovary lobe	χ^2	d.f.	p	Total oocyte count	Total area analyzed [b] (mm²)
Early developing[a]	8	Left	3.65	2	0.1610	6 525	273.6
		Right	2.63	2	0.2683	6 435	
Developing	4	Left	6.57	4	0.1602	2 906	136.8
		Right	4.04	4	0.4006	2 971	
Spawning capable	5	Left	12.2	8	0.1423	3 545	171.0
		Right	19.04	8	0.0146*	3 555	
Actively spawning[a]	6	Left	10.65	10	0.3858	2 958	205.2
		Right	7.42	10	0.6852	2 844	

[a] = reproductive sub-phases; n = number of females analyzed; χ^2 = chi-square goodness-of-fit statistic; d.f. = degrees of freedom (r-1)(c-1), with r=anterior, middle and posterior regions of each right and left lobes of ovaries, and c=oocyte stages (see Table1), for a chi-square calculated from contingency table data; p = probability value; [b] = three 3.8 mm² microscopic fields x three ovary regions x n. * = significant statistical difference (P < 0.05).

of the right lobe (χ^2 = 7.83; d.f. = 6; P = 0.2510). Oocyte frequency distribution between lobes was essentially uniform, no matter the reproductive phase or sub-phase (χ^2, 0.1459 ≤ P ≤ 0.7094; Table 4).

DISCUSSION

Most of females analyzed in the present study exhibited a high degree of homogeneity in oocyte distribution within and between the ovary lobes. The only observed difference in distribution was between different regions of the right lobe in SC phase females. These females had low Vtg3 oocyte densities in both lobes, as well as notable variation in the frequencies of this oocyte stage among the regions of the right lobe. This variation was apparently one of the possible factors causing the heterogeneity observed in the SC phase females. During the spawning season of *L. maximus* from the Eastern Gulf of Mexico, Collins and McBride (2015) observed a diel pattern of oocyte maturation in females: germinal vesicle migration

TABLE 3

Replicated goodness-of-fit test (heterogeneity G-test) results for oocyte density frequencies in anterior, middle
and posterior regions of the right ovary lobe of five *Lachnolaimus maximus* females in
spawning capable (SC) reproductive phase. Total area analyzed = 57.0 mm^2

Ovary region	Total oocyte counts						Test	G	d.f.	p
	PG	CA	Vtg1	Vtg2	Vtg3	OM				
Anterior	894	124	84	32	12	-		1 915.88	4	0.0001*
Middle	942	138	81	16	5	-		2 157.78	4	0.0001*
Posterior	970	131	92	33	1	-		2 177.94	4	0.0001*
							Total G	6 251.60	12	< 0.05
Pooled	2 806	393	257	81	18	-	Pooled G	6 231.11	4	< 0.05
							Heterogeneity G	20.49	8	0.0086

PG = primary growth oocyte; CA = cortical alveolar oocyte; Vtg1 = primary vitellogenic oocyte; Vtg2 = secondary
vitellogenic oocyte; Vtg3 = tertiary vitellogenic oocyte; OM = oocyte maturation; G = G statistic; d.f. = degrees of freedom;
p = probability value; * = highly significant statistical difference (P < 0.05).

TABLE 4

Chi-square goodness-of-fit test results for oocyte density frequencies in left and right ovary lobes of *Lachnolaimus
maximus* females in four reproductive phases and sub-phases

Female reproductive phase	n	χ^2	d.f.	p	Total oocyte counts	Total area analyzed [b] (mm^2)
Early developing[a]	8	0.60	1	0.4390	12 960	547.2
Developing	4	3.85	2	0.1459	5 877	273.6
Spawning capable	5	6.20	4	0.1844	7 100	342.0
Actively spawning[a]	6	2.94	5	0.7094	5 802	410.4

[a] = reproductive sub-phases; n =number of females analyzed; χ^2 = chi-square goodness-of-fit statistic; d.f. = degrees of
freedom (r-1)(c-1), with r=left or right lobes of the ovaries, and c=oocytes stages (see Table 1), p = probability value; [b] =
three 3.8 mm^2 microscopic fields x three ovary regions x two lobes x n.

of the vitellogenic oocytes occurs in the late
morning-early afternoon preceding spaw-
ning day. As a consequence, few vitellogenic
oocytes without any evidence of maturation
were present in the ovaries at mid-day. Not-
withstanding, SC phase females analyzed in the
present study presented neither oocytes under-
going early nor late germinal vesicle migration.
These females were not undergoing OM and
probably were barely entering the SC phase
and therefore displaying very few oocytes pro-
gressing towards the final vitellogenesis stage.

In this latter case, then females classified
as being in the SC phase, may not necessarily
be representative of this stage, and data from
them must be used with caution. Larger sample
sizes of SC phase females collected throughout
this species' reproductive season would help to
eliminate any uncertainty about Vtg3 oocyte

distribution during this phase. However, even
if Vtg3 oocyte distribution is heterogeneous in
the right lobe during this phase, it did not affect
the protocol for estimating fecundity. Female *L.
maximus* have asynchronous oocyte develop-
ment and engage in successive batch spawning
(Claro et al., 1989; McBride & Johnson, 2007).
This means that only females in the AS sub-
phase, not exhibiting newly collapsed POFs
in the ovary, should be selected to estimate
batch fecundity (Murua et al., 2003). In the
present data, AS sub-phase females exhibited a
homogeneous distribution of all oocyte stages,
including OM, which are those considered
when estimating batch fecundity.

Two previous studies have been done
on oocyte development homogeneity in *L.
maximus*, both with a Florida population
(Davis, 1976; McBride & Johnson, 2007).

Over 40 years ago, Davis (1976) has addressed oocyte development in the anterior, middle and posterior regions of the same lobe and between right and left lobes. Using contingency tables based on the frequency distributions and supported with χ^2 data, this author analyzed if the distribution of oocytes in three diameter ranges differed between regions of the same lobe and/or between lobes. No differences were observed in oocyte counts in any of the three diameter classes between regions or lobes. However, this analysis was not particularly robust since the sample size was small (N = 2; 29.6 and 30.4 cm FL), and the oocyte diameter ranges were unrelated to reproductive phase, being essentially random. McBride and Johnson (2007) also studied histological sections from three sections of ovary lobes in *L. maximus*, and found no differences in oocyte distribution. However, these authors reached their conclusion by analyzing the oocytes stages present, tunica appearance, and presence or absence of atretic, rather than attempting to count cell numbers per stage, as was done in the present study (McBride, personal communication, May 19, 2016).

Oocyte distribution patterns in teleosts vary by species, and appear unrelated to taxonomic level or any other ecological or biogeographic characteristic. The pattern in *L. maximus* of largely homogeneous distribution of oocyte stages within and between ovary lobes coincides with reports for other females teleosts. It has been observed in species from both temperate waters [e.g. *Clupea harengus* (Linnaeus, 1758) (Ma et al., 1998), *Spicara maena* (Linnaeus, 1758) (Matić-Skoko et al., 2004), *Gadus morhua* and *Merluccius merluccius* (Linnaeus, 1758) (Witthames et al., 2009), *Epinephelus marginatus* (Lowe, 1834) (Reñones et al., 2010), and *Pseudopleuronectes americanus* (Walbaum, 1792) (McElroy et al., 2013)]; tropical and subtropical waters [e.g. *Chaetodon multicinctus* (Garrett, 1863) (Tricas & Hiramoto, 1989), *Leiognathus equulus* (Forsskål, 1775) (Lee et al., 2005), *Spratelloides gracilis* (Temminck, & Schlegel, 1846)

(Weng et al., 2005), *Tylosurus acus melanotus* (Bleeker, 1850) (Liao & Chang, 2011), and *Psenopsis anomala* (Temminck, & Schlegel, 1844) (Wu et al., 2012)]; and temperate and tropical waters [e.g. *Helicolenus dactylopterus* (Delaroche, 1809) (Sequeira et al., 2012) and *Trachinocephalus myops* (Forster, 1801) (Yang et al., 2013)]. In contrast, other teleosts exhibit a heterogeneous oocyte distribution pattern in the ovary, as in the temperate marine flatfish species *Solea solea* (Linnaeus, 1758) (Witthames & Greer Walker, 1995), *Limanda aspera* (Pallas, 1814) (Nichol & Acuna, 2001) and *Pleuronectes platessa* (Linnaeus, 1758) (Kennedy et al., 2007), and the freshwater species *Prochilodus lineatus* (Valenciennes, 1837) from Brazil (Hainfellner et al., 2011). In another example, significant differences in average oocyte size were observed along the anterior-posterior axis and between the center and periphery of ovary lobes in bigeye tuna *Parathunnus sibi* (= *Thunnus obesus*; Lowe, 1839) (Yuen, 1955) and bluefin tuna *Thunnus thynnus* (Linnaeus, 1758) (Baglin, 1982).

Given the homogeneous oocyte distribution pattern within and between the ovary lobes in *L. maximus* females in the AS sub-phase, no systematization is required of the gonad histological sampling protocol to estimate species batch fecundity. Stereological methods in tandem with digital image processing can therefore be used to make accurate measurements of oocyte size, shape, count, area and volume in the ovaries of *L. maximus*, and generate unbiased fecundity estimates in this species. However, ovary sampling for characterizing the reproductive cycle in this species, based on analysis of the seasonal development of reproductive phase and sub-phase frequency, needs to be done with caution due to the potential for heterogeneity among Vtg3 oocytes in the right ovary lobe of females in the SC phase. If this is a possible risk in a given sample, the present results suggest it is best to systematically take sections of any region in the left ovary when conducting a study encompassing all of a species' reproductive aspects.

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RESUMEN

La distribución de los ovocitos en y entre los lóbulos ováricos de *Lachnolaimus maximus* **(Perciformes: Labridae) es generalmente homogénea.** Para el estudio de la reproducción de los peces es necesario, un conocimiento previo sobre la distribución de los ovocitos en el ovario, así como estimaciones cuantitativas del número de ovocitos para estimar la fecundidad por lote o anual. Una distribución heterogénea exige tener un protocolo de muestreo estandarizado para prevenir sesgos en las estimaciones, mientras que una distribución homogénea permite utilizar muestras de cualquier parte de las gónadas sin riesgo de sesgo. Nosotros estudiamos el patrón de distribución de los ovocitos de la población de la doncella de pluma *Lachnolaimus maximus* del sur del Golfo de México. Para este propósito, 23 hembras maduras fueron seleccionadas de un total de 47 individuos que presentaron ovocitos observables a simple vista en los ovarios. Estas hembras fueron clasificadas según su fase o sub-fase reproductiva (desarrollo temprano-DT, desarrollo-D, aptitud para desovar-AD y desove activo-DA). Seis secciones histológicas fueron realizadas de las regiones anterior, media y posterior de los lóbulos derecho e izquierdo del ovario de cada individuo. Un procesamiento digital de imágenes (AxioVision e Image ProPlus) fue utilizado para estimar la densidad de los ovocitos, en diferentes estadios de desarrollo, por unidad de área. Las frecuencias de distribución de los ovocitos fueron analizadas entre regiones de un mismo lóbulo y entre lóbulos de cada ovario por medio de tablas de contingencia. Este análisis involucró la aplicación de la prueba de bondad de ajuste del χ^2 de Pearson y de la prueba de G replicada en el caso de observar una distribución heterogénea. La mayoría de las 23 hembras analizadas presentó una distribución homogénea de los diferentes estadios de ovocito, en cualquier lóbulo considerado. En el lóbulo ovárico izquierdo, la distribución de los ovocitos fue similar en las tres regiones muestreadas, en cualquier fase o sub-fase de las hembras. En el lóbulo ovárico derecho, las frecuencias de los ovocitos fueron semejantes para las hembras en fase y sub-fases de DT, D y DA; sin embargo, durante la sub-fase de AD, una heterogeneidad en el desarrollo de los ovocitos en vitelogénesis terciaria-Vtg3 fue observada, especialmente en las muestras de las regiones media y posterior de este lóbulo. Las hembras en sub-fase de DA son usualmente utilizadas para estimar la fecundidad por lote en las especies de peces como *L. maximus*, la cual presenta un desarrollo asincrónico de los ovocitos y realiza desoves sucesivos por lote. Debido al patrón de distribución homogéneo de los ovocitos en y entre los lóbulos ováricos de las hembras en sub-fase de DA, no se requiere estandarizar un protocolo de muestreo histológico de las gónadas para estimar la fecundidad por lote de la especie. Sin embargo, debido a la distribución heterogénea de los ovocitos en Vtg3 en el lóbulo ovárico derecho de las hembras en fase de DA, es preferible tomar sistemáticamente secciones de cualquier región del lóbulo ovárico izquierdo cuando se realiza un estudio que incluye todo los aspectos reproductivos de la especie.

Palabras clave: reproducción, homogeneidad del ovario, doncella de pluma, Golfo de México.

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