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DETERMINATION OF FREQUENCY DISTRIBUTION OF OOCYTES AT DIFFERENT MATURITY PHASES IN THE OVARIES OF ROACH, *RUTILUS RUTILUS* (L.)

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Background. The frequency distribution of oocytes at different maturity phases can be used to assess environmental effects on gametogenesis in teleosts. As oocyte counting is labour-intensive, little attention is generally paid to changes in oocyte numbers in oogenesis analysis.

Material and methods. Anterior, middle, and posterior ovarian segments from three pre-spawning female roach, *Rutilus rutilus* (L.) were fixed in buffered formalin, dehydrated, and embedded in paraffin. Serial 10-µm sections were cut with a microtome and stained with haematoxylin and eosin. This paper compares two methods for determining the frequency distribution of oocytes at different maturity phases and their occurrence in particular segments of the ovaries in roach, *R. rutilus*: counting oocytes in serial microtome sections and the use of Abercrombie's formula.

Results. Differences between frequency distributions determined by the two methods were statistically insignificant ($P > 0.05$), as were the frequency distributions of oocytes in different ovarian segments.

Conclusion. Determination of frequency distribution of oocytes at different maturity phases can therefore be achieved with the less time- and labour-intensive method of using Abercrombie's formula.

Key words: fish, *Rutilus rutilus*, ovary, percentage of oocytes, oocyte counting methods

INTRODUCTION

The ovaries of most teleosts are long, paired organs suspended on the mesovarium adhering to the swim bladder. Oocytes are clustered in lamellae — epithelial folds lining the internal ovarian wall. During sexual maturation and reproduction in females, a cohort of previtellogenic oocytes undergoes vitellogenesis. Mature oocytes

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are then spawned, while remaining previtellogenic oocytes are supplemented with new generations of oocytes produced through mitotic proliferation of oogonia.

The development of vitellogenic and previtellogenic oocytes depends, to a different degree, on the activity of reproductive hormones. Hypophysectomy in maturing female of guppy, *Poecilia reticulata* Peters, 1859 (cf. Pandey 1969) and stinging catfish, *Heteropneustes fossilis* (Bloch, 1794) (cf. Sundararaj et al. 1972a, b) has shown that vitellogenic oocytes cannot exist without the activity of gonadotropins. Through their activity, estradiol (E_2) is synthesized in follicular tunicas of oocytes formed at the end of previtellogenesis (Jalabert 1976) as well as in ovarian interstitial cells (Upadhyay et al. 1978). Estradiol stimulates yolk formation in oocytes through hepatic receptors and synthesis of vitellogenin, a precursor to egg yolk proteins (Ng and Idler 1983, Wallace 1985). The number of vitellogenic oocytes depends on protein and fat reserves in the fish, as well as on food availability (Billard 1992). Oogonial divisions, and thus oocyte number, are affected by gonadotropic and steroid hormones (Barr 1963, Yamazaki 1965, Sakun 1972a, b). Exogenous factors such as temperature and light also effect qualitative changes in gametogenesis (Taranger et al. 1999, Koger et al. 1999, Lukšiené et al. 2000, Rodríguez et al. 2001).

At known absolute fecundity, determination of the frequency distribution of oocytes at different maturity phases makes it possible to determine the number of oocytes. For quantitative determination of oocyte number, stereology can also be used (Medina et al. 2002).

The principal method, hitherto used for determining distribution of oocytes of various stages in fish ovaries, consisted in counting oocytes on serial microtome sections of whole ovaries (Yamamoto and Yamazaki 1961, Yamamoto and Yoshioka 1964). The present authors attempted to introduce another method, previously used for counting somatic cells of uniform diameters—the Abercrombie's formula (Marrable 1962). The novelty of the present work is the application of Abercrombie's formula to cells of diversified diameters.

This paper compares the values of percentage shares of oocytes representing various maturity stages and determined based on the two methods mentioned above. The model for this study was roach, *Rutilus rutilus* (Linnaeus, 1758), a species characterized by a periodic rhythm of oocyte development. In Poland, vitellogenesis in roach starts in September and proceeds up to spawning (Długosz 1986), which generally occurs in May (Załachowski 2000). Roach females spawn a single time per spawning season.

MATERIAL AND METHODS

Anterior, middle, and posterior ovarian segments from three pre-spawning female roach were fixed in buffered formalin, dehydrated, and embedded in paraffin. Serial 10-µm sections of the embedded ovaries were then cut with a Leica microtome and stained with haematoxylin and eosin (Zawistowski 1986). The sections from the left ovary of one female were used for comparing the counting of oocytes in serial sections with the use of Abercrombie's formula (after Marrable 1962) for determination of the proportions of vitellogenic and previtellogenic oocytes, as outlined in the following two

paragraphs. Abercrombie's formula was also used to determine oocyte proportions in the right ovary of this female and in both ovaries of the remaining two females.

For the counting of oocytes in serial sections, 60 sections were cut from both the anterior and posterior ovarian segments and 120 sections were cut from the middle segment. Images of these sections were prepared and used to count both vitellogenic and previtellogenic oocytes. The number of sections of complete oocytes and the section thickness were used to calculate oocyte diameters. The number of incomplete oocytes in the anterior and posterior areas of each segment was estimated from the number of oocyte sections obtained and their diameters. In the case of previtellogenic oocytes from the middle ovarian segment, oocytes were only counted in seven areas selected from within the 120 serial sections.

In order to calculate oocyte number with Abercrombie's formula, oocyte sections were counted in three sections of each ovarian segment and oocyte diameters measured to 0.005 mm. The data were used in the formula:
where:

$$N = \frac{T}{T + D}\, n$$

N, number of oocytes in a selected phase,
T, section thickness,
D, arithmetic mean of diameters of 20 oocytes in a selected phase,
n, number of sections of oocytes in a selected phase in three serial sections.

The test of differences between two percentage distributions (Stanisz 1998) was used to determine the statistical significance of differences in frequency distributions of vitellogenic and previtellogenic oocytes determined by the two different methods. The statistical significance of differences in frequency distributions of oocytes in particular ovarian segments was determined using the chi-square test. Differences were deemed to be insignificant at $P > 0.05$.

RESULTS

Comparison of counting oocytes in serial sections with the use of Abercrombie's formula

Oocyte diameters measured from the serial sections ranged from 330 to 540 μm for vitellogenic oocytes and from ⩽ 10 to 110 μm for previtellogenic oocytes. A total of 107 complete vitellogenic oocytes were found in the middle ovarian segment, sectioned into 4477 fragments (Table 1). An additional 2033 incomplete oocyte fragments were found in the anterior and posterior areas of the middle segment, constituting another 50 vitellogenic oocytes based on the diameter limits observed for complete oocytes. Thus, in total, 157 vitellogenic oocytes occurred in the 120 sections of the middle ovarian segment. A total of 235 previtellogenic oocytes, cut into 972 fragments, were found in the seven areas selected from these same 120 sections (Table 2). Extrapolating to the

entire 120 sections gives a total of 5433 previtellogenic oocytes cut into 22 476 fragments. The frequency distribution of vitellogenic and previtellogenic oocytes in the middle ovarian segment was therefore 2.8% and 97.2%, respectively.

In the anterior segment of the ovary, 22 complete vitellogenic oocytes were found (Table 1), as well as 1649 incomplete fragments accounting for an additional 38 oocytes, giving a total of 60 vitellogenic oocytes. As well, 7597 fragments of previtellogenic oocytes, corresponding to 1836 oocytes, were counted. The frequency distribution of vitellogenic and previtellogenic oocytes in the 60 sections of the anterior ovarian segment was therefore 3.2% and 96.8%, respectively. In the posterior segment, 21 complete vitellogenic oocytes were found (Table 1), as well as 722 incomplete fragments accounting for an additional 17 oocytes and, thus, a total of 38 vitellogenic oocytes. As well, 3531 fragments of previtellogenic oocytes, corresponding to 853 oocytes, were counted. The frequency distribution of vitellogenic and previtellogenic oocytes in the 60 sections of the posterior ovarian segment was therefore 4.3% and 95.7%, respectively.

Table 1

Diameter, number of complete vitellogenic oocytes, and number of oocyte sections in a series of sections from the middle, anterior, and posterior segments of the left ovary of a roach female

Oocyte diameter [µm]	Ovary segment					
	Middle		Anterior		Posterior	
	No. of oocytes	No. of oocyte sections	No. of oocytes	No. of oocyte sections	No. of oocytes	No. of oocyte sections
330	2	66	—	—	—	—
340	1	34	—	—	—	—
350	3	105	1	35	—	—
360	3	108	1	36	2	72
370	6	222	1	37	1	37
380	4	152	—	—	2	76
390	3	117	1	39	5	195
400	13	520	4	160	1	40
410	8	328	3	123	3	123
420	12	504	1	42	2	84
430	17	731	1	43	3	129
440	12	528	2	88	1	44
450	11	495	3	135	1	45
460	7	322	—	—	—	—
470	3	141	1	47	—	—
480	—	—	2	96	—	—
500	1	50	—	—	—	—
520	—	—	1	52	—	—
540	1	54	—	—	—	—
Total	107	4477	22	933	21	845

Table 2

Diameter, number of previtellogenic oocytes, and number of their sections in selected areas

Oocyte diameter [μm]	Number of oocytes								No. of oocyte sections
	Selected areas in a series of sections								
	I	II	III	IV	V	VI	VII	Σ	
≤10	—	2	4	2	5	9	4	26	26
20	1	6	8	6	2	12	4	39	78
30	—	3	6	8	2	6	6	31	93
40	1	4	12	4	—	8	10	39	156
50	8	3	7	7	2	5	5	37	185
60	3	2	5	5	—	4	4	23	138
70	2	6	8	4	1	3	7	31	217
80	—	1	1	1	—	—	1	4	32
90	—	1	1	—	—	1	1	4	36
110	—	—	1	—	—	—	—	1	11
			Total					235	972

The frequency distributions of vitellogenic and previtellogenic oocytes, as determined with Abercrombie's formula, ranged from 2.3 to 4.3% and from 97.7 to 95.7%, respectively (Table 3). These values were not significantly different from those obtained by counting oocytes in serial sections.

Table 3

Percentage distribution of vitellogenic (v) and previtellogenic (p) oocytes in the left ovary of roach determined by the method of counting oocytes with the use of the Abercrombie's formula

Ovary segment, No. of section	Pooled number of oocyte sections		Oocyte diameter [μm]		No. of oocytes		Percentage distribution of oocytes	
	v	p	v	p	v	p	v	p
			$\bar{x}$ ± SD	$\bar{x}$ ± SD				
Middle								
8,9,10	173	561	1129 ± 69.4	112 ± 40.6	1.5	46.0	3.1	96.9
40,41,42	166	567	1060 ± 101.6	91 ± 44.0	1.5	56.1	2.6	97.4
60,61,62	163	778	1090 ± 84.8	110 ± 51.4	1.5	64.8	2.3	97.7
91,92,93	155	485	1112 ± 76.0	90 ± 33.6	1.4	48.5	2.8	97.2
Anterior								
1,2,3	123	406	1117 ± 97.7	92 ± 36.5	1.1	39.8	2.7	97.3
29,30,31	131	338	1099 ± 105.8	88 ± 33.4	1.2	34.5	3.4	96.6
43,44,45	147	400	1141 ± 80.7	100 ± 52.3	1.3	36.4	3.4	96.6
50,51,52	130	415	1177 ± 69.6	106 ± 46.1	1.1	35.8	3.0	97.0
Posterior								
32,33,34	85	196	1144 ± 76.5	93 ± 36.0	0.7	19.0	3.6	96.4
40,41,42	77	168	1151 ± 61.7	97 ± 36.3	0.7	15.7	4.3	95.7
50,51,52	72	204	1140 ± 69.3	82 ± 33.5	0.6	22.2	2.6	97.4
58,59,60	72	218	1129 ± 77.7	75 ± 29.3	0.6	25.6	2.3	97.7

Frequency distribution of vitellogenic and previtellogenic oocytes in different ovarian segments

The frequency distributions of vitellogenic and previtellogenic oocytes in different ovarian segments, as determined with Abercrombie's formula, are shown in Table 4. There were no statistically significant differences among these frequency distributions.

Table 4

Percentage distribution of vitellogenic (v) and previtellogenic (p) oocytes in particular segments of roach ovaries

Body length SL [cm]	Body weight [g]		Ovary segment					
			Right ovary			Left ovary		
			Anterior	Middle	Posterior	Anterior	Middle	Posterior
11.6	31	v	3.5	3.0	4.9	2.7–3.4	2.3–3.1	2.3–4.3
		p	96.5	97.0	95.1	97.3–96.6	97.7–96.9	97.7–95.7
14.5	50	v	3.0	2.5	2.8	2.9	2.5	4.2
		p	97.0	97.5	97.2	97.1	97.5	95.8
26.2	410	v	11.3	13.4	10.7	10.7	10.7	14.3
		p	88.7	86.6	89.3	89.3	89.3	85.7

DISCUSSION

Previous studies of the rhythm of oocyte development in goldfish, *Carassius auratus* (Linnaeus, 1758) (cf. Yamamoto and Yamazaki 1961) and in Japanese rise fish, *Oryzias latipes* (Temminck et Schlegel, 1846) (cf. Yamamoto and Yoshioka 1964) were based on counting oocytes in 10-µm sections of the entire ovaries. The current study demonstrates that this procedure can be simplified by applying Abercrombie's formula to a smaller number of sections. The frequency distributions of vitellogenic and previtellogenic oocytes in roach ovaries were not significantly different when determined by these two methods. However, there were differences in oocyte diameters, with a larger mean diameter obtained with the latter method. These differences resulted from the technique of section preparation, which involved moistening of the cut surface of the paraffin wax and caused hydration of the oocytes in the plane perpendicular to the cutting direction to a depth of ca. 30 µm. These differences in measured oocyte diameters did not affect the estimation of oocyte frequency distributions.

The conclusion of the present study is that determination of frequency distribution of oocytes at different maturity phases can be achieved with the less time- and labour-intensive method of using Abercrombie's formula.

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