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Review

Effects of ethinylestradiol (EE2) and an organophosphorus flame retardant (TCPP) on gonadal maturation in sea urchin, Paracentrotus lividus.

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Table 1. Mean gametogenic stages[1] of the sea urchins exposed to different treatments in the 2016 and 2017 experiments.

Treatment		2016				2017			
		Females		Males		Females		Males	
		t7	t28	t7	t28	t7	t28	t7	t28
SW		1.9	2.0	2.07	1.77	3.17	3.31	2.38	2.86
SC		1.92	2.0	1.67	1.5	3.38	3.17	2.42	2.13
TCPP (µg/L)	0.2					3.1	2.79	2.50	2.20
	1	2.27	2.0	1.6	2.0	3.1	3.64	2.60	2.44
	5					2.67	3.08	1.75	3.00
	10	2.0	2.18	2.0	2.0				
EE2 (ng/L)	10					3.00	3.27	1.14	2.67

[1] $Mean\ GS = \frac{Proportion\ Stage\ I \times 1 + Prop.\ II \times 2 + Prop.\ III \times 3 + Prop.\ IV \times 4 + Prop.V \times 5 + Prop.VI \times 6}{n^{o}\ GS\ (6)}$

Table 2. Mean of seawater and environmental temperature (± S.D.) maximum and minimum in the 2016 and 2017 experiments.

Date	Air Temperature T ºC				Seawater T ºC			
	Mean[(1)]	± S.D.	Min[(2)]	Max[(3)]	Mean[(1)]	± S.D.	Min[(2)]	Max[(3)]
08/2016	18.7	2.7	13.3	32.2	16.6	1.5	13.0	19.8
09/2016	17.4	2.0	12.9	24.0	16.8	1.0	14.7	19.2
10/2016	16.0	2.1	10.3	24.6	15.7	0.5	14.5	17.2
11/2016	13.0	2.6	6.4	19.7	15.5	0.7	14.1	17.6
08/2017	18.8	2.0	13.6	28.3	17.5	1.4	14.2	20.8
09/2017	16.7	1.9	11.4	23.1	16.2	0.8	14.1	18.6
10/2017	16.7	3.2	10.2	32.1	16.0	0.6	14.5	17.7
11/2017	13.1	2.9	4.8	21.5	14.2	0.7	12.8	15.7

(1) Average of ten-minute data collected in a month by the ocean-meteorological station of ECIMAT (Marine Science Station of Toralla, Vigo-SP). https://torallamar.info/

(2) Minimum temperature recorded in a month.

(3) Maximum temperature recorded in a month.

Figure 1. Structures of the compounds used in this study: (a) EE2 ethinylestradiol; (b) TCPP mixture of isomers: mainly tris(1-chloro-2-propyl) phosphate 66%, minor components: bis(1-chloro-2-propyl) (2-chloropropyl) phosphate and (1-chloro-2-propyl) bis(2-chloropropyl) phosphate.

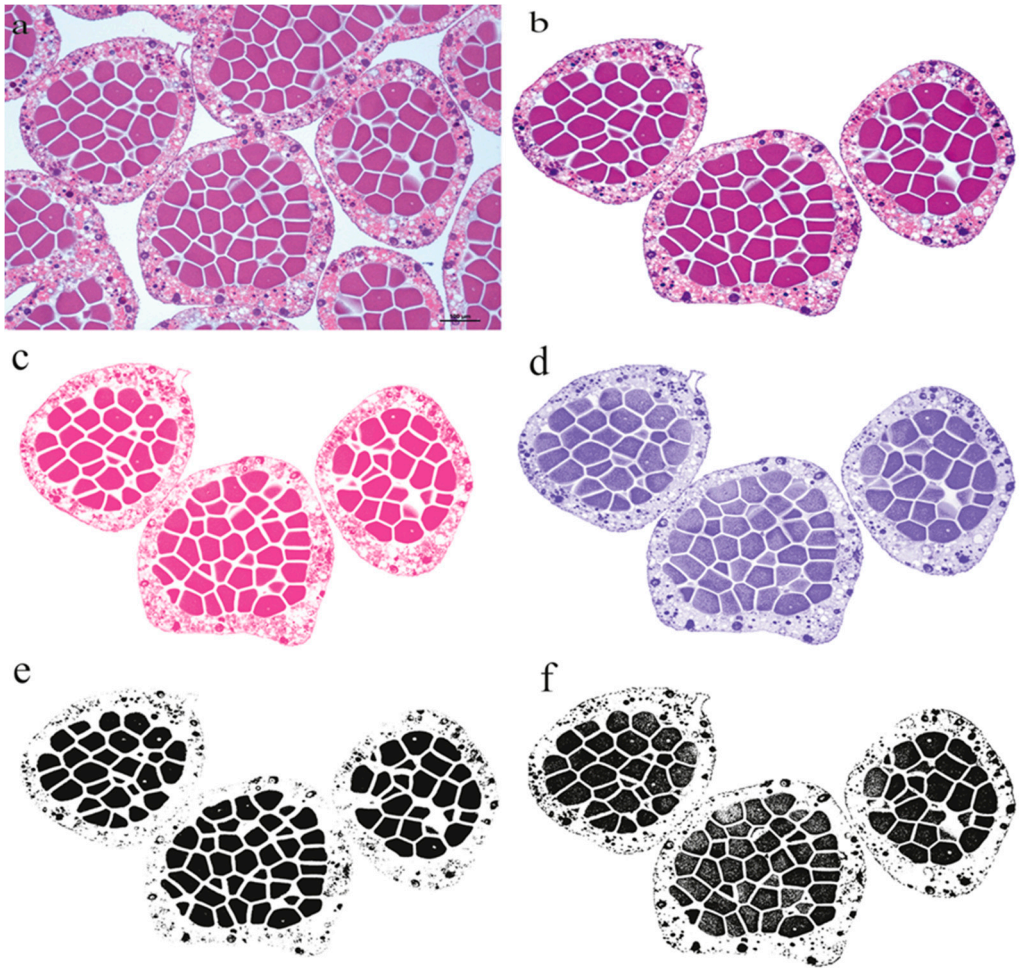

Figure 2. Sequence of image processing to quantify the Pixelar Index: original image in HE dye (a), bar = 100 µm. Image without incomplete follicles after the cleaning process (b) with Adobe Photoshop. From b, the Colour Deconvolution plugin (v.3.0.2, ImageJ, FIJI), generates a pink layer (c), and a violet layer (d) and the respective binary images (e, f). The binary images obtained were quantified by the CellProfiler software (Carpenter et al. 2006; Lamprecht et al. 2007) creating a pipeline effect and using the Measure Image Area Occupied tool.

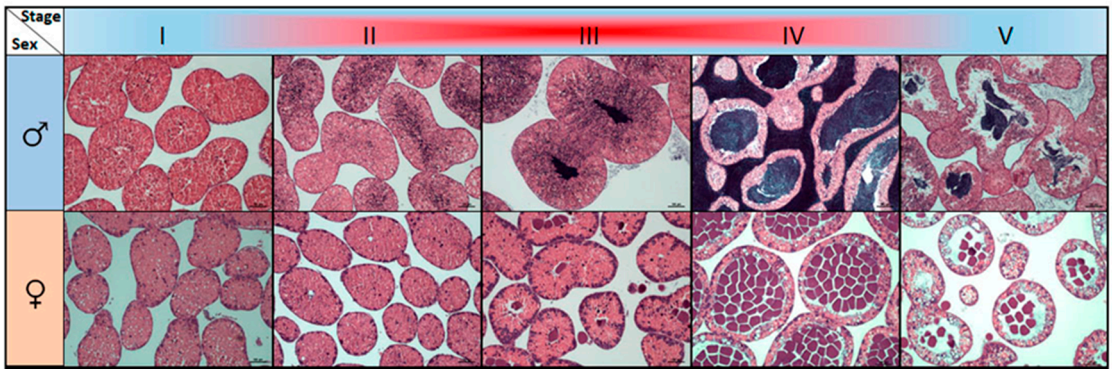

Figure 3. Sea urchin, *Paracentrotus lividus*, developing stages of males (top) and females (bottom): I, recovery; II, growing; III, premature; IV, mature; V, partly spawned (ECIMAT histology service).

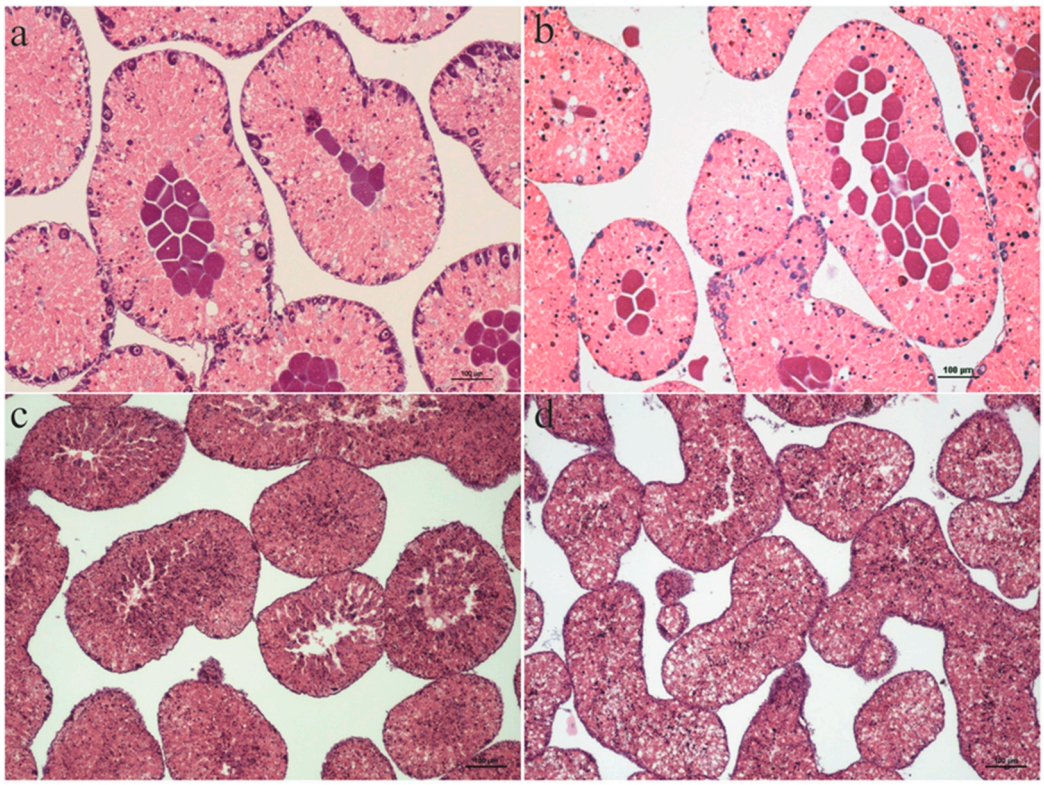

Figure 4. Examples of individuals histological slides of significant data on gametogenic stages. a: Female stage III, premature, CTRL in 2016 (t = 7 d). b: Female stage III, premature, TCPP 1 µg/L in 2016 (t = 7 d). c: Male in recovery condition, stage I, EE2 0.010µg/L in 2017 (t = 7 d). d: Male in recovery condition, stage I, TCPP 5µg/L in 2017 (t = 7 d). Bar = 100 µm.

References

Carpenter AE, Jones TR, Lamprecht MR, Clarke C, Kang IH, Friman O, Guertin DA, Chang JH, Lindquist RA, Moffat J, Golland P, Sabatini DM (2006) CellProfiler: image analysis software for identifying and quantifying cell phenotypes. Genome Biology 7 (10): R100.

Lamprecht MR, Sabatini DM, Carpenter AE (2007) CellProfiler™: free, versatile software for automated biological image analysis. BioTechniques 42 (1):71-75.