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

Firstname Lastname 1, Firstname Lastname 2 and Firstname Lastname 2,*

1 Ecology and Animal Biology Department, University of Vigo, Vigo, Galicia 36310, Spain; estefania.pereira.pinto@uvigo.es(E.P-P.); leomantilla@uvigo.es(L.M-A.); rbeiras@uvigo.es(R.B.)
2 Toralla Marine Station (ECIMAT), University of Vigo, Vigo, Galicia 36331, Spain
* Correspondence: pcampoy@uvigo.es

Received: 28 June 2020; Accepted: 13 August 2020; Published: date

Table 1. Mean gametogenic stages1 of the sea urchins exposed to different treatments in the 2016 and 2017 experiments.

| Treatment | 2016 Females | 2016 Males | 2017 Females | 2017 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       | 2.27 | 2.0 | 1.6 | 2.0 | 3.1 | 2.79 | 2.5 | 2.2 |
| 1         | 2.67 | 3.08 | 1.75 | 3.00 |
| 5         | 2.67 | 3.08 | 1.75 | 3.00 |
| 10        | 2.0 | 2.18 | 2.0 | 2.0 | 3.00 | 3.27 | 1.14 | 2.67 |

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

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

| Date   | Air Temperature T °C | Seawater T °C |
|--------|----------------------|---------------|
|        | 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).  \[\text{https://torallamar.info/}\]
(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).

| Stage | Sex | I | II | III | IV | V |
|-------|-----|---|----|-----|----|---|
| I     | ♂   | ![Image](image1) | ![Image](image2) | ![Image](image3) | ![Image](image4) | ![Image](image5) |
| II    | ♂   | ![Image](image6) | ![Image](image7) | ![Image](image8) | ![Image](image9) | ![Image](image10) |
| III   | ♂   | ![Image](image11) | ![Image](image12) | ![Image](image13) | ![Image](image14) | ![Image](image15) |
| IV    | ♂   | ![Image](image16) | ![Image](image17) | ![Image](image18) | ![Image](image19) | ![Image](image20) |
| V     | ♂   | ![Image](image21) | ![Image](image22) | ![Image](image23) | ![Image](image24) | ![Image](image25) |
| I     | ♀   | ![Image](image26) | ![Image](image27) | ![Image](image28) | ![Image](image29) | ![Image](image30) |
| II    | ♀   | ![Image](image31) | ![Image](image32) | ![Image](image33) | ![Image](image34) | ![Image](image35) |
| III   | ♀   | ![Image](image36) | ![Image](image37) | ![Image](image38) | ![Image](image39) | ![Image](image40) |
| IV    | ♀   | ![Image](image41) | ![Image](image42) | ![Image](image43) | ![Image](image44) | ![Image](image45) |
| V     | ♀   | ![Image](image46) | ![Image](image47) | ![Image](image48) | ![Image](image49) | ![Image](image50) |

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.

© 2020 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).