Supplemental information for "Smallmouth bass gonadal development in the absence and presence of EE2"

Table S1. Monitored ion transitions (m/z) for LC-MS ethinylestradiol quantification (Experiment 2)

Compound	Precursor Ion (m/z)	Product Ion(s) (m/z)[a]
EE2	279.2	**133.0**, 159.2
EE2-d4	283.3	**155.1**
E2-^{13}C	261.2	**159.1**

[a]Quantifier ion in bold

Table S2. Experiment 1 analytical chemistry results (ELISA)

Nominal exposure concentrations are based on mean measured stock solution concentration and dilution pump speeds.

Sample type	Mean, ng/L	Std. dev	Nominal, ng/L
Control water (LSW)	<1.0		0.0
LSW matrix blank	<1.0		0.0
Low EE2 exposure	1.2	0.7	2.0
Low EE2 matrix spike	1.8	1.0	2.1
High EE2 exposure	5.1	2.0	10.0
High EE2 matrix spike	9.5	3.9	10.3
EE2 stock solution	3698	951	3860

Table S3. Experiment 2 analytical chemistry results (LC-MS)

Nominal exposure concentrations are based on mean measured stock solution concentration and dilution pump speeds.

Sample type	Mean, ng/L	Std. dev	Nominal, ng/L
Control water (LSW)	< 0.3		0
LSW matrix blank	< 0.3		0
0.1 ng/L EE2 exposure	Not measured		0.1
0.3 ng/L EE2 exposure	Not measured		0.4
1 ng/L EE2 exposure	1.03	0.18	1.1
1 ng/L EE2 matrix spikes	1.28	0.22	1.1
3 ng/L EE2 exposure	3.01	0.78	3.3
3 ng/L EE2 matrix spikes	3.65	0.80	3.0
EE2 stock solution	1088	49	1008

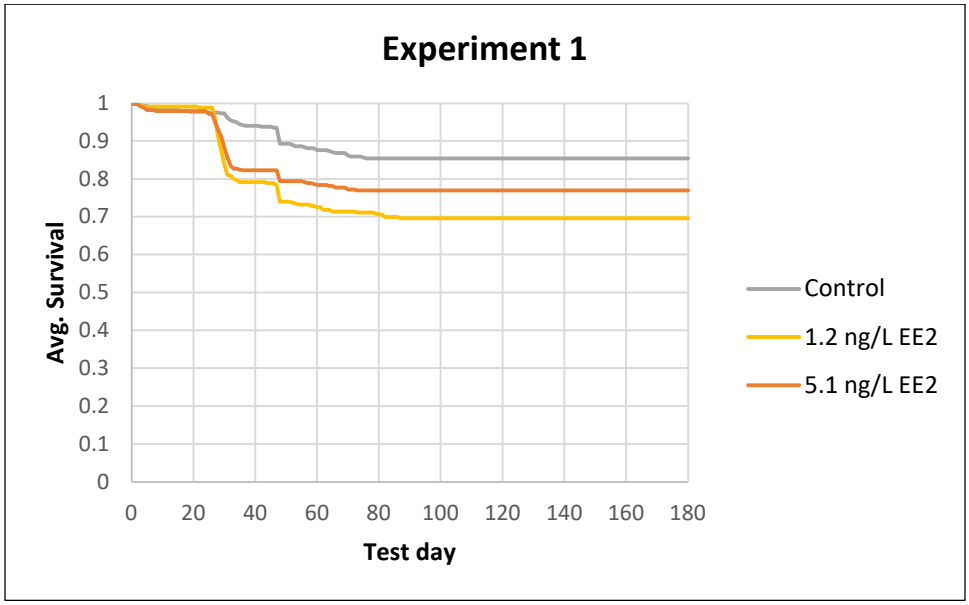
Table S4. Timeline of gonadal characteristics and germ cell types observed in smallmouth bass fry reared for 180 days in the absence and presence of EE2 (1.2 ng/L measured) during Experiment 1.

<table>
<tr><th rowspan="2">Test day</th><th colspan="3">CONTROL</th><th rowspan="2">EE2-EXPOSED (first 90 days)</th></tr>
<tr><th>Undifferentiated</th><th>Ovaries/oogenesis</th><th>Testes/spermatogenesis</th></tr>
<tr><td>24</td><td>Small gonads; few germ cells; small somatic projections (n = 6 of 12)</td><td>Undifferentiated characteristics</td><td rowspan="6">Undifferentiated characteristics</td><td rowspan="6">Ovarian and undifferentiated characteristics similar to control groups; no testicular characteristics</td></tr>
<tr><td>32</td><td rowspan="5">Slightly ↑ gonad size, blood vessel size, and germ cell n</td><td>Somatic projections form ovarian cavity; ↑ germ cell n</td></tr>
<tr><td>40</td><td>Clusters of meiotic germ cells</td></tr>
<tr><td>49</td><td>Few chromatin nuclear oocytes (CN)</td></tr>
<tr><td>63</td><td>CN oocytes predominant; folliculogenesis</td></tr>
<tr><td>74</td><td>Early and late stage perinucleolar (PN) oocytes</td></tr>
<tr><td>92</td><td rowspan="5">No undifferentiated gonads</td><td>Late stage PN oocytes predominant; balbiani bodies</td><td>Small spermatic tubules</td><td rowspan="4">Abnormal characteristics (n = 26 of 68): small ovary size, few oocytes, fibrosis</td></tr>
<tr><td>120</td><td>Vacuolated perinuclear stage; ↑ PN oocyte size</td><td>Pre-meiotic germ cells, spermatogenesis</td></tr>
<tr><td>151</td><td rowspan="3">↑ PN oocyte and ovary size</td><td>Cysts in multiple stages of spermatogenesis</td></tr>
<tr><td>165</td><td rowspan="2">Mature spermatozoa in tubules (low number)</td></tr>
<tr><td>180</td><td>Intersex (n = 1 of 12)</td></tr>
</table>

Figure S1. Survival

Average cumulative survival, among replicate exposure chambers of each treatment group in Experiment 1 (A) and Experiment 2 (B)

A.



B.

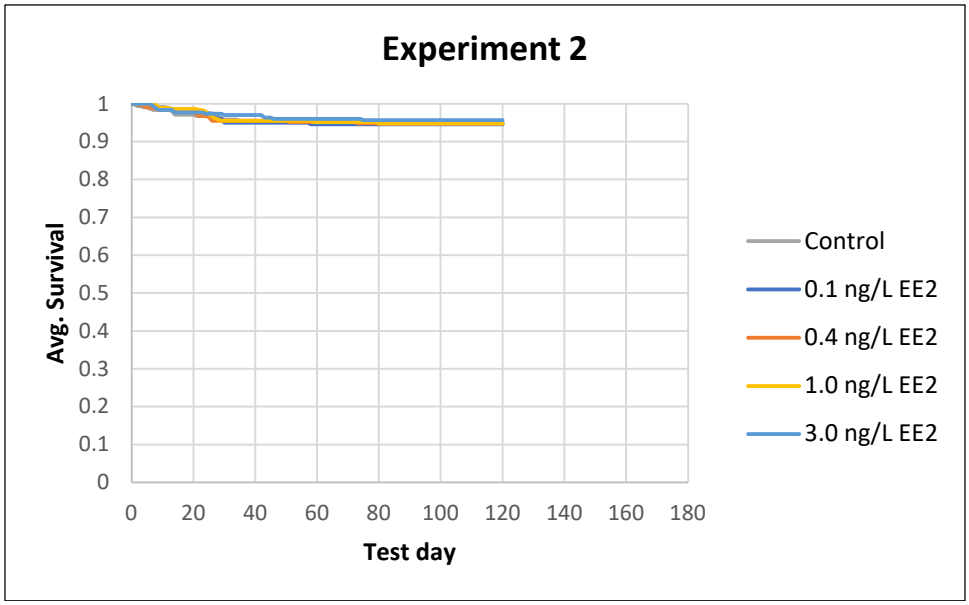
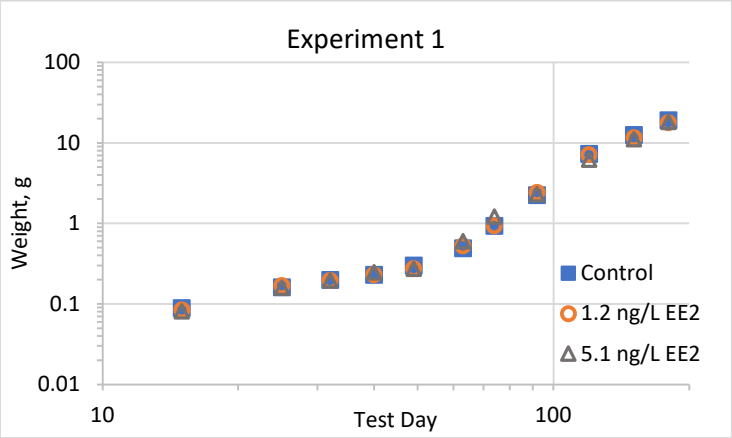


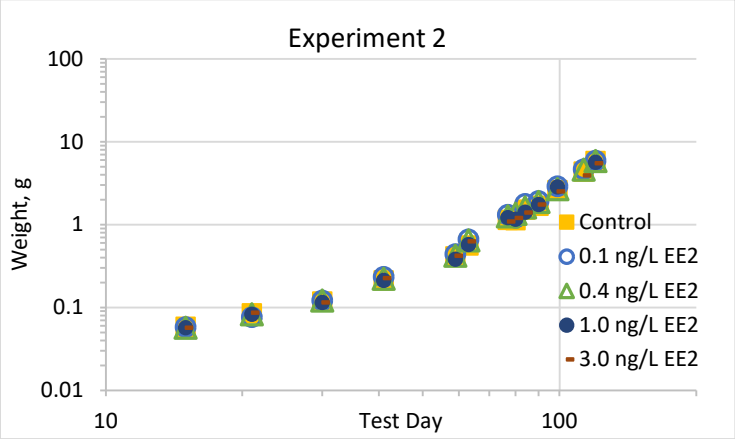
Figure S2. Growth

Average wet weight among replicate exposure chambers of each treatment group in Experiment 1 (A) and Experiment 2 (B), and average (range) wet weight among all treatments in Experiment 1 and 2 (C)

A.



B.



C.

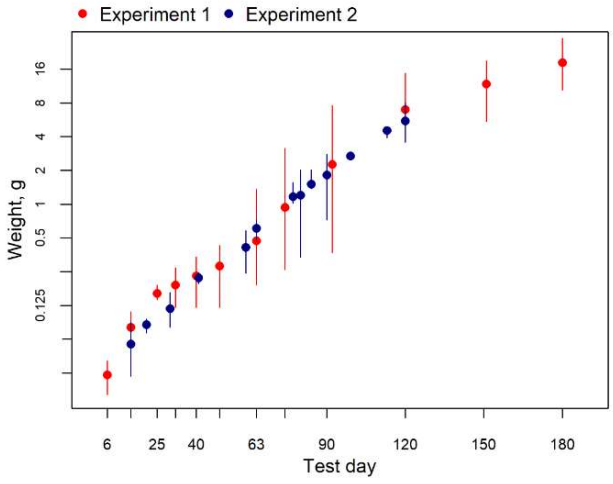


Figure S3. Transverse section of smallmouth bass abdomen collected on test day 32 of Experiment 1. Section shows typical position of early stage gonads (g) in relation to the peritoneal wall (pw), swim bladder (sb), liver (lv), and stomach (s). Image is oriented with dorsal side on top.