"Oyster Biomonitor of Estrogenic Endocrine Disrupting Chemicals in Australian Coastal Waterways"

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Background

❑ Endocrine disrupting chemicals (EDCs)

❑ What are Endocrine disrupting chemicals (EDCs)?

Definition: Some exogenous chemicals that are hormonally active, and can bind with receptors in the endocrine system and can disrupt the normal function, are known as Endocrine disrupting chemicals (EDCs).

Examples: Polychlorinated biphenyls, insecticides, pesticides, herbicides, antifouling agents, and plasticizers, steroid compounds-17β-ethinyl-estradiol (EE2), Bisphenol A, 4-*t*-octylphenol (OP), and 4-Nonylphenol (4-NP) etc.

❑ Estrogenic EDCs: A subgroup of EDCs that can interact with the estrogen receptors.

(i) Natural EDCs: Estrone (E1), 17β-estradiol (E2), Estriol (E3)
(ii) Synthetic EDCs: 17α-ethinylestradiol (EE2)
(iii) Industrial chemicals: Bisphenol-A (BPA), 4-*t*-Octyl phenol (4-*t*-OP), 4-Nonylphenol (4-NP), etc.

Solecki et al., 2016; Snyder, 2003; Rutkowska et al., 2016

Background

❑ Major sources of common EDCs

Livestock → Estrone (E1), 17β-estradiol (E2), Estriol (E3)

Women → Estrone (E1), 17β-estradiol (E2), Estriol (E3)

Pharmaceuticals (Women & livestock) → 17α-ethinylestradiol (EE2)

Plastic materials → Bisphenol A (BPA)

Industry →

4-*t*-octylphenol (4-*t*-OP) (i.e. Emulsifiers, paints, paper, inks, adhesives carpet backings, textile, leather, pesticides

4-Nonylphenol (4-NP) (i.e.Non-ionic surfactant, lubricating oil, additives, laundry, dish detergents, emulsifiers, and solubilisers)

Tran et al. 2019; Adeel et al., 2017

Background

❑ Contamination pipelines of EDCs

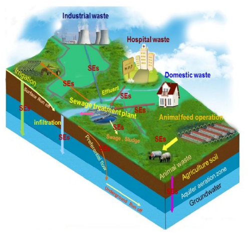


Fig: Schematic illustration of main sources of estrogens release to the environment

Global scale

Human contribution :

- ✓ 30 Tons/yr. (approx.) of natural steroidal estrogens (E1, E2, and E3).
- ✓ 0.7 Tons/yr. (approx.) of synthetic estrogens (EE2) solely from birth control pill practices.

Livestock contribution:

- ✓ 83 Tons/yr. (approx.) natural-E1, E2, and E3, and synthetic estrogens (EE2).

Industrial Chemicals:

- ✓ 2.2-4.7 million Tons/yr. (approx.) of BPA
- ✓ 0.5 million Tons/yr. (approx.) of APEs (NP & OP)

Adeel et al., 2017; De Falco et al., 2015

Background

❑ Max. Level of estrogenic contamination in Australia vs Global scale

Estrogenic Compounds		Level of Estrogenic Compounds (ng/L)	
		Receiving water/Surface water	
Name	**Symbol**	**Australia**	**Globally**
Estrone	E1	14.5	180
17β-estradiol	E2	7.3	175
Estriol	E3	<5	94
17α-ethinylestradiol	EE2	<5	34
Biphenol A	BPA	560	1,096
4-*t*-octylphenol	4-*t*-OP	483	916
4-nonylphenol	4-NP	5,270	5,270

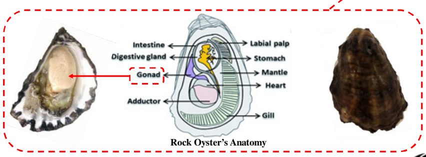
Williams et al., 2007; Leusch et al., 2010; Scott et al., 2014; Tran et al. 2019

Background

❑ Sydney rock oyster (*Saccostrea glomerata*)

- ✓ One of the most important seafoods of the world, especially in Australia.
- ✓ In local conditions, they generally reach 40-60g.
- ✓ About 60% of prime eating oysters are female.
- ✓ NSW produced approx. 5,000 tonnes for 2013/2014 fiscal year (Market value of approx. $32 million)

Filter- approx. 190L water/day/oyster
Bioindicator of estrogenic load



Rock Oyster's Anatomy

Trenaman et al., 2014; Van In et al., 2016; Alex Kasprak, 2018

Background

❑ Sydney rock oyster (*Saccostrea glomerata*)

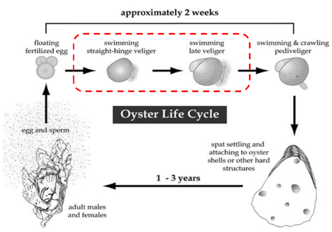


Fig: Life cycle of Rock Oyster

Background

❑ Vitellogenin (Vtg) as a biomarker

❑ What Vitellogenin (Vtg)?

Definition: *Vitellogenin* is an egg yolk precursor protein (lipoproteins and phosphoproteins) expressed in the females of nearly all oviparous species including fish, amphibians, reptiles, birds, most invertebrates, and monotremes.

In the presence of estrogenic endocrine disruptive chemicals (EDCs), **Not only females but also males** fish/mollusc can express the Vtg gene in a dose dependent manner.

Thus, Vtg gene expression in fish/mollusc can be used as a molecular biomarker of exposure to estrogenic EDCs.

Richard, 2008; Tran et al. 2019

Estrogenic exposure vs Vitellogenin (Vtg) responses

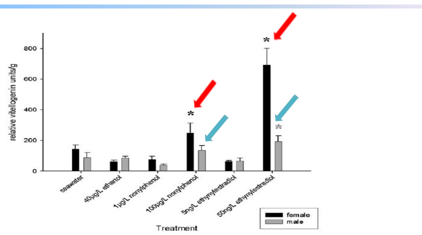


Fig: Vitellogenin (VTG) (relative units/g) measured in gonadal tissue of *S. glomerata*, exposed to 4 **nonylphenol (1µg/L and 100µg/L)** and **17α-ethynylestradiol (5 ng/L and 50 ng/L)** over an **8-weeks exposure** period in experimental aquaria (mean ± standard error, n = 14), *p < 0.05.

Andrew et al. 2008; Aquatic Toxicology, Vol. 88, pp.39-47

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Estrogenic exposure vs Sex ratio changes

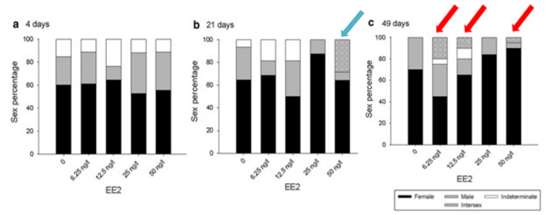

Fig: Sex ratio comparison of Sydney rock oysters, *S. glomerata* following exposure to EE2 (0, 6.25, 12.5, 25 and 50 ng/l) in experimental aquaria at **(a)** 4 days exposure, n = 17–18, **(b)** 21 days exposure, n = 15–16 per treatment and **(c)** 49 days exposure, n = 18–20 per treatment.

Andrew et al. 2010; Ecotoxicology, Vol. 19, pp. 1440-1451

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Estrogenic exposure vs Vitellogenin (Vtg) responses

Estrogenic compound/activity	Solids extraction (ng/L)		Liquid extraction (ng/L)	
	Mean ± SE	Range	Mean ± SE	Range
Estrone (E1)	1.08 ± 1.08	0–11.90	0.34 ± 0.34	0–3.74
17β estradiol (E2)	0 (<LOQ)	0	0.69 ± 0.49	0–5.12
Estriol (E3)	0.57 ± 0.41	0–4.12	3.26 ± 1.99	0–21.01
17α-Ethynylestradiol (EE2)	0 (<LOQ)	0	0.56 ± 0.56	0–6.19
Bisphenol A (BPA)	2.46 ± 0.97	0–9.37	61.73 ± 7.66	6.16–92.83
4-n-Nonylphenol (NP)	1.63 ± 0.92	0–9	5.88 ± 2.61	0–25.26
4-tert-Octylphenol (OP)	0.98 ± 0.73	0–7.78	4.95 ± 1.37	0–11.30
Estradiol equivalents (EEQ)	1.05 ± 0.39	0.11–3.66	3.43 ± 0.92	0.73–8.70

Average concentrations of selected estrogenic compounds, measured via GCMS, and estrogenic activity, measured via the YES® assay, in effluent collected from Burwood Beach wastewater treatment plant.

Andrew et al. 2012; Aquatic Toxicology, Vol. 120-121, pp. 99-108

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Estrogenic exposure vs Vitellogenin (Vtg) responses

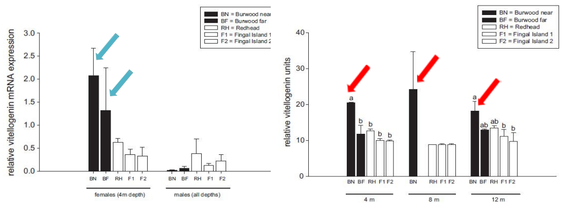

Fig: Vitellogenin gene expression in gonadal tissue of *S. glomerata*, following **6 weeks** deployment **(a)** Location and sex effects; **(b)** Location and depth-4, 8 and 12 m at Burwood beach (Near-< 50m; Far-100-150m) and controlled locations

Andrew et al. 2012; Aquatic Toxicology, Vol. 120-121, pp. 99-108

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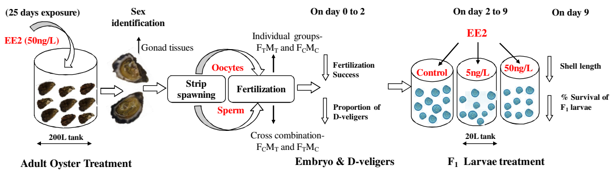
Estrogenic exposure vs Transgenerational study



Islam et al. 2019 (Under review at Ecotoxicology and Environmental Safety)

Estrogenic exposure vs Transgenerational Study

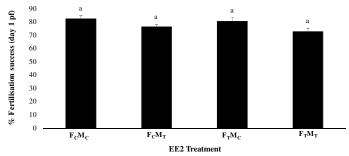


Fig. Percent fertilization success among parental exposure treatments (50 ng/L) on day 1 pf. Fertilisation identified by the consideration of cell cleavage (*n* =3, mean ± SE).

Islam et al. 2019 (Under review at Ecotoxicology and Environmental Safety)

Estrogenic exposure vs Transgenerational Study

Table: Percentage of early larvae morphs and unfertilized eggs in treatments on day 1 pf (*n=3*, mean ± SE)

Group (s)	Total observation (in triplicates)	Avg. D-veligers (D's) (%)	Avg. Deformed D's (%)	Avg. Trochophore (%)	Avg. unfertilized eggs (%)
F_CM_C	93	71.80±14.32	0.011±0.01	2.22+3.33	24.83±12.52
F_CM_T	101	71.95±3.20	0.008±0.01	1.11±1.11	26.12±2.79
F_TM_C	90	78.89±6.19	0.011±0.01	3.33±3.33	16.67±6.94
F_TM_T	95	56.90±2.01	0.010±0.01	10.77±4.62	31.32±6.04
P values		0.505	0.999	0.182	0.708

Islam et al. 2019 (Under review at Ecotoxicology and Environmental Safety)

Estrogenic exposure vs Transgenerational Study

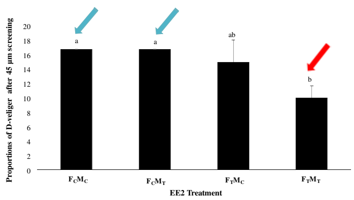


Fig. F_1 Proportions of D-veliger larvae on day 2 pf after screening with 45µm mesh. (*n*=3, mean ± SE). Similar letters denote statistically similar treatments via one-way ANOVA and Tukey's post-hoc test.

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Estrogenic exposure vs Transgenerational Study

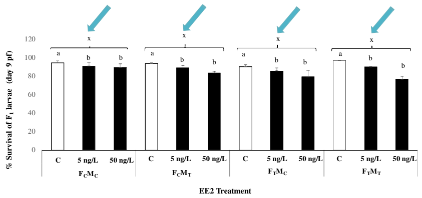

Figure 5: Percentage survival of F_1 larvae on day 9 pf among parental (50 ng/L) and offspring (5, 50 ng/L) EE2 exposure treatments (*n*=3, mean ± SE). Different letters denote statistically significant in treatments via two-way ANOVA and Tukey's post-hoc test.

Islam et al. 2019 (Under review at Ecotoxicology and Environmental Safety)

Estrogenic exposure vs Transgenerational Study

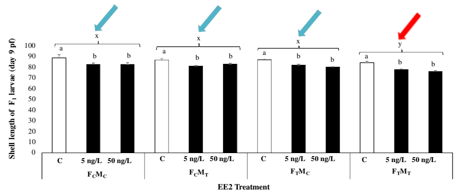

Fig. Average shell length (µm) of F_1 larvae on day 9 pf among parental (50 ng/L) and offspring (5, 50 ng/L) EE2 exposure treatments (*n=3*, mean ± SE). Different letters denote statistically significantly different each other via two-way ANOVA and Tukey's post-hoc test.

Islam et al. 2019 (Under review at Ecotoxicology and Environmental Safety)

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