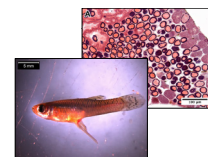


Investigation of endocrine disruption in Australian rivers using an *in situ* approach

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INTRODUCTION

- Although there are more than 40,000 chemical compounds registered for use in Australia¹, only a handful have been monitored in aquatic receiving environments.
- Trace organic contaminants (TrOCs) and endocrine active compounds (EACs) may negatively impact fish in Australian rivers:
 - Some monitored concentrations are cause of concern (e.g., ethinylestradiol, estrone, bisphenol A)^{2,3}.
 - Some evidence of endocrine disruption (reduced gonopodial length) in male mosquitofish (*Gambusia holbrooki*) at point (WWTP effluent) and non-point (agricultural and residential) sources^{4,5}, but other studies report no adverse endocrine effects in mosquitofish⁶.

Study Objective: To more accurately determine the risk of endocrine disruption in Australian rivers.

Approach: Collect mosquitofish and water samples from six sites in Southeast Queensland and measure a variety of biomarkers to assess endocrine and overall health.

- Identified four hotspots and two undeveloped sites out of 73 previously monitored^{2,3}. Site selection based on recent monitoring (“exposure”) and *in vivo* (“hazard”) data.
- Sites sampled: two agricultural (AGR1 and AGR2), one WWTP, one landfill (LNDF) and two undeveloped (“reference”) sites (REF1 and REF2).

RESULTS

Mosquitofish

1) Histopathology and morphology (Table 1)

- No histological or morphological evidence of endocrine disruption.

2) Chemical body burden [QuEChERS + LCMSMS]

- Detection limits for 38 compounds ranged from 4 to 61 ng/g ww.
- At least one compound detected in 60% of fish (52 of 87).
- Max number of compounds in a fish = 8 out of 38 (Site WWTP).
- Highest detection frequency at a site = 91% (Site WWTP).
- Highest average number of compounds per fish = 2.6 ± 0.4 (Site WWTP).
- Highest concentration = 7,703 ng/g ww (TCEP; Site WWTP).
- TCEP, omeprazole, chlorpyrifos, androsterone, dihydrotestosterone present at concentrations >1,000 ng/g ww.
- **Pharmaceuticals detected in 40% of mosquitofish.**
 - Clozapine was the most frequently detected pharmaceutical (16%)
 - Fluoxetine, omeprazole and caffeine also detected.
- **Pesticides detected in 25% of mosquitofish.**
 - Chlorpyrifos detected in 19% of fish.
- **Natural and/or synthetic hormones detected in 29% of mosquitofish.**
 - Various hormones and EE2 (@ AGR2 and REF2; max = 37 ng/g ww) detected.

Water analysis

3) Chemistry [SPE + LCMSMS / GCMSMS]

- Max number of compounds per sample = 6 of 50 (Site WWTP).
- Samples from sites AGR1 and REF2 contained three compounds each, LNDF and REF1 had one each, and no TrOCs were detected in water from AGR2.
- Carbamazepine, gemfibrozil, paracetamol, salicylic acid, caffeine and triclosan detected (but all concentrations < PNEC).
- EE2 was detected twice (0.07 and 0.11 ng/L at REF2 and LNDF, respectively; PNEC = 0.1 ng/L).

4) *In vitro* endocrine activity [SPE + GeneBLazer]

- All 6 endocrine responses detected in at least one sample (Table 2).
- ER activity >1 ng/L EEQ at AGR1 site

Table 1. Summary of morphological and histological analyses for mosquitofish (*Gambusia holbrooki*) collected at six sites in Southeast Queensland. All data with reported error are presented as average ± standard error of the mean.

	Impacted sites			Undeveloped sites		
	AGR1	WWTP	LNDF	AGR2	REF1	REF2
Sample size for						
Histology (n) ^a	9 (2/7)	15 (9/6)	7 (5/2)	0	6 (2/4)	22 (11/11)
Vitellogenin (n) ^b	6	11	10	5	5	10
QuEChERS (n)	13 (5/8)	21 (9/12)	14 (7/7)	9 (4/5)	13 (3/10)	14 (6/8)
Morphology						
Male (n)	13	29	22	9	10	27
Mass (mg)	198.6 ± 11.1	164.5 ± 6.7	296.9 ± 84.2	208.0 ± 9.1	182.9 ± 22.1	174.6 ± 8.1
Standard length (mm)	23.9 ± 0.4	23.96 ± 0.3	24.8 ± 0.4	24.1 ± 0.6	22.9 ± 1.03	22.7 ± 0.4
Condition factor (K) ^c	1.45 ± 0.03	1.34 ± 0.02	1.94 ± 0.58	1.48 ± 0.05	1.48 ± 0.04	1.53 ± 0.09
Gonopodium index ^d	0.31 ± 0.01	0.31 ± 0.004	0.32 ± 0.004	0.32 ± 0.01	0.304 ± 0.02	0.32 ± 0.01
Female (n)	15	18	9	5	14	19
Mass (mg)	340.3 ± 29.7	189.4 ± 13.1	569.03 ± 63.9	321.2 ± 39	311.6 ± 56.2	277.1 ± 35
Standard length (mm)	27.5 ± 0.7	23.5 ± 0.5	32.9 ± 0.99	27.5 ± 1.3	26.2 ± 1.6	24.9 ± 1.2
Condition factor (K)	1.58 ± 0.05	1.42 ± 0.02	1.56 ± 0.08	1.53 ± 0.07	1.53 ± 0.04	1.83 ± 0.22
Histopathology						
Gonads						
n (male/female)	5 (2/3)	12 (9/3)	4 (2/2)	0	4 (2/2)	21 (11/8)
Condition	Healthy	Healthy	Healthy	N/A	Healthy	Healthy
Liver						
n (male/female)	8 (2/6)	15 (9/6)	7 (5/2)	0	6 (2/4)	19 (10/9)
Fatty (lacy)	25.6 ± 4.8	50 ± 3.8	36 ± 6.9	N/A	38.7 ± 2.8	20.3 ± 3.7
Haem/Liq	6.4 ± 3.6	2.9 ± 0.6	4.1 ± 1.7	N/A	4.5 ± 1.7	4.7 ± 1.9
Kidney						
n (male/female)	8 (2/6)	14 (8/6)	5 (4/1)	0	6 (2/4)	17 (9/8)
Haem/Liq	15.03 ± 4.7	23.1 ± 5.2	17.9 ± 2.7	N/A	32.9 ± 11.1	24.9 ± 3.4
Inclusion	3.1 ± 1.1	4.2 ± 0.8	5.8 ± 1.5	N/A	1.3 ± 0.7	1.8 ± 0.5

Condition factor (K) for males from WWTF was significantly different from REF2 (Kruskal-Wallis; p = 0.010).

^(a) total n (male n/female n); ^(b) male fish only; ^(c) Condition factor (K) calculated as K=100,000×W/L³, where W is the wet weight (in g) and L is the standard length (in mm); ^(d) Gonopodium index = gonopodium length (in mm)/standard length (in mm). “WWTF” = Wastewater treatment plant. “Haem/Liq” = percentage of area of kidney affected by haemorrhage and liquefaction.

Table 2. Endocrine activity of water samples prior to and at the time of fish sampling, determined by GeneBLazer (2013, this study) or CALUX (2011-2012²) assay.

	unit	Impacted sites						Undeveloped sites				
		AGR1		WWTP		LNDF		AGR2		REF1	REF2	
<i>In vitro</i> endpoint		2011-12	May 2013	2011-12	May 2013	2011-12	May 2013	2011-12	May 2013	2011-12	May 2013	May 2013
Estrogenic	ng/L EEQ	<0.1 - 0.52	1.18	<0.1 - 1.16	0.89	<0.1 - 0.28	0.1	<0.1	0.78	<0.1	0.04	<0.02
Anti-estrogenic	µg/L TMXEQ	<20	2.44	<20	<1	<20	1.46	<20	<1	<20	<1	2.74
Androgenic	ng/L DHTEQ	<7	<9	<7	<9	<7	<9	<7	<9	<7	<9	<9
Anti-androgenic	µg/L FluEQ	<60	96	<60	90	<60	83	<60	80	<60	90	73
Progestagenic	ng/L LevoEQ	<5	0.09	<5	<0.06	<5	0.14	<5	<0.06	<5	<0.06	0.11
Anti-progestagenic	µg/L MifeEQ	<8	4.2	<8	2.2	<8	3.5	<8	<1.8	<8	1.8	2.1

CONCLUSIONS

- No morphological abnormalities associated with endocrine disruption at sites with moderate estrogenicity (up to 1.18 ng/L EEQ) – but vitellogenin analysis pending.
- Anthropogenic chemical loads may increase chemical body burden and stress on organs (e.g., liver and kidneys).
- Results suggest that endocrine disruption in fish unlikely at most of the 73 sites sampled in 2011-12²; but what is the effect of body burden residues on overall health?

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