

# **FRST Enterprise Scholarship**

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## **Final Report**

### **Identification and characterization of endocrine disrupting compounds (EDCs) in treated sewage water**

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## A. PROGRAMME OF STUDY

This project was entirely research-based.

## B. RESEARCH

### B.1. Introduction

In the mid-1980s, anglers in the United Kingdom reported the presence of hermaphrodite roach (*Rutilus rutilus*) in sewage treatment plant (STP) lagoons (Purdom *et al.* 1994). Follow-up studies by Thames Water and the United Kingdom Environment Agency discovered a high incidence of ovotestis (the presence of both male and female sex cells in the gonads of an individual) in roach captured in several English rivers (reviewed in Desbrow *et al.* 1996, IEH 1999). Roach are a gonochoristic species, in other words they normally have very distinct male and female sexes. The presence of intersex individuals was thus a clear indication that normal sexual development was being negatively affected in wild populations and prompted a concerted effort to identify the cause of this dysfunction (IEH 1999). Sexual development in most vertebrates is directed by hormones, and steroid hormones such as estrogens and androgens play a critical role in sex differentiation. The discovery that treated sewage effluent was estrogenic to fish led to the hypothesis that compounds in treated sewage were interfering with the normal function of these sex hormones (Purdom *et al.* 1994, Harries *et al.* 1996). A toxicity identification and evaluation (TIE) approach revealed that natural and synthetic hormones excreted by humans as well as some alkylphenolic industrial chemicals present in treated sewage were responsible for the majority of the estrogenic activity (Desbrow *et al.* 1998, Routledge *et al.* 1998). This problem was not confined to the United Kingdom, and studies in continental Europe, Japan, and North America quickly confirmed that treated sewage there also contains chemicals with estrogenic activity (Solé *et al.* 2000, Körner *et al.* 2001, Onda *et al.* 2002) and that these may be impacting a wide range of wild fish species (Folmar *et al.* 1996, Jobling *et al.* 1998, Hashimoto *et al.* 2000, Folmar *et al.* 2001, Christiansen *et al.* 2002). Since the discovery of androgenic hormones in treated sewage (Kirk *et al.* 2002) and river water (Thomas *et al.* 2002), there has also been concern about androgenic effects of treated sewage. Due to broad socioeconomic and climatic differences, data generated in Europe or North America cannot necessarily be extrapolated to Australia and/or New Zealand. For example, Layton *et al.* (2000) showed that temperature can significantly affect the rate of degradation of hormones in activated sludge. To our knowledge, there are to date no other studies on the estrogenicity or androgenicity profile of sewage during treatment for Australia or New Zealand. This research aimed to address this knowledge gap. The estrogenic and androgenic activity of sewage during treatment at several municipal STPs was monitored using a battery of complimentary bioassays.

We also examined mosquitofish (*Gambusia holbrooki*, Girard 1859) for signs of exposure to androgenic or estrogenic chemicals. Males are much smaller than the females and have an elongated anal fin, the gonopodium, which is used as an intromittent organ during copulation. Gonopodium development is under androgenic stimulation from the testis in the final stages of sexual maturation (Turner 1941), and can be inhibited by castration (Turner 1947) and to a lesser extent by exposure to estrogenic chemicals (17 $\beta$ -estradiol, 100ng/L) (Doyle and Lim 2002). Conversely, laboratory exposure of juvenile females to androgenic stimulation (11-ketotestosterone, 20 $\mu$ g/g) results in gonopodium-like elongation of the anal fin typical of juvenile males (Angus *et al.* 2001). This hormone-dependent morphological attribute, along with their restricted home-range, abundance, and widespread distribution in Australia make the mosquitofish an ideal local indicator species for exposure to EDCs (Bortone and Davis 1994, Overstreet *et al.* 1996).

### **B.2. Overall purpose of the research programme**

The purpose of this research programme was to determine the endocrine disrupting potential of treated sewage and wastewater in Australia and New Zealand using laboratory bioassays and field bioindicator species. A solid-phase extraction (SPE) method was used to extract organic analytes from wastewater samples. The samples were collected at different stages during treatment at several municipal sewage treatment plants (STP) in south Queensland and Canterbury. Bioassays were then used to evaluate the extent to which those extracted samples were capable of interfering with the endocrine system. The aim of this approach was to assess the efficacy of different technologies to remove endocrine disrupting compounds (EDCs) from domestic sewage and the levels of EDCs discharged into the environment in the treated effluents. It was concluded that while raw sewage contained significant levels of both estrogenic and androgenic activity, treatment (in particular activated sludge) was effective at reducing that activity and that the levels in the final treated effluent were unlikely to cause significant effects in exposed wildlife.

In the field, mosquitofish (*Gambusia holbrooki*) were collected from two south Queensland sites receiving undiluted secondary-treated sewage (one a wetland for tertiary treatment of sewage, and the other a holding pond), as well as a reference site. Anal fins and gonads were examined for evidence of endocrine disruption. Fish at one sewage effluent site exhibited slightly elongated anal fins suggestive of androgenic stimulation, but the effect was not evident at sites further downstream, indicating it was related to a short-lived chemical. It was concluded that final treated effluent was unlikely to cause adverse effects in mosquitofish, but concerns were raised about the sensitivity of this species to low levels of contaminants and further research with more sensitive biomarkers is required to provide an accurate assessment of the effect of very low levels of EDCs on endemic fish populations.

### **B.3. Objectives**

This project had five main objectives:

- To develop *in vitro* bioassays and chemical extraction techniques (using locally available resources) to evaluate the potential of EDCs in complex wastewater samples (Objective 1A).
- To develop techniques to measure biomarkers of exposure to EDCs in mosquitofish (Objective 1B).
- Building on Objectives 1A&B, to measure and characterize EDCs in sewage waters from Australia and New Zealand (Objective 2A), and evaluate their potential impacts in exposed mosquitofish populations (Objective 2B).
- To determine the efficacy of different sewage treatment technologies to eliminate EDCs (Objective 3).

### B.3.1. Objective 1A – Methods development for *in vitro* bioassays

#### B.3.1.a. Intent

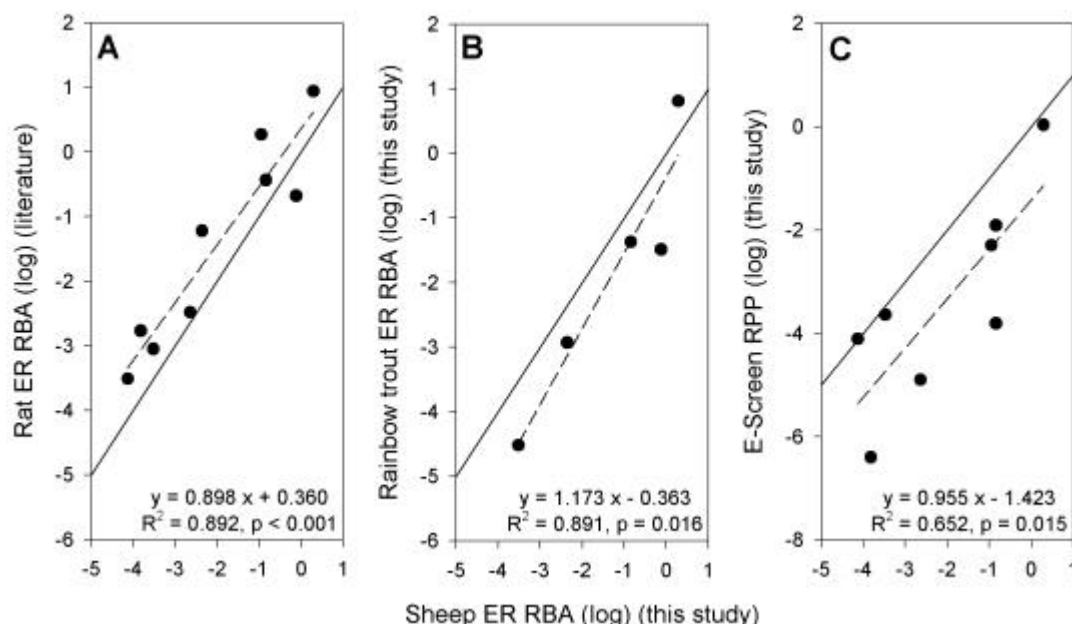
To develop *in vitro* bioassays and extraction techniques (using locally available resources) to evaluate the potential of EDCs in complex wastewater samples.

#### B.3.1.b. Results

We have developed several assays to examine the effects of EDCs at different biological levels. At the molecular levels, we developed a sheep estrogen receptor (ER) binding assay at Lincoln University. A rainbow trout liver ER binding assay and a rainbow trout brain androgen receptor (AR) binding assay were developed in collaboration with Mike van den Heuvel and Emil Bandelij (Forest Research, Rotorua, NZ). The results of the two ER binding assays (sheep and rainbow trout) were compared with literature values of the more commonly used rat ER binding assay, and there was a good correlation between the results of the different assays (Fig. 1A and 1B). This suggests that the sheep ER binding assay is a reliable alternative to the more established rat ER binding assay.

We compared the results of our molecular bioassays with those of a cellular bioassay (E-Screen) developed by Anna Eriksson (Landcare Research, Lincoln, NZ) and found a good correlation between the results with the two assays (Fig. 1C), suggesting that binding to the ER is a good predictor of the whole cellular response. For this project, we therefore relied mostly on the results of the sheep ER and rainbow trout AR binding assays, as that would be the most likely mode of action of most EDCs in municipal sewage (such as natural and synthetic hormones).

To extract organics from sewage samples, several solvents and reversed solid-phase extraction (SPE) cartridges were tested. In the end, Waters Oasis HLB cartridges were deemed to be the most efficient and reliable (data not shown), and methanol was selected as elution and carrier solvent.



**Figure 2.** Comparison of relative binding affinities in the sheep ERBA (abscissa) vs. (A) the rat ERBA, data from Kuiper *et al.* (1997) and Fang *et al.* (2000); (B) the rainbow trout ERBA; and (C) the relative proliferative effect in the E-Screen. The unbroken line is the isometric line. The equation, R<sup>2</sup>, and p value at the bottom right of each graph are those of the best regression line (dashed line).

### B.3.2. Objective 1B – Methods development for *in vivo* biomarkers

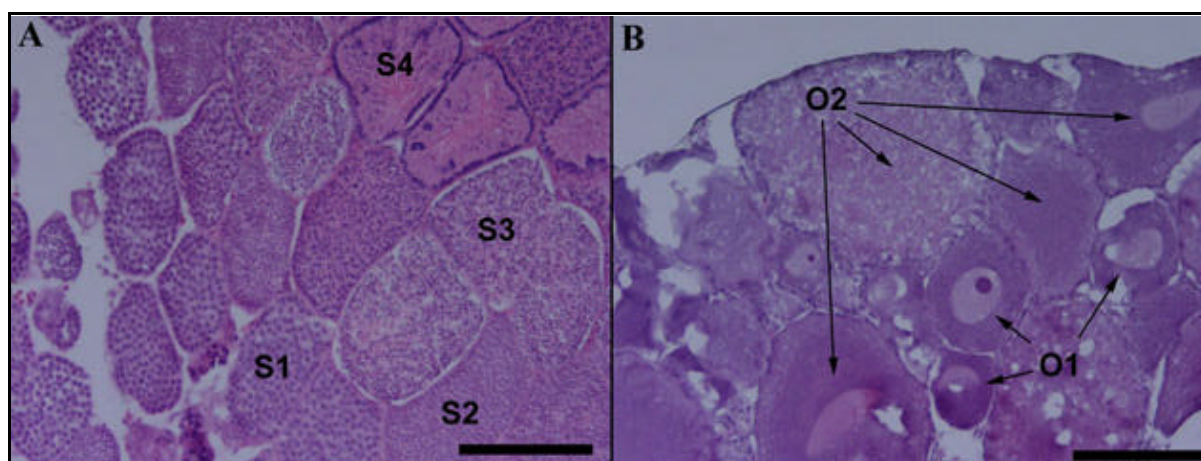
#### B.3.2.a. Intent

To develop techniques to measure biomarkers of exposure to EDCs in mosquitofish.

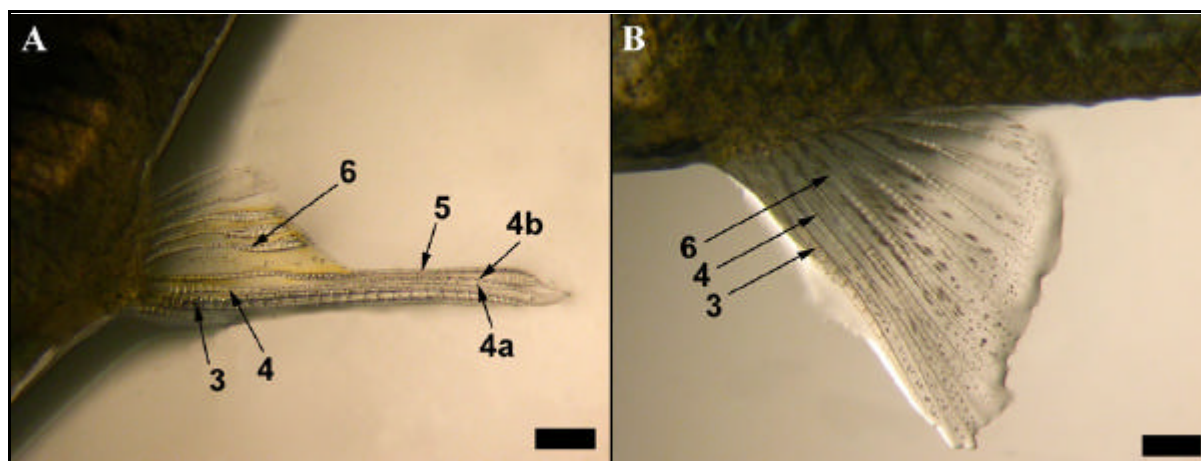
#### B.3.2.b. Results

An ecotoxicology laboratory was set up at Griffith University (Brisbane, Qld) and digital microscope techniques (to measure the anal fin of mosquitofish) were adapted from Richard Lim's group at the University in Sydney (UTS, Sydney, NSW). A protocol was also developed to prepare histology sections of the gonads of mosquitofish for examination, based on the recommendations of an international panel of experts (OECD 2002). Typical gonad structures from a male and female mosquitofish are presented in Fig. 2, and the typical morphology of a male and female anal fin is described in Fig. 3.

A novel reverse-transcription real-time polymerase chain reaction (RT-PCR) assay to measure vitellogenin mRNA was also developed, using a gene sequence for vitellogenin provided by Andrew Laurie (Landcare Research, Lincoln, NZ). We did not have time to apply this novel technique to field measurements during this project, but it is expected to be more sensitive than the morphological biomarkers (anal fin and gonadal histology) and will be of use for future work with mosquitofish.



**Figure 2.** Gonadal histology *G. holbrooki*. Bar = 100µm. **A:** Typical male gonad, with different stages of spermatocyst development, from spermatocytes (S1), to spermatids (S2), to early (S3) and late stages of spermiogenesis (S4). **B:** Typical juvenile female gonad, showing primary (O1) and early and late cortical alveoli oocytes (O2).



**Figure 3.** Anal fin of *G. holbrooki*. Bar = 1mm. **A:** Adult male anal fin, with rays 3 to 6 labelled. **B:** Adult female anal fin, with rays 3,4, and 6 labelled.

### **B.3.3. Objective 2A – EDCs in sewage**

#### *B.3.3.a. Intent*

To measure and characterize EDCs in sewage waters from Australia and New Zealand.

#### *B.3.3.b. Results*

Using the techniques developed in Objective 1A, we measured the estrogenic and androgenic activity of sewage samples from 13 STPs in south Queensland and 2 STPs in Canterbury (Table 1).

**Estrogenic activity:** All raw sewage samples tested in this study displayed significant estrogenic and androgenic activity (Table 1, “Raw”) at levels comparable to those reported by previous studies for municipal sewage treatment plants abroad (Desbrow et al. 1998, Shore and Shemesh 2003). As in previous studies, most of the activity was associated with the most polar fraction (data not shown), where natural and synthetic steroids are found (Desbrow et al. 1998, Snyder et al. 2001). In municipal sewage, these compounds originate from human excretion. Treatment was very effective at removing the estrogenic activity, and estrogenicity in the final effluent was below detection limit (< 1ng/L) at 9 of the 15 STPs tested, below quantification limit (< 4ng/L) at 5 of the STPs, and at 4.2ng/L EEq at one of the STPs tested (plant S6A, Table 1).

In laboratory exposures, the median effective concentration of 17 $\beta$ -estradiol required for a significant induction of the egg-yolk precursor vitellogenin in juvenile rainbow trout after 2 weeks of exposure was estimated to be approximately 10-20 ng/L (Routledge et al. 1998, Thorpe et al. 2003). In this study, estrogenic activity in final effluents (expressed in estradiol equivalents, EEq) was well below this level. Dilution in the environment upon discharge would further lower these concentrations, suggesting that the potential for the effluent to induce estrogenic effects in exposed wildlife is minimal, at least in the short term.

**Androgenic activity:** Fewer STPs were tested for androgenicity than for estrogenicity, and androgenic activity of all sewage samples tested with the androgen receptor (AR) binding assays is summarized in Table 1. Androgenic activity in raw and treated sewage was much higher than estrogenic activity (Table 1), and levels in raw sewage were similar to those reported for several STPs in the United Kingdom (Kirk et al. 2002). The androgenic activity in the plant effluents were, with the exception of plant F1A, similar or lower than levels reported overseas (Kirk et al. 2002, Thomas et al. 2002). With regards to the high levels of androgenicity in the effluent from plant F1A, it should be emphasized that the effluent from this particular plant flows through a constructed wetland for tertiary treatment before being discharged into the environment. Passage through the wetland, which takes approximately 14 days, was very efficient at removing the estrogenic activity of the plant effluent before final discharge (Table 1, EEq).

Little is known about the effect of exposure to androgenic chemicals in fish, and more research needs to be done to determine if the levels reported in the present study might induce effects in exposed fish populations.

**Table 1.** Estradiol and testosterone equivalents (EEq and TEq, respectively) for all 15 sewage treatment plants determined with sheep uterine estrogen and rainbow trout brain androgen receptor binding assays (ERBA and ARBA, respectively). Raw = raw sewage; 1°Treat = primary-treated sewage; 2°Treat = secondary-treated sewage; Plt Eff = plant effluent; L/HP/W = lagoon, holding pond, or wetland; Fin Eff = final effluent. NA = not available.

STP	STP Type <sup>a</sup>	Flow (m <sup>3</sup> /d)	Head (people)	Source <sup>b</sup>	Sample date	Air temp. <sup>c</sup> (°C)	Rain <sup>c</sup> (mm)	EEq <sup>d</sup> (ng/L ± SE)				TEq <sup>e</sup> (ng/L)			
								Raw	1°Treat	2°Treat	Plt Eff	L/HP/W	Fin Eff	Raw	Plt Eff
S1A <sup>f</sup>	S	NA	NA	D	7/03	15 – 23	2.2	50 ± 20			BDL	BDL	3360	196	
S1A <sup>f</sup>	S	NA	NA	D	7/03	14 – 23	1.5	29 ± 12			BDL	BDL	4890	208	
S2A <sup>f</sup>	S	NA	NA	D	7/03	15 – 23	2.2	< 4			BDL				
S2A <sup>f</sup>	S	NA	NA	D	7/03	14 – 23	1.5	15 ± 6.8			BDL		2570	BDL	
S3A	S	980	3 800 <sup>g</sup>	D	8/00	9 – 25	0.0	54 ± 26	80 ± 12	BDL	BDL				
S3A	S	980	3 800 <sup>g</sup>	D	8/02	8 – 22	0.0	76 ± 11							
S3A	S	980	3 800 <sup>g</sup>	D	7/03	9 – 23	0.0	93 ± 5.0			< 4		1920	105	
S4A	S	1 500	6 000	D	7/03	8 – 20	0.2	125 ± 8.5			< 4	BDL	6810	603	
S5A	S	5 900	23 000 <sup>g</sup>	D	7/03	14 – 22	4.2	137 ± 16	61 ± 13	6.0 ± 4.1	BDL				
S6A	S	3 500	13 000 <sup>g</sup>	D	7/03	14 – 22	4.2	163 ± 1.5		< 4	4.2 ± 1.7		6920	BDL	
S7A	S	58 000	220 000	D, I	8/02	7 – 24	0.0	62 ± 15							
S7A	S	58 000	220 000	D, I	7/03	9 – 22	0.0	64 ± 9.3	75 ± 17	< 4			9330		
S8A	S	6 800	26 000 <sup>g</sup>	D, i, b	7/03	11 – 21	0.2	81 ± 30	89 ± 29	< 4	< 4		2630	94.9	
S9A	S	130 000	750 000	D, I	7/03	13 – 21	0.0	51 ± 6.9	64 ± 19		BDL		4630	84.5	
S10A	S	12 000	45 000	D, I	7/03	11 – 21	0.2	66 ± 18 <sup>g</sup>			BDL		4150	736	
S11A	S	26 000	100 000 <sup>g</sup>	D	7/03	5 – 23	2.8		77 ± 3.4		< 4				
S12A	S	60 000	230 000 <sup>g</sup>	D, i	7/03	6 – 22	4.2				< 4		2460	214	
F1A	F	570	2 200	D	6/03	14 – 21	1.6	83 ± 30			6.4 ± 3.0	< 4	BDL	5340	2290
F2N	F	161 000	320 000	D, i	9/03	4 – 14	2.0	127 ± 14	55 ± 23	143 ± 15 <sup>g</sup>		BDL			
O1N	L	4 000	10 500	D	9/03	4 – 14	2.0	185 ± 31				BDL	BDL		

<sup>a</sup> The different types of secondary treatment were: (F) fixed film systems such as trickling filters; (S) suspended film systems such as activated sludge reactors; and (O) oxidation ponds.

<sup>b</sup> Source of influent are domestic (D), industrial (I), and bio medical (B). A lowercase letter indicates this type of wastewater contributes less than 15% of the total flow.

<sup>c</sup> Minimum and maximum temperature and rainfall at the closest monitoring station over a 48h period prior to sampling. Australian data courtesy of the Commonwealth Bureau of Meteorology; New Zealand data courtesy of the National Institute of Water and Atmospheric Research.

<sup>d</sup> Values are the mean of assays with receptors isolated from 2 different sheep, ± SE. Accurate quantification limit was 4 ng/L, and method detection limit was 1 ng/L.

Samples below detection limit are indicated as “BDL”, samples where activity was detected but was too low to be accurately quantified are marked as “< 4”.

<sup>e</sup> Value is the mean of duplicates in an assay with pooled receptors from several male rainbow trouts. Method detection limit was 6.5 ng/L. BDL = below detection limit.

<sup>f</sup> Indicates this plant is located in a high-tourism area, and flow can vary depending on tourist season.

<sup>g</sup> Actual head not available, equivalent population (EP) estimated based on average daily water use per person in Australia (260L/person/d).

<sup>h</sup> 24 h-composite sample.

### **B.3.4. Objective 2B – Impact on resident mosquitofish populations**

#### *B.3.4.a. Intent*

To measure the potential impacts of STP effluents on exposed mosquitofish populations.

#### *B.3.4.b. Results*

Male and female mosquitofish were sampled in July 2003 at two test sites (sites A and B) receiving undiluted secondary-treated sewage from two STPs (F1A and S11A in Table 1) near Brisbane in south Queensland, Australia. A reference site was also sampled and consisted of a wetland constructed for educational and recreational uses which receives water from a water storage reservoir located in a mostly forested catchment. Sewage effluent site A is an artificial wetland that provides tertiary treatment for a small municipal STP (plant F1A in Table 1). Three cells were sampled at site A: A1, A2, and A3 (Table 2). Secondary-treated sewage enters the wetland in cell A1 and is further treated as it slowly flows into cells A2 and then A3. The tertiary-treated sewage in cell A3 is then discharged into the environment. Water residence time in the wetland is approximately 14 days. Sewage effluent site B is a holding pond for a much larger municipal STP (plant S11A in Table 1).

There was no evidence of ovotestis (“intersex”) in fish at any of the sites sampled, and no gross morphological differences between fish from test and reference sites. While the treated domestic sewage water discharged from STP S11A (site B) did not significantly affect any of the endpoints used in this study (Table 3, Fig. 4), significant differences in anal fin elongation were observed at the site A. Adult males captured immediately downstream of the effluent discharge by plant F1A into the wetland (site A1) had the most elongated anal fins (“GP4”) of all mosquitofish sampled (Fig. 4A), and gonopodia of small adult males were significantly longer than those captured at the reference site (Table 3). This is similar to the results of Angus et al. (2002) who found that western mosquitofish (*G. affinis*) captured downstream of a North American STP had significantly elongated gonopodia and larger testes (when corrected for total body weight) than those at a reference site, both of which are suggestive of an androgenic effect. It is important to note however that the site A wetland from which the mosquitofish were sampled is a part of the treatment train of STP F1A. A closer look at sites A2 and A3 reveals a trend towards normalisation of gonopodium morphology downstream from site A1, and the anal fins of fish captured at these sites did not differ significantly from those of fish at the reference site (Table 3 and Fig. 4). This suggests that the wetland efficiently removed the androgenic activity still present in the secondary-treated effluent and that by the time the wastewater has flowed through the entire wetland (approximately 14 days), the activity has been significantly lowered to a level where it no longer causes concern. There were also no significant differences in the length of the 4<sup>th</sup> anal fin ray in females at any of the sites (Fig. 4B).

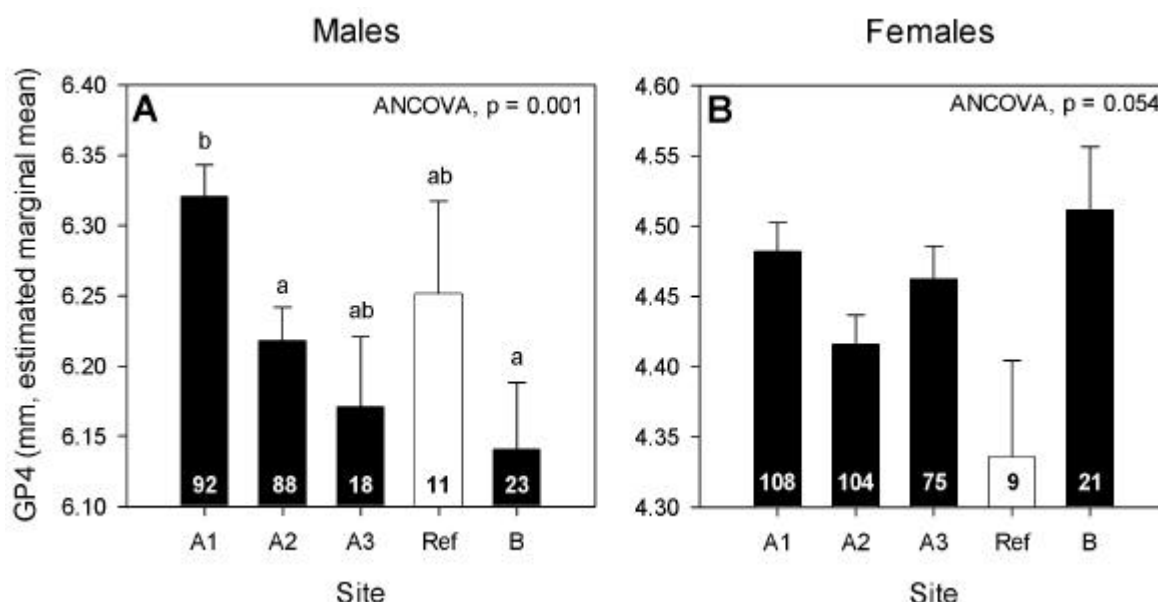
Although mosquitofish have been shown to be a relevant organism to measure exposure to hormonally-active chemicals in wildlife (Bortone and Davis 1994, Batty and Lim 1999), it is possible that the morphological endpoints used in this study were not sensitive enough to detect more subtle effects. More research needs to be done to examine the effect of trace levels (ng/L) of hormonally-active chemicals on those morphological endpoints, as well as in the development of more sensitive biomarkers.

**Table 2.** Sampling site description, including description of the sewage treatment plants (STP) at the two tests sites. Letters in bold in the STP treatment train column highlight differences between the two STPs. Plant ID refers to STP identifiers used in Table 1. Steps of the treatment train: S = screens; GGC = grit and grease chamber; PS = primary settling tank; TF = trickling filter; AS = activated sludge bioreactor; SS = secondary settling tank; CC = chlorine contact tanks; Wt = wetland; HP = holding pond.

Site	Water type	Water temp (°C)	STP treatment	Flow (ML/d)
Ref	Reservoir dam	16 – 19	Not applicable	NA
A	A1	13 – 19	Plant F1A: S, GGC,	26
	A2		PS, <b>TF</b> , SS, CC, <b>Wt</b>	
	A3			
B	Secondary-treated sewage	15 – 20	Plant S11A: S, GGC, PS, <b>AS</b> , SS, CC, <b>HP</b>	0.57

**Table 3.** Length of the 4<sup>th</sup> anal fin ray (GP4) and relative GP4 extension (GPx) for adult male mosquitofish (identified by the presence of a fully -developed gonopodium) arranged in three different size classes. Values are means at a site within a size class  $\pm$  SE. The value in brackets is the sample size. Different letters indicate statistically significant differences among sites within that size class (one-way ANOVA followed by Bonferroni's test,  $\alpha = 0.05$ ). NA = not available.

Site	Size class		
	16.3 - 19.1mm	19.2 - 21.9mm	22.0 - 24.7mm
A1	6.11 $\pm$ 0.05 (13) b	6.36 $\pm$ 0.03 (69)	6.90 $\pm$ 0.08 (10)
A2	5.85 $\pm$ 0.05 (37) a	6.19 $\pm$ 0.05 (45)	6.72 $\pm$ 0.07 (6)
A3	5.96 $\pm$ 0.08 (3) ab	6.28 $\pm$ 0.09 (15)	NA
Reference	5.72 $\pm$ 0.04 (6) a	6.26 $\pm$ 0.13 (5)	NA
B	5.89 $\pm$ 0.03 (2) ab	6.28 $\pm$ 0.06 (11)	6.86 $\pm$ 0.08 (10)



**Figure 4.** Length of the 4<sup>th</sup> anal fin ray (GP4) in male (left) and female (right) *G. holbrooki* collected at two sites receiving secondary-treated sewage (A and B) and a reference site (Ref). Values are estimated marginal means corrected for standard body length at each site  $\pm$  SE. The value at the bottom of each bar is the sample size. Different letters indicate statistically significant differences among different sites (one-way ANCOVA with standard body length as covariate followed by Bonferroni's test,  $\alpha = 0.05$ ).

### **B.3.5. Objective 3 – Efficacy of treatment**

#### *B.3.5.a. Intent*

To examine various sewage treatment steps and determine the efficacy of these technologies to eliminate EDCs.

#### *B.3.5.b. Results*

**Estrogenic activity:** The estrogenic activity in primary-treated sewage as determined by the ER binding assay was often higher than in raw sewage (Table 1, “1° Treat”), indicating the formation of estrogenic chemicals during this treatment step. A combination of reactivation of steroid estrogens (which are excreted in an inactive form) during primary treatment (Ternes et al. 1999a) with the degradation of 17 $\beta$ -estradiol into the less potent estrogen estrone early in activated sludge treatment (Ternes et al. 1999b) could be one explanation for this phenomenon. Secondary treatment, and in particular activated sludge treatment (Table 1, “STP type” S), was very effective at reducing the estrogenic activity in sewage. With the exception of plant F2N, secondary treatment removed more than 90% of the activity in primary-treated sewage, in most cases to levels below quantification limit (< 4ng/L EEq). Overall, STPs with activated sludge treatment removed 92 to >99% of the estrogenic activity in the raw sewage (Table 1). These figures are similar to those reported for municipal STPs in other parts of the world (Shore and Shemesh 2003), and clearly indicate that activated sludge treatment is very effective at removing estrogenic activity from sewage water. In a study on the fate of estrogens during sewage treatment, Andersen et al. (2003) showed that most estrogens were either eliminated or bound to sludge during activated sludge treatment. Steroid concentrations in the sludge were not measured in this study, but further studies will investigate estrogenic activity in the sludge. The removal efficiency at plants with trickling filters was much more variable. The Australian trickling filter STP (F1A) removed 92% of the estrogenic activity, while the New Zealand trickling filter STP (F2N) actually caused an increase in estrogenic activity (Table 1). These results are similar to those reported in Giger et al. (1984, cited in Angus et al. 2002), where trickling filters were found to be less efficient than activated sludge systems at removing lipophilic contaminants. The poor efficacy of the New Zealand plant may be related to the lower temperature compared to the Australian plant (Table 1). Mann and Reid (1971) showed that degradation of a lipophilic contaminant in trickling filters decreased from 80% at 15°C to 20% at 5°C. In most cases, the plant effluent was discharged into a holding pond or tertiary wetland before discharge into the environment, where further degradation of the estrogenic chemicals took place (Table 1). Natural hormones (such as 17 $\beta$ -estradiol and estrone) and synthetic hormones (such as ethinylestradiol), which are responsible for most of the estrogenic activity in domestic sewage in the United Kingdom (Desbrow et al. 1998, Routledge et al. 1998), have been shown to be sensitive to photodegradation (Jürgens et al. 2002). Oxidation ponds at the New Zealand STPs (F2N and O1N) and the wetland at plant F1A were very effective at removing the estrogenic activity in municipal sewage and the levels in the final effluents were below detection limit (< 1ng/L EEq) (Table 1).

**Androgenic activity:** As was the case with the estrogenic activity, STPs with activated sludge treatment were more effective than trickling filters at removing the androgenic activity, with 82 to >99% removal in activated sludge plants vs. 47% in the trickling filter plant (F1A) (Table 1). Androgens have very similar physico-chemical properties as estrogens, and it is likely that sorption to activated sludge plays a major part in removing androgens from the aqueous phase as it does with estrogens.

**B.4. Overall conclusions**

The levels of estrogenic and androgenic activity in treated municipal sewage from 15 different plants in south Queensland (Australia) and Canterbury (New Zealand) were below those reported by researchers in the United Kingdom. Trickling filter technology is widely used in the United Kingdom (Angus et al. 2002). The lower levels of estrogenic and androgenic activities in treated sewage in Australia is likely due to the wider usage of activated sludge systems, which have been shown to be more efficient at removing endocrine disruptors from sewage. Further studies need to be undertaken to examine the potential long-term effects of treated sewage containing trace concentrations (ppt) of estrogenic and androgenic chemicals on exposed wildlife, as well as to determine if the unique Australian and New Zealand wildlife is more susceptible to hormonally-active chemicals.

## **B.5. Publications**

### **B.5.1. Papers submitted for publication**

**Leusch FDL**, Chapman HF, Körner W, Gooneratne SR, and Tremblay LA. Efficacy of an advanced biological and nutrient removal plant in Queensland (Australia) to remove estrogenic chemicals. Submitted to Environ. Sci. Technol. (Oct 2004).

### **B.5.2. Manuscripts ready for submission**

**Leusch FDL**, Eriksson AE, van den Heuvel MR, Chapman HF, Gooneratne SR, and Tremblay LA. Comparison of bioassays and solid-phase extraction cartridges for quantification of estrogenic and androgenic activity of wastewater samples. To be submitted to Ecotox. Environ. Saf.

**Leusch FDL**, Chapman HF, Kay GW, Gooneratne SR, and Tremblay LA. Gonadal histopathology and anal fin morphology in *Gambusia holbrooki* exposed to treated municipal sewage effluent. To be submitted to Arch. Environ. Contam. Toxicol.

**Leusch FDL**, Chapman HF, van den Heuvel MR, Gooneratne SR, and Tremblay LA. Estrogenic and androgenic activity of sewage from 15 municipal treatment plants in Australia and New Zealand. To be submitted to Ecotox. Environ. Saf.

**Leusch FDL**, van den Heuvel MR, Laurie AD, Chapman HF, Gooneratne SR, and Tremblay LA. Quantification of vitellogenin mRNA induction in *Gambusia affinis* by reverse transcription real-time polymerase chain reaction. To be submitted to Biomarkers.

### **B.5.3. Conference presentations**

**Leusch FDL**, Chapman HF, Gooneratne SR, and Tremblay LA. Use of bioassays in the study of endocrine disruption: a case study with sewage. APEC workshop MRC02/2004, 12-16 Jul 2004, Gold Coast, Qld, Australia.

**Leusch FDL**, Chapman HF, van den Heuvel MR., Tan B, Gooneratne SR, and Tremblay LA. A survey of EDCs in treated sewage water in Australia/New Zealand using chemical analysis methods and integrative bioassays. Interact 2004, 4-8 Jul 2004, Gold Coast, Qld, Australia.

**Leusch FDL**, Chapman HF, Gooneratne SR, and Tremblay LA. Survey of the endocrine-disrupting potential of sewage water in Australia and New Zealand using solid-phase extraction and in vitro bioassays. SETAC Europe 2004, 18-22 Apr 2004, Prague, Czech Republic.

**Leusch FDL**, Chapman HF, Gooneratne SR, and Tremblay LA. Gonadal histopathology of *Gambusia affinis* exposed to sewage treatment effluent. SETAC/ASE 2003, 28 Sep - 1 Oct 2003, Christchurch, New Zealand.

**Leusch FDL**, Chapman HF, Gooneratne SR, and Tremblay LA. Assessing the endocrine-disrupting potential of sewage water in Australasia. Limnological Society of New Zealand Annual Meeting 2002, 10-14 November 2002, Shantytown, New Zealand.

**Leusch FDL**, Chapman HF, Gooneratne SR, and Tremblay LA. Biological methods to determine the presence of compounds with estrogenic activity in treated sewage water. Interact 2002, 21-25 July 2002, Sydney, NSW, Australia.

## **C. SKILLS DEVELOPMENT AND FUTURE CAREER PLANS**

### ***C.1. Skills development***

The approach to this project was, by necessity, multi-disciplinary. Over the past 3 years, I have delved into areas of biochemistry, environmental toxicology, histology, molecular biology, and toxicogenomics, and tried to adapt techniques from these fields to advance the project. Needless to say, this experience has been most enriching. I have had to learn many new laboratory and field techniques, as well as develop new assays, which can be transferred to other institutions to further their research possibilities. The sheep ER binding assay for example is now in use at Landcare Research (Lincoln, NZ), the National Research Centre for Environmental Toxicology (University of Queensland, Brisbane, Qld, Australia), and is being set up at the University of Sydney (Sydney, NSW, Australia). The project was a collaborative effort involving researchers and institutions in Europe, Australia, and New Zealand. This international team effort has helped me develop communication and organizational skills which are the cornerstone of successful multinational projects.

### ***C.2. Future career plans***

I have been offered a post-doctoral position with the Cooperative Research Centre for Water Quality and Treatment in Brisbane, Qld, Australia. The main objectives of this two-year appointment will be to review available bioassays to measure estrogenic activity in wastewater samples, and develop a toolbox of bioassays and protocols for the water industry.

## **D. COMMENTS OF SCHOLAR'S TERTIARY EDUCATION MENTOR**

Fred Leusch is completing his PhD research on "An assessment of the endocrine-disrupting potential of sewage water in Australasia". He hopes to submit his thesis by end December 2004.

During the 3 years I have known him, I found Fred to be a mature, hard working, motivated and a responsible person with a positive attitude. He sets high standards for himself and is prepared to put in the time required to achieve them. Fred has shown academic maturity and excellent research skills and analytical skills.

He has got some exciting research findings on endocrine disruptor assessment methods and his travel to several conferences has afforded him an excellent opportunity to showcase the endocrine disruptor findings from an Australasian perspective. This study also gave him an opportunity to collaborate with other scientists and students in the field of endocrine disruption in Europe where most of the research related to his field is done.

I am extremely pleased with Fred's progress. Based on my assessment from what I have seen and read so far from the drafts of his thesis, I expect him to submit a high quality PhD thesis.

Ravi Gooneratne BVSc, Dip. Tox., PhD, FRCPath  
**Associate Professor in Toxicology**

## **E. SIGNATURES**

\_\_\_\_\_  
 Frederic DL Leusch  
 Fellow

Date: \_\_\_ / \_\_\_ / \_\_\_\_\_

\_\_\_\_\_  
 S Ravi Gooneratne  
 Mentor

Date: \_\_\_ / \_\_\_ / \_\_\_\_\_

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