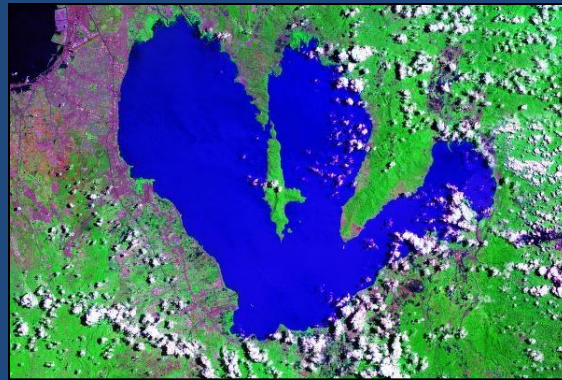


ESTROGENIC CONTAMINATION OF LAGUNA DE BAY AND ITS POTENTIAL IMPACT ON FISH HEALTH



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Environmental Services

PRODUCTIVE

- Fisheries
- Water source for irrigation
- Power generation
- Industrial cooling

CONSUMER

- Recreation
- Domestic use

Environmental Services

DISPOSAL

- Flood reservoir
- Absorptive sink for residuals of human activities



Sources of waste

- Domestic*
- Industries
- Agriculture - cropland areas, livestock & poultry production, fishery activities



Endocrine Disruptor

“An exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations.”

WHO, 2003

Environmental Estrogens

NATURAL ESTROGENS

- 17β -estradiol (E_2)
- Estriol
- Estrone
- Phytoestrogens

SYNTHETIC ESTROGENS

- 17α -ethinylestradiol
- Phthalates
- Organochlorine pesticides

Total Population	13.6 M
Projected Annual Population Growth Rate	2.76%

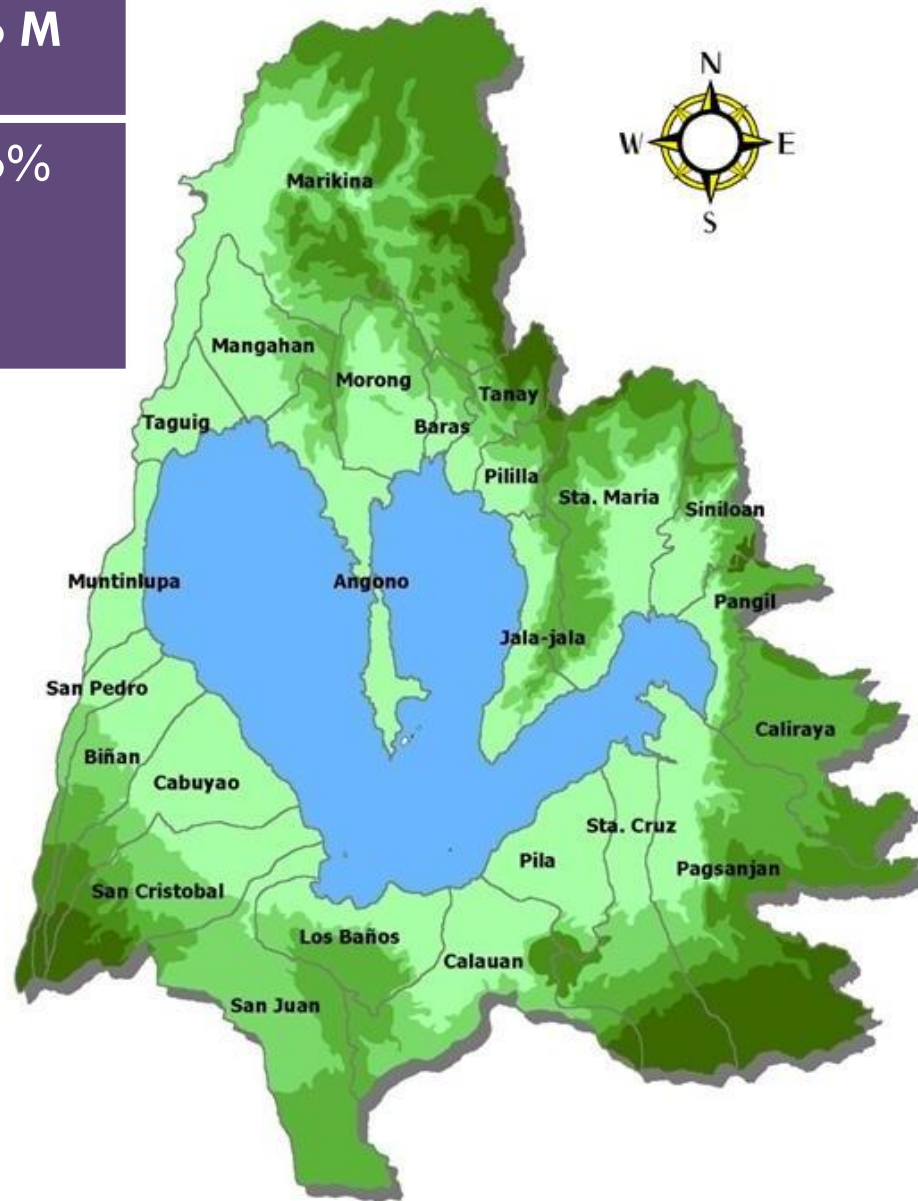
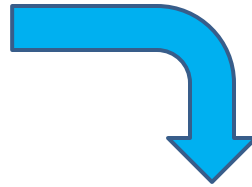
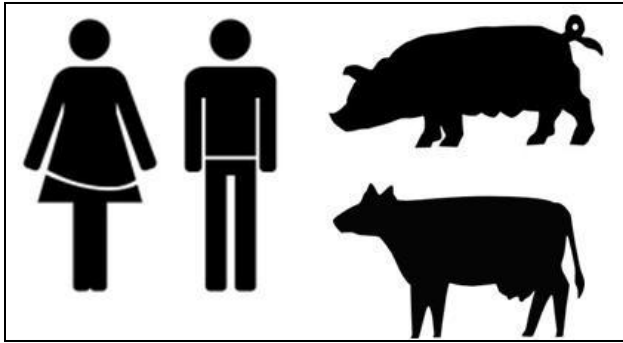


Figure 1. The Laguna Lake watershed and its 24 sub-basins (source: LLDA, 9/22/10).

SUB-BASIN	DOMESTIC POPULATION
Marikina	393,353
Mangahan	100,311
Angono	83,516
Morong	50,941
Baras	6,207
Tanay	10,992
Pililla	8,626
Jala jala	12,789
Sta. Maria	19,489
Siniloan	15,448
Pangil	19,068
Caliraya	13,890
Pagsanjan	45,805
Sta. Cruz	55,727
Pila	41,292
Calauan	98,561
Los Baños	57,816
San Juan	112,967
San Cristobal	120,880
Sta. Rosa/Cabuyao	160,643
Biñan	130,529
San Pedro	65,023
Muntinlupa	226,983
Taguig	123,384
Total	1,974,239

Table 1. Households in the Laguna Lake watershed without access to septic tanks (LLDA, 2007)



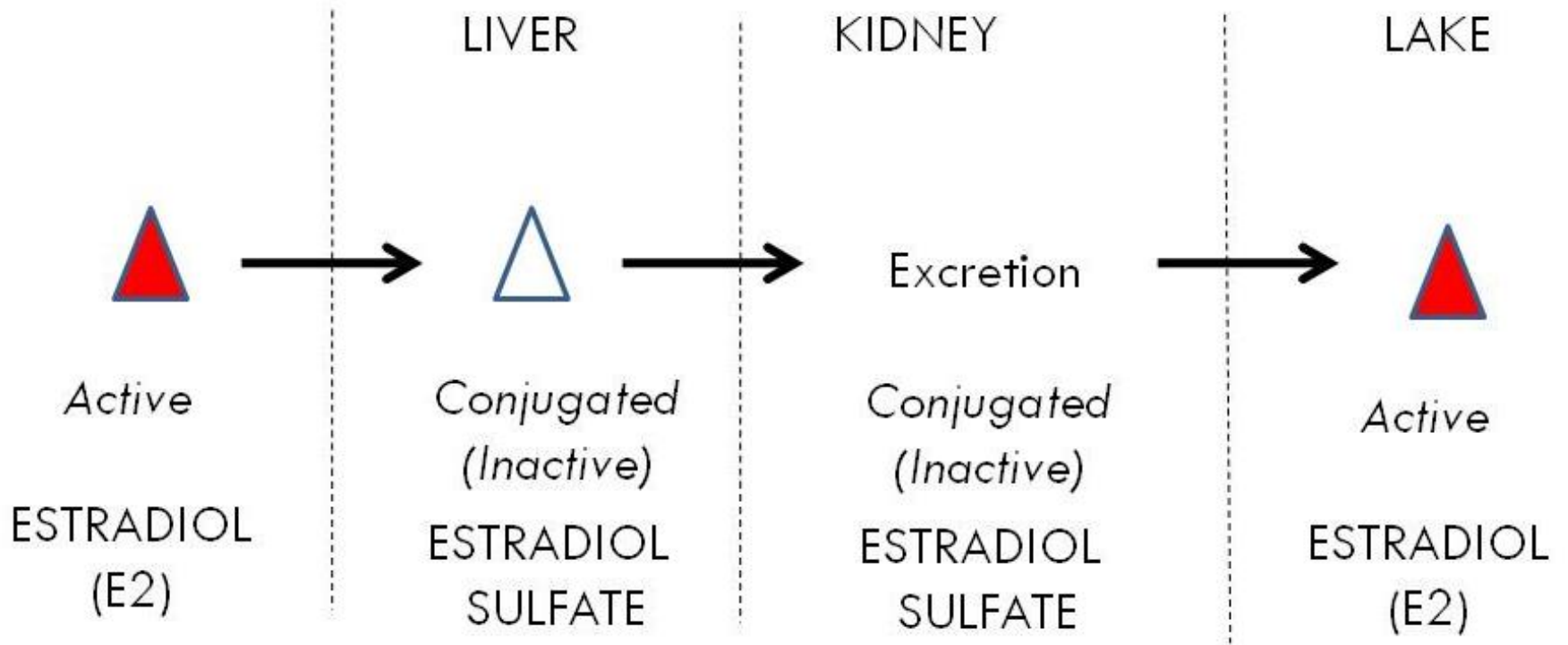
Source of natural
estrogens



Wastewater
treatment plants



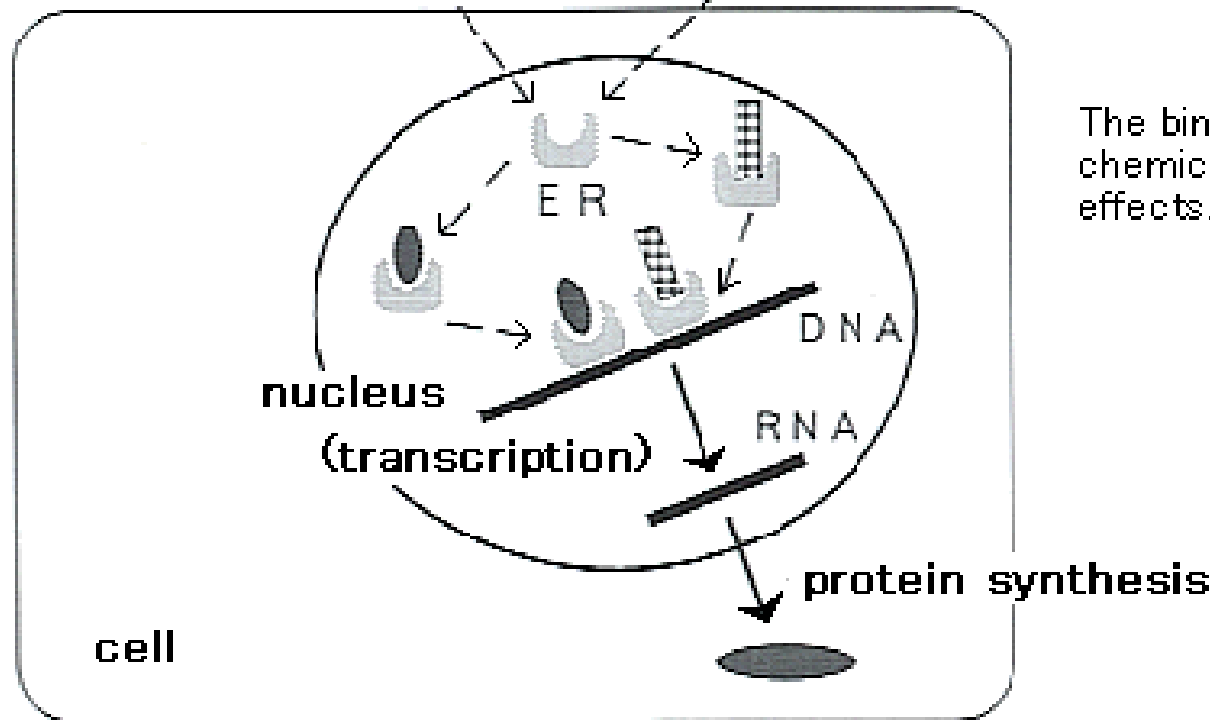
Receiving water
body



estrogen

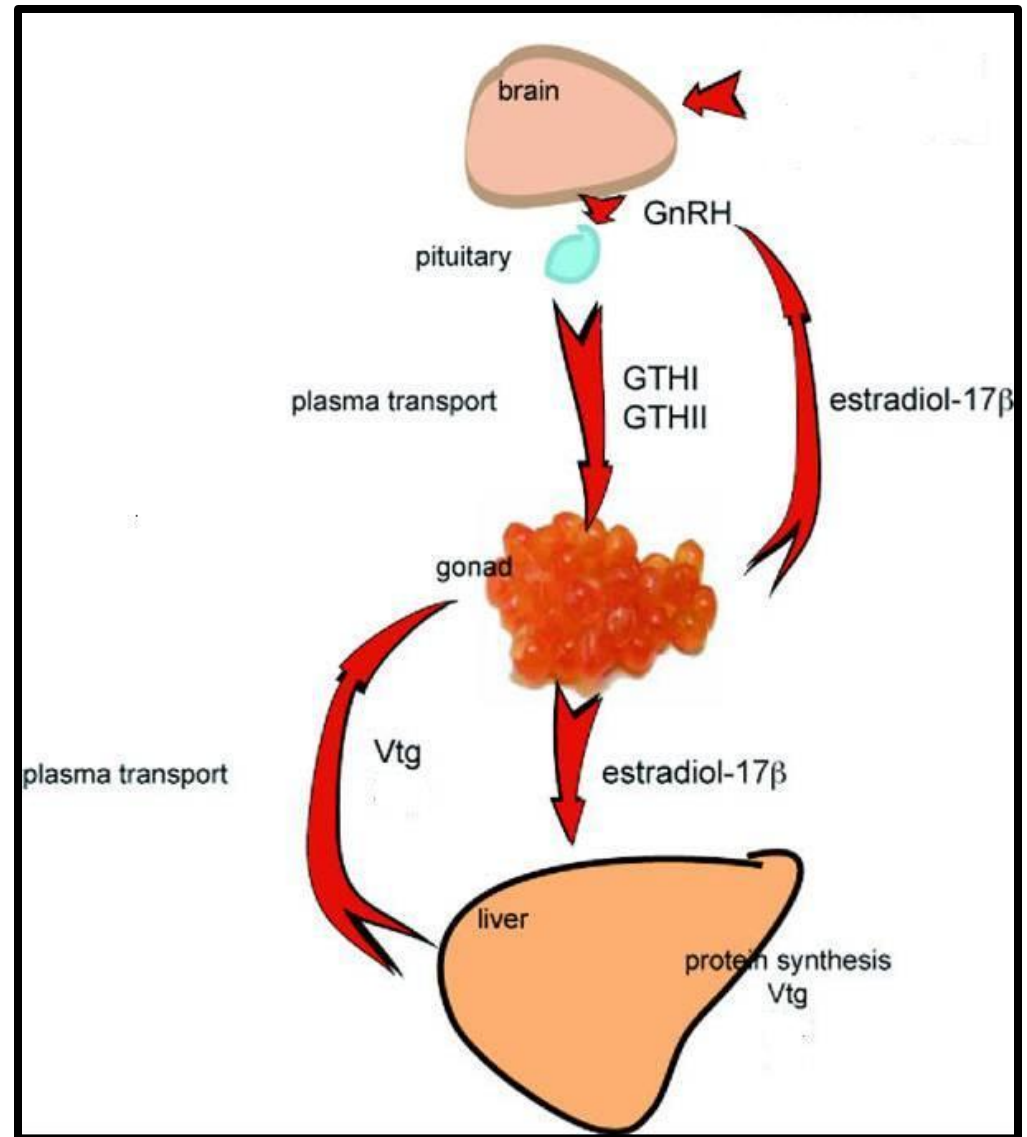
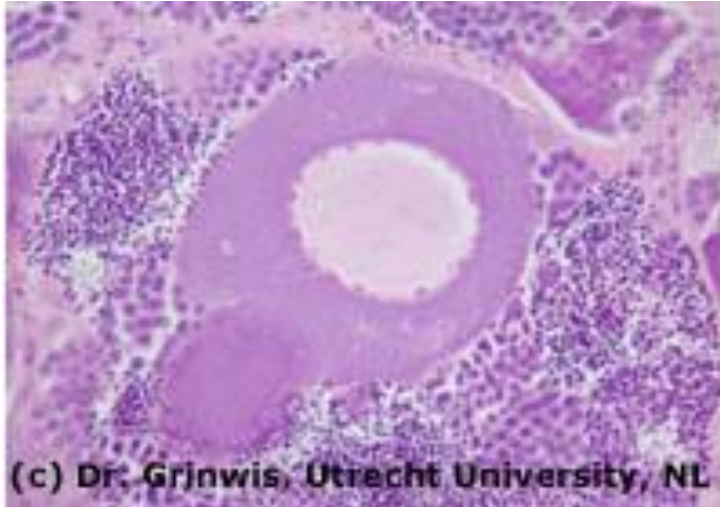
endocrine disrupting chemicals

(bisphenol A, nonylphenol,
phthalate, DDT, etc)



The binding of endocrine disrupting chemicals to ER results in estrogenic effects.





Environmental estrogens are linked to reproductive abnormalities in humans and in wildlife

CONCERNS



- Is Laguna de Bay contaminated with endocrine disruptors?
- Do these compounds pose a threat to fish health?



Methodology

Water analysis for E₂

Sampling
points: 16

Distance
between
sampling
points:
 ≥ 100 m



Fish cage studies





UPLB Limnological Research Station



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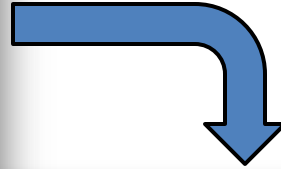
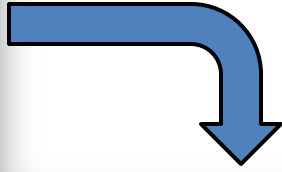
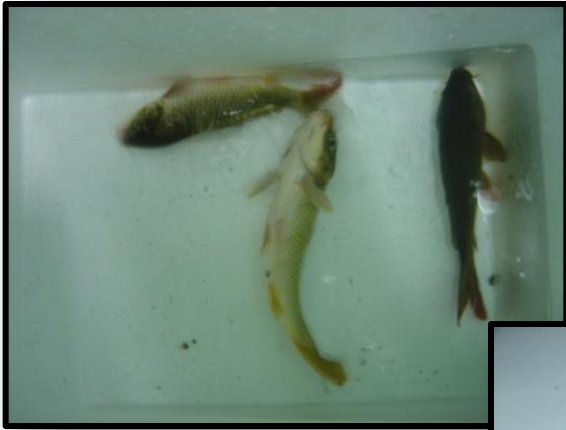
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Caging study (30 days)



Investigation of Biomarkers

1. Condition indicators
2. Immunological parameter
→ melanomacrophage centers
3. Endocrine Parameter
→ Vitellogenin levels
4. Histopathology
→ Testicular lesions



Condition indices

- Condition Factor

$$CF = [\text{total wt (g)} / \text{length(cm)}^3] \times 100$$

- Hepatosomatic index

$$HSI = [\text{liver weight(g)} / (\text{total wt(g)})] \times 100$$

- Gonadosomatic index

$$GSI = [\text{testis weight(g)} / (\text{total wt(g)})] \times 100$$

A horizontal bar at the top of the slide, divided into a red section on the left and a blue section on the right.

RESULTS

Table 2. Levels of 17-beta estradiol in water samples from the east and west bay of Laguna Lake.

Sampling site	Measured concentration (mean \pm SD) ($\mu\text{g/l}$)	
	August	September
East Bay (n=8)	0.29 \pm 0.07	0.39 \pm 0.15
West Bay (n=7)	0.37 \pm 0.12	0.40 \pm 0.16

Table 3. Plasma vitellogenin (VTG) levels in the reference and caged groups.

Sampling Site	Number of fish	VTG levels ($\mu\text{g/ml}$)
West Bay	9	8.33 ± 0.40^a
East Bay	12	8.77 ± 0.60^a
Reference	12	3.53 ± 4.85^c

Values are presented as means \pm SD.

Values within a column with different superscripts are significantly different at $p < 0.01$.

East and West Bay values are not significantly different using t-test.

Table 4. Morphometric data of reference and caged fish (mean \pm SD).

Sampling Site	GSI	HSI	CF
West Bay (n=12)	3.27 \pm 2.65	0.70 \pm 0.37 ^a	2.98 \pm 1.89
East Bay (n=12)	4.78 \pm 1.76	0.46 \pm 0.10 ^b	3.15 \pm 0.55
Reference (n=11)	5.83 \pm 2.56	1.61 \pm 2.54 ^a	4.03 \pm 2.26

GSI = gonadosomatic index; HSI = hepatosomatic index; CF = condition factor
 Values with different superscripts within a column are significantly different at $p < 0.05$ using ANOVA

East and West Bay values for HSI were significantly different using t-test.

Table 5. Developmental stage of testes from the reference and from the caged groups

Gonadal Staging	Reference group (n=11)	West bay (n=12)	East bay (n=12)
Juvenile	--	--	--
Stage 0	--	--	--
Stage 1	--	1	1
Stage 2	--	1	--
Stage 3	11	12	11
Stage 4	--	--	--

Values are presented as number of observations.

Table 6. Frequency of testicular abnormalities in reference fish (n=11) and those exposed to the east (n=12) and west bay (n=12) of Laguna Lake.

Diagnostic criteria	Reference group	West bay	East bay
<i>Primary</i>			
Presence of testis-ova	--	--	--
Increased proportion of spermatogonia	--	3	3
Testicular degeneration	--	--	--
<i>Secondary</i>			
Decreased proportion of spermatogonia	--	--	--
Interstitial fibrosis	--	1	--
Increased vascular or interstitial proteinaceous fluid	--	3	4
Altered proportions of spermatozoa or spermatocytes	--	1	2
Macrophage aggregates	--	8	8

Values are presented as number of observations.

(--) Not Detected.

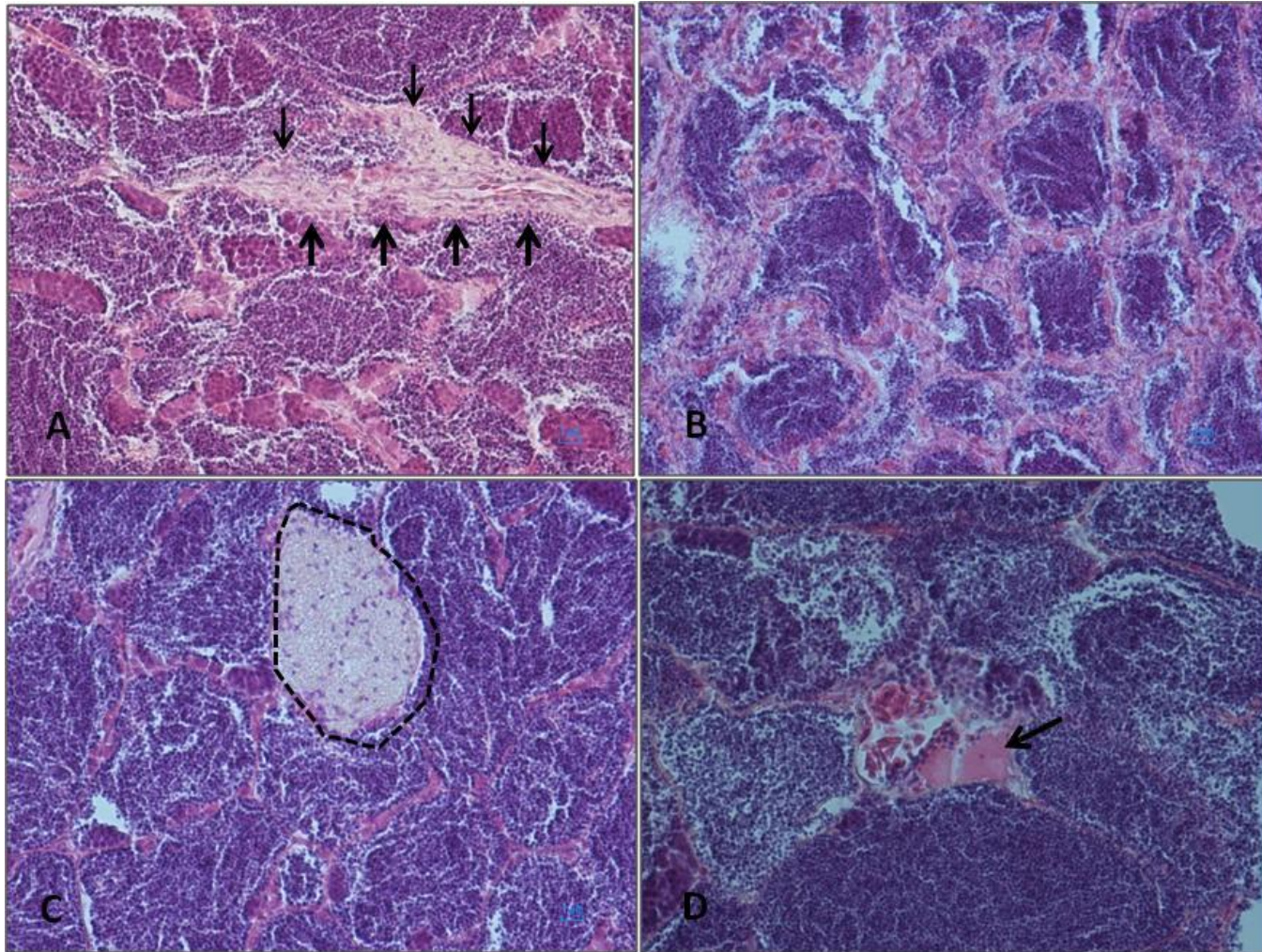


Fig. 1. Photomicrographs of testicular lesions in common carp caged in the east and west bay of Laguna Lake: (A) interstitial fibrosis (arrows); (B) increased spermatogonia; (C) macrophage aggregate (dotted lines) and (D) vitellogenin (arrow). H and E. Bar: 1 μ m.



Table 7. MMC number and size in the liver of reference and caged fish (mean \pm SD).

Sampling sites	Number of fish	Number *	Size (μm)**
West Bay	9	33.56 \pm 14.70 ^a	54.61 \pm 29.07 ^a
East Bay	9	29.78 \pm 15.57 ^a	48.61 \pm 32.36 ^a
Reference	7	10 \pm 3.27 ^c	17.17 \pm 9.73 ^b

*Values within a column with different superscripts are significantly different at $p < 0.01$.

**Values within column with different superscripts are significantly different at $p < 0.05$.

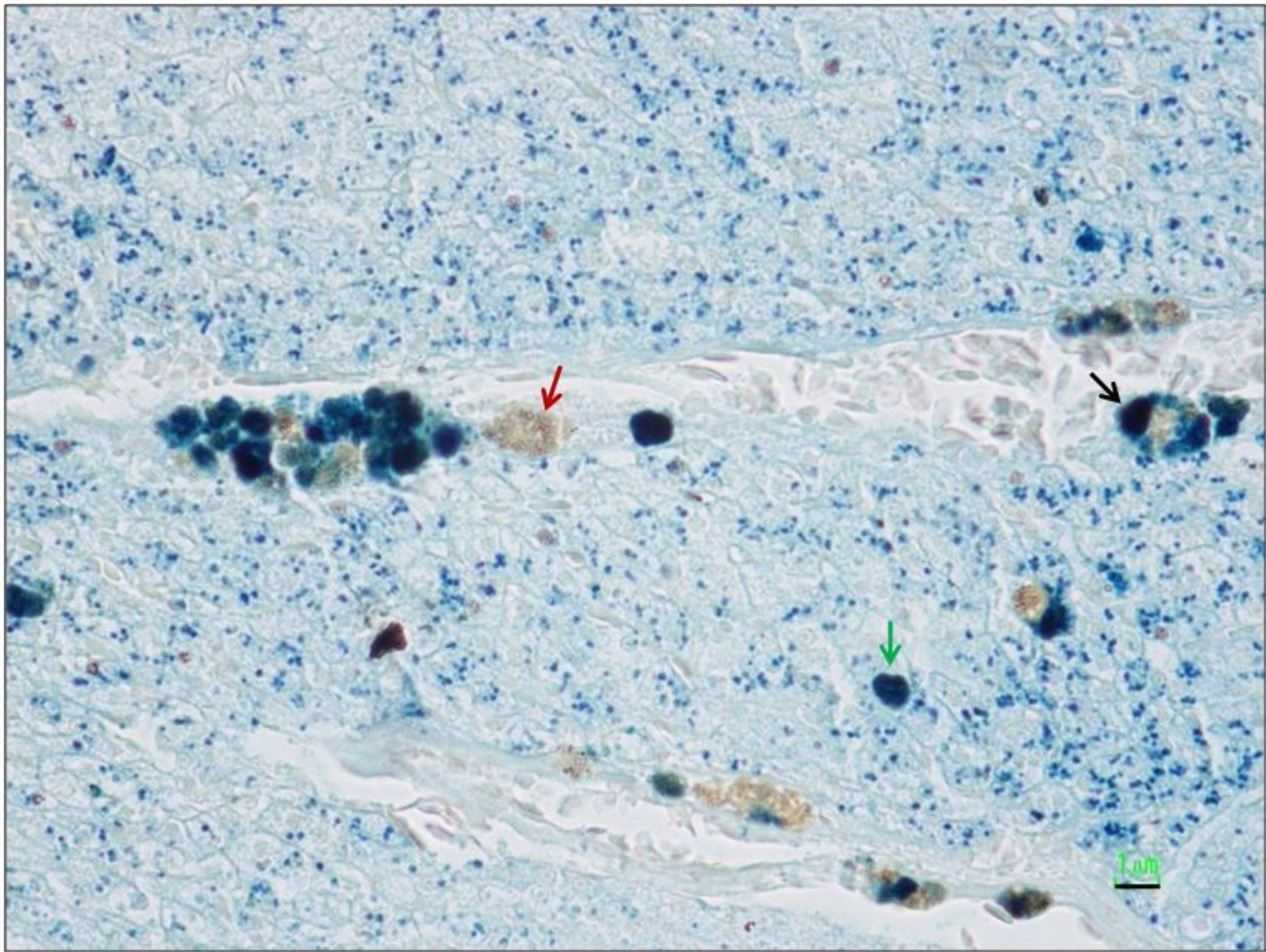


Fig. 2. Liver section of common carp caged in the west bay of Laguna Lake. Arrows point to pigmented melanomacrophage centers (MMCs). Lipofuscin (red arrow), melanin (black arrow) and hemosiderin (blue arrow). Perl's stain. Bar: 1 μ m.

Table 8. Pigment distribution (%) of MMCs in the liver of reference fish and caged fish (means \pm SD)

Sampling sites	Number of fish	Hemosiderin/iron (blue)	Lipofuscin (yellowish-tan)	Melanin (black)
West Bay	9	2.56 \pm 2.35	3.11 \pm 2.09	1.89 \pm 2.09^a
East Bay	9	1.78 \pm 1.86	2.22 \pm 1.79	0.89 \pm 1.36^a
Reference Site	7	0.43 \pm 0.53	1.86 \pm 1.35	0.14 \pm 0.38^b

Values with different superscripts within a column are significantly different at $p < 0.05$ using ANOVA.


East and West Bay values for all pigments are not significantly different using t-test.

Summary

- Biochemical responses reflecting the potential of contaminants in the lake to impair physiological processes in caged fish were analyzed
- Emphasis was given to those induced by 17 beta-estradiol (E_2) as part of a complex environmental mixture
 - Condition indicators: CF, HSI, GSI
 - Endocrine Parameter: VTG levels
 - Histopathology: Testicular lesions
 - Immunological parameter: MMCs

Conclusion

- Hormonal excretions of both humans and animals origin contaminate the lake
- Male fish have manifested effects of estrogenic exposure (i.e., testicular abnormalities, VTG synthesis in males)

- 
- Observations in MMC size and frequency imply the presence of a compound/s in the water that exert immunomodulatory or immunotoxicologic effects
 - Whether or not this biological response was induced by the steroidal estrogen as part of a complex environmental mixture should be a subject of future research

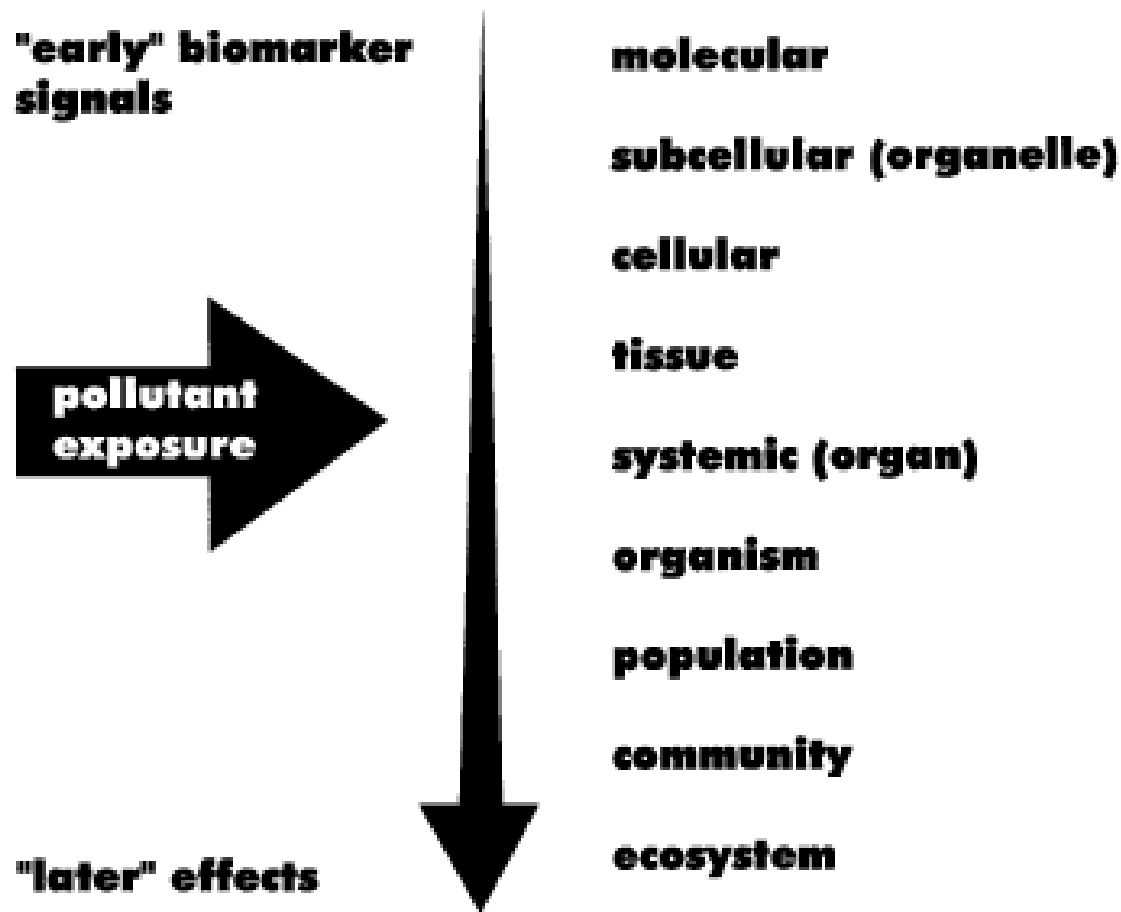




Fig. 3. Schematic representation of the sequential order of responses to pollutant stress within a biological system (van der Oost et al., 2003)

Recommendations

- Strict implementation of environmental laws, standards & regulations
- Control or prevent future emissions in the watershed
 - ▣ *Incorporation of E2 removal in the discharge permits*

- 
- Establishment of basic sewage treatment facility in each home
 - ▣ *provision of a sewage treatment facility at the municipal level*

 - Implementation of an effluent testing program for existing STPs

- 
- Monitoring to evaluate changes in environmental quality especially those that are associated with endocrine disruption
 - ▣ e.g. *vitellogenin, histology and population parameters*

THANK YOU FOR YOUR ATTENTION

