Hypoxia:
Problems and Scientific Challenges

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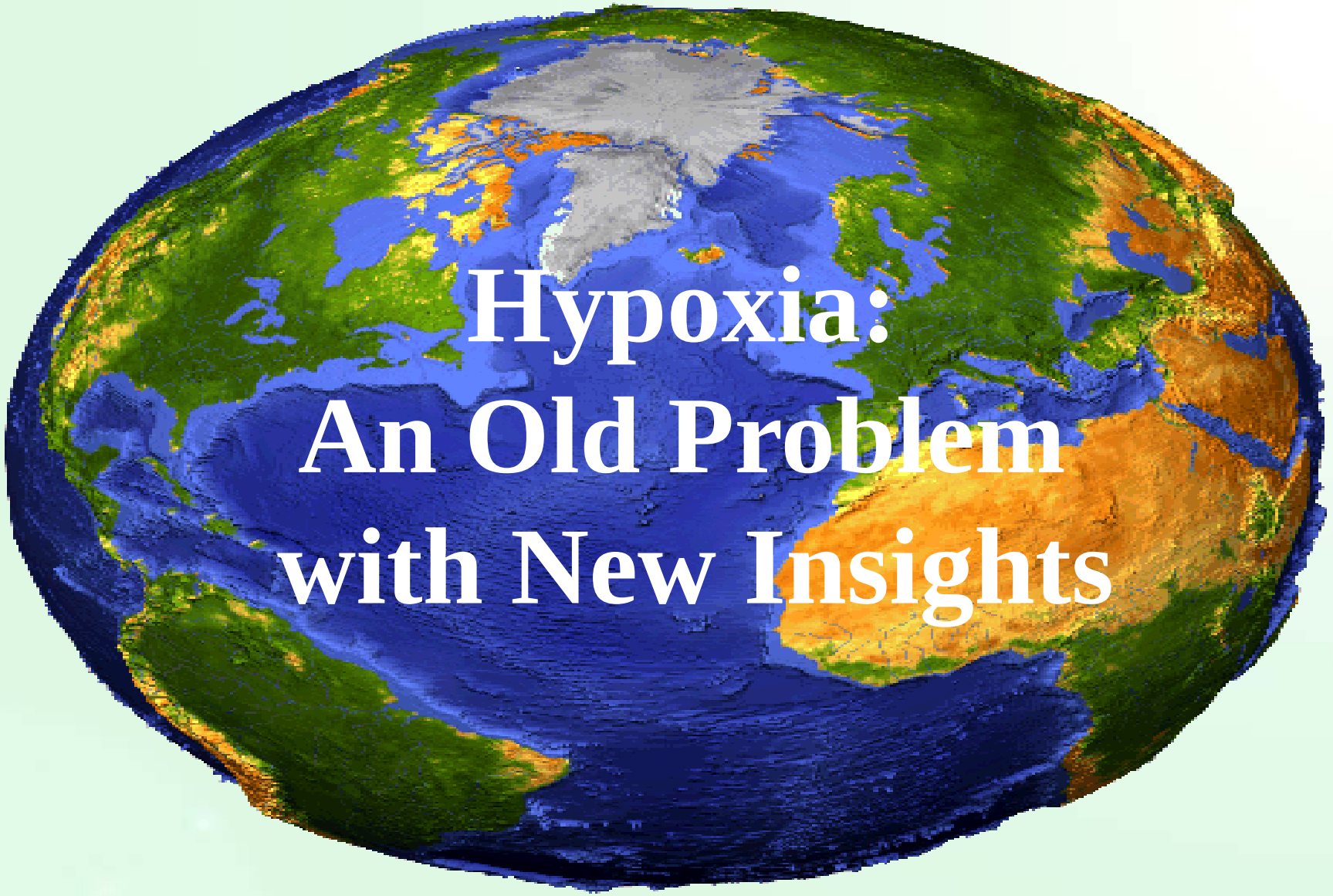

Hypoxia: An Old Problem with New Insights

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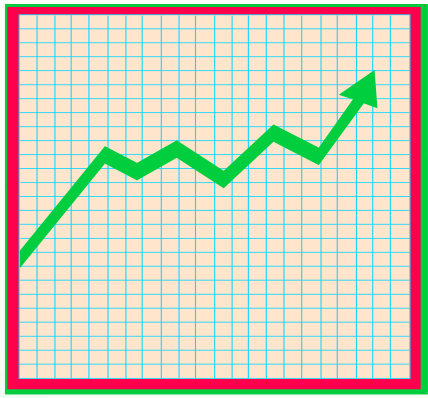
Hypoxia: Trend

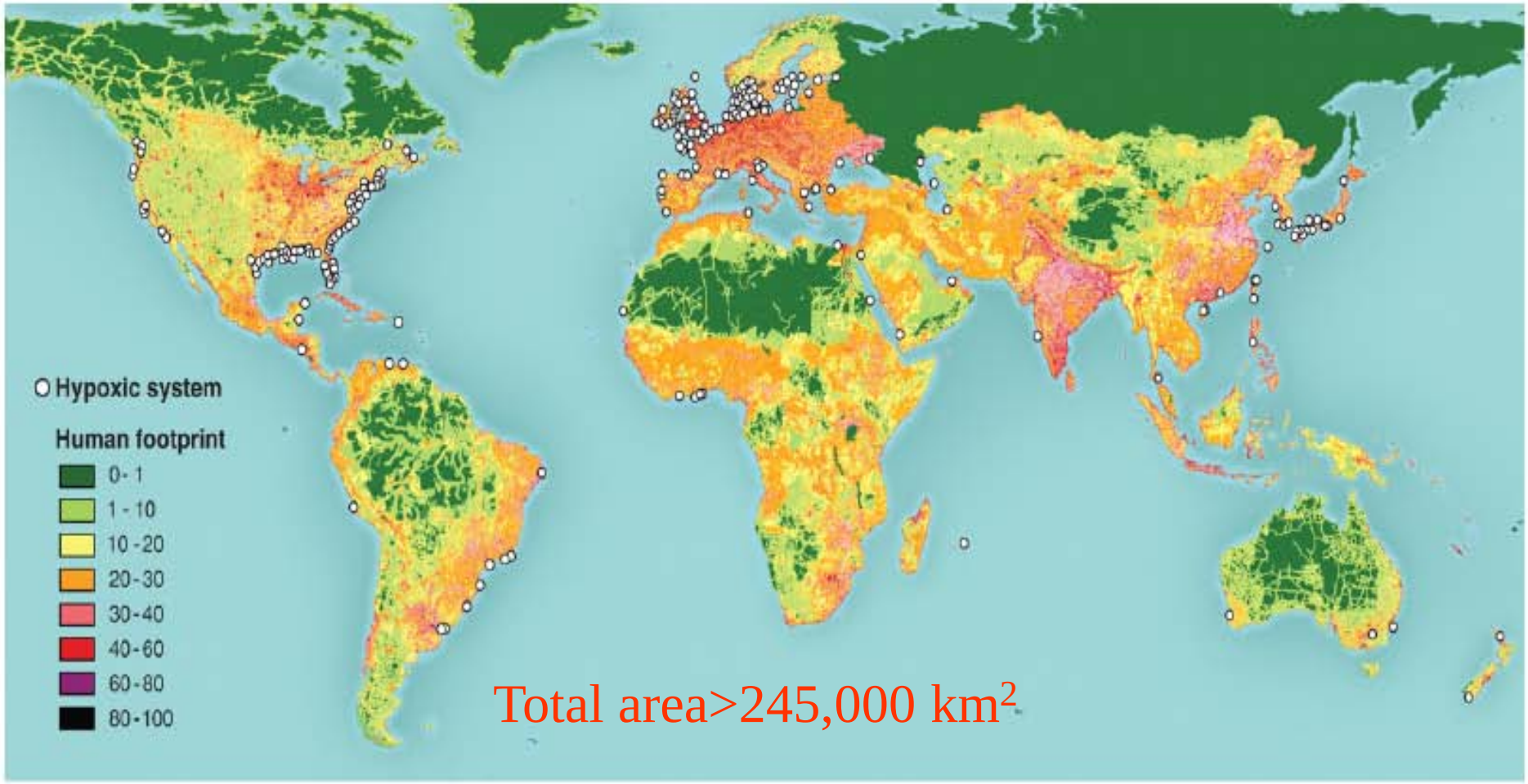
During the last few decades, anthropogenic input of nutrients into our coastal environment has increased ca. three folds, and is expected to double or triple if no action is taken

Hypoxia: Trend



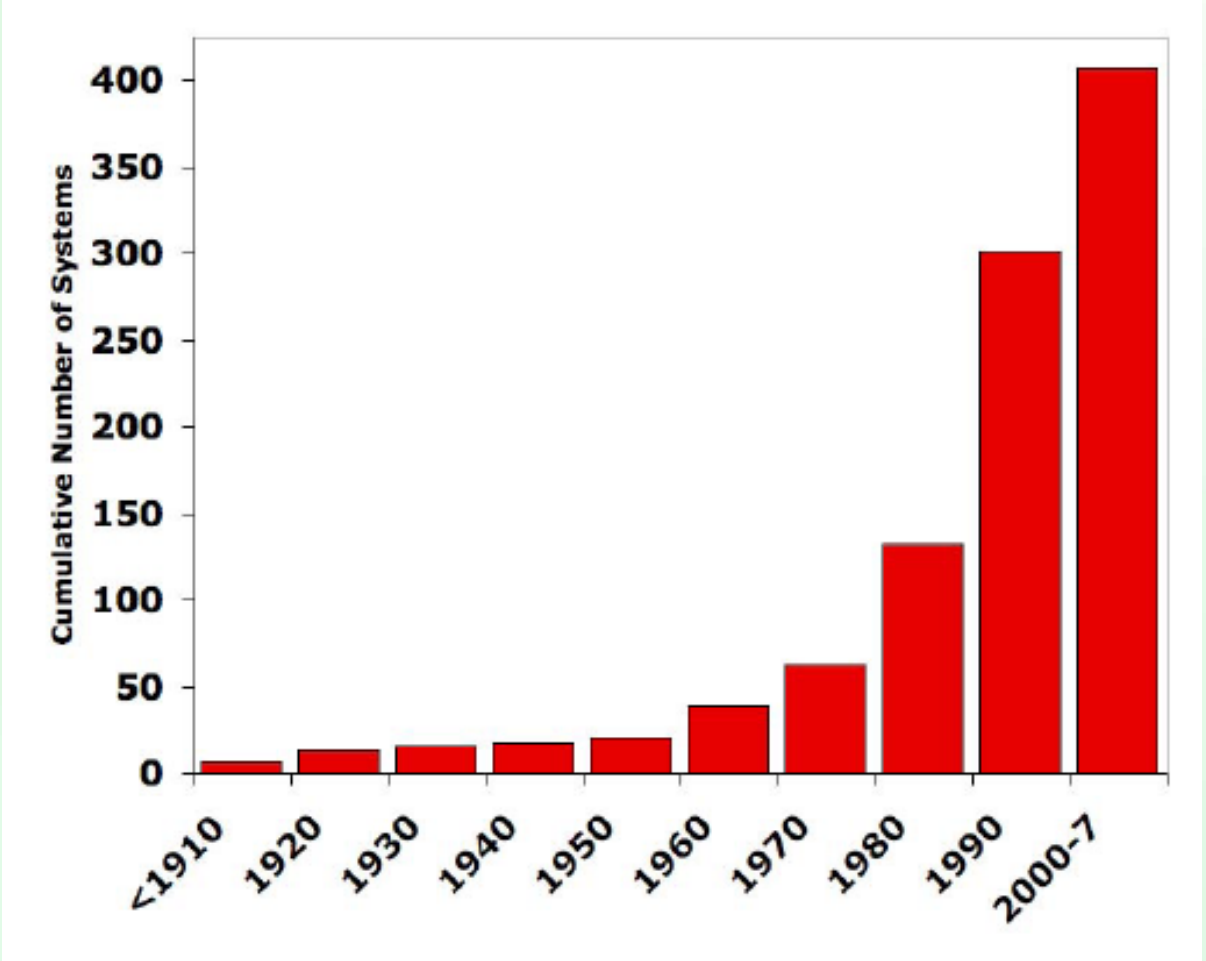
Decrease in dissolved oxygen recorded over large coastal areas worldwide (including USA, China, Norway, UK, Sweden, Germany, Denmark, the Black Sea, Adriatic Seas) in the last 30-80 years

Diaz & Rosenberg, 1995; Rabalais, 2001



Global distribution of 400-plus systems that have scientifically reported accounts of being eutrophication-associated dead zones and global human footprint. Adapted from (Diaz & Rosenberg 2008).

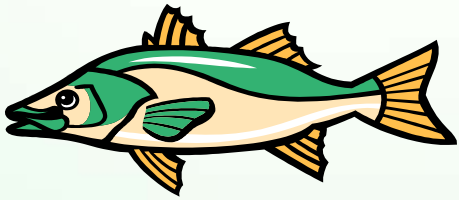
Number of Dead Zones doubled every 10 years since the 1960s

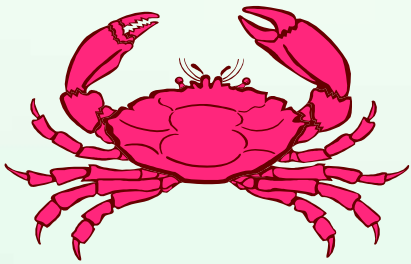


Diaz & Rosenberg, 2008

Hypoxia Has Caused Major Changes in Structure and Functions of Ecosystems

- Mass mortality of fish and benthos
- Changes in species composition
- Changes in trophic relationships
- Decrease in biodiversity and species richness
- Decrease in fisheries production





Phil, 1994; Wu, 2002; Gray et al., 2002

Wu, 2002; Gray et al., 2002

Generalized Changes in Structure and Functions of Ecosystems

Normoxia → Hypoxia

Suspended feeders
Diversity, Species richness
Demersal fish
Larger body size
Predator

Deposit feeders
Meiofauna,
Nanoplankton
Pelagic fish
Short life cycle

Wu, 2002

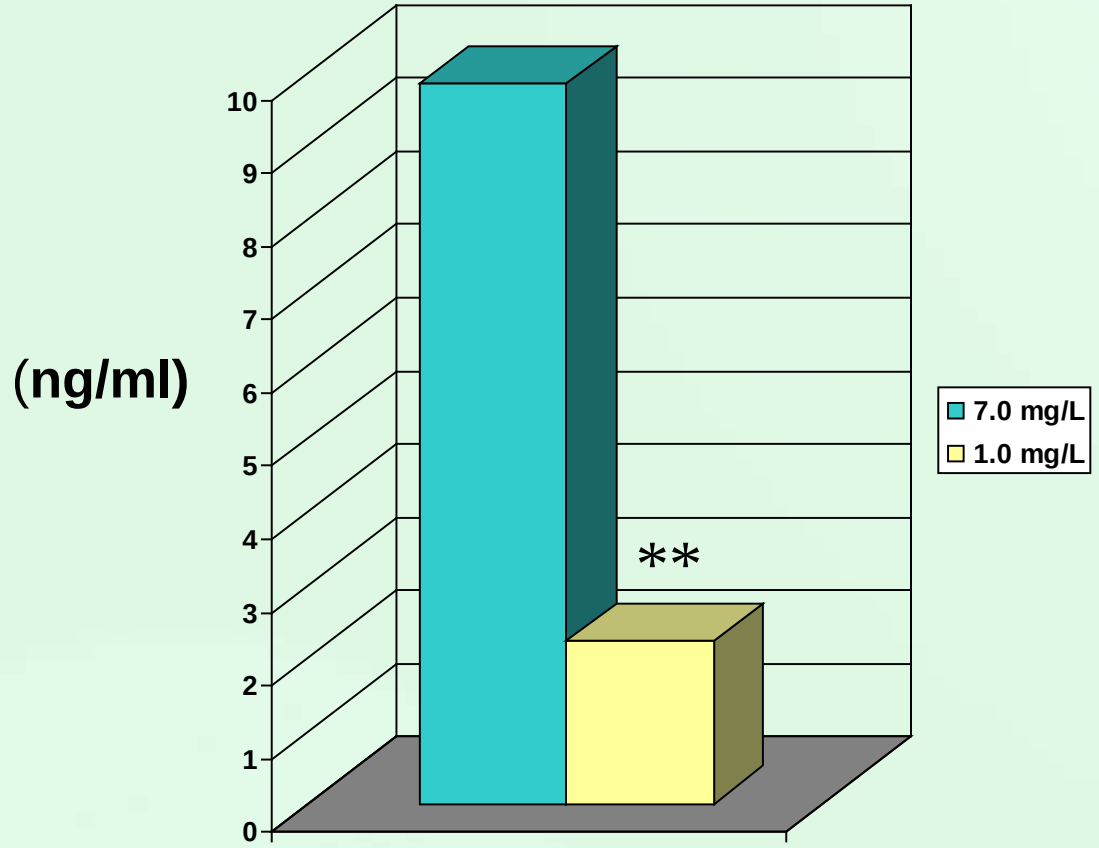
New Scientific Evidence further show that…..

Hypoxia is an endocrine disruptor

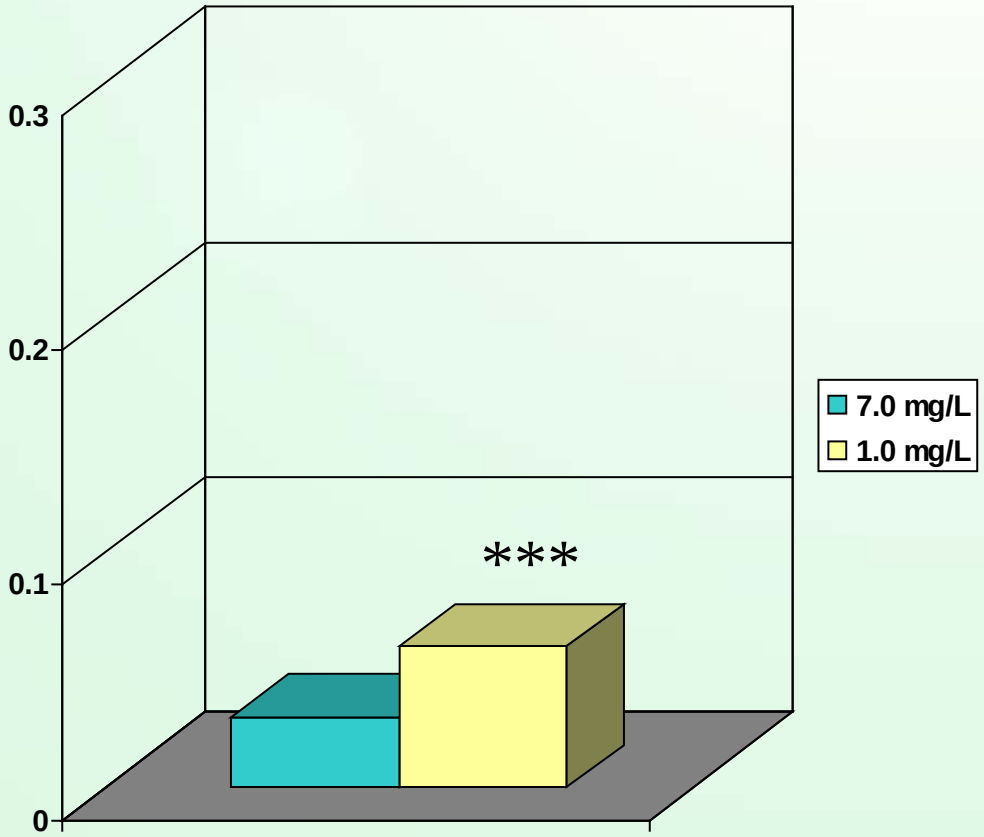
Common carp (*Cyprinus carpio*)

Male

Testosterone



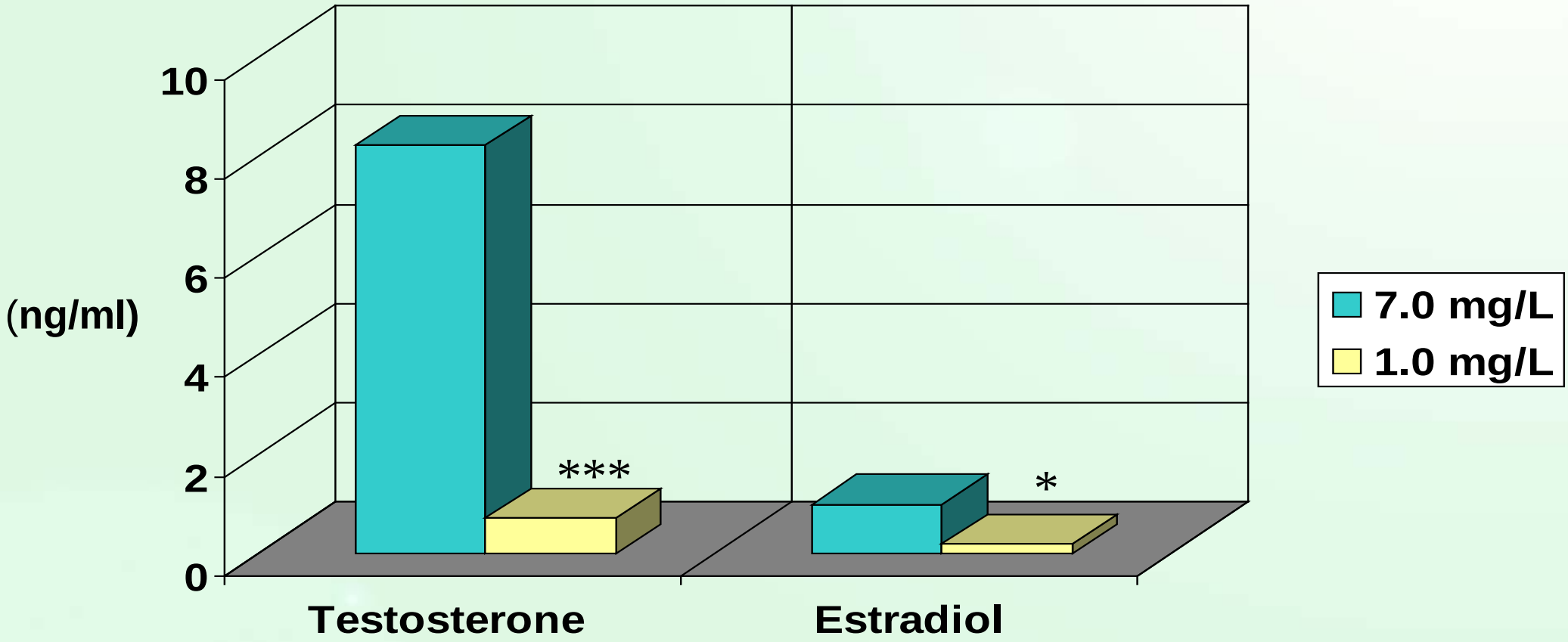
Estradiol



** $p<0.01$; *** $p<0.001$

Wu et al, 2003

Female



* $p<0.05$; *** $p<0.001$

Wu et al, 2003

Hypoxia impairs fish reproduction

- Gonadal development
- Gametogenesis
- Spawning
- Fecundity
- Fertility
- Gamete quality
- Offspring survival
- Reproductive behavior

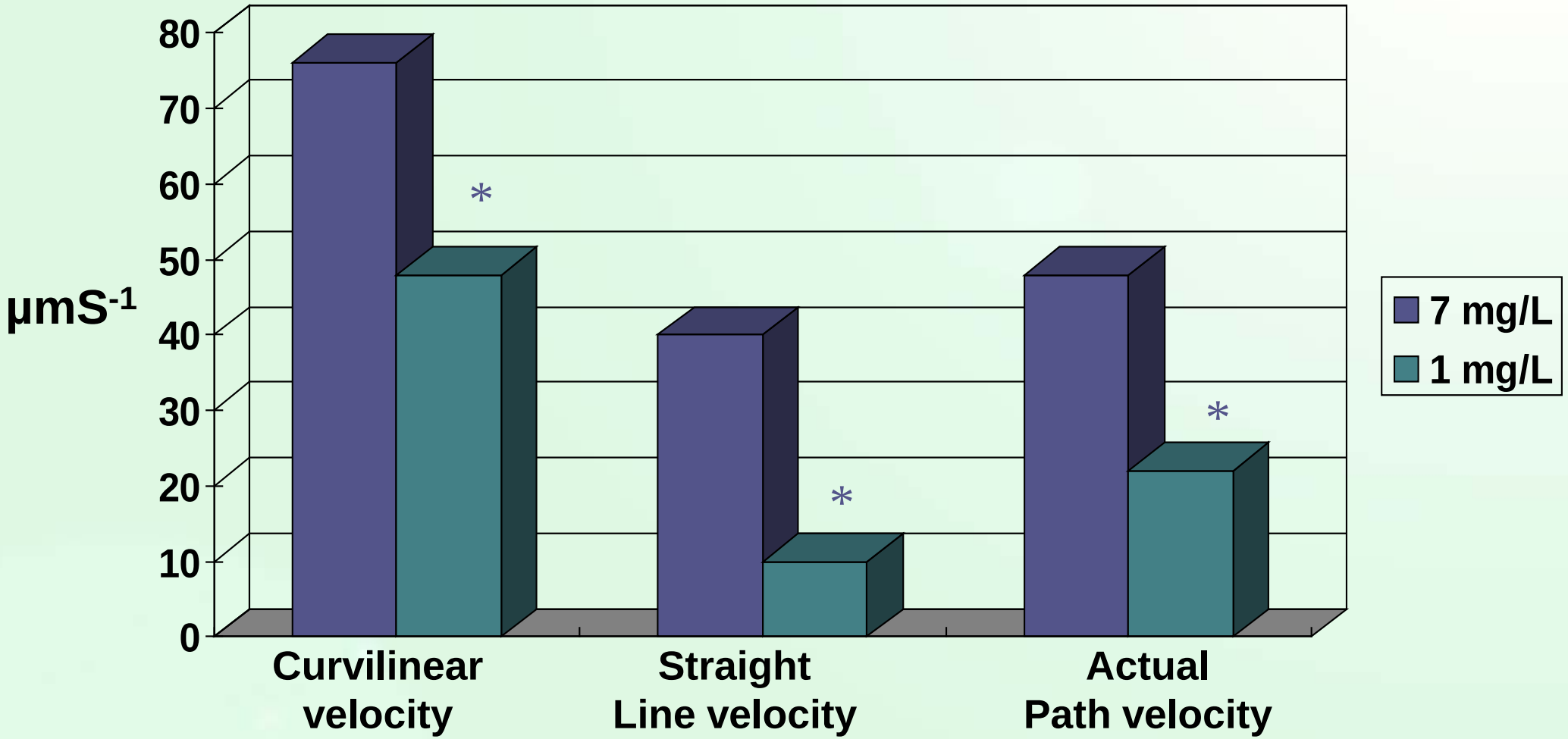
Common carp (Wu et al. 2003)

Zebrafish (Shang et al. 2006)

Atlantic croaker (Thomas et al. 2006,2007)

Gulf killifish (Landry et al. 2007)

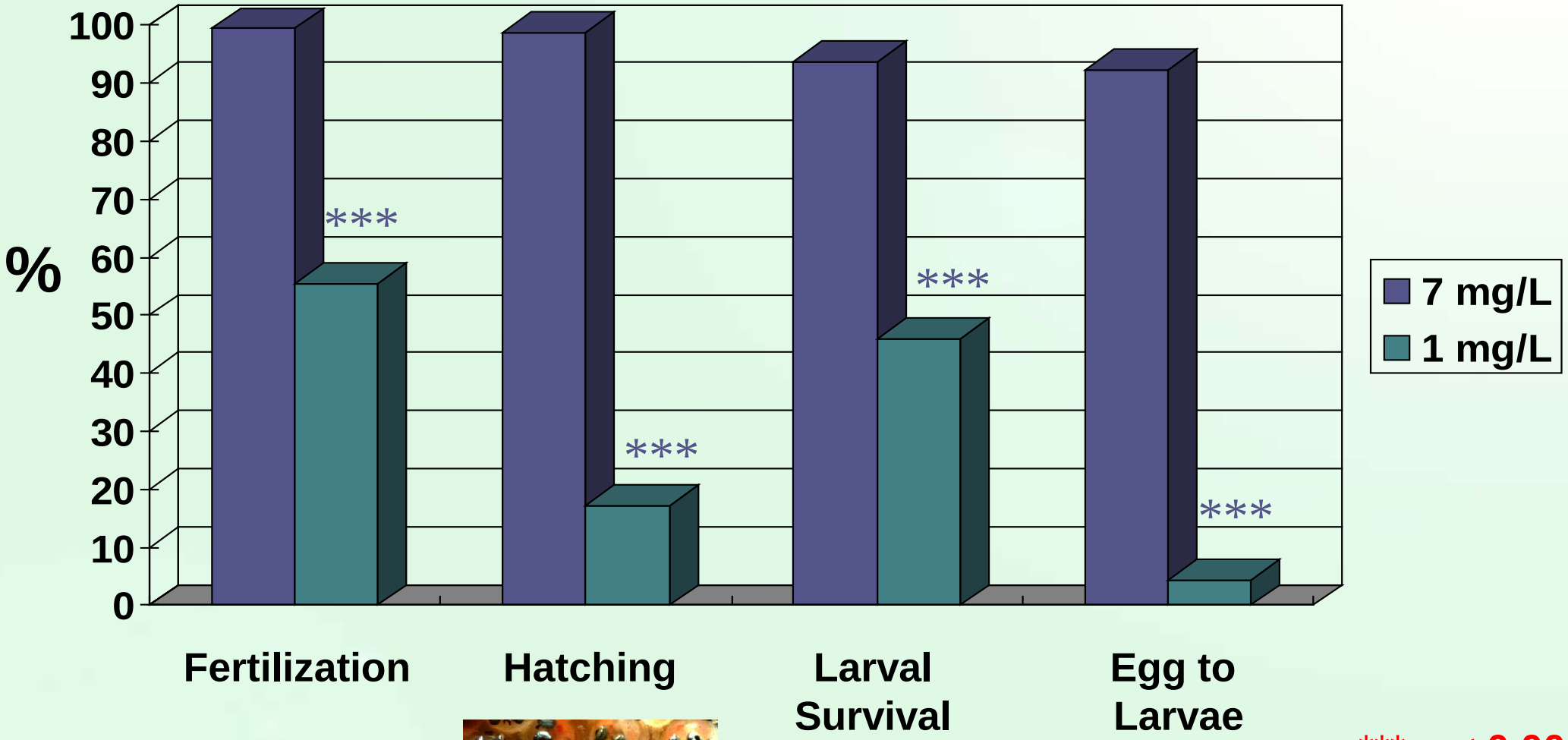
Sperm Motility



* $p < 0.05$

Wu et al, 2003

Reproductive Impairment



*** $p < 0.001$

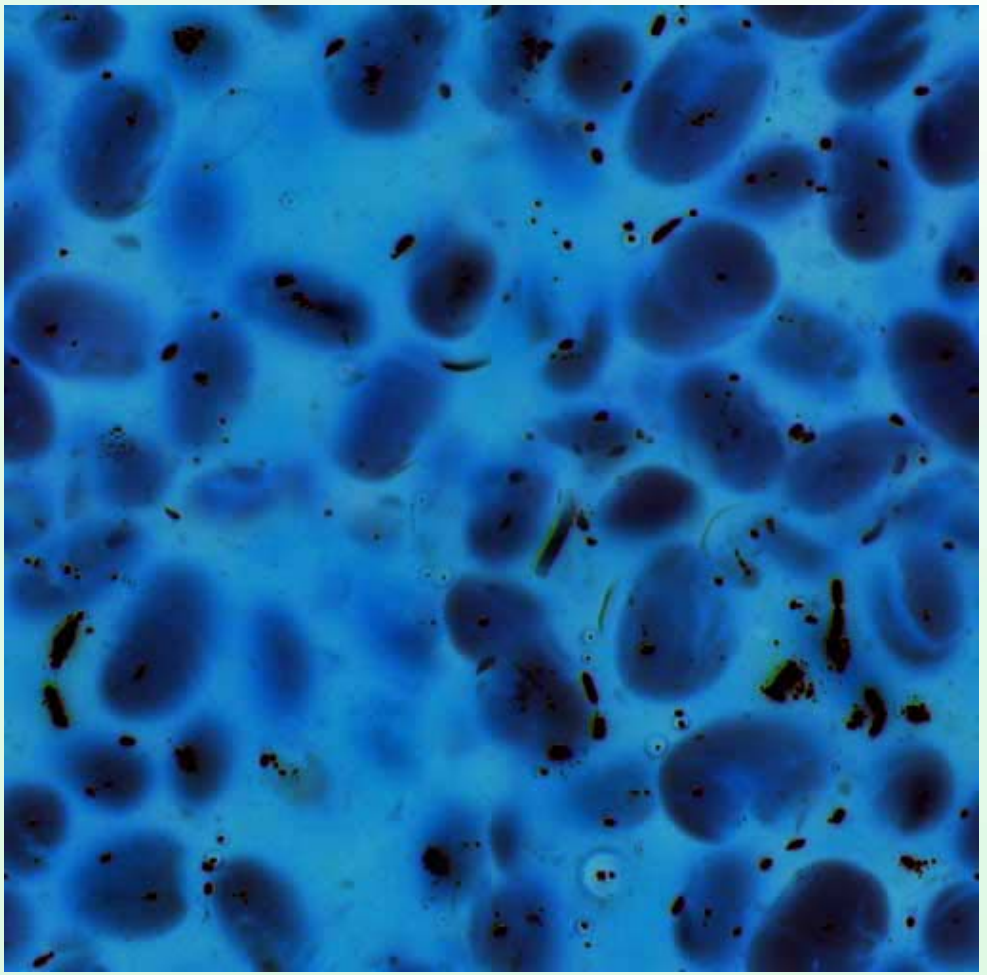
Wu et al, 2003

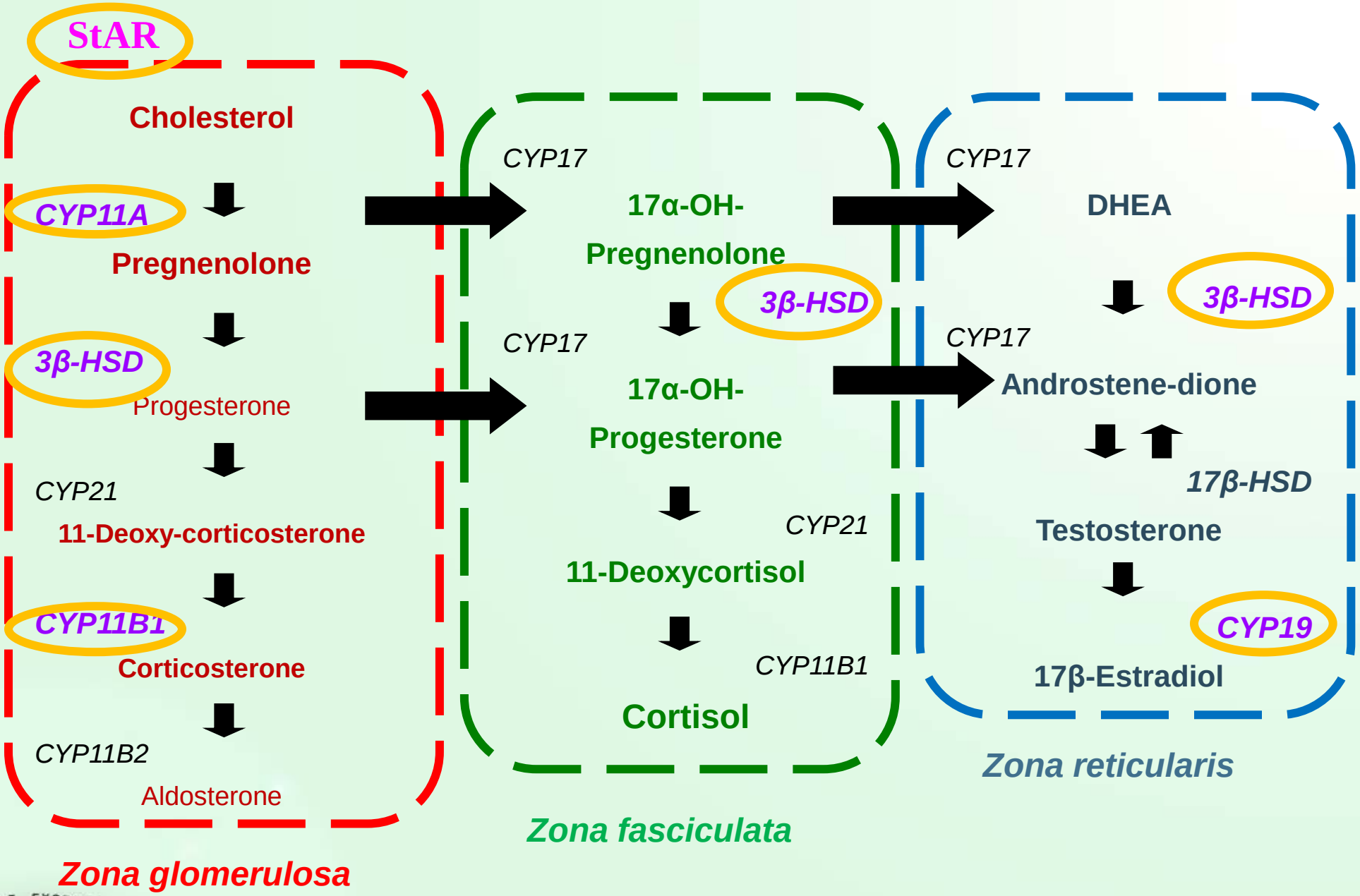
Follow-up questions: What caused the observed endocrine disruption?

- A smaller gonad and reduced hormone production due to reduced energy intake and reduced growth?
- Hypoxia affects synthesis and metabolism of sex hormones?
- Hypoxia affects GnRH and gonadotropins?

In vitro evidence

- *In vitro* studies using H295R human adrenocortical carcinoma cell line and primary cell culture of medaka gonads

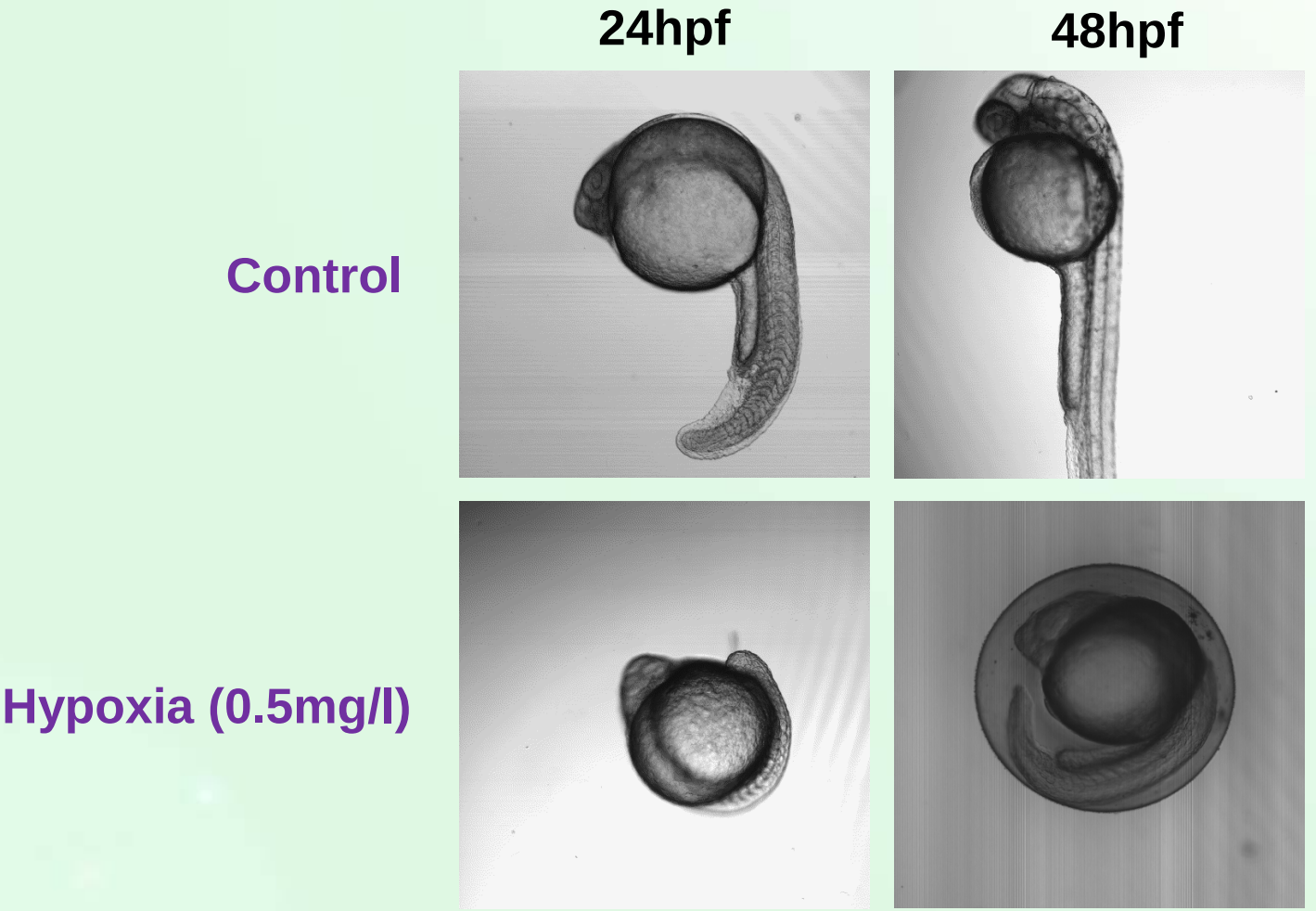



StAR
Cholesterol
CYP11A
Pregnenolone
3β-HSD
Progesterone
CYP21
11-Deoxy-corticosterone
CYP11B1
Corticosterone
CYP11B2
Aldosterone
Zona glomerulosa
CYP17
17α-OH-
Pregnenolone
3β-HSD
CYP17
17α-OH-
Progesterone
CYP21
11-Deoxycortisol
CYP11B1
Cortisol
Zona fasciculata
CYP17
DHEA
3β-HSD
CYP17
Androstene-dione
17β-HSD
Testosterone
CYP19
17β-Estradiol
Zona reticularis

Hypoxia is a teratogen

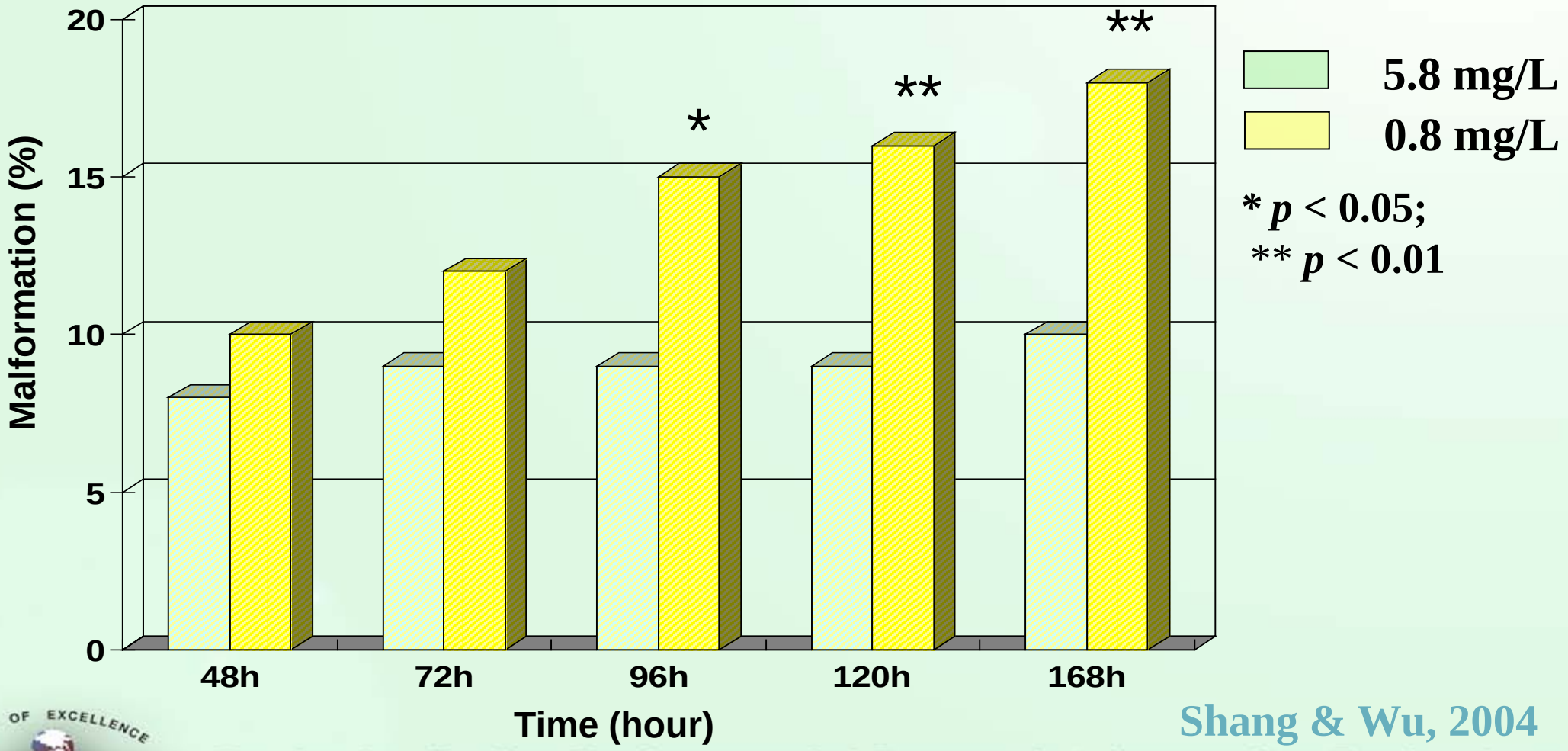
Zebrafish (*Danio rerio*)

Hypoxia delays development



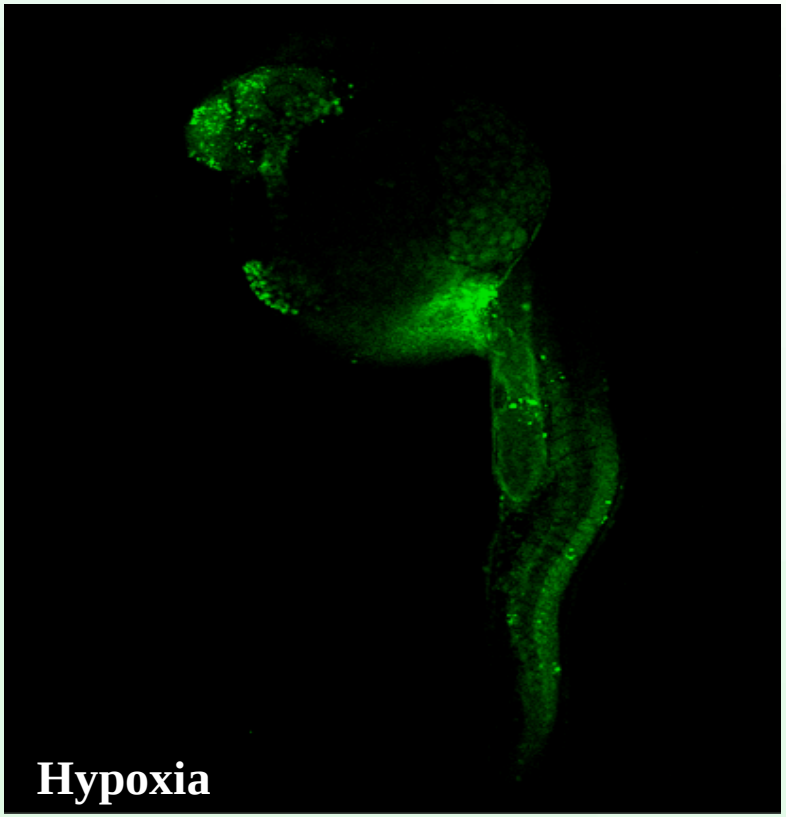
Shang & Wu, 2004

Hypoxia caused spine and cardiac malformations

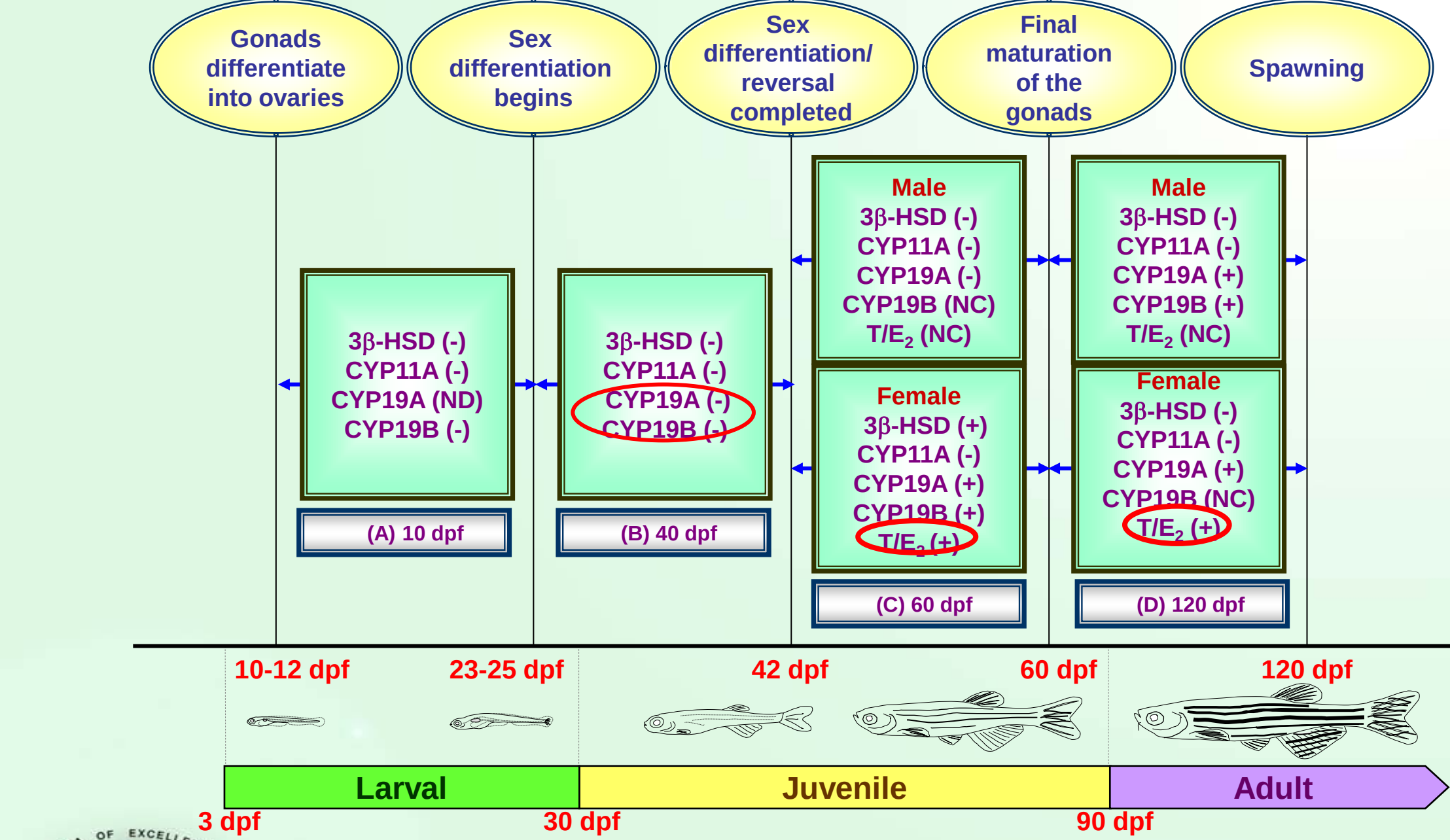


Shang & Wu, 2004

Under hypoxia, apoptosis concentrates in the head but not the tail



Shang & Wu, 2004



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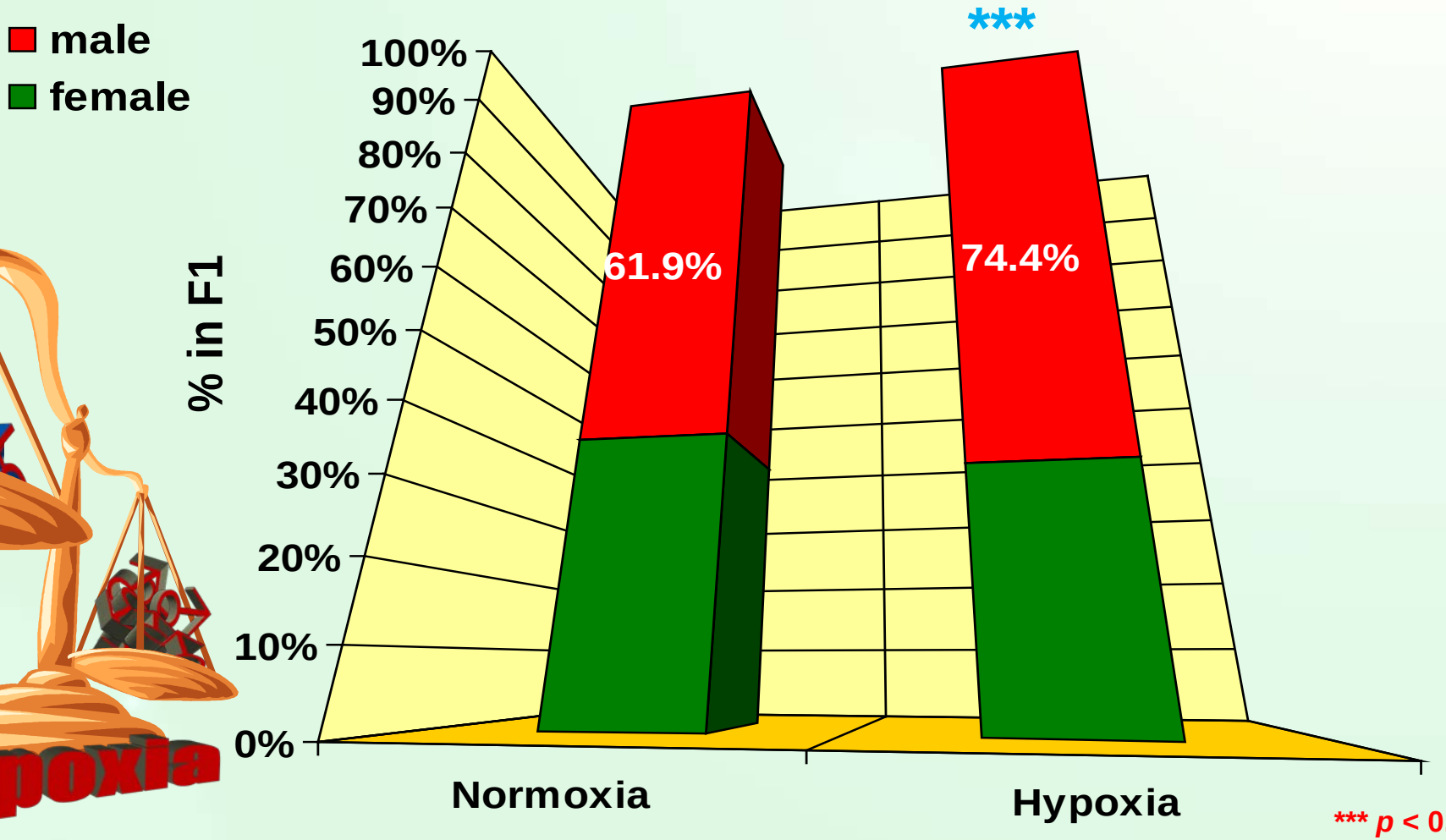
Shang et al, 2006

Hypoxia tips sex balance & favors a male biased population



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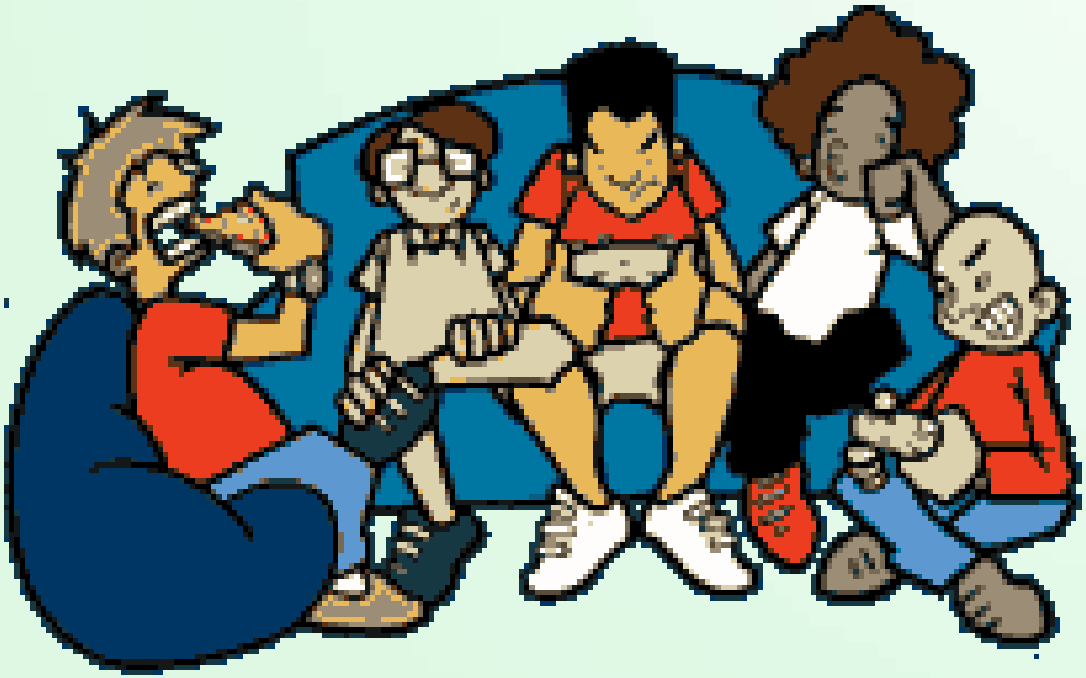
Shang, Yu & Wu, 2006

Where the girls are?



Will this affect reproductive success of the natural population?

Is the observed male biased phenotypic or geneotypic?

Will the same happens to species with XY chromosomes?

Phenotypic & gonadal Sex in genotypic females (*O. latipes*) are altered by hypoxia

Treatment (mgO_2/L)	Genotypic XX			Genotypic XY		
	Phenotypic Sex			Phenotypic Sex		
	Female	Male	Sex reversal	Female	Male	Sex reversal
≧ 5.8	35	0	0%	3	24	11%
1.5 ± 0.1	6	20	77% ***	2	17	11%

Treatment (mgO_2/L)	Genotypic XX			Genotypic XY		
	Gonad Sex			Gonad Sex		
	Ovary	Testis	Sex reversal	Ovary	Testis	Sex reversal
≧ 5.8	34	1	3%	1	26	4%
1.5 ± 0.1	12	14	54% ***	0	19	0%

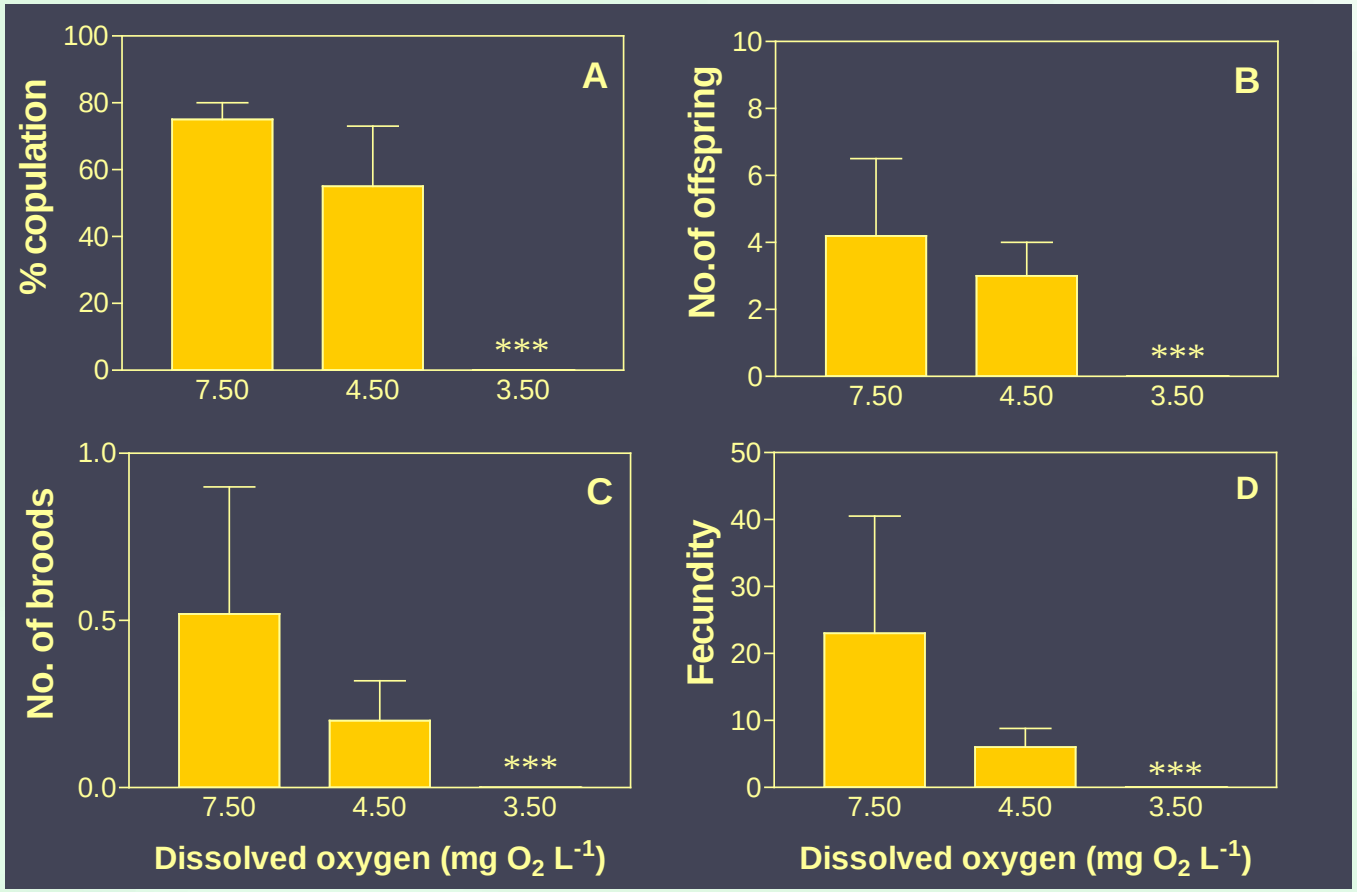
Cheung, 2006; Wu, 2009

Will the same occur in higher vertebrates?

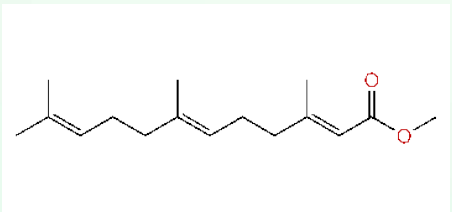
- Salamanders (*Ambystoma sp.*): delayed development and hatching, less developed and deformed embryos
- Australian frog (*Crinia georgiana*): delayed embryonic development, increased malformation
- Male albino rats: reduced numbers of sertoli cells and Leydig cells in testis
- Male Wistar rats: lower levels of LH and testosterone

Seymonr et al., 2000; Shevantaeva & Kosyuga, 2006; Farias et al., 2007

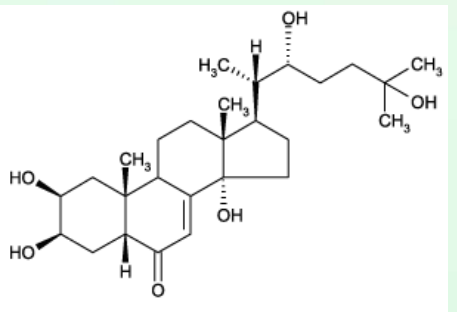
Will the same occur in invertebrates?



*** $p<0.005$



Methyl farnesoate?



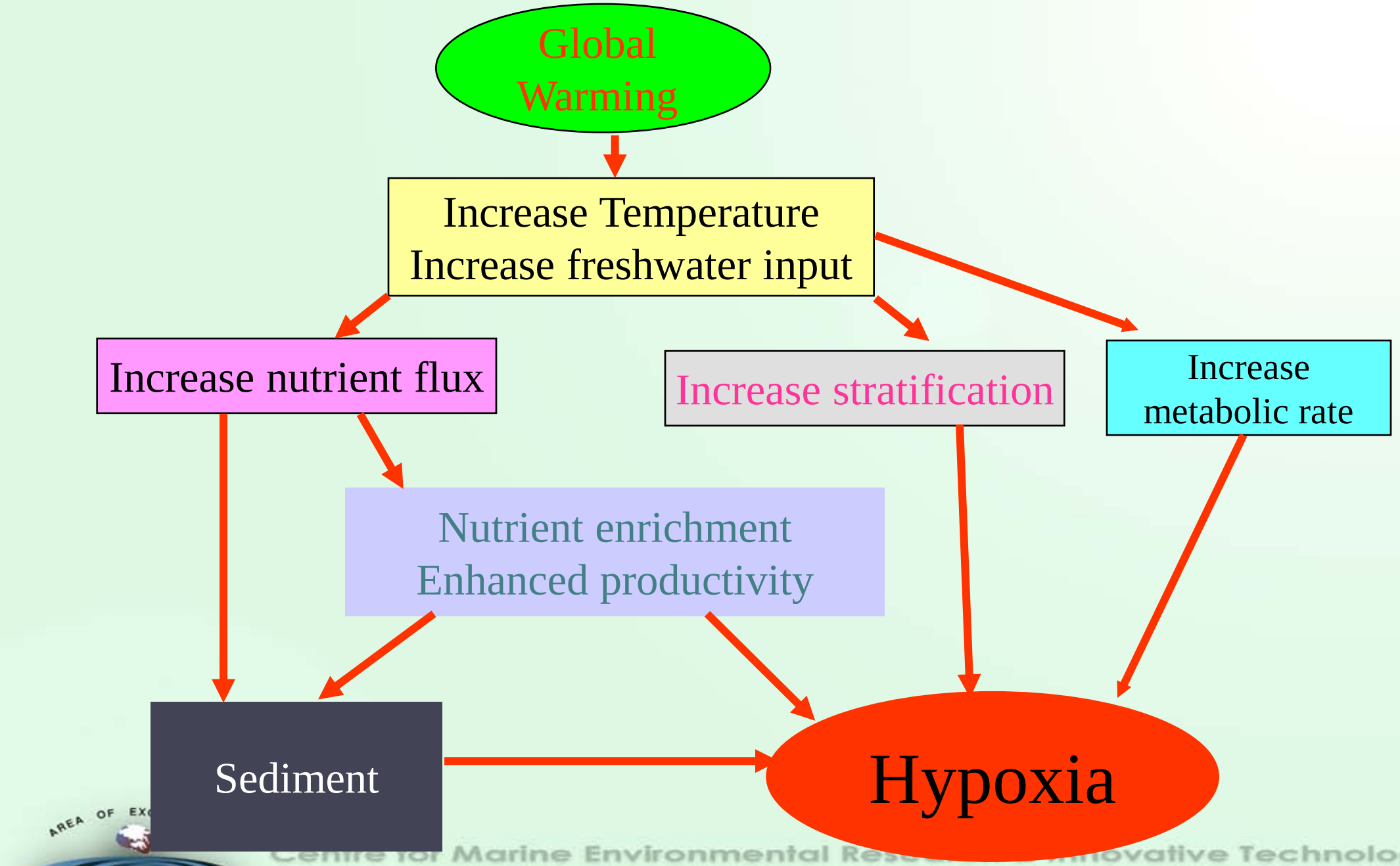
Ecdysteroid?

Wu & Orr, 2005

Is the situation getting better or getting worse?

The situation is likely to get worse because…..

- Growth of treatment facilities is unlikely to catch up with growth of population and industry, especially in developing counties
- Contributions from atmospheric fallout and non-point source are significant
- Trans-boundary issues are difficult to resolve



Risk Assessment

• Ecological consequence	Very serious
• Area affected	Very large
• Socioeconomic loss	Very big
• No. people affected	Very large
• Probability of occurrence	Very high
• Trend	Getting worse

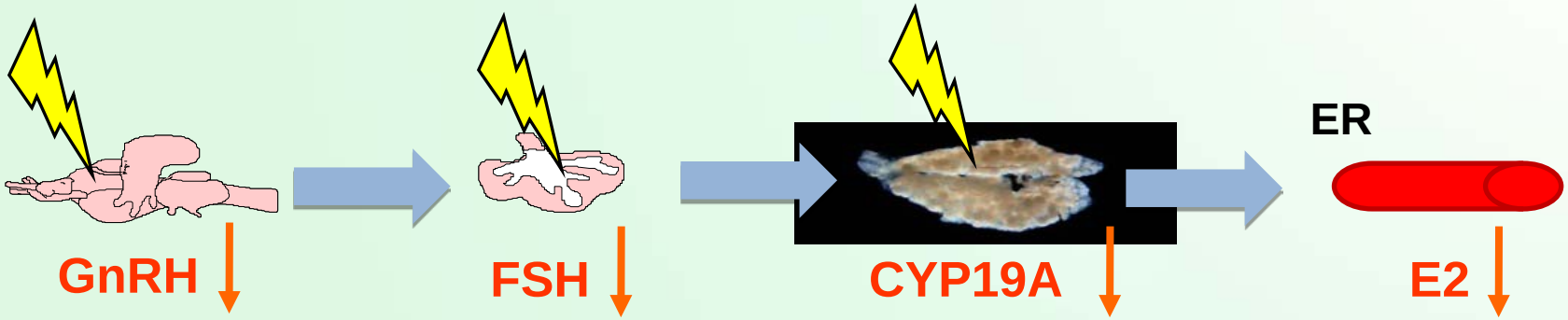
The new scientific evidence presented here calls for an urgent re-assessment of this old problem

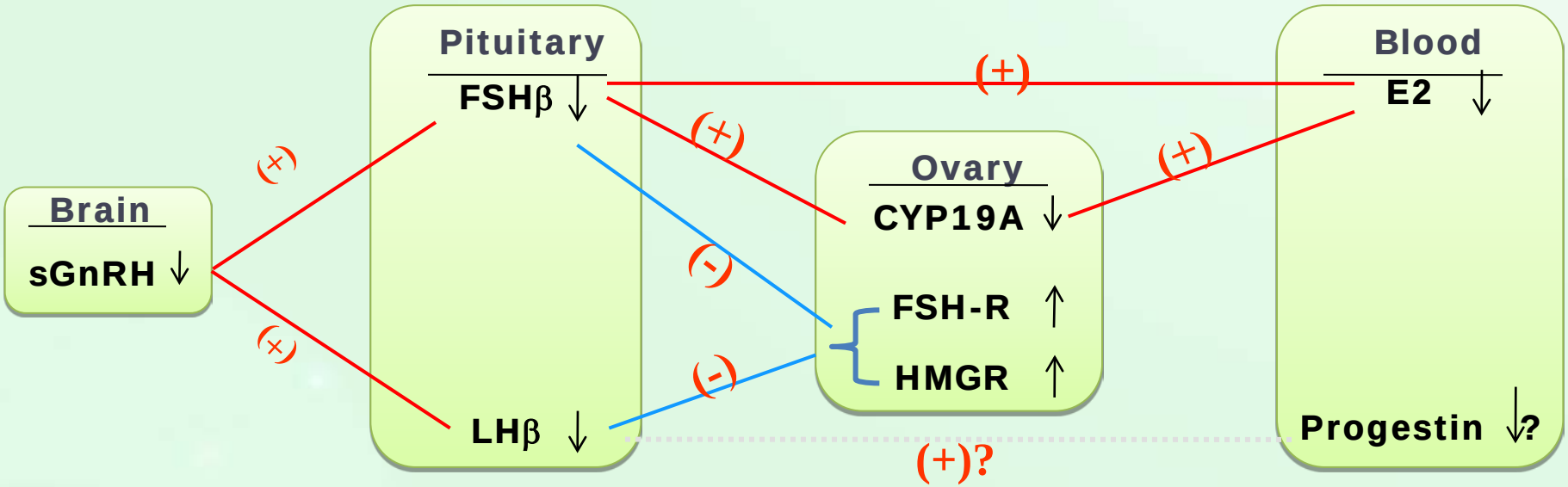
Thank you

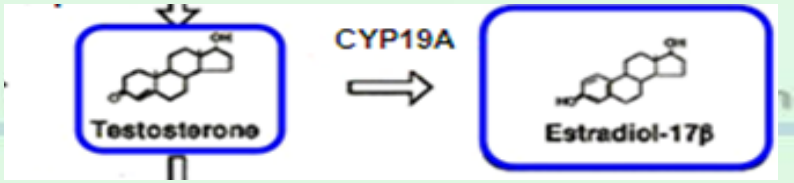
Will the same happen in humans?

- Patients suffering from sleeping apnea have lower sex drive and testosterone level (Saaresranta & Polo, 2003)
- Sex ratio in human depends on level of sex hormones of father and mother during conception, and high testosterone level during conception favors subsequent birth of sons (James, 2004)

Summary of effects of hypoxia on The HPG axis in female zebrafish







Proposed Work

Collaborate with fisheries authorities and undertake a scientific global review, focusing on :

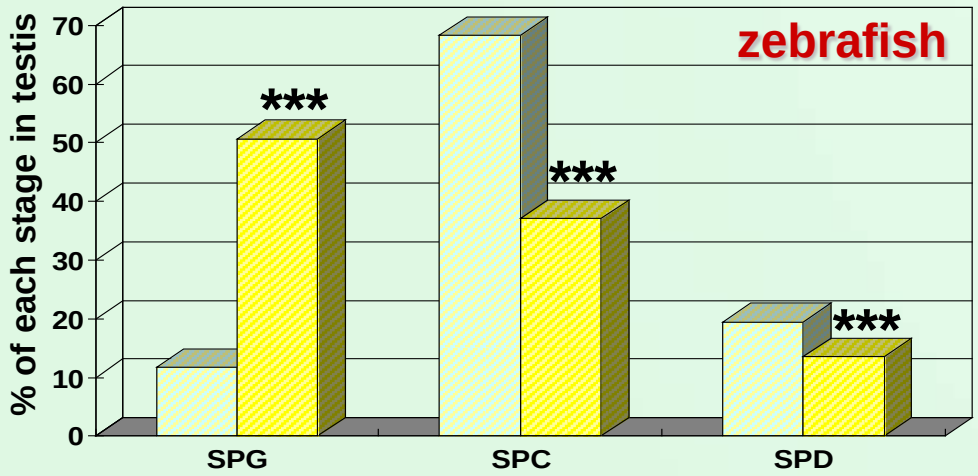
- Trend analysis (spatial and level in the last 50 years)
- Changes in structure and trophodynamics of marine communities (plankton, benthos, fish)
- Identification of sensitive groups (bioindicators)

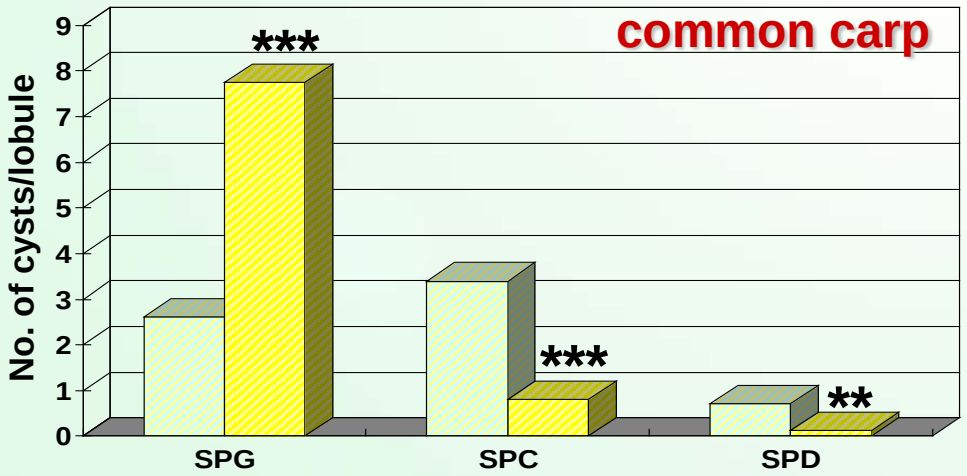
Proposed Work

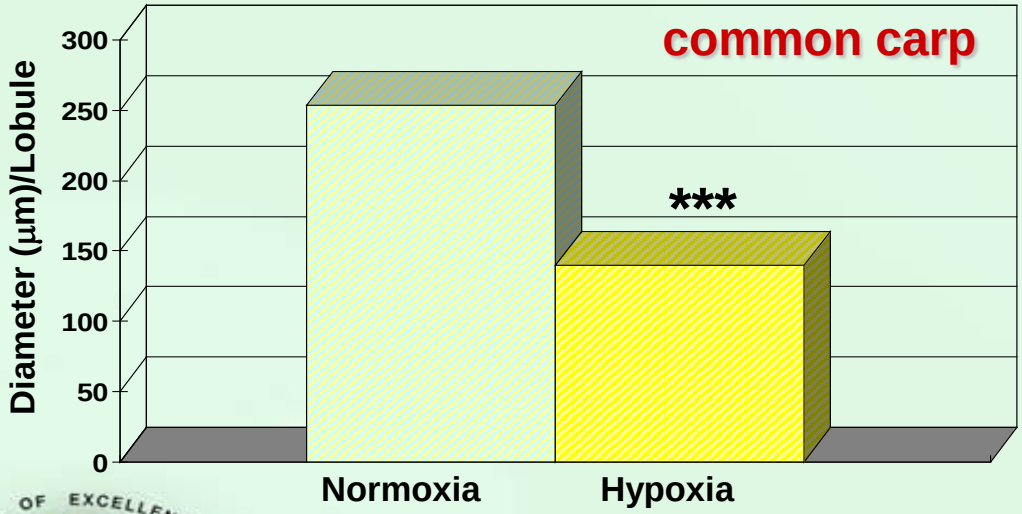
Collaborate with fisheries authorities and undertake a scientific global review, focusing on :

- Reproductive status and reproductive impairment of fish in hypoxic areas vs normoxic areas
- Endocrine disruption, malformation, sex ratio
- Deciphering effects of hypoxia from those caused by other anthropogenic activities (chemicals) prevailing simultaneously in the marine environment??
- Identifying information gaps and further studies

Hypoxia affects spermatogenesis







Normoxia Hypoxia

** $p < 0.01$; *** $p < 0.001$

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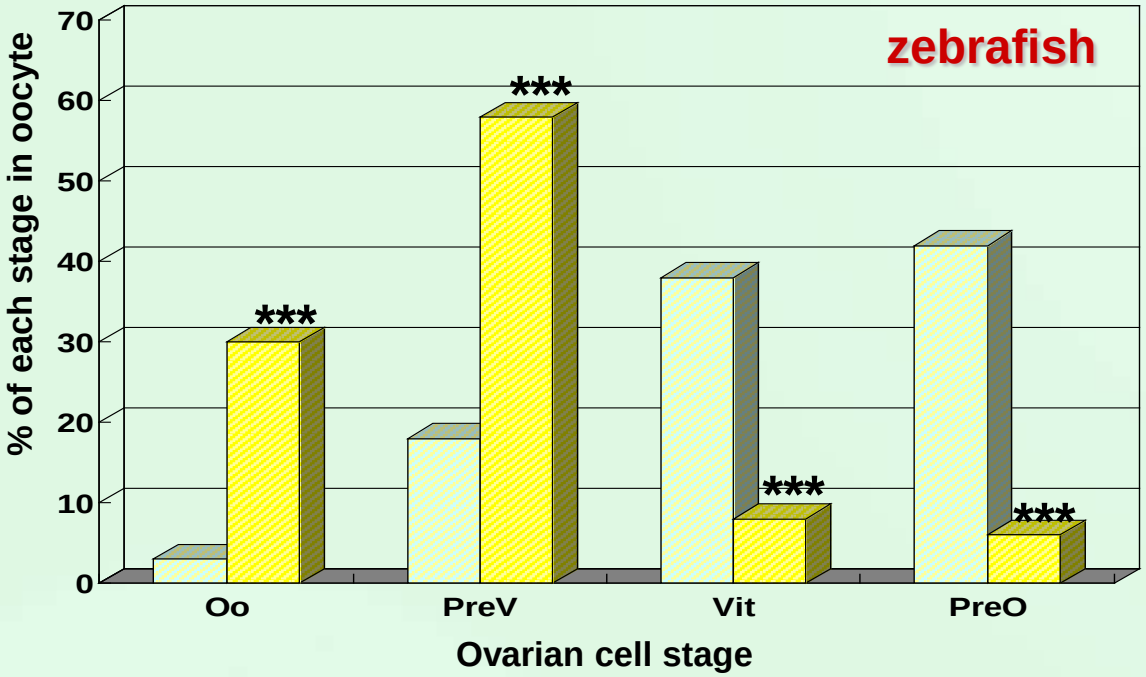
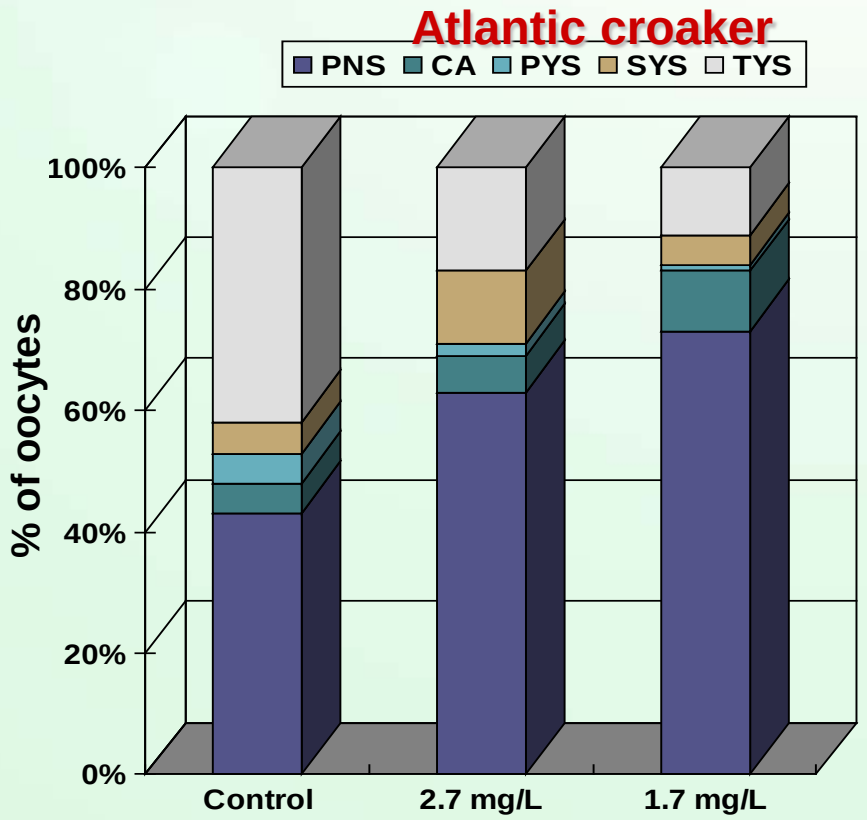
Wu et al., 2003; Shang et al., 2006

Hypoxia affects Oogenesis



Normoxia Hypoxia

*** $p < 0.001$



Shang et al., 2006; Thomas et al., 2007 Landry et al., 2007