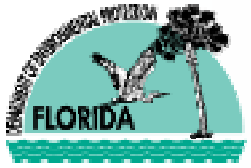


Florida Department of Environmental Protection

Pharmaceuticals in the Aquatic Environment:

Understanding the Potential for Adverse Biological Effects in Springs

Bureau of Laboratories

David Whiting, Program Administrator

February 2010

Pharmaceuticals and Personal Care Products

PPCPs are a highly diverse group of chemicals

Basically all prescription and over-the-counter drugs
Illicit Drugs
Diagnostic agents
Dietary Supplements
Fragrances, soaps, conditioners, sunscreens, cosmetics…
Caffeine
Nicotine

Common Routes of Entry Into the Environment Include

- **Wastewater**
  - **Domestic and industrial wastewater, sewer overflows, septage, and storm water**
  - **Either excreted, rinsed, or purposely flushed**
- **Municipal landfills**
  - **Leachate to ground water**
- **Manure, biosolids and pet waste**
  - **Concentrated animal feedlot operations**
  - **Land application then through groundwater or runoff**

Science progresses in stages

Historical Approach

- Analytical methods had to be developed to detect PPCPs in environmental media (e.g., water, soil, sediment, organisms)
- This is often quite difficult at low concentrations
- Receiving water concentrations are typically in the ppt to low ppb range
- Extracting and measuring contaminants in soils, sediments, and tissues is more difficult than pure water.
- The signal is often harder to pick up given all the extra noise created by the interferences in the environmental media.

1 part per billion =

1¢ in $10,000,000 or

1 second in 32 years or

1 drop of gas in the largest gas tanker truck

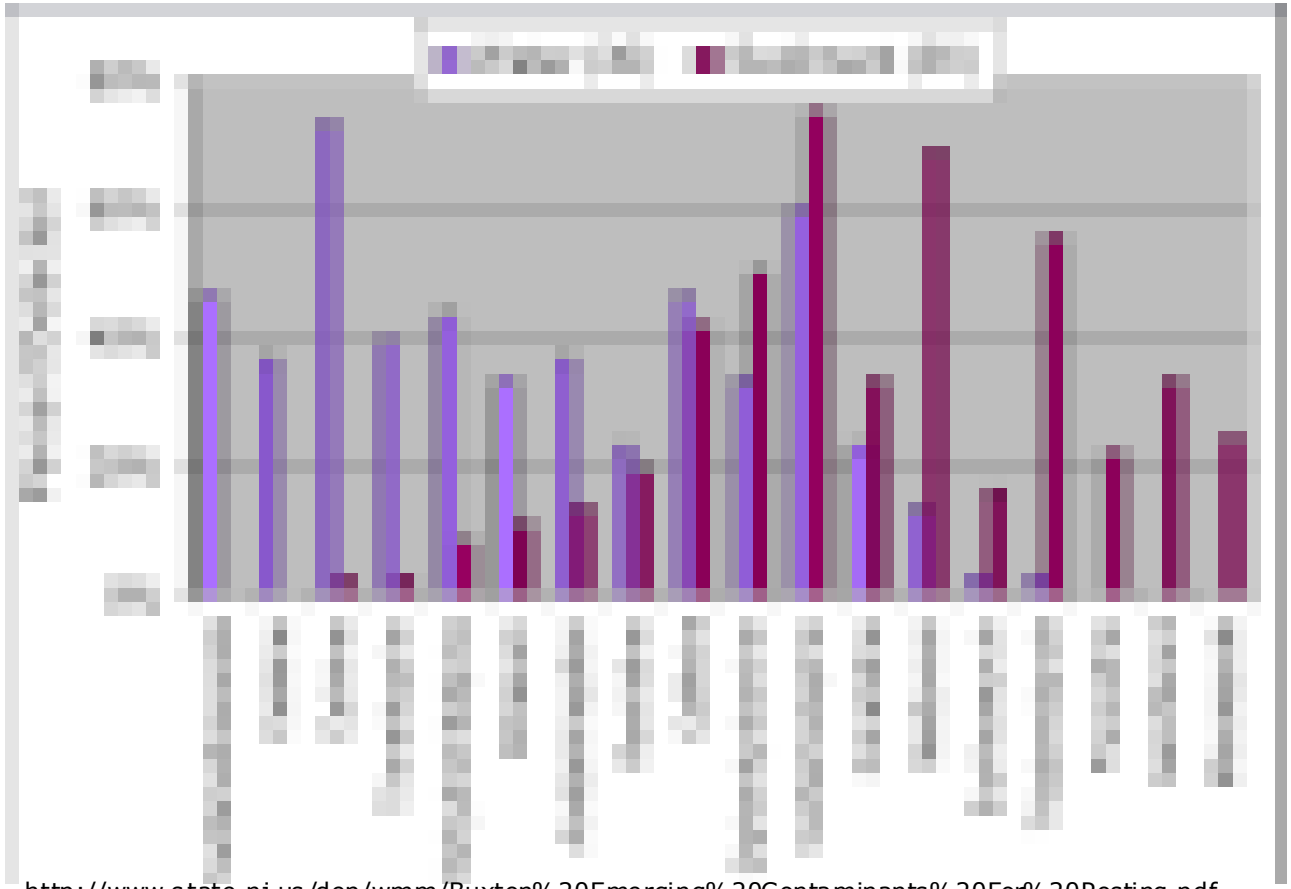
1 part per trillion =

1 trillion seconds ago, it was around 30,000 BC

1 drop of ink divided into 20 Olympic-size swimming pools

Science progresses in stages, cont.

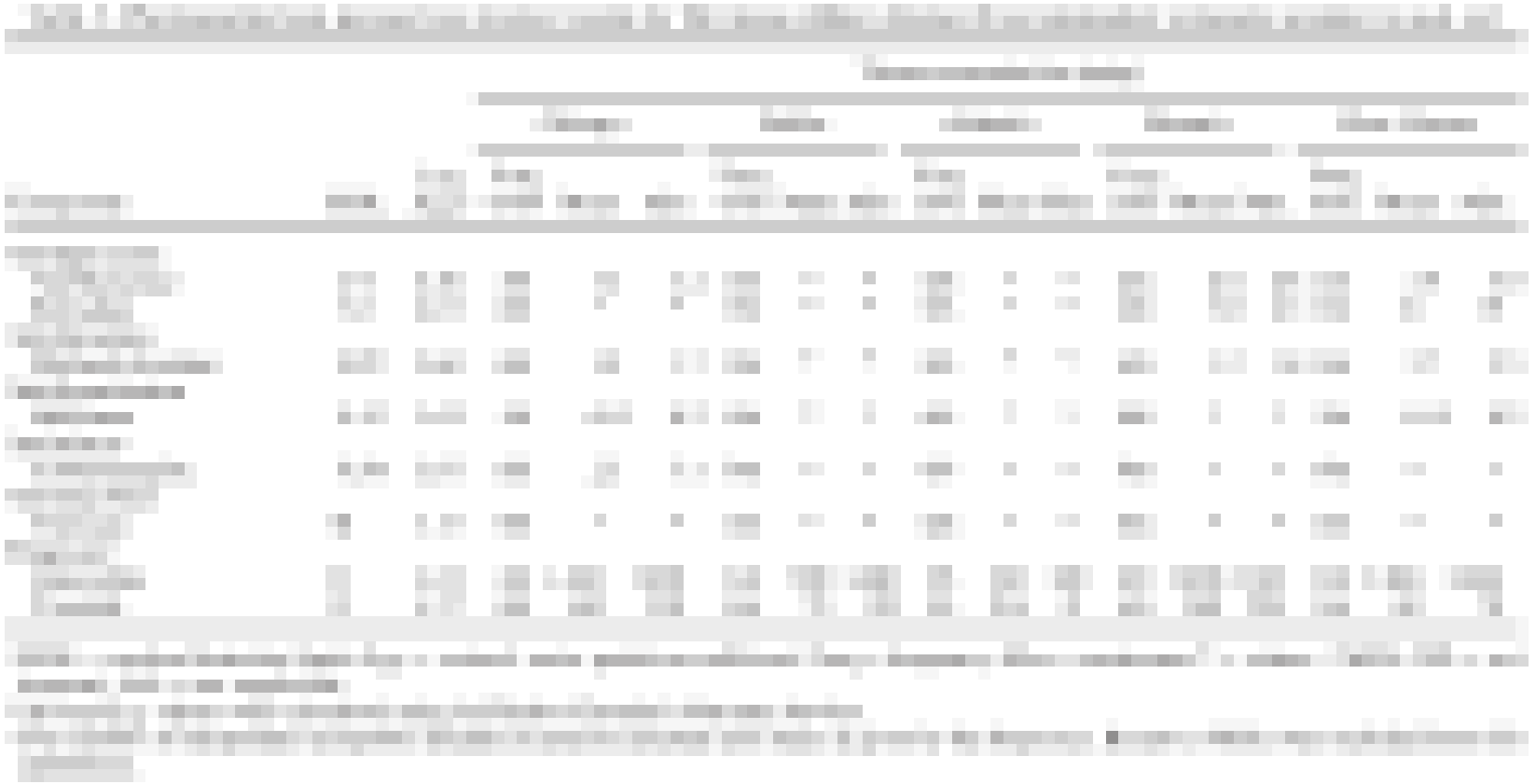
- Once the chemical could be detected, its fate had to be determined
  - Does it rapidly degrade, persist, or accumulate?
  - Does it pass through WWTPs?
  - Does it bind readily with soil?
  - Does it partition to the sediment or biological tissues?



http://www.state.nj.us/dep/wmm/Buxton%20Emerging%20Contaminants%20For%20Posting.pdf

Science progresses in stages, cont.

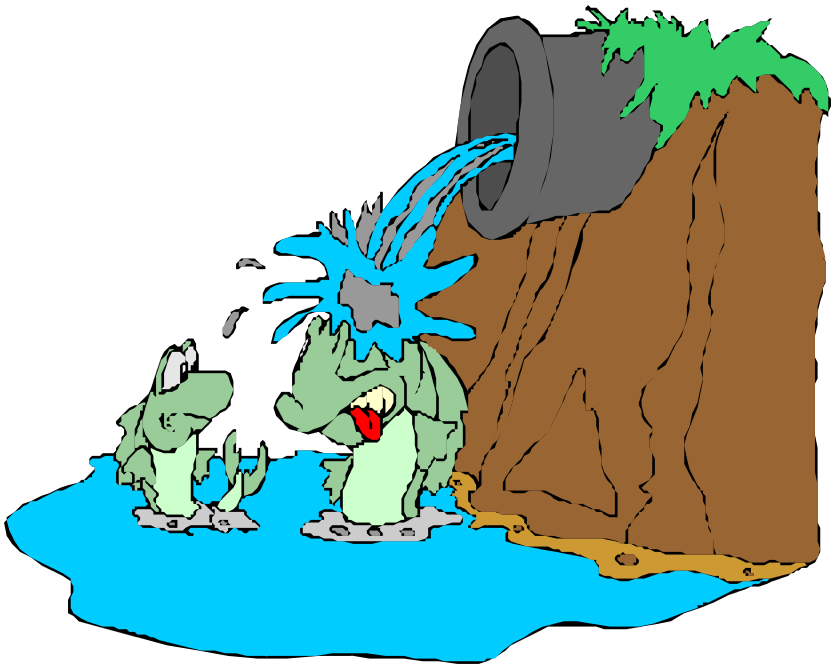
Ramierez, A J. et al., 2009



Environmental Toxicology and Chemistry, Vol. 28, No. 12, pp. 2587–2597, 2009

Science progresses in stages, cont.

- Once a compound could be detected and something was known about its fate, research could begin measuring the exposure of organisms to the chemical and determining what effects could be observed **at environmentally relevant concentrations**
- Studies documenting the biological effects of PPCPs in natural systems have only started being published fairly recently



Fate and Effects of PPCPs in the Aquatic Environment

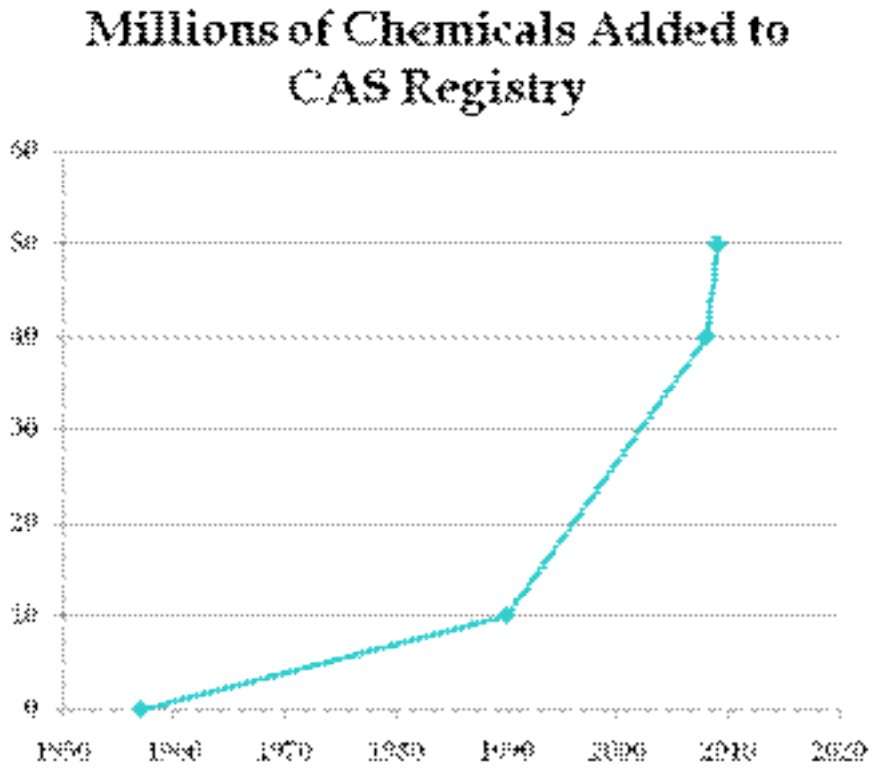
- Field studies are often difficult to interpret due to the lack of ability to control many environmental variables
- Translating the biological effects of exposure to organisms in laboratory experiments to actual environmental effects in natural systems can be very difficult as well

Table 2 | Reported subtle effects of pharmaceutical compounds on aquatic and terrestrial organisms

Substance(s)	Medicine class	Reported effect	Reference
Fenfluramine	Anorexic	Enhances release of serotonin (5-HT) in crayfish which in turn triggers the release of ovary-simulating hormone resulting in larger oocytes with enhances amounts of vitellin In fiddler crabs, stimulates the production of gonad-stimulating hormone accelerating testicular maturation	Daughton & Ternes, 1999
17a-Ethinylestradiol	Synthetic steroid	Endocrine-disrupting effects on fish, reptiles and invertebrates	Young *et al*, 2002
Methyltestosterone	Synthetic steroid	Impersex, reduced fecundity, oogenesis, spermatogenesis in snails	Schulte-Oehlmann *et al*, 2004
Avermectins	Parasiticide	Adults insects: loss of water balance, disruption of feeding and reduced fat accumulation, delayed ovarian development, decreased fecundity and impaired mating Juvenile insects: delayed development, reduced growth rates, development of physical abnormalities, impairment of pupariation or emergence and a loss of developmental symmetry	Floate *et al*, 2005
Tetracyclines, macrolides and streptomycin	Antibacterials	Antibacterial resistance measured in soil bacteria obtained from sites treated with pig slurry	Sengelov *et al*, 2003
Cypermethrin	Ectoparasiticide	Impact on dung decomposition	Sommer & Bibby, 2002
Fenbendazole	Parasiticide	Impact on dung decomposition	Sommer & Bibby, 2002
Tylosin	Antibacterial	Impacts on the structure of soil microbial communities	Westergaard *et al*, 2003
Erythromycin	Antibacterial	Inhibition of growth cyanobacteria and aquatic plants	Pomati *et al*, 2004
Tetracycline	Antibacterial	Inhibition of growth cyanobacteria and aquatic plants	Pomati *et al*, 2004
Ibuprofen	Anti-inflammatory	Stimulation of growth of cyanobacteria and inhibition of growth of aquatic plants	Pomati *et al*, 2004
Fenofibrate	Lipid regulator	Inhibition of basal EROD activity in cultures of rainbow trout hepatocytes	Laville *et al*, 2004
Carbamazepine	Analgesic	Inhibition of basal EROD activity in cultures of rainbow trout hepatocytes Inhibition of emergence of *Chironomus riparius*	Laville *et al*, 2004; Nentwig *et al*, 2004
Diclofenac	Analgesic	Inhibition of basal EROD activity in cultures of rainbow trout hepatocytes	Laville *et al*, 2004
Propanolol	Beta blocker	Weak EROD inducer in cultures of rainbow trout hepatocytes	Laville *et al*, 2004
Sulphamethazole	Antibacterial	Inhibition of basal EROD activity in cultures of rainbow trout hepatocytes	Laville *et al*, 2004
Clofibrate	Lipid regulator	Inhibition of basal EROD activity in cultures of rainbow trout hepatocytes	Laville *et al*, 2004
Diazepam	Antianxiety drug	Inhibition in the ability of dissected polyps from the cnidarian *Hydra Vulgaris* to regenerate a hypostome, tentacles and a foot	Pascoe *et al*, 2003
Digoxin	Cardiac glycoside	Inhibition in the ability of dissected polyps from the cnidarian *Hydra Vulgaris* to regenerate a hypostome, tentacles and a foot	Pascoe *et al*, 2003
Amlodipine	Calcium channel blocker	Inhibition in the ability of dissected polyps from the cnidarian *Hydra Vulgaris* to regenerate a hypostome, tentacles and a foot	Pascoe *et al*, 2003

Rapid Rate of New Chemical Production

- New chemicals (including pharmaceuticals) are being developed and produced at an unprecedented rate
- Chemical Abstracts Service registers new chemicals
- It took 33 years for CAS to register the 10 millionth compound in 1990. CAS registered the 40 millionth compound in November 2008. Nine months later, CAS registered its 50 millionth chemical compound



Potential Revised Approach

- Supplement analysis for specific commonly used PPCP compounds with analyses for indicators of combined effects for specific classes of compounds
- Test for overall estrogenicity (feminizing) or androgenicity (masculinizing)
- Measure abnormalities in reproductive tissues
- Measure hormones and biomarkers in fish serum

Photographer: Jo Ellen Hinck (vog.sgsu@kcnihj), U.S. Geological Survey

Types of Biological Effects Observed

PPCPs are capable of producing a wide variety of intentional and unintentional effects in humans, animals, and plants.

- **Direct toxicity (death, reduced growth or reproduction)**
- **Disruption of the Endocrine System**
  - **Endocrine Disrupting Compounds (EDCs) cause changes in hormone levels that, depending upon the exposed organism's stage of development, exposure to EDCs may cause temporary or permanent effects**
- **Suppression of Immune System**
  - **Reduced ability to fend off disease**
- **Decreased predator avoidance behavior**
- **Increased Antibiotic Resistance**
  - **Bacterial populations become less susceptible to antibiotics through repeated sub-lethal exposures**

Endocrine Disrupting Compounds

Effects from EDCs are typically the easiest to observe

- Birth control pills contain natural and/or synthetic hormones and are designed specifically to regulate hormone levels in women
- It is not uncommon for these hormones to pass through wastewater treatment plants in the low part per trillion range (typically less than 5 ppt)
- Laboratory studies have demonstrated reduced reproductive success in fishes exposed to <5 ppt of the synthetic estrogen 17∂-ethynylestradiol (EE2) and feminization of male fishes have been observed in waterways receiving wastewater plant effluent, but direct cause and effect evidence in natural systems was lacking.

Canadian Lake Study

- Karen Kidd *et al,* 2007 conducted a 7-year whole lake study in Ontario, Canada and demonstrated that chronic exposure to 5-6 ppt of EE2 resulted in the near total collapse of the exposed fathead minnow population

Canadian Lake Study, cont.

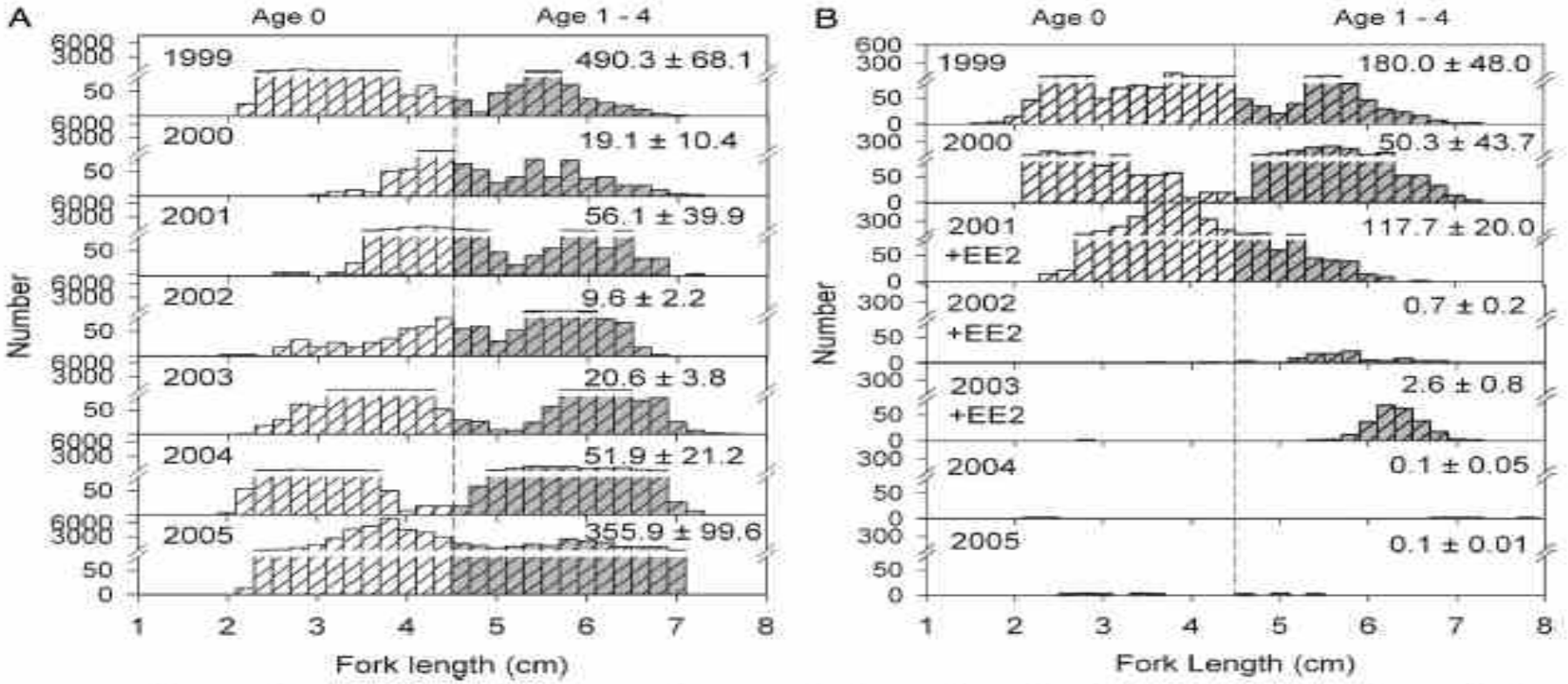
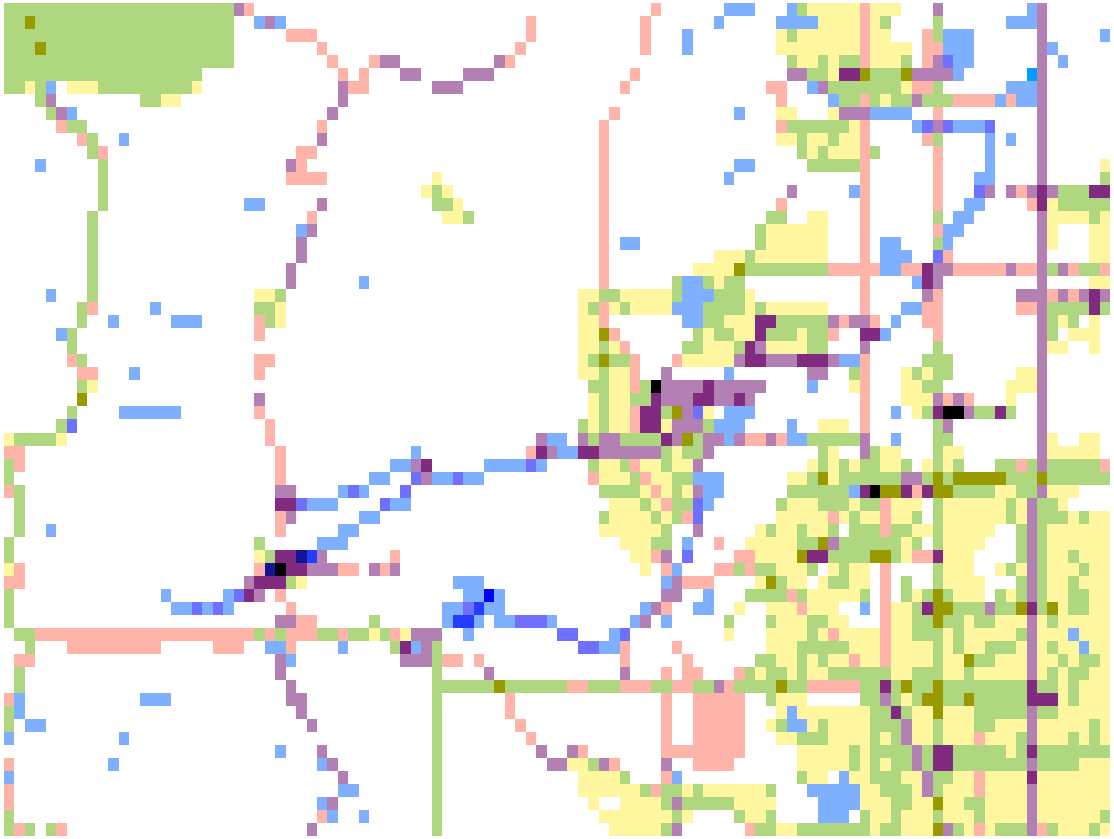


Fig. 3. Length frequency distributions of fathead minnow captured in trap nets in reference Lake 442 (*A*) and Lake 260 (*B*) (amended with 5–6 ng·L^{-1} of EE2 in 2001–2003) during the fall of 1999–2005. Distributions for each fall have been standardized to 100 trap-net days. Mean ± SE daily trap-net CPUE data for adults and juveniles for the fall catches are shown in the panels.

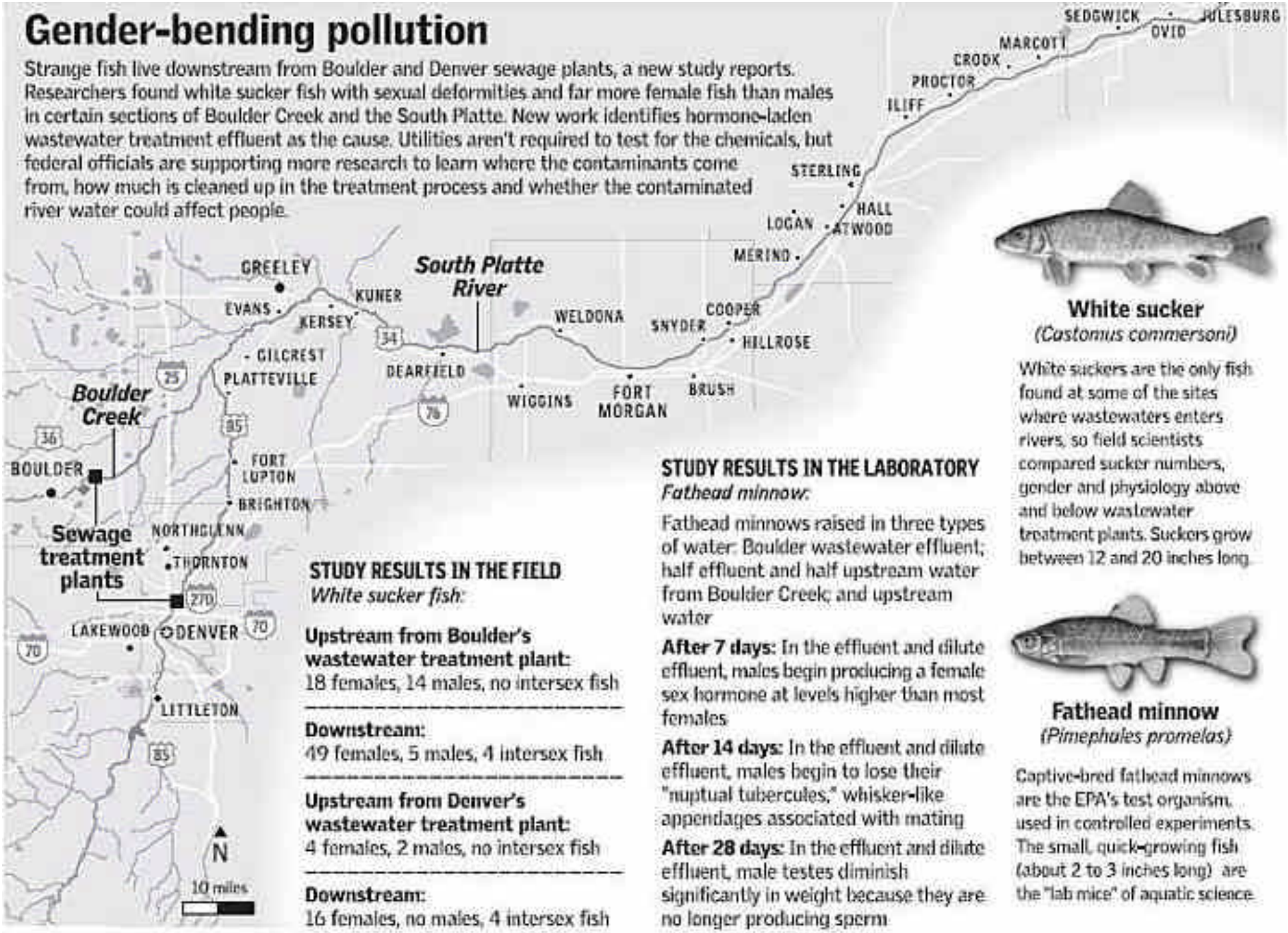
Boulder Creek, Colorado

- USGS and Colorado University studies on the fate and effects of PPCP's in a WWTP discharging into Boulder Creek, near Boulder , CO
- White Sucker population downstream of municipal wastewater outfall found to have highly skewed sex ratio (proportion of male to female fish) when compared to upstream reference sites



Gender-bending pollution

Strange fish live downstream from Boulder and Denver sewage plants, a new study reports. Researchers found white sucker fish with sexual deformities and far more female fish than males in certain sections of Boulder Creek and the South Platte. New work identifies hormone-laden wastewater treatment effluent as the cause. Utilities aren't required to test for the chemicals, but federal officials are supporting more research to learn where the contaminants come from, how much is cleaned up in the treatment process and whether the contaminated river water could affect people.



STUDY RESULTS IN THE FIELD

White sucker fish:

Upstream from Boulder's wastewater treatment plant:
18 females, 14 males, no intersex fish

Downstream:
49 females, 5 males, 4 intersex fish

Upstream from Denver's wastewater treatment plant:
4 females, 2 males, no intersex fish

Downstream:
16 females, no males, 4 intersex fish

STUDY RESULTS IN THE LABORATORY

Fathead minnow:

Fathead minnows raised in three types of water: Boulder wastewater effluent; half effluent and half upstream water from Boulder Creek; and upstream water

After 7 days: In the effluent and dilute effluent, males begin producing a female sex hormone at levels higher than most females

After 14 days: In the effluent and dilute effluent, males begin to lose their "nuptual tubercules," whisker-like appendages associated with mating

After 28 days: In the effluent and dilute effluent, male testes diminish significantly in weight because they are no longer producing sperm

White sucker
(Castomus commersoni)

White suckers are the only fish found at some of the sites where wastewaters enters rivers, so field scientists compared sucker numbers, gender and physiology above and below wastewater treatment plants. Suckers grow between 12 and 20 inches long.

Fathead minnow
(Pimephales promelas)

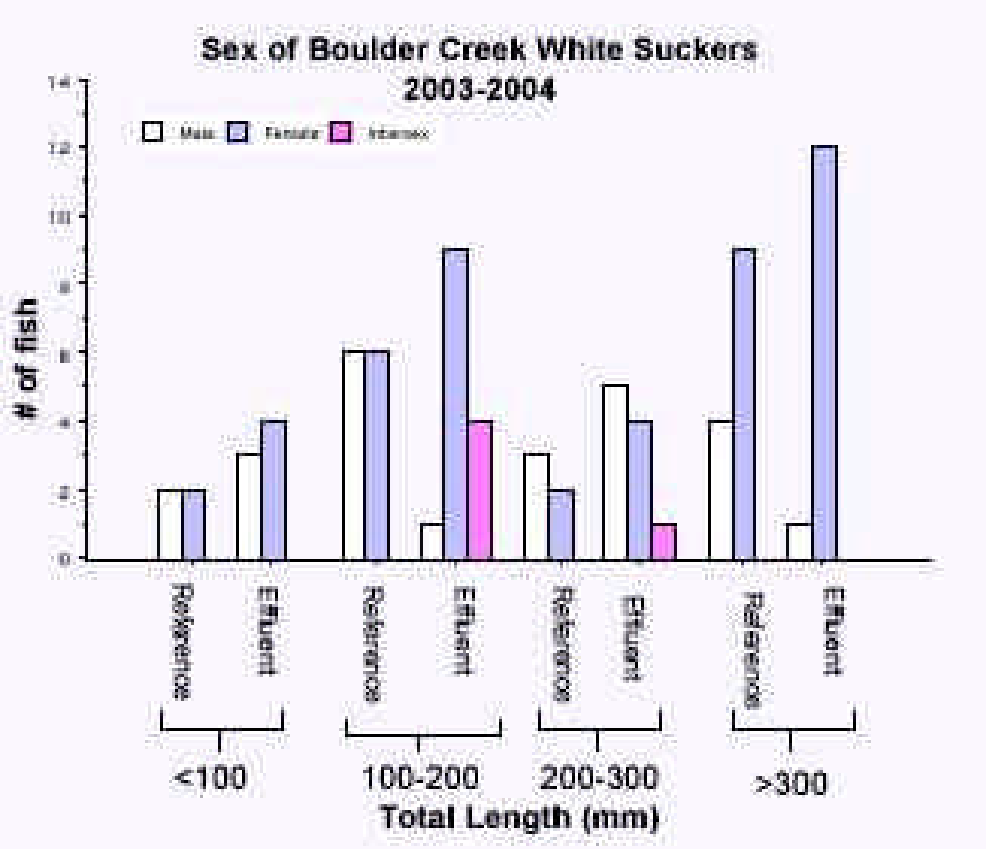
Captive-bred fathead minnows are the EPA's test organism used in controlled experiments. The small, quick-growing fish (about 2 to 3 inches long) are the "lab mice" of aquatic science.

Sources: David Norris, University of Colorado, Comparative Biochemistry and Physiology

The Denver Post

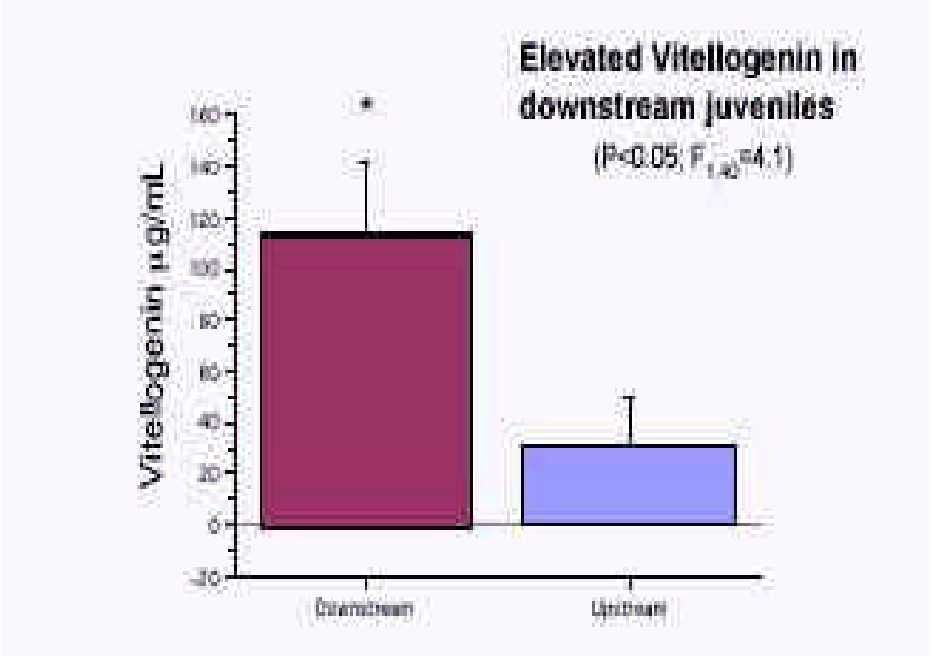
Boulder Creek, Colorado

- Intersexed males (male fish with oocytes in testes) not found at reference sites but were found downstream of wastewater plant outfall
- Skewed sex ratios found predominantly downstream of discharge

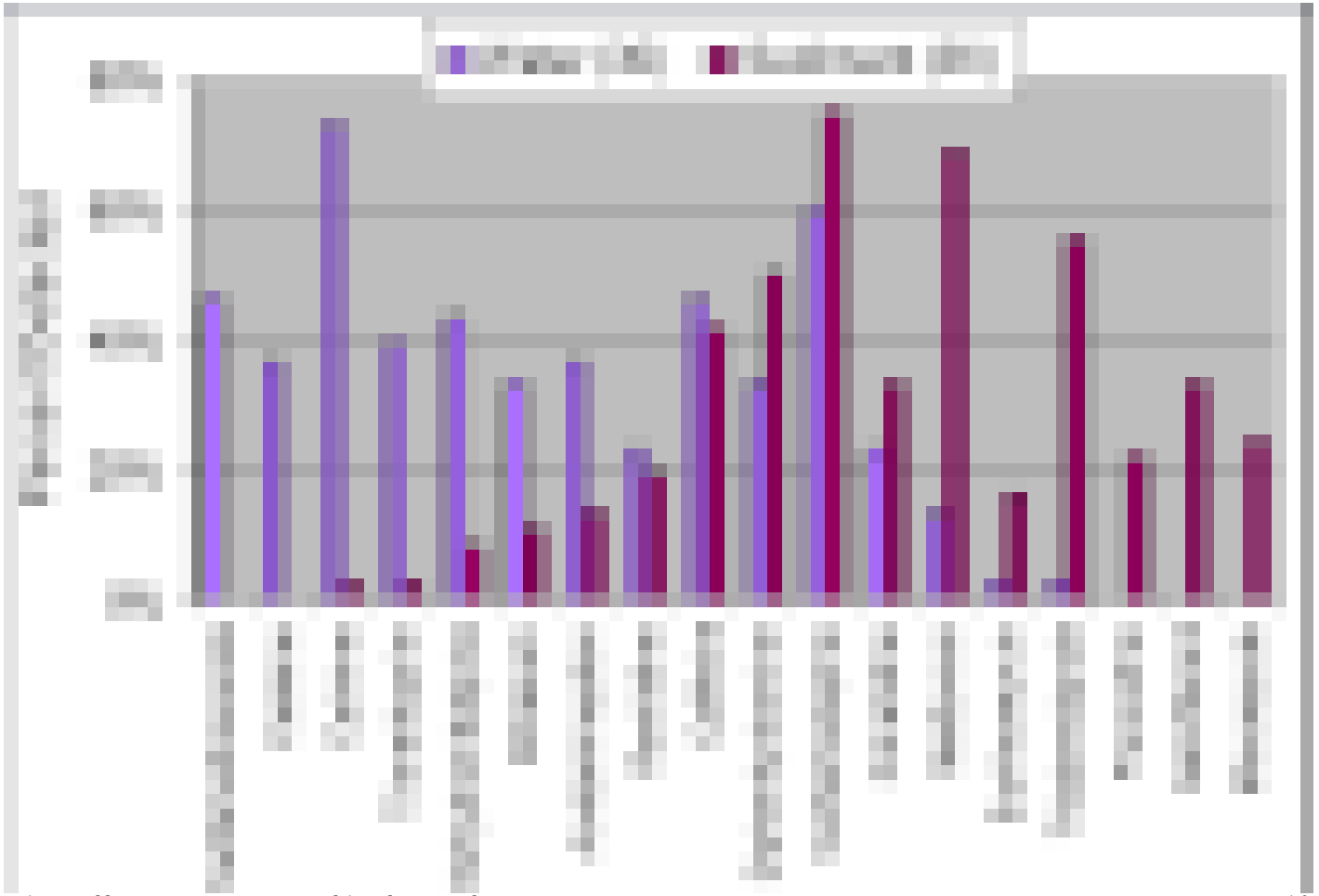


Boulder Creek, Colorado

- Elevated vitellogenin observed in downstream juvenile white suckers
- Vitellogenin is normally produced by the liver in female fishes and is transported to the ovaries via the bloodstream where it stimulates egg production and becomes egg yolk
- Juveniles and males should have very low or no vitellogenin in their blood plasma



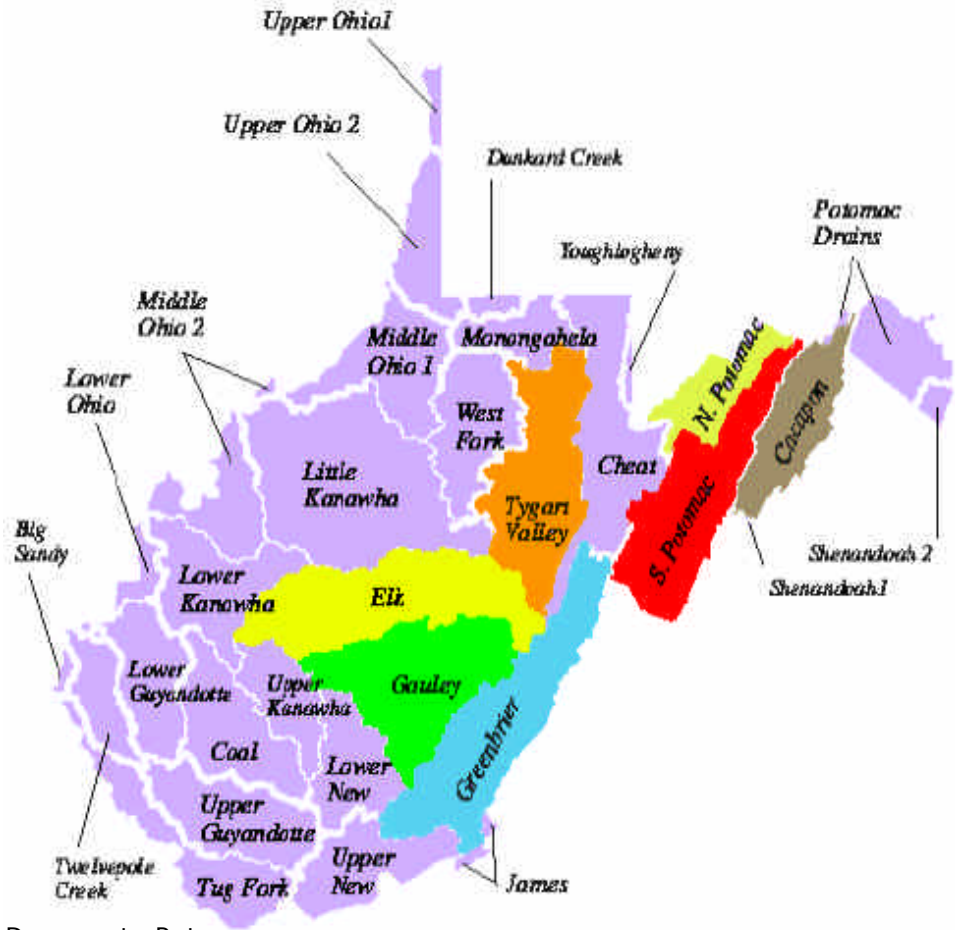
Boulder Creek, Colorado



http://www.state.nj.us/dep/wmm/Buxton%20Emerging%20Contaminants%20For%20Posting.pdf

Potomac Drainage Studies

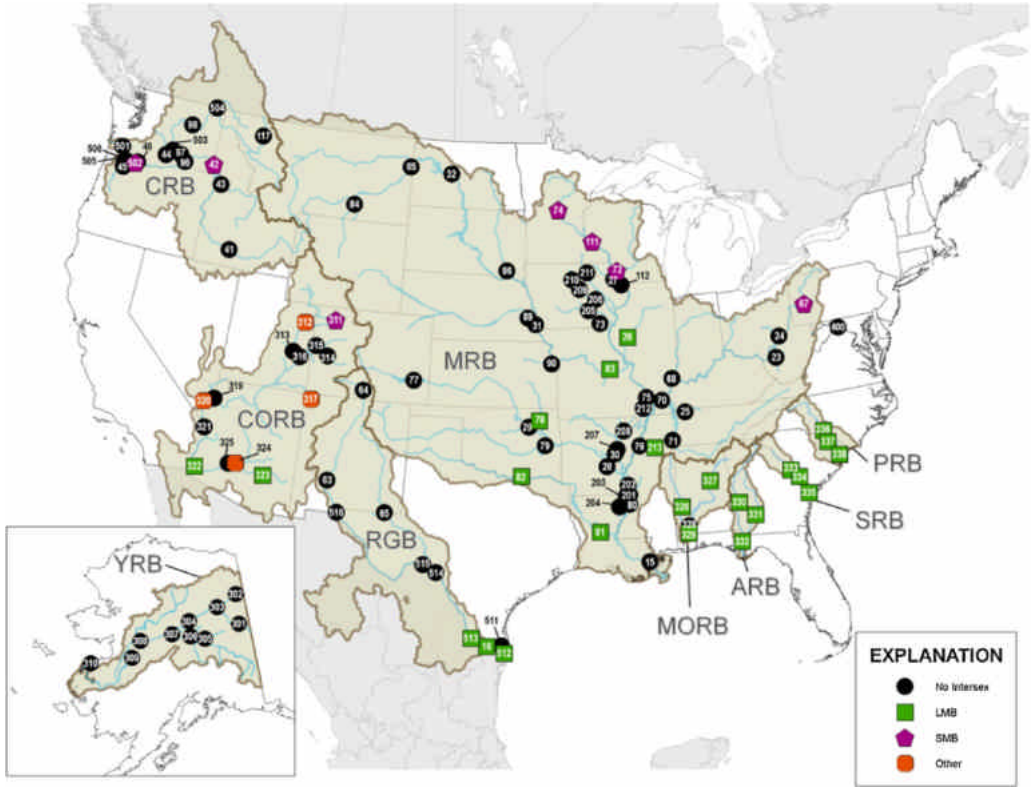
- Higher incidences of intersexed and infected fishes in developed drainages when compared to less disturbed drainages
- Male fishes with elevated Vitellogenin
- Estrogenic activity 532 to 748-fold greater in sediments than in overlying water, suggesting strong partitioning and accumulation in sediments



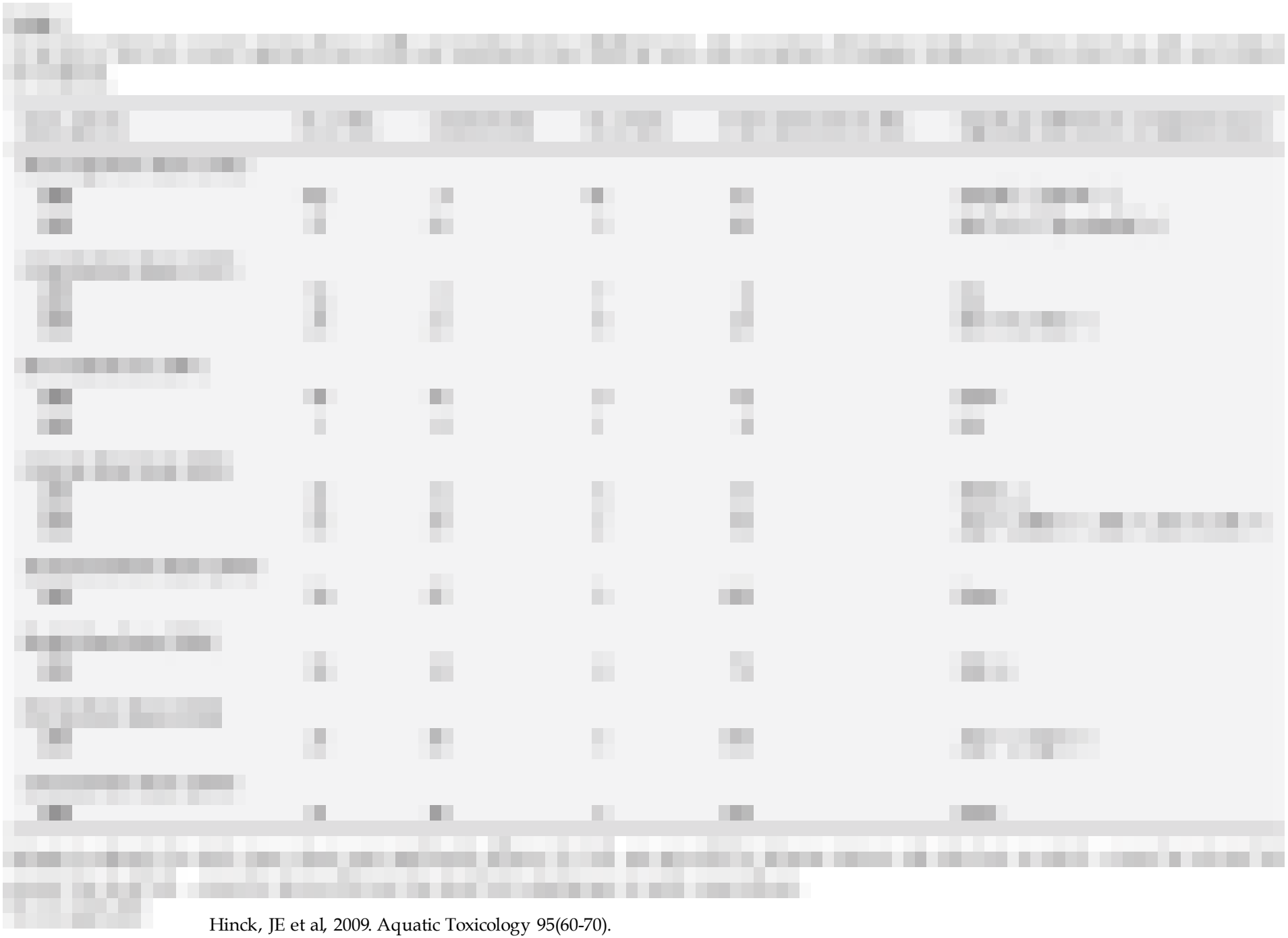
www.dep.wv.gov_WWE_watershed_wqmonitoring_Documents_Potomac-Intersex_USGS_FishHealthReproductiveIssuesPotomac_2006.pdf

USGS Report on Intersex Black Bass in U.S. Rivers 1995-2004

- Incidence of intersex greatest in Southeastern United States, why?



Hinck, JE et al, 2009. Aquatic Toxicology 95(60-70).



Hinck, JE et al, 2009. Aquatic Toxicology 95(60-70).

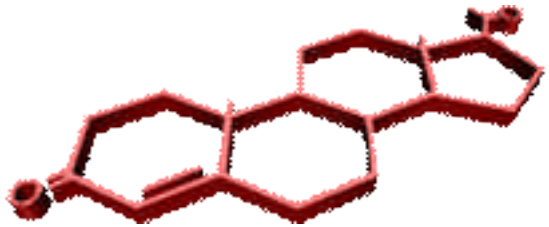
USGS Report on Intersex Black Bass in U.S. Rivers 1995-2004

- Did not observe significant correlations between incidents of intersex at Apalachicola sites and other EDC exposure endpoints (vitellogenin, 17β-estradiol)
- Intersex did not aways occur in areas with suspected sources of EDCs
- Exposures to EDCs intermittent? Other causes?

Widespread Endocrine Disruption and Reproductive Impairment in Atlantic croaker Exposed to Seasonal Hypoxia

Peter Thomas

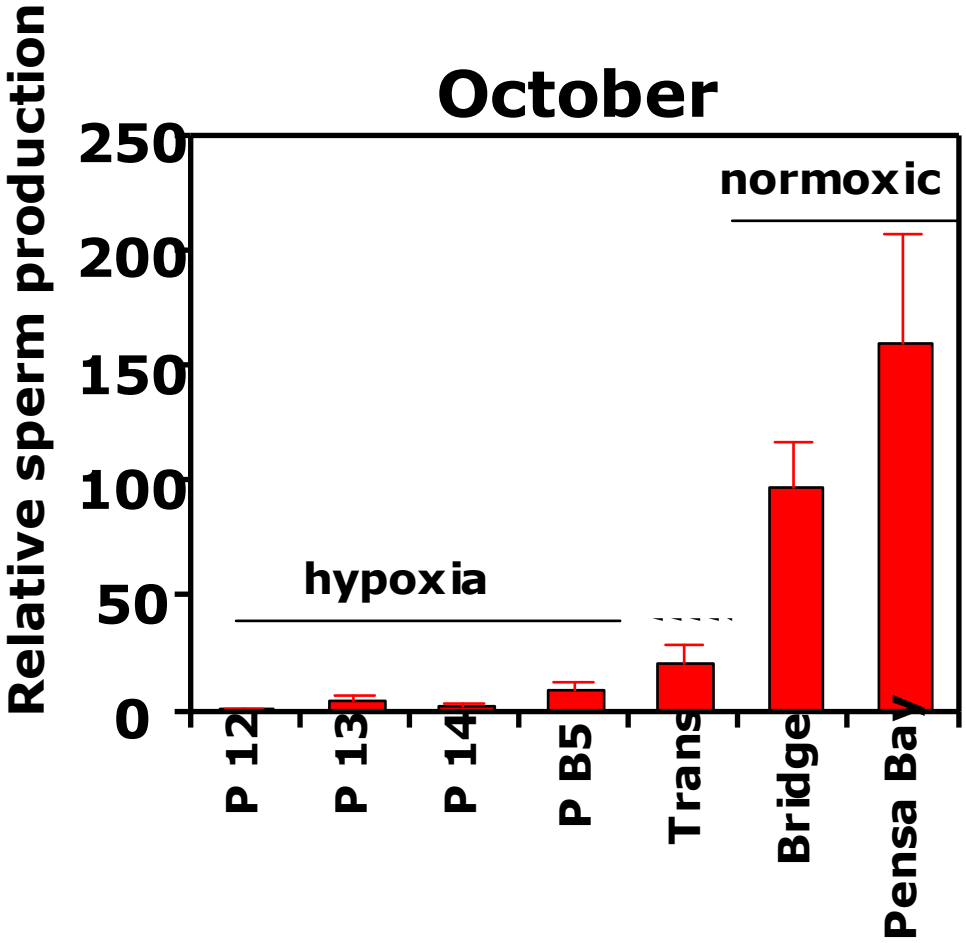
University of Texas at Austin



Collaborators:
Dr. Md.S. Rahman
J. Kummer
Dr. I.Khan

Endocrine Disruption and Hypoxia

- Through a series of field and laboratory experiments, Univ. of Texas researchers were able to demonstrate that exposure to low dissolved oxygen waters can cause disruption of endocrine system and lead to reproductive effects
- Must use caution when interpreting endocrine disruption effects



Conclusions

- There is currently a shortage of studies specifically designed to measure the responses of biological communities to PPCPs
- Laboratory and field studies to date indicate that there is potential for environmental impacts, but more research needs to be done to determine effects at environmentally relevant concentrations and in complex mixtures
- Science Progresses in Stages
- We are at the stage where studies to determine causes and effects in natural systems is possible
- Due to the rapid increase in the number of new PPCPs and other contaminants entering the environment, compound specific testing is not sufficient, can't keep up
- Need to look for indicators of exposure to classes of contaminants (e.g., measuring overall estrogenicity)

Conclusions, cont.

- New studies should examine effects to populations or communities (both animal and human) exposed to PPCPs at environmentally relevant concentrations
- Causal relationships between PPCP exposure and effects should be demonstrated using a combination of laboratory and field experiments
- Research also needs to be conducted to develop better wastewater treatment technologies that improve removal efficiencies for PPCPs
- All of this takes money. Let your representatives know that this is important to you.

Contact

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