

# Intersex and other forms of reproductive disruption in feral white sucker (*Catostomus commersoni*) downstream of wastewater treatment plant effluent in Boulder, Colorado



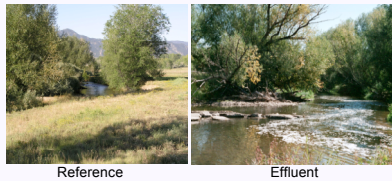
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## Abstract

Feral white suckers (*Catostomus commersoni*) were collected on Boulder Creek downstream from wastewater treatment plant (WWTP) effluent and from reference sites. This effluent is known to contain endocrine-active compounds including alkylphenols, bisphenol A, and reproductive steroids. We found gonadal intersex and other forms of reproductive disruption in white suckers collected downstream of WWTP effluent but not at reference sites. The male to female ratio was skewed toward females at the downstream site. Abnormalities in gonadal morphology, including smaller ovaries, less developed oocytes, and asynchronous follicular development were noted in female white suckers downstream of the WWTP. We also report elevated plasma vitellogenin in downstream juvenile white suckers. The reproductive potential of native fishes may be compromised in stream reaches of western states where large volumes of treated wastewater are discharged into relatively small-sized streams.

## Study Sites

Boulder Creek was sampled above and below the effluent of the City of Boulder wastewater treatment plant (WWTP). Boulder Creek upstream of the Boulder WWTP flows through the city of Boulder and receives all storm water runoff from the municipal area. Pristine comparison sites were not available within the distributional range of the target species. Upstream reference sites were downstream of city of Boulder storm water runoff and upstream of all WWTP effluent.



Reference

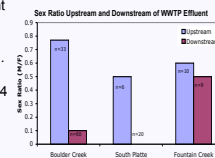
Effluent

## Results

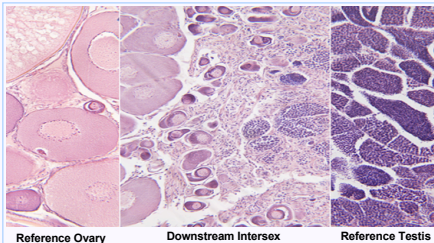
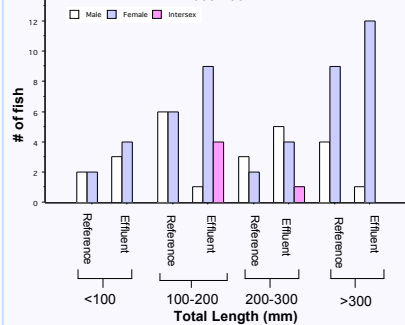
- Sex ratio skewed towards females downstream of WWTP effluent.
- Asynchronous follicular development in downstream females.
- Intersex gonads, composed primarily of ovarian tissue, were found in fishes only downstream of WWTP effluent.
- Elevated vitellogenin in plasma of juvenile fish downstream of WWTP effluent.
- Reduced gonadosomatic index in downstream females.

## Sex ratio skewed toward females

We identified skewed sex ratios downstream of WWTP effluent in Boulder, Denver, and Colorado Springs in 2001 (Woodling et al., unpublished). Further sampling of Boulder Creek in 2002, 2003, and 2004 found the effect of effluent on sex ratio and occurrence of intersex to be size specific.

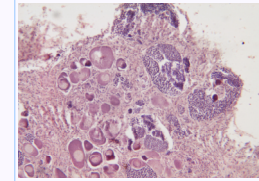


## Sex of Boulder Creek White Suckers 2003-2004

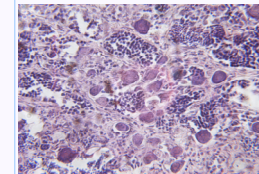


Reference Ovary Downstream Intersex Reference Testis

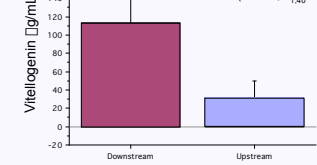
## Representative intersex gonads and evidence of effluent endocrine-activity



Intersex gonads from white suckers collected downstream of WWTP effluent



## Elevated Vitellogenin in downstream juveniles (P<0.05; F<sub>1,40</sub>=4.1)

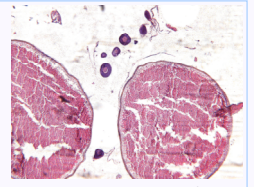
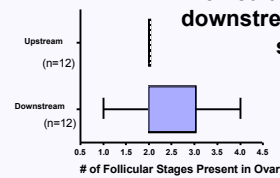


## Endocrine-Active Wastewater Constituents in Boulder Creek \*

Compound	Concentration	Endocrine Activity
17β-Estradiol	2.4 ng/L	Estrogenic
17β-Estradiol	24 ng/L	Estrogenic
Estrone	3.1 ng/L	Estrogenic
Bisphenol A	5.6 ng/L	Estrogenic
4-Nonylphenol	240 ng/L	Estrogenic
Total Nonylphenoethoxy-carboxylates	200 µg/L	

\* Murphy et al. 2003 and Larry Barber, pers. comm.

## Follicular asynchrony in downstream female white suckers

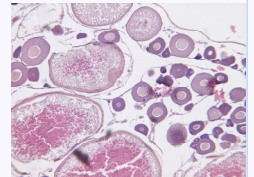


Above: synchronous oocyte development in reference ovary  
Below: asynchronous oocyte development in downstream ovary

A characterization of follicular development in ovaries from size-matched (>200mm) females shows that downstream fish possess significantly more simultaneous ovarian follicular stages than reference fish (P<0.01).

All upstream fish examined possess only two follicular stages, one pre-vitellogenic and one post-vitellogenic.

Downstream fish possess between one and four simultaneous follicular stages.



## Future Directions

To further characterize the extent of reproductive disruption in the fishes of Colorado's front range rivers we are continuing our survey of 3 rivers and incorporating laboratory exposure experiments to determine the role of effluent in generating gonadal phenotype.

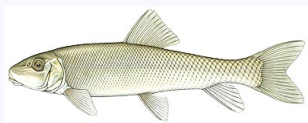
- Determine whether gonadal phenotype in white suckers is influenced by exogenous estrogens.
- Determine whether the intersex condition and follicular asynchrony downstream of WWTP effluent is inducible by laboratory exposure to effluent.
- Determine whether disruption of follicular synchrony is accompanied by other modifications of life-history phenotype among downstream fish.
- Determine whether reproductive disruption in fishes can be detected using non-lethal means.

## Literature Cited

- Goodbred, S.L., R.J. Gilliom, T.S. Gross, N.P. Denlow, W.L. Bryant, and T.R. Schoeb. 1997. Reconnaissance of 17β-estradiol, 11-ketotestosterone, vitellogenin and gonad histopathology in common carp of United States streams: potential for contaminant induced endocrine disruption. U.S. Geological Survey, Open file report 96-627. Sacramento, California.
- Murphy, S.F., Verplanck, P.L. and Barber, L.B. 2003. Comprehensive water quality of the Boulder Creek Watershed, Colorado, during high-flow and low-flow conditions, 2000. USGS Water-resources Investigations Report 03-4045. Denver, CO.
- Propst, D.L. 1982. Warmwater fishes of the Platte River Basin, Colorado: distribution, ecology and community dynamics. Dissertation (PhD), Colorado State University.
- Presnell, J.K., M.P. Schreibman. 1997. Humason's Animal Tissue Techniques. Johns Hopkins University Press, Baltimore.

## Acknowledgements

Funding: US EPA Region, 8 NPDES WQCA #CP988934-01  
In-kind support from the Colorado Division of Wildlife



## White Sucker (*Catostomus commersoni*)

A long-lived species, the white sucker (*Catostomus commersoni*) was selected as the target species. White suckers tolerate a wide variety of conditions including river stretches greatly enriched from domestic sewage treatment plant effluents. White suckers have been collected at sites immediately downstream of WWTP effluents in Colorado. Propst (1982) often found the white sucker to be the most common fish at such locations, although numbers were lower than at similar unpolluted sites. Spring sampling dates coincide with spawning in white sucker while fall sampling dates coincide with reduced stream flows and with male and female gametogenesis.

