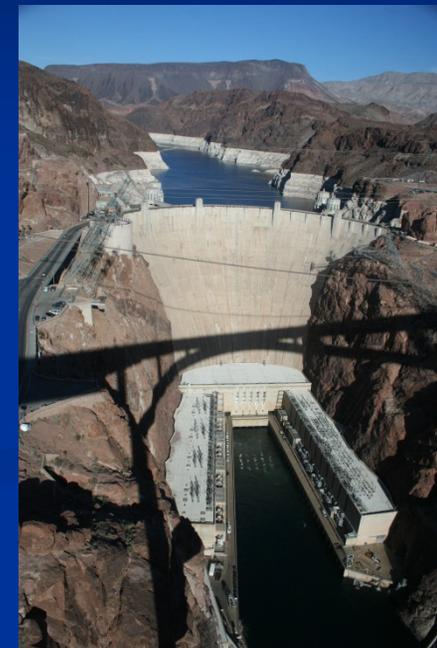


How evaluations of low concentrations of organic chemical contaminants are important in managing the Lake Mead ecosystem



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Presentation Outline

- What are Endocrine Disrupting Compounds (EDCs)?
- Why are EDCs important?
- Why study EDCs in Lake Mead?
- History of EDC Work at Lake Mead
- Have Things Changed Over Time?



Endocrine Disruption

Process by which a chemical from outside an organism causes **ADVERSE** health effects by changing the endocrine function of the organism

For more about endocrine disruption:

Sumpter and Johnson (2005) ES&T, v39, pp 4321- 4332

Jobling and Tyler (2006) EHP, v 114, supplement 1

<http://www.tmc.tulane.edu/ecme/eehome/>

Classes of EDCs



Effluents	STW, pulp & paper mills
Flame Retardants	PBDEs
Fungicides	Vinclozolin
Herbicides	Atrazine
Insecticides	Methoxychlor
Metals	Tributyltin
Pharmaceuticals	Ethinyl Estradiol
Phenols	Bisphenol A
Plasticizers	Phthalates
Industry	PCBs, dioxin
Soy Products	Genistein
Surfactants	Alkylphenol
	Ethoxylates



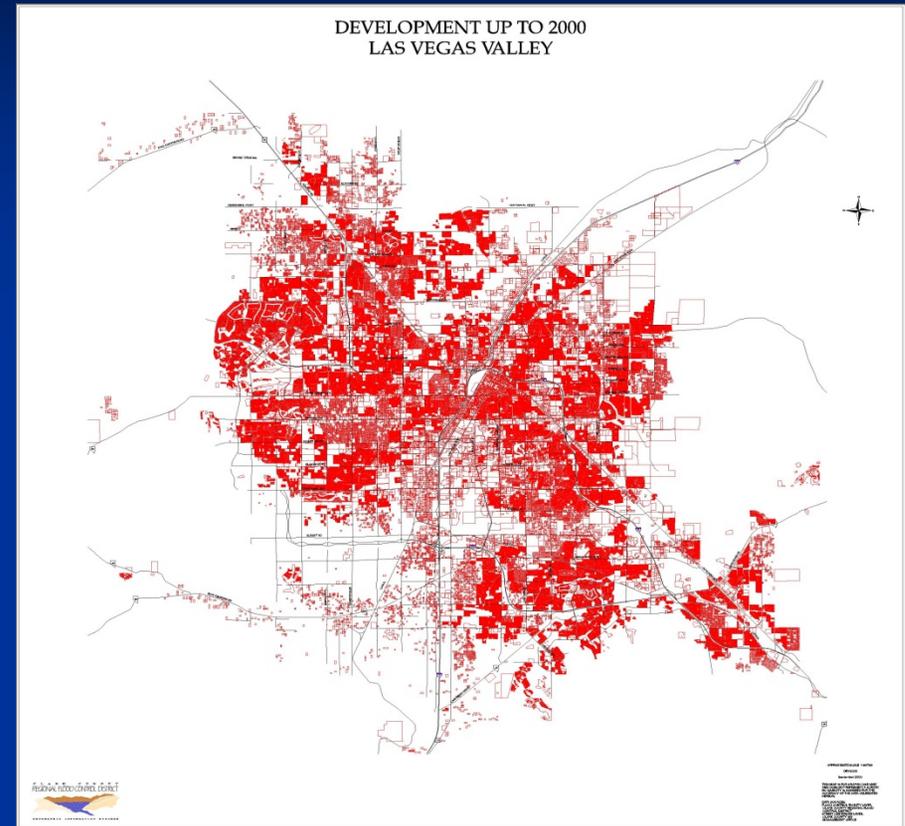
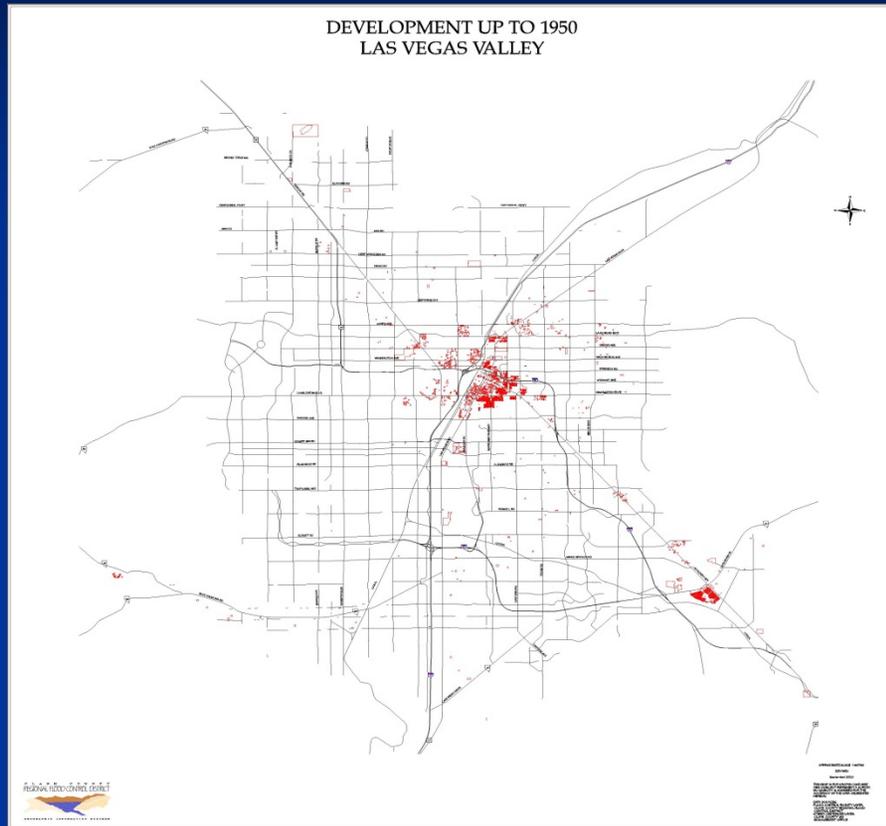
Why are EDCs Important?

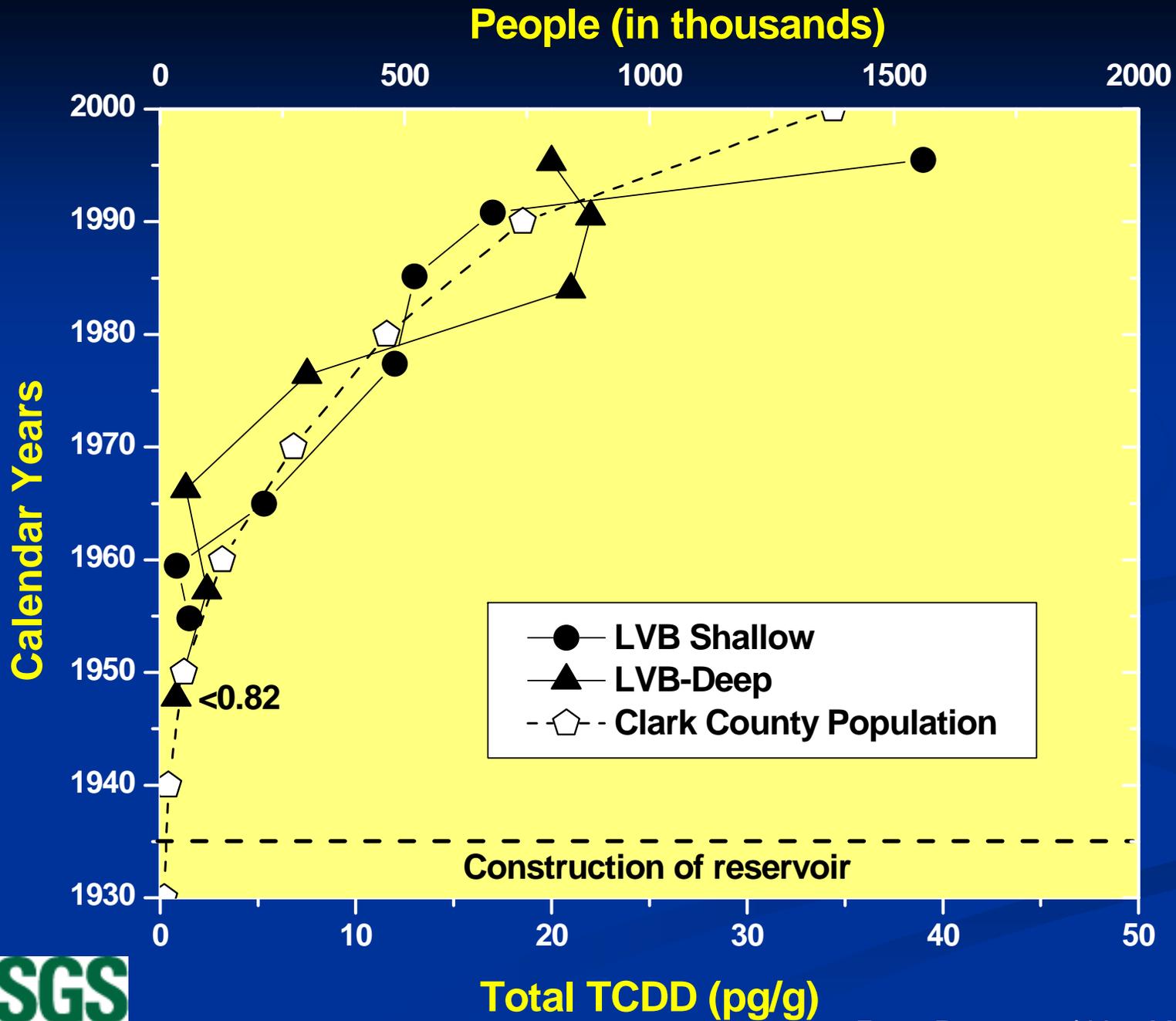
- Since the 1990's there has been evidence that synthetic organic compounds cause disruption to the endocrine system of fish and other aquatic organisms
- These findings were controversial, but were substantiated in multiple studies over many years and different locations
- This then led to the controversy of whether EDCs released to the environment affect Human health
- There is still controversy about whether there is an affect on Human health through water

Chronic Exposures of Aquatic Wildlife to EDCs

- Wildlife do not have the benefit of analyzing and treating their food and water prior to consumption
- Aquatic organisms are particularly susceptible to pollutants in the water: They are exposed constantly
- Fetal development is regulated by the endocrine system
- Low doses of ECs at critical points during fetal development may have profound impacts on the development which may last through maturation

Population Growth in Las Vegas Valley





Why Study Lake Mead



CSI: Las Vegas – “The case of the cross-dressing carp”

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How did the USGS get involved

- As part of the National Water Quality Assessment (NAWQA) program Lake Mead was studied for water quality
- Relatively new types of fish tissue research was also included in the water quality sampling
- Results were published in a USGS report in 1996 →
- Results were not met with overwhelming enthusiasm....



U.S. Department of the Interior—U.S. Geological Survey

SYNTHETIC ORGANIC COMPOUNDS AND CARP ENDOCRINOLOGY AND HISTOLOGY IN LAS VEGAS WASH AND LAS VEGAS AND CALLVILLE BAYS OF LAKE MEAD, NEVADA, 1992 AND 1995

Water-Resources Investigations Report 96-4266

Nevada Basin and Range Study Unit
National Water-Quality Assessment Program

By Hugh E. Bevans¹, Steven L. Goodbred², John F. Miesner³, Sharon A. Watkins¹, Timothy S. Gross⁴, Nancy D. Denslow⁴, and Trenton Schoeb⁴

ABSTRACT

The Nevada Basin and Range study unit of the National Water-Quality Assessment Program, U.S. Geological Survey, in cooperation with the National Park Service, National Biological Service, and U.S. Fish and Wildlife Service, investigated the occurrence of organochlorines and semivolatile industrial compounds in the water column, bottom sediment, and carp (*Cyprinus carpio*) tissue at five sites in Las Vegas Wash and Lake Mead. Endocrine systems of carp were assessed by analyzing concentrations of female and male sex-steroid hormones, 17 β -estradiol and 11-ketotestosterone, and vitellogenin (an estrogen-controlled egg protein) in blood-plasma samples. The histology of carp gonads, hepatopancreas, kidney, gill, and lower intestine were analyzed for effects that can result from endocrine disruption or exposure to toxicants. Organochlorines (pesticides and industrial compounds) and semivolatile industrial compounds were detected in semipermeable membrane devices and bottom-sediment samples; only organochlorines were detected in carp-tissue samples. Concentrations of organochlorines were higher in Las Vegas Wash and Bay than in Callville Bay (the reference site) for the three media that were sampled. Results of a carp-tissue bioassay indicated the presence of dioxins or furans with low toxic-equivalent factors relative to 2,3,7,8-tetrachlorodibenzo- β -dioxin in Las Vegas Wash and Las Vegas and Callville Bays. Patterns of necrosis observed in hepatopancreas and kidney samples from carp are consistent with long-term subchronic exposure to toxicants. Polycyclic aromatic hydrocarbons, phthalates, and phenols also were detected at higher concentrations in bottom-sediment samples from Las Vegas Bay than in a comparable sample from Callville Bay. Polycyclic aromatic hydrocarbons were detected in samples from semipermeable membrane devices from all sites.

Endocrine disruption in carp from Las Vegas Wash and Bay, as compared to Callville Bay, is evidenced by high concentrations of 11-ketotestosterone levels in blood-plasma samples of female carp in Las Vegas Wash, low concentrations in male carp from Las Vegas Bay, and low 17 β -estradiol concentrations in male carp from Las Vegas Bay. The most compelling evidence of endocrine disruption is the presence of vitellogenin in blood-plasma samples of male carp from Las Vegas Wash and Bay and elevated concentrations in female carp from Las Vegas Bay.

Many of the organochlorines and semivolatile industrial compounds detected in semipermeable membrane devices, bottom sediment, and carp tissue from Las Vegas Wash and Bay have been linked to endocrine disruption in fish by previous investigations of other areas. The endocrine disruption observed in carp from Las Vegas Wash and Bay could be due to the presence of these compounds.



Aerial view of lower Las Vegas Wash and Las Vegas Bay of Lake Mead. View to the northwest, Oct. 12, 1995. Photograph by A.S. VanDenburgh.

¹U.S. Geological Survey, Carson City, Nev.
²U.S. Geological Survey (formerly National Biological Service), Sacramento, Calif.
³U.S. Fish and Wildlife Service, Reno, Nev.
⁴University of Florida, Gainesville (Biotechnology for Evolutionary, Ecological, and Conservation Services Program).

ABSTRACT 1

Possible EDC Effects seen at Lake Mead from 1996 USGS report

- Genital and gender characteristics abnormalities
- Gonadal tissue irregularities ("intersex")
- Vitellogenin induction in males
- Skewed sex-ratios
- Bad Temper!

Cartoon from Las Vegas newspaper in 1996
when EDCs were first reported in Lake Mead



Are EDCs important in Lake Mead?

- Associated Press article on pharmaceuticals in water supplies and their effect on aquatic organisms created quite a bit of interest in EDCs
- Senate hearing held in April 2008

Reno Gazette-Journal March 11, 2008

Many of those studies refer to the heralded research at Lake Mead. There, on a recent



Carla Wieser, fishery biologist with the U.S. Geological Survey, takes a blood sample from a carp Nov. 5 in the Lake Mead National Recreation Area near Boulder City to study effects of pharmaceuticals in water on fish.

Drugs also harming fish

Unclean water causing reproduction problems

BY JEFF DONN,
MARTHA MENDOZA AND
JUSTIN PRITCHARD
ASSOCIATED PRESS

LAKE MEAD, Nev. — On this brisk, glittering morning, a flat-bottomed boat glides across the massive reservoir that provides Las Vegas its drinking water. An ominous rumble grows beneath the boat as its two long, electrified claws extend into the depths.

Moments later, dozens of stunned fish float to the surface. Federal scientists scoop them up and transfer them into 50-quart Coleman ice chests for transport to a makeshift lab on the dusty lakeshore. Within the hour, researchers will submerge seven-pound common carp to death, draw their blood, snip out their gonads and pack them in aluminum foil and dry ice.

The specimens will be flown

across the country to laboratories where aquatic toxicologists are studying what happens to fish that live in water contaminated with at least 13 different medications — from over-the-counter pain killers to prescription antibiotics and mood stabilizers.

More often than not these days, the laboratory tests bring unwelcome results.

A five-month Associated Press investigation has determined that trace amounts of many of the pharmaceuticals we take to stay healthy are seeping into drinking water supplies, and a growing body of research indicates that this could harm humans.

But people aren't the only ones who consume that water. There is more and more evidence that some animals that live in or drink from streams and lakes are seriously affected.

Pharmaceuticals in the wa-

ter are being blamed for severe reproductive problems in many types of fish: The endangered razorback sucker and male fathead minnow have been found with lower sperm counts and damaged sperm; some walleyes and male carp have become what are called feminized fish, producing egg yolk proteins typically made only by females.

Meanwhile, female fish have developed male genital organs. There are problems with other wildlife as well: kidney failure in cultures, impaired reproduction in mussels, inhibited growth in algae.

"We have no reason to think that this is a unique situation," said Erik Orsak, an environmental contaminants specialist with the U.S. Fish and Wildlife Service, pulling off rubber gloves splattered with fish blood at Lake Mead.

"We find pretty much anywhere we look, these com-

pounds are ubiquitous." In November, at the annual Society of Environmental Toxicology and Chemistry meeting in Milwaukee, 30 new studies related to pharmaceuticals in the environment were presented — hormones found in the Chicago River; abnormalities in Japanese zebra fish; ibuprofen, gemfibrozil, triclosan and naproxen in the lower Great Lakes.

Many of those studies refer to the heralded research at Lake Mead. There, on a recent morning, Steven Goodbread struggled to hold a large wriggling carp with both hands. On the outside, the carp looked fine, vibrant and strong, but the U.S. Geological Survey scientist assumed the worst.

"Typically, we see low levels of sex steroids, limited testicular function, low sperm count, that kind of thing," he said slipping the fish into a holding tank and closing the lid. "We'll have to wait and see about this fellow."

In 2009 the Center for Biological Diversity Petitioned NDEP to list some waters of Lake Mead



CENTER for BIOLOGICAL DIVERSITY

Because life is good.

For Immediate Release, November 16, 2009

Endocrine-disrupting Chemicals, Risk to Human Health and Wildlife Survival, are Polluting Lake Mead

CBD petitions Nevada Division of Environmental Protection to list Lake Mead, Las Vegas Wash and Bay on 303(d) list of impaired waters



Lake Mead timeline of EDC understanding

Publication of USGS WRIR on EDCs in Lake Mead



1996

Media and local government reaction was not positive



2001

Snyder et al 2001, and other references indicates there is no toxicological relevance to organic chemicals in Lake Mead. One drop in a swimming pool analogy. Caged fish also show no effects.

Publication of Patiño et al, 2003 showing histopathological effects on carp



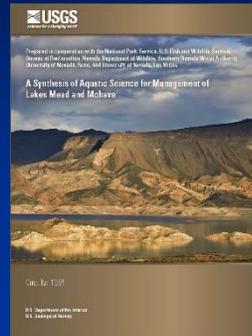
2003

New USGS studies from 2005 to 2010 show continued effect on Lake Mead. But with smaller impact

2005

2006

2009



Publication of USGS circular on Lakes Mead and Mohave – cooperation of all agencies involved in Lake Mead

Publication of USGS Video >250,000 hits

2013

CBD requests listing of LM to EPA 303d list



Two USGS journal articles show impacts on both Carp and Large Mouth Bass in Lake Mead
Total of 19 pubs by USGS on EDCs

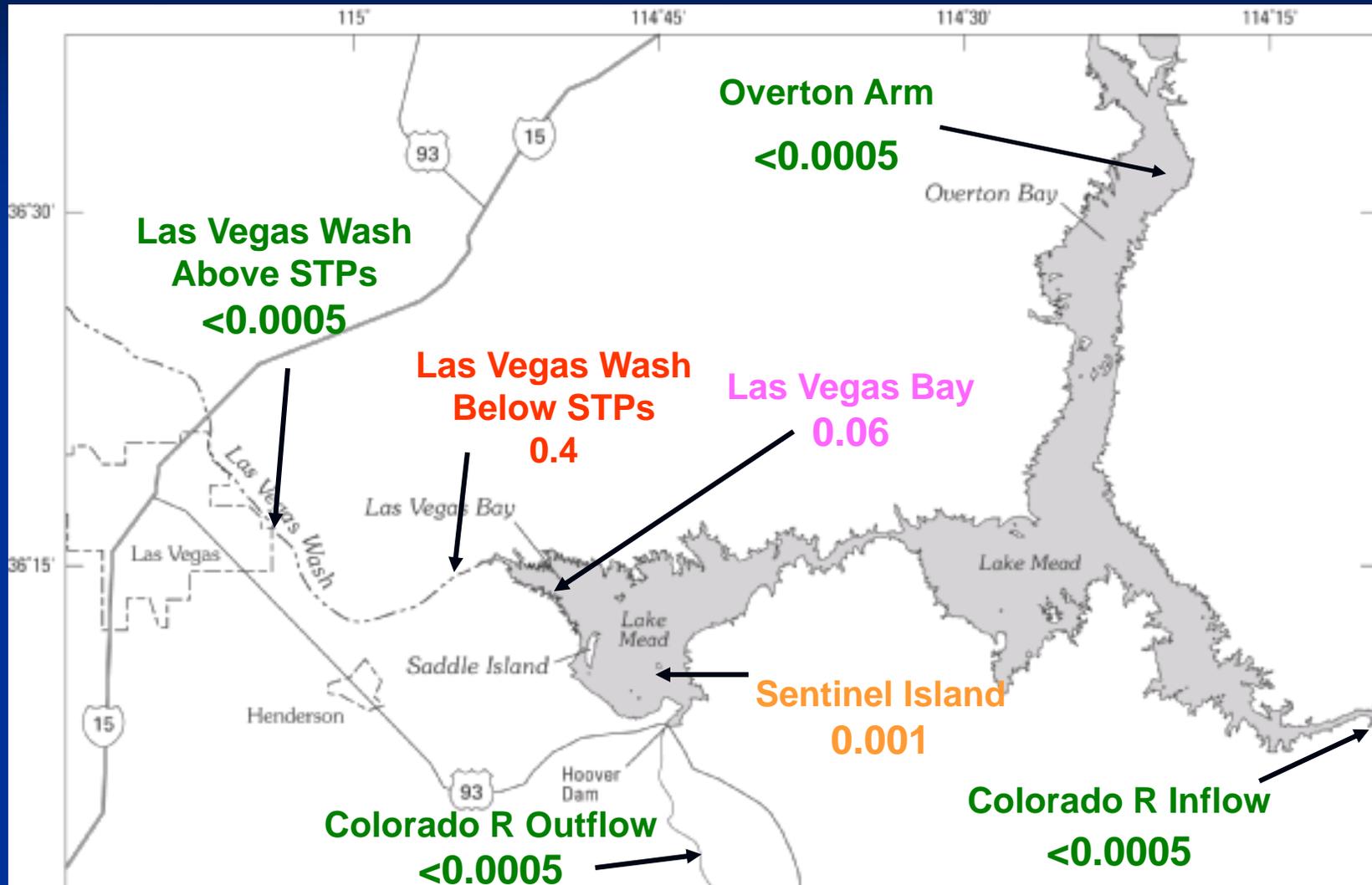
Recognition that aquatic wildlife may be at risk begins at Lake Mead

2012

2015

Where are EDCs Coming From?

Example of Galaxolide ($\mu\text{g/L}$) at 8 m depth in Lake Mead



Compounds Detected in Las Vegas Wash Upstream of WWTPs (21 different detections)

PCPs

- Caffeine
- 4-tert-octylphenol

PAHs

- Pyrene
- Various naphthalene compounds
- Phenanthrene
- Benzo (a) pyrene

Pesticides/herbicides

- Chlorpyrifos
- Dacthal
- Hexachlorobenzene
- Trifluralin, Benfluralin

Industrial

- BDE (47,99)
- Pentachloroanisole (degradation of PCP)
- 5-methyl-1H-benzotriazole
- *para*-cresol
- Tris (2-butoxyethyl) phosphate



Compounds Detected in Las Vegas Wash Downstream of WWTPs (35 different detections)

PCPs

- Galoxolide
- Tonalide
- 4-tert-octylphenol
- Triclosan

PAHs

- Pyrene
- Anthracene
- Phenanthrene
- Benzo (a) pyrene

Pesticides

- Chlorpyrifos
- o,p' DDE
- Trifluralin
- HCB
- Trans & Cis Chlordane

Industrial

- BDE (47,99,100,138,
146,180)

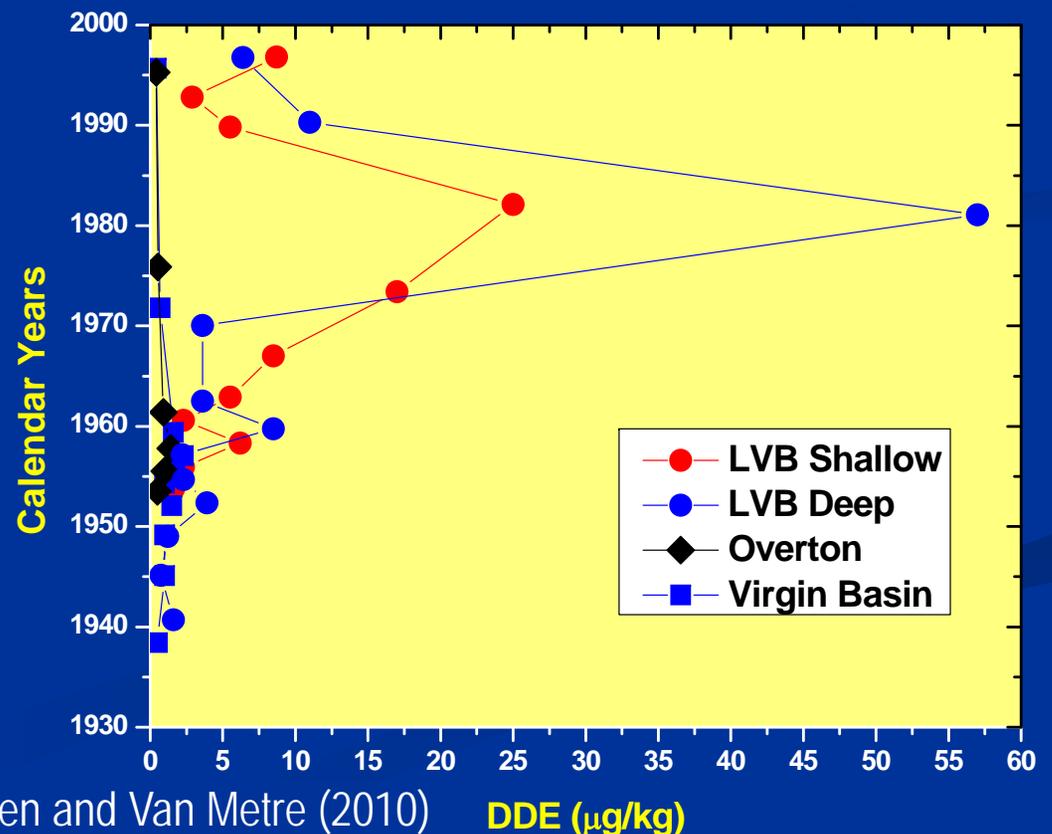
And others

Organic Contaminants in 1998 core

More than 50 compounds detected at each site in Las Vegas Bay
Approximately 30 compounds detected at Overton and Virgin Basin

Compounds detected were: organochlorine pesticides, PCBs, furans, dioxins, PAHs, and phenol

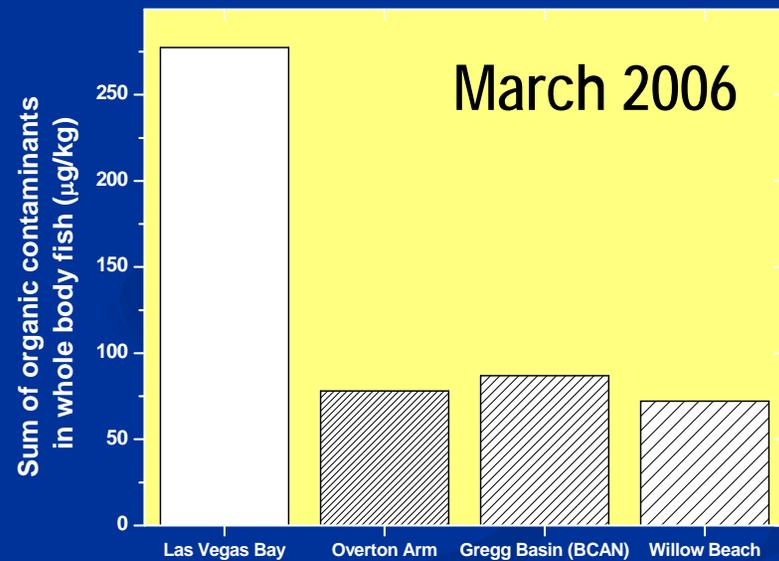
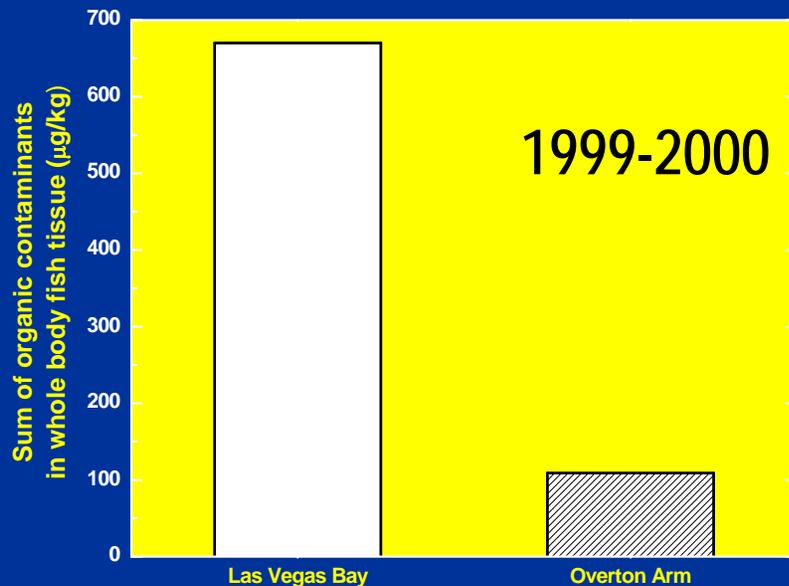
However emerging contaminants
(e.g. triclosan, tonalide,
galaxolide, ethynylestradiol, etc.)
were not analyzed



Effects in Male Carp

Tumors and Intersex fish found in Willow Beach Carp

EDCs found in carp include
Methyl Triclosan, PBDEs,
DDT (and metabolites), Fragrances



From Goodbred et al. (2007)

From high to low, reproductive condition by site can be classified as:

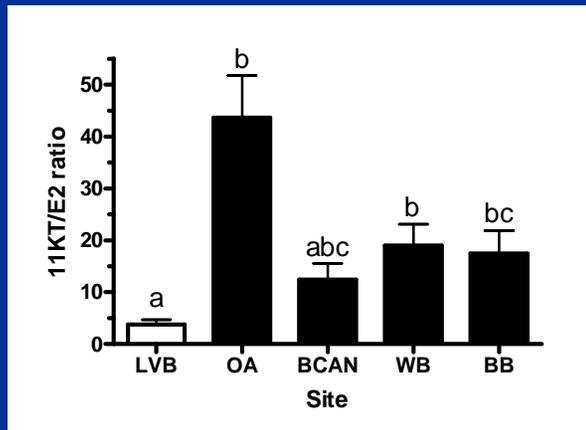
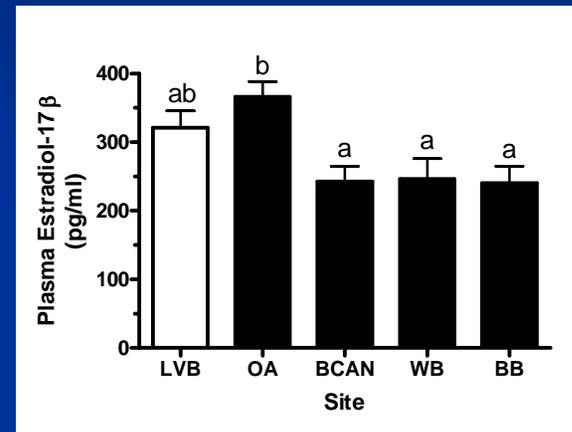
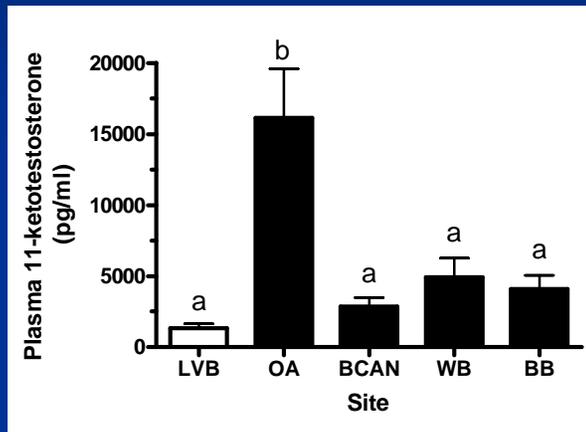
Derived from Patiño et al. (2015)



OA > LVB > LVW > WB

March 2006 Results – Males

Reproductive hormones

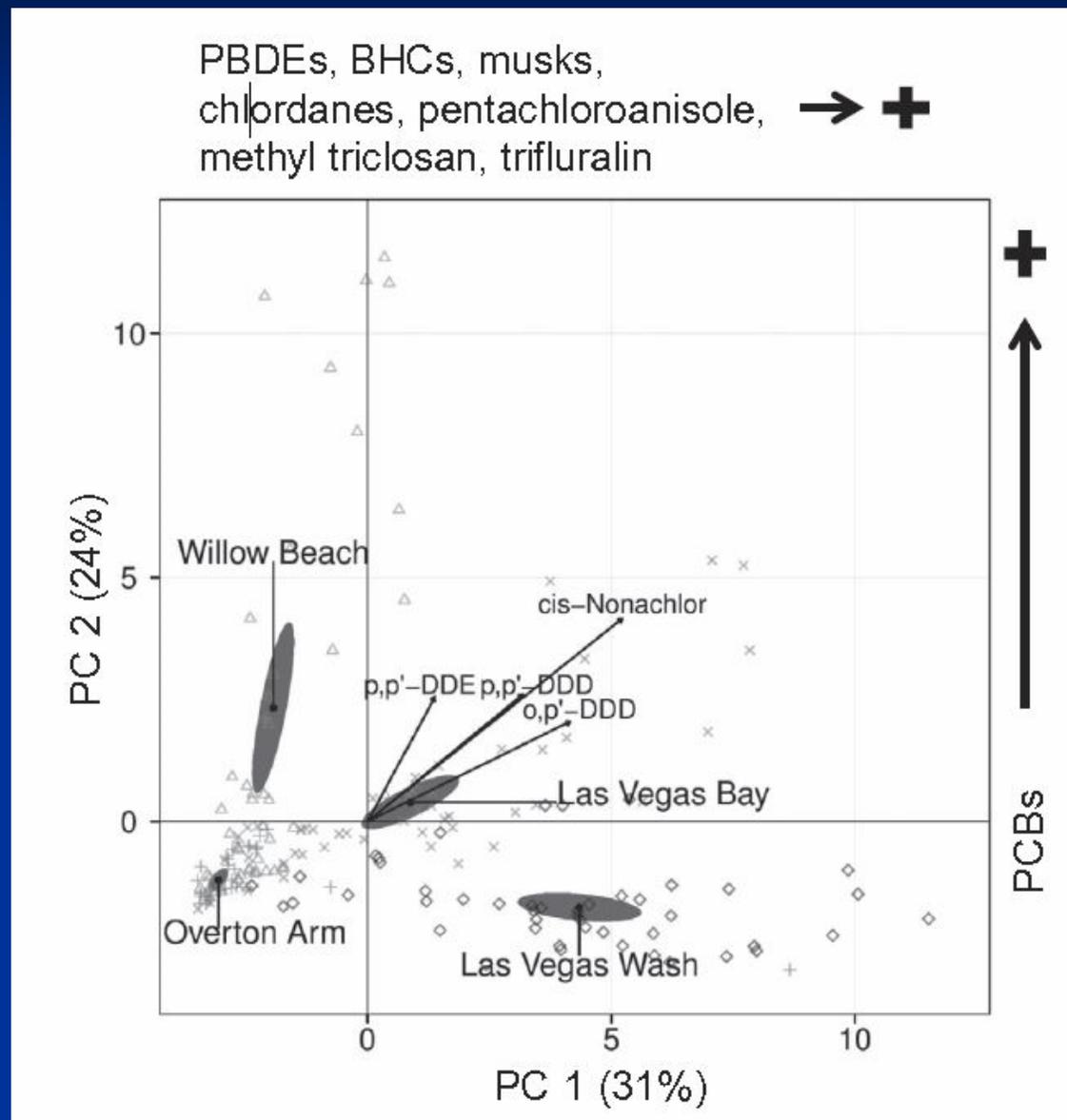


Comments

- Highest levels of plasma 11KT and 11KT/E2 ratios found in OA males
- Lowest levels of plasma 11KT and 11KT/E2 ratios found in LVB males

Principle component analysis of Carp in LMNRA

Showing differences between sites and chemical influences



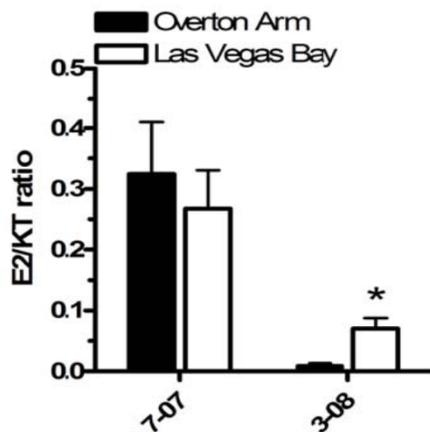
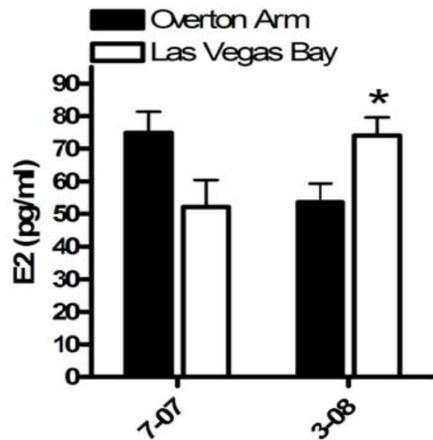
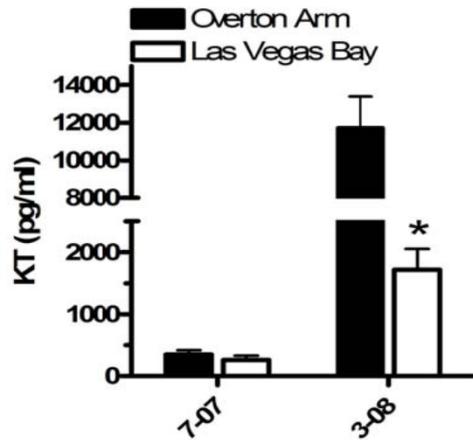
From Patiño et al.
(2015)

Effects in Male Largemouth Bass

Only two sites have bass samples collected
chemical data show similar trends to carp

The differences between sites were most
evident just prior spawning period
(March 2008)

Relatively low KT levels and high
E2/KT ratios in Las Vegas Bay males
are consistent with reduced health
and reproductive condition



From Goodbred et al. (2015)

Motility of Razorback Sucker sperm

	Total (%)	Duration (s)
Las Vegas Bay	72.3 (4.1)	40.2 (1.1)
Reference Site	87.1 (1.4)	42.8 (1.6)



From data in Jenkins et al. (2011)



How widespread is this issue ?

GLOBAL ASSESSMENT OF THE STATE-OF-THE-SCIENCE OF ENDOCRINE DISRUPTORS, International Programme on Chemical Safety 2002

Damstra et al., editors.

4.5.3 Conclusion

This brief review shows that the endocrine disruption that is undoubtedly occurring in wild fish populations in North America, Asia, Australia, and Europe is caused through a variety of mechanisms including hormone receptor interactions, interference with the biosynthesis of sex steroids, and perturbations of the hormonal control by the pituitary on reproductive and adrenal processes. However, in most cases the precise modes of action are still poorly understood, and data are largely confined to the gonochoristic species. The compounds responsible for the observed effects may be due to both synthetic and natural compounds. Currently, there is limited understanding of how the existing endocrine disruption affects population fitness.

Conclusions

- As more and more studies have been published since the 1990s, it became difficult to continue to assert that there was no affect on aquatic wildlife, even when chemical concentrations were at “low” levels
- Affects on human health are less certain, but there are likely much more direct and concentrated routes that could affect human health
- Studies in Lake Mead and other places have shown that EDCs can affect aquatic wildlife, and these studies have helped to improve the way wastewater is treated in large cities, including Las Vegas

References listed in presentation

1. Goodbred, S.L., Leiker, T.J., Patiño, R., Jenkins, J.A., Denslow, N.D., Orsak, E., and Rosen, M.R., 2007, Organic chemical concentrations and reproductive biomarkers in common carp (*Cyprinus carpio*) collected from two areas in Lake Mead, Nevada, May 1999–May 2000: U.S. Geological Survey Data Series 286, 18 p.
2. Goodbred, S.L., Patino, R., Torres, L., Echols, K.R., Jenkins, J.A., Rosen, M.R., and Orsak, E., 2015, Are endocrine and reproductive biomarkers altered in contaminant-exposed wild male Largemouth Bass (*Micropterus salmoides*) of Lake Mead, Nevada/Arizona, USA? *General and Comparative Endocrinology*, 219: 125–135. <http://dx.doi.org/10.1016/j.ygcen.2015.02.015>
3. Jenkins, J.A., Eilts, B.E., Guitreau, A.M., Figiel, C.R., Draugelis-Dale, R.O., and Tiersch, T.R., 2011, Sperm quality assessments for endangered razorback suckers *Xyrauchen texanus*. *Reproduction*, 141, 55–65.
4. Rosen M.R. and Van Metre, P.C., 2010, Assessment of multiple sources of anthropogenic and natural chemical inputs to a morphologically complex basin, Lake Mead, USA. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 294, 30-43.
5. Patiño, R., VanLandeghem, M.M., Orsak, E., Jenkins, J.A., Goodbred, S.L., Echols, KR, Rosen, M.R., and Torres, L., 2015, Novel associations between contaminant body burdens and reproductive condition of male Common Carp along multiple gradients of contaminant exposure in Lake Mead National Recreation Area, USA. *General and Comparative Endocrinology*, 219: 112–124. <http://dx.doi.org/10.1016/j.ygcen.2014.12.013>
6. Rosen, M.R., Alvarez, D.A., Goodbred, S.L., Leiker, T.J., and Patiño, R., 2010, Sources and distribution of organic compounds using passive samplers in Lake Mead National Recreation Area, Nevada and Arizona, and their implications for potential effects on aquatic biota *Journal of Environmental Quality*, 39, 1161-1172.
7. Rosen, M.R., Turner, K., Goodbred, S.L., and Miller, J.M., eds., 2012, *A synthesis of aquatic science for management of Lakes Mead and Mohave: U.S. Geological Survey Circular 1381*, 168 p. <http://pubs.usgs.gov/circ/1381/>
8. Wessells, S. and Rosen, M.R. 2013, *Lake Mead Clear and Vital*, U.S. Geological Survey General Information Product 148, 13 minute DVD, available at: http://www.usgs.gov/blogs/features/usgs_top_story/lake-mead-video-documents-healthy-ecosystem/

Other USGS Lake Mead References

Alvarez, D.A., Rosen, M.R., Perkins, S.D., Cranor, W.L., Schroeder, V.L., and Jones-Lepp, T.L., 2012, Bottom sediment as a source of organic contaminants in Lake Mead, Nevada, USA. *Chemosphere*, 88, 605–611.

Bevans, H.E., Goodbred, S.L., Meisner, J.F., Watkins, S.A., Gross, T.S., Denslow, N.D., and Scheob, T., 1996, Synthetic organic compounds and carp endocrinology and histology in Las Vegas Wash and Las Vegas and Callville Bays of Lake Mead, Nevada, 1991 and 1995, U.S. Geological Survey Water Research Investigation Report 96-4266, 12p.

Boyd, R.A., and Furlong, E.T., 2002, Human-Health Pharmaceutical Compounds in Lake Mead, Nevada and Arizona, and Las Vegas Wash, Nevada, October 2000–August 2001: U.S. Geological Survey Open-File Report 02-385.

Caldwell, T.J., Rosen, M.R., Chandra, S., Acharya, K., Caires, A.M., Davis, C.J., Thaw, M., Webster, D. 2015, Temporal and basin-specific population trends of quagga mussels on soft sediment of a multi-basin reservoir. /n Wong WH, Gerstenberger SL (eds) *Biology and Management of Invasive Quagga and Zebra Mussels in the Western United States*, CRC Press

Covay, K.J., and Beck, D.A., 2001, Sediment deposition rates and organic compounds in bottom sediment at four sites in Lake Mead, Nevada, U.S. Geological Survey, Open File Report 01-282, 34p.

Covay, K.J., and Leiker, T.J., 1998, Synthetic organic compounds in water and bottom sediment from streams, detention basins, and sewage-treatment plant outfalls in Las Vegas Valley, Nevada, 1997: U.S. Geological Survey Open-File Report 98–633, 15 p.

Jenkins, J. 2011. Male Germplasm in Relation to Environmental Conditions: Synoptic Focus on DNA, Chapter 16. In: *Cryopreservation in Aquatic Species*, 2nd Edition. T. R. Tiersch. and C. C. Green, editors. World Aquaculture Society, Baton Rouge, Louisiana. Pp. 227-239

Leiker, T.J., Abney, S.R., Goodbred, S.L., and Rosen, M.R., 2009, Identification of methyl triclosan and halogenated analogues in both male common carp (*Cyprinus carpio*) from Las Vegas Bay and Semipermeable Membrane Devices from Las Vegas Wash, Nevada. *Science of the Total Environment*, 407, 2102-2114.

Linder, G., Little, E.E. 2009 Competing Risks and the Development of Adaptive Management Plans for Water Resources: Field Reconnaissance Investigation of Risks to Fishes and Other Aquatic Biota Exposed to Endocrine Disrupting Chemicals (EDCs) in Lake Mead, Nevada USA. *ASCE Conf. Proc.* 342, 567, DOI:10.1061/41036(342)567.

Patiño R., Goodbred, S.L., Draugelis-Dale, R., Barry, C.E., Foott, J.S., Waincott, M.R., Gross, T.S., and Covay, K.J., 2003, Morphometric and histopathological parameters of gonadal development in adult common carp from contaminated and reference sites in Lake Mead, Nevada. *Journal Aquatic Animal Health* 15:55-68.

Patiño, R., Rosen, M.R., Orsak, E., Goodbred, S.L., May, T.W., Alvarez D., Echols, K.R., Wieser, C.M., Ruessler, S., and Torres, L., 2012, Patterns of metal composition and morpho-physiological condition and their association in male common carp across an environmental contaminant gradient in Lake Mead National Recreation Area, Nevada and Arizona, USA. *Science of the Total Environment*. 416, 215–224.

Rosen, M.R., Goodbred, S.L., Patiño, R., Leiker, T.J., and Orsak E., 2006, Investigations of the effects of synthetic chemicals on the endocrine system of common carp in Lake Mead, Nevada and Arizona, U.S. Geological Survey Fact Sheet 2006-3131, 4 p.
Permanent URL: <http://pubs.usgs.gov/fs/2006/3131/>

Umek, J., Chandra, S., Rosen, M.R., Wittmann, M., and Orsak E., 2010, Importance of benthic production to fish populations in Lake Mead prior to the establishment of quagga mussels. *Lake and Reservoir Management*, 26, 293–305.

Wittmann, M.E., Chandra, S., Caires, A., Denton, M., Rosen, M.R., Wong, W.H., Teitjen, T., Turner, K., Roefer, P. and Holdren, C., 2010, Early invasion population structure of quagga mussel and associated benthic invertebrate community composition on soft sediment in a large reservoir, *Lake and Reservoir Management*, 26, 316–327.



Questions?