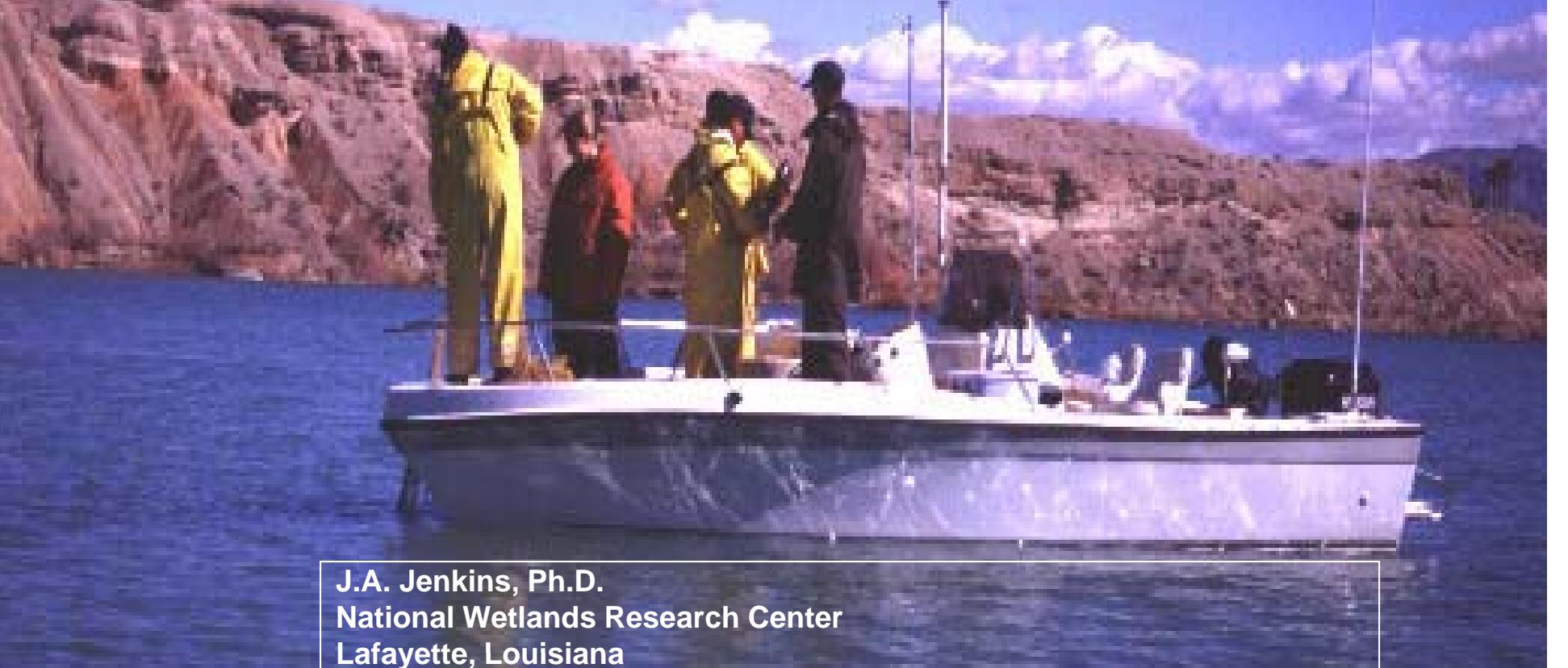


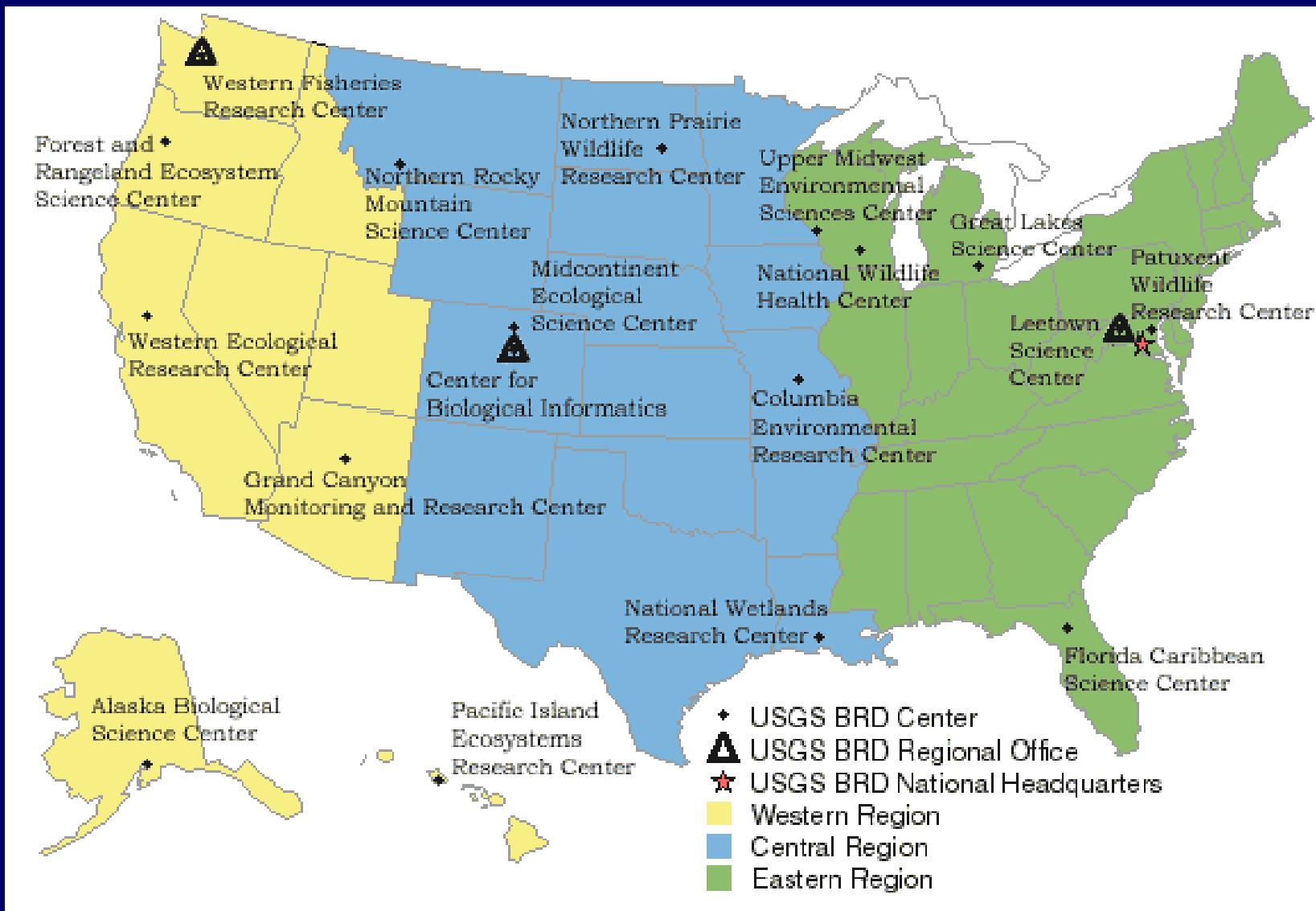


Biomarkers for Endocrine Disruption in Feral Fish from the U.S.



A photograph showing a group of researchers in yellow raincoats standing on a white boat. They are positioned in front of a large, rocky, coastal area under a clear sky. The boat has some equipment and chairs visible on its deck.

J.A. Jenkins, Ph.D.
National Wetlands Research Center
Lafayette, Louisiana





USGS. A multi-disciplinary science organization that focuses on **biology**, **geography**, **geology**, **geospatial information**, and **water**. Dedicated to the timely, relevant, and impartial study of the landscape, our natural resources, and the natural hazards that threaten us.

BRD Mission. Provide the scientific understanding & technologies needed to support the sound management & conservation of our Nation's biological resources.



National Wetlands Research Center
Lafayette, LA

National Water-Quality Assessment Program (NAWQA)

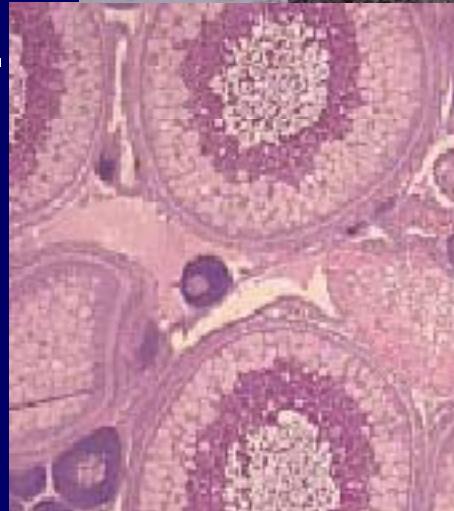


The long-term goals:

- to describe the status and trends in the quality of a large, representative part of the Nation's surface- and ground-water resources**
- to provide a sound, scientific understanding of the primary factors affecting the quality of these resources.**

Biomonitoring of Environmental Status and Trends (BEST) Program

BEST is the only long-term monitoring program documenting biological effects resulting from contaminants at broad temporal and geographical scales.



Methods Used in BEST

- Cytochrome P450-1A induction
- Necropsy-based fish health assessments
- Histopathological assessments
- Immune system indicators
- Condition factor and GSI
- OC and elemental analysis of tissue
- Reproductive and endocrine bioindicators



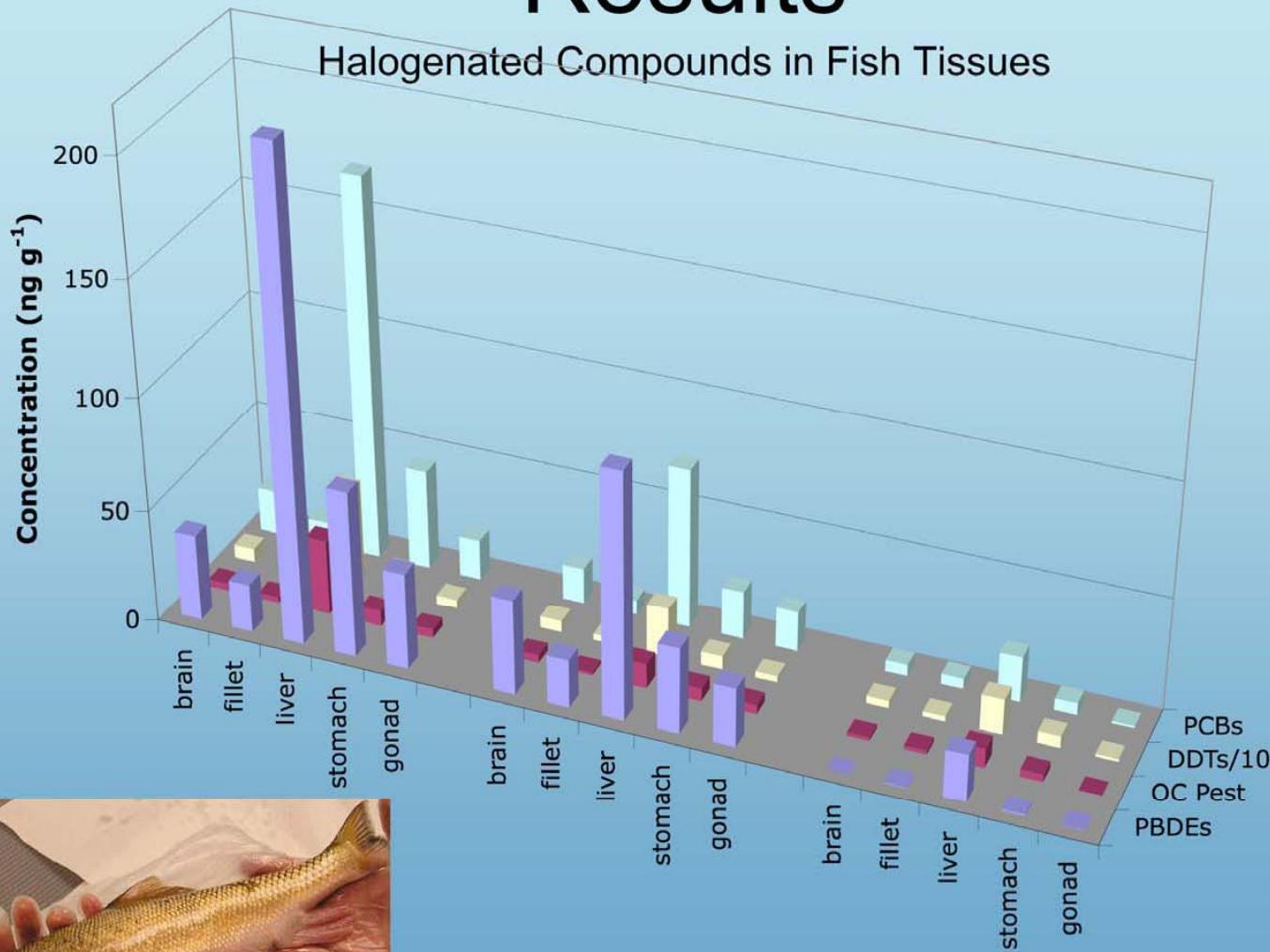
Type Chemical Examples

1. anti-estrogenic: PCBs
2. estrogenic: BDEs, artificial estrogen
3. anti-androgenic: pp-DDE
4. androgenic: some pharmaceuticals
and personal care products

Halogenated Compound Classes	isoborneol OPEO-1
PBDEs DDTs PCBs	menthol 4-cumylphenol
BDE71 Trifluralin Oxyfluorfen p,p-DDE PCB101	methyl salicylate bromacil
BDE47 Benfluralin trans-Nonachlor p,p-DDD	isoquinoline galoxolide (HHCB)
PCB110	dichlorovos metalaxyl
BDE66 Tefluthrin Tetradifon p,p-DDT PCB146	2-methylnaphthalene anthraquinone
BDE100 Desulfnylfipronil Cyhalothrin PCB118	indole metolachlor
BDE99 Chlорpyrifos Cyfluthrin PCB138	3,4-dichlorophenyl NPEO1-total
BDE85 DCPA Pentachloronitrobenzene PCB149	1-methylnaphthalene chlorpyrifos
BDE154 Oxychlordane Hexachlorobenzene (HCB)	skatol triclosan
PCB187	2,6-dimethylnaphthalene bisphenol A
BDE153 Fipronil_Sulfide Pentachloroaniline (PCA)	butylated hydroxyanisole (BHA) OPEO-2
PCB183	5-methyl-1H-benzotriazole NPEO2-total
BDE138 Fipronil Octachlorostyrene PCB174	N,N-diethyl-meta-toluamide (DEET)
BDE183 trans-Chlordane Triclosan PCB180	tri(dichloroisopropyl) phosphate
Endosulfan_I Pentabromotoluene PCB194	4-tert-octylphenol triphenyl phosphate
cis-Chlordane Triclosan_methoxy PCB206	benzophenone tris (2-butoxyethyl phosphate)
cis-Nonachlor Fire_master PCB177	tributylphosphate benzo(a)pyrene
Dieldrin Dechlorane_plus PCB170	ethyl citrate 3-beta-coprostanol
PCB151	cotinine cholesterol
Organochlorine Pesticides	para-nonylphenol beta-sitosterol
Anthropogenic Waste Indicators (AWIs)	Pharmaceuticals
cumene atrazine	Cotinine Diphenhydramine
bromoform pentachlorophenol	Albuterol Diltiazem
phenol 4-octylphenol	Acetaminophen Carbamazapine
1,4-dichlorobenzene tri(2-chloroethyl phosphate)	Ranitidine Fluoxetine
d-limonene anthracene	1,7-dimethylxanthine Dehydronifedipine
acetophenone diazinon	Codeine Warfarin
para-cresol carbazole	Caffeine Miconazole
isophorone caffiene	Trimethoprim Erythromycin
camphor tonalide (AHTN)	Thiabendazole Azithromycin
	Sulfamethoxazole Cimetidine

Results

Halogenated Compounds in Fish Tissues



Biomarkers at Varying Levels of Biological Organization



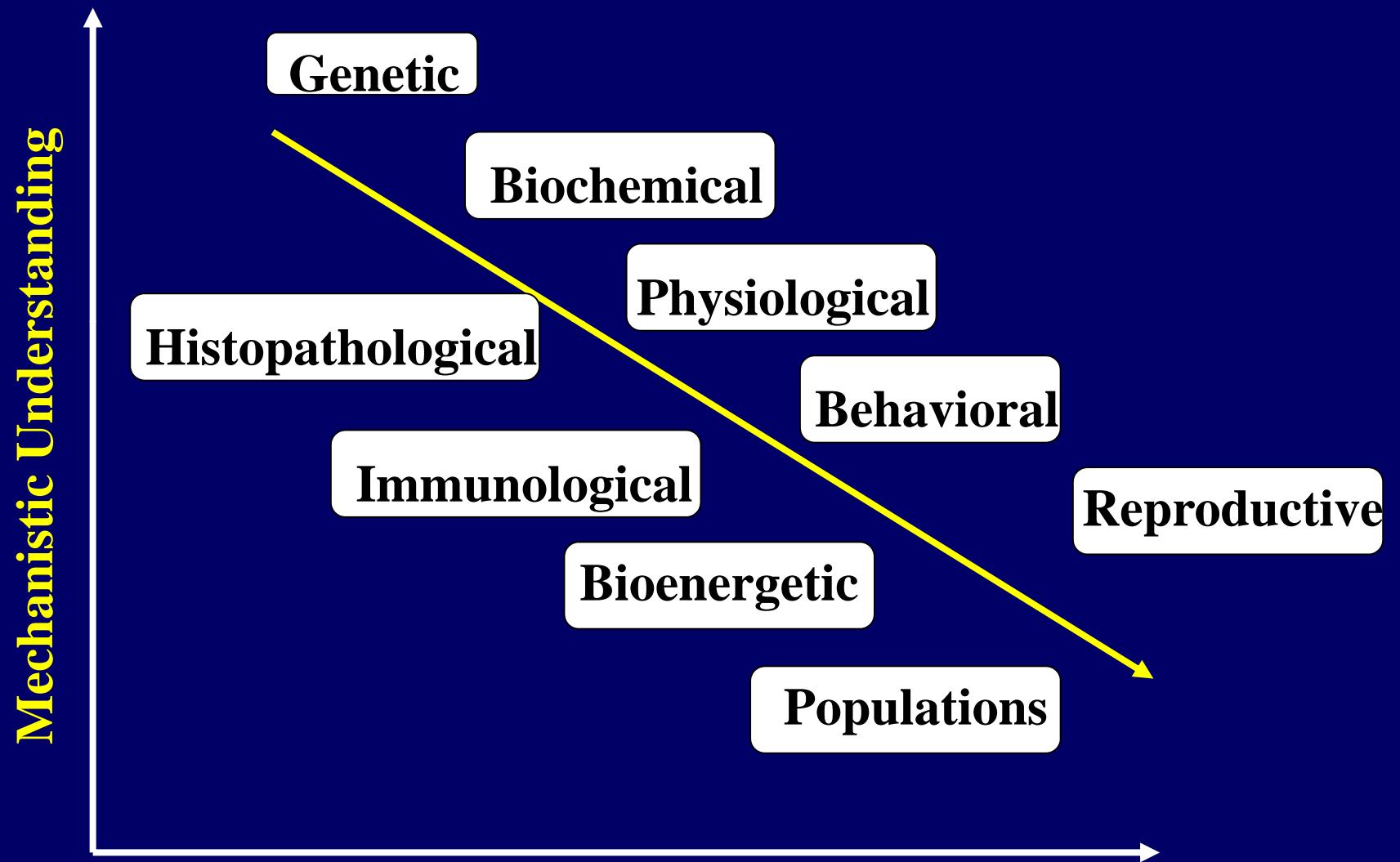
Endocrine function: liver, hypoth., gonad

Genetics: diversity, development

Reproductive condition:
sex steroids, GSI, Vtg

Together, provide more power, confidence that
biologically relevant assessments are made.

Biomarkers at Varying Levels of Biological Organization



Reproductive Biomarkers

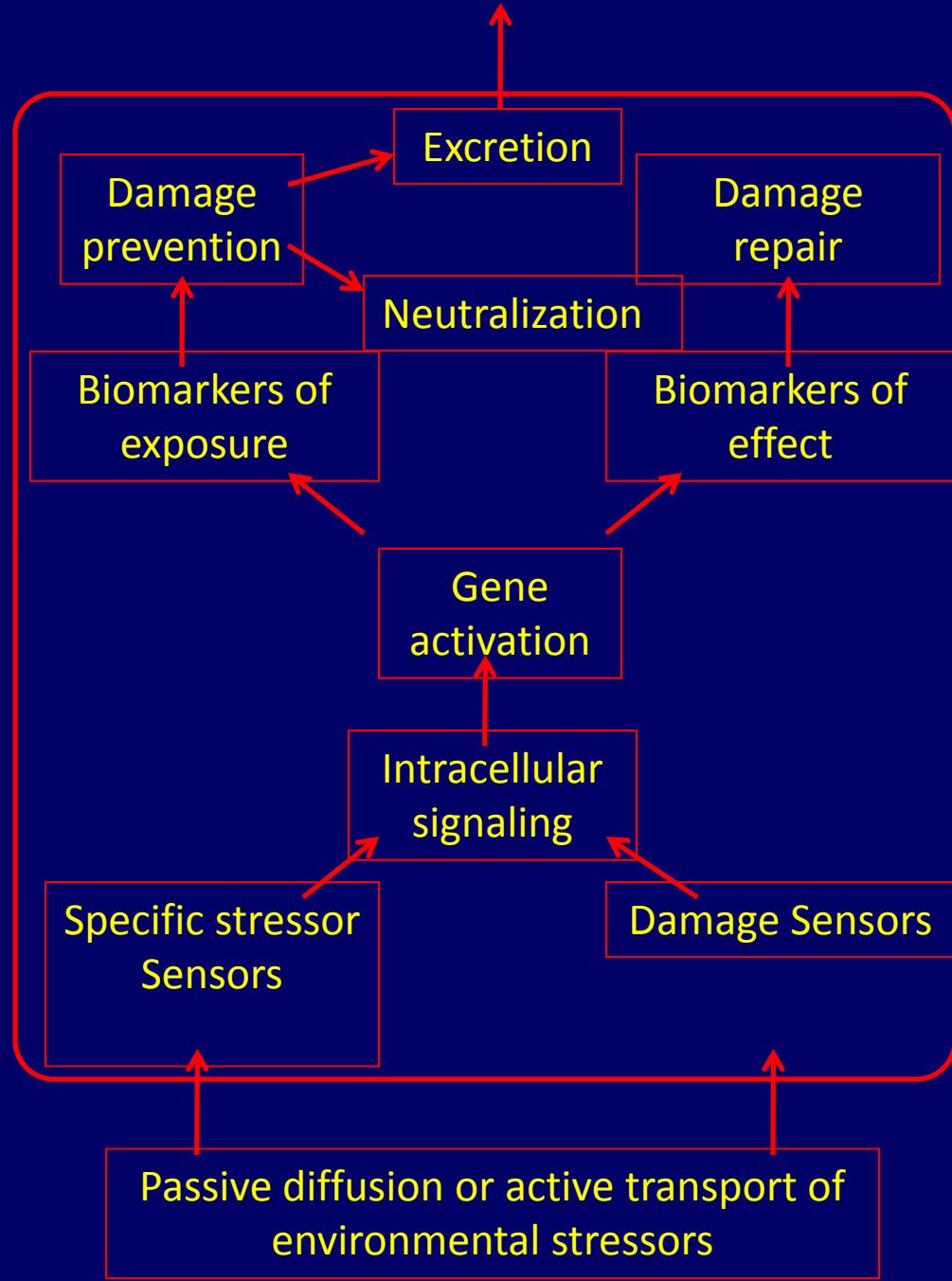
1. GSI
2. VTG
3. Gametes (egg)
4. Sex Steroids

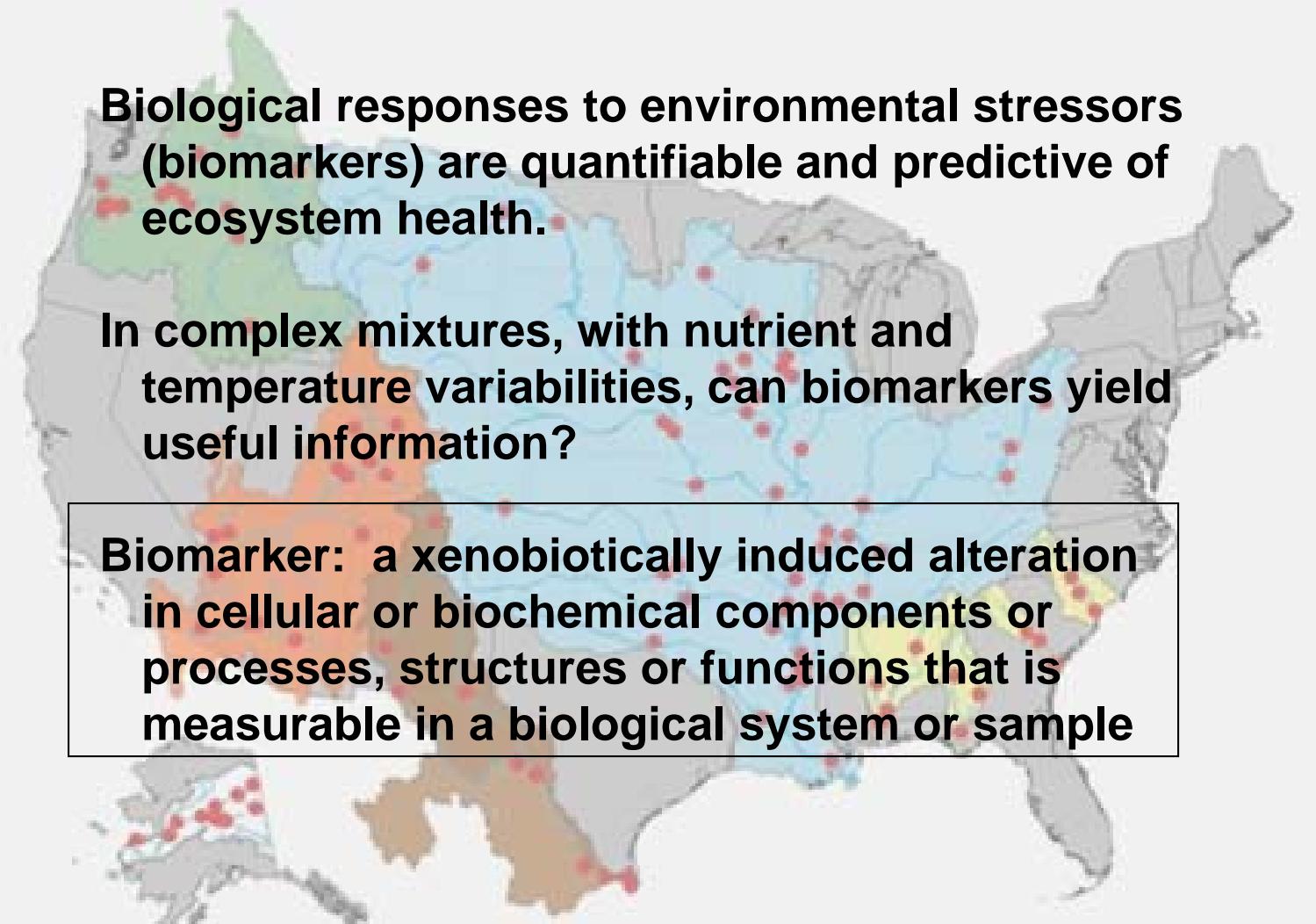
Estradiol: egg growth & development

11-KT: spermatogenesis

Testosterone: precursor for E & KT

Progesterone: egg maturation





Biological responses to environmental stressors (biomarkers) are quantifiable and predictive of ecosystem health.

In complex mixtures, with nutrient and temperature variabilities, can biomarkers yield useful information?

Biomarker: a xenobiotically induced alteration in cellular or biochemical components or processes, structures or functions that is measurable in a biological system or sample

Hypotheses: Are the populations of animals showing differences among sites?

Razorback Suckers (*Xyrauchen texanus*)



Common Carp

(Cyprinus carpio)

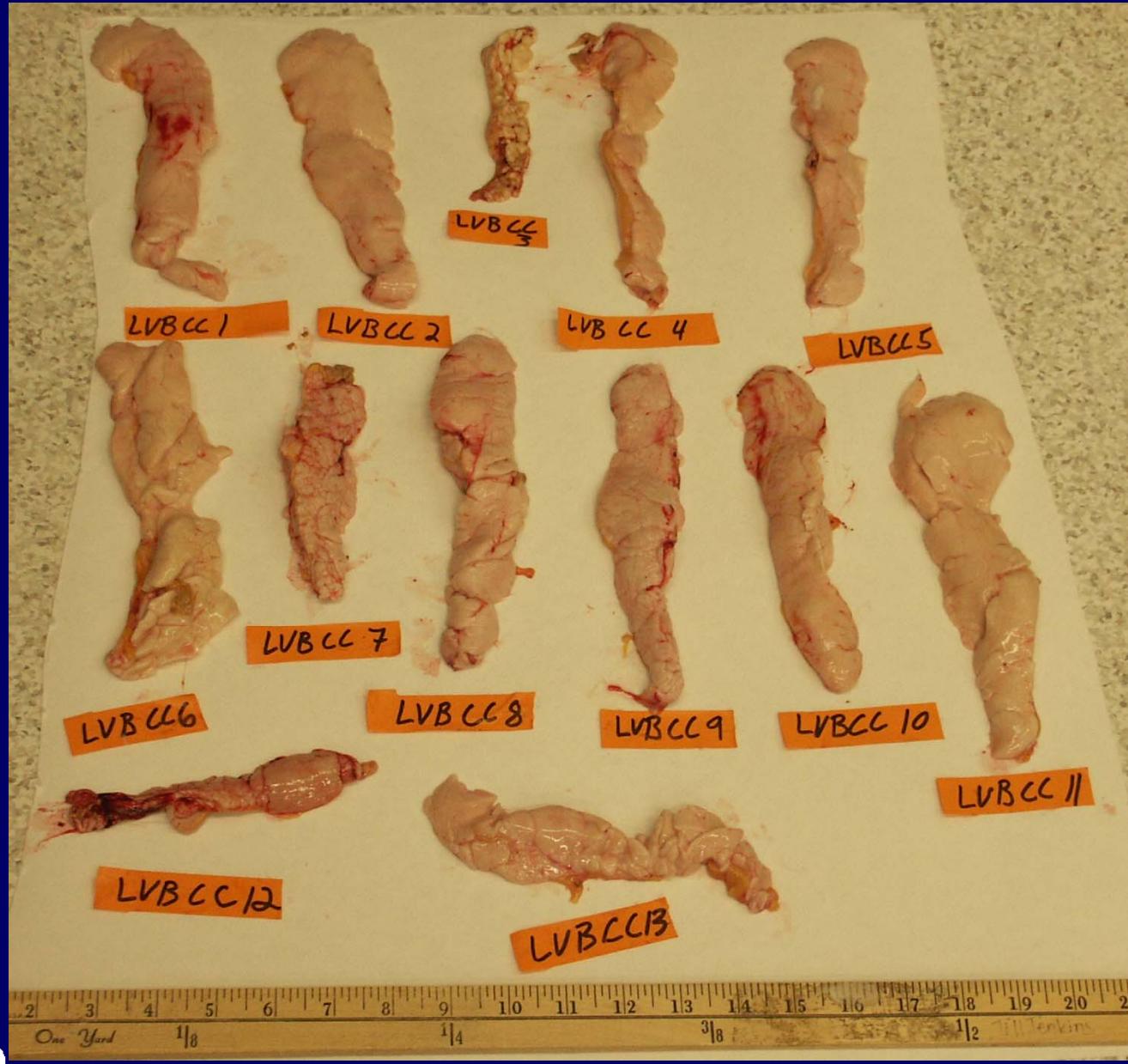


Common Carp (*Cyprinus carpio*)



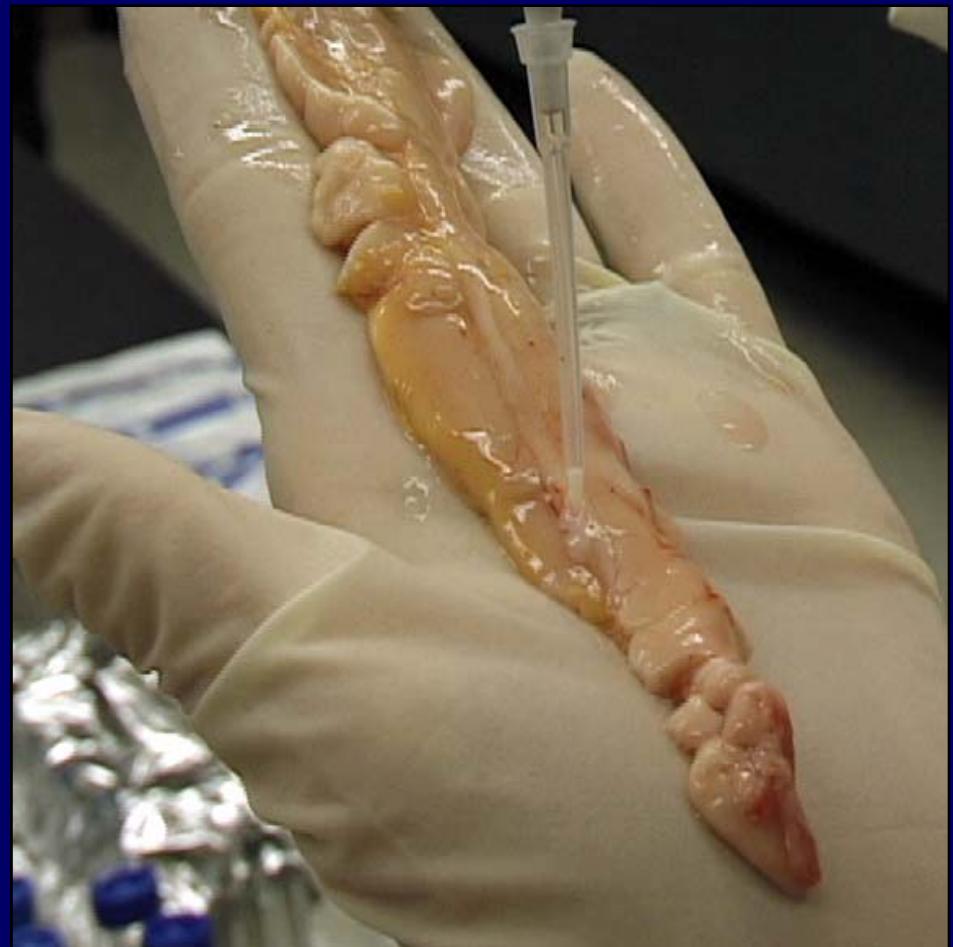


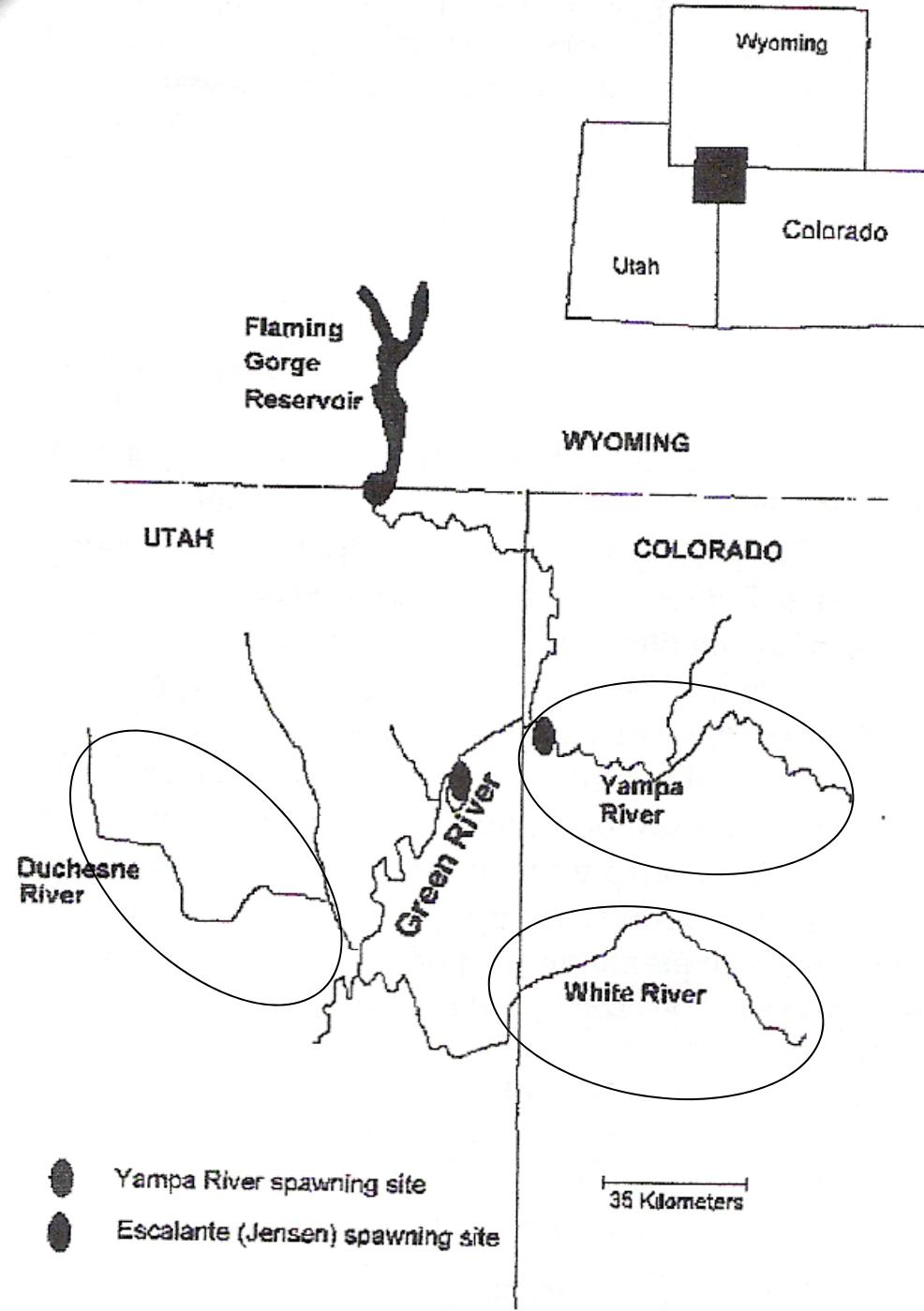
2007

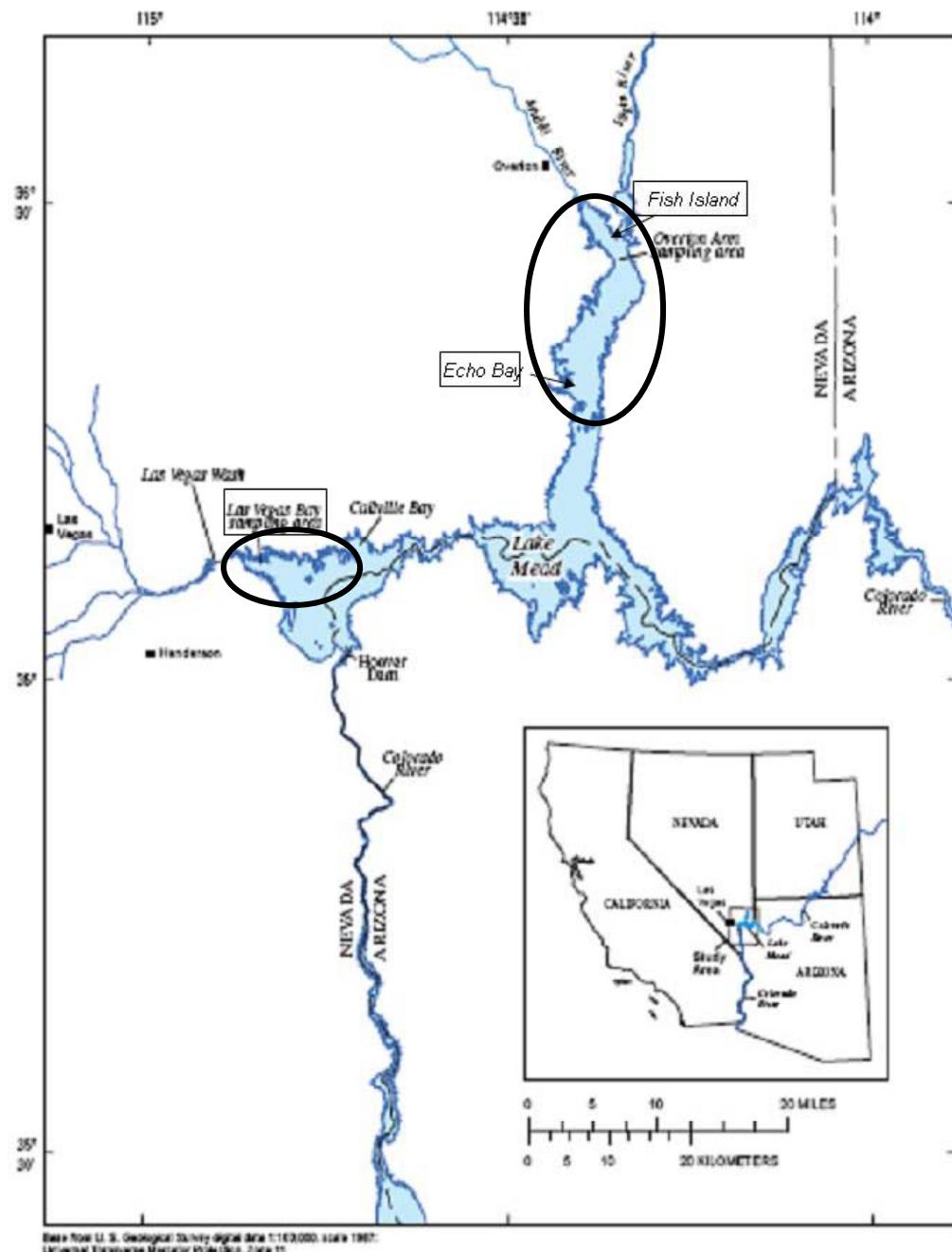




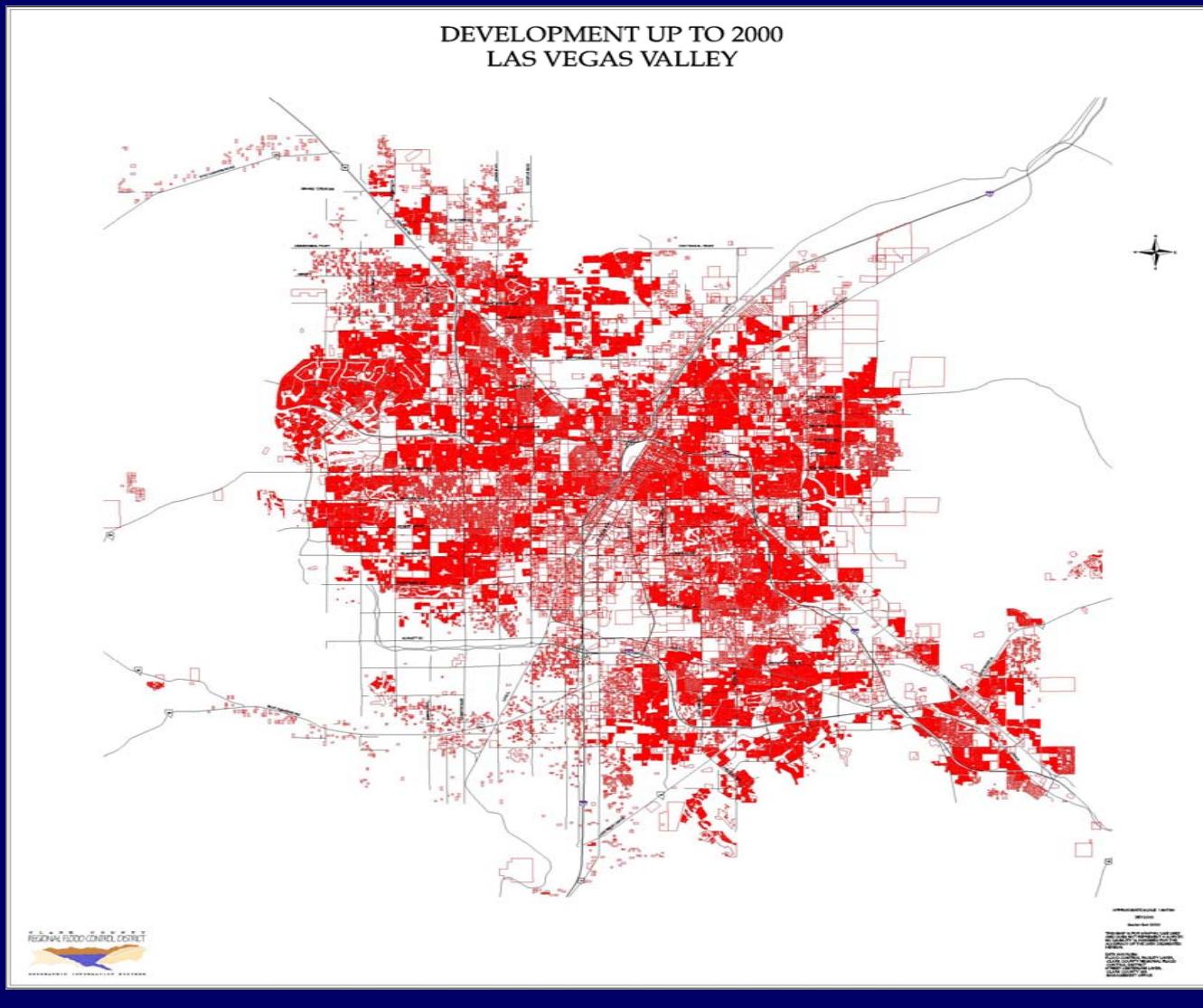
Extracting Milt from Carp Testis







Population Growth for Las Vegas Valley



1950
Pop 47,000

1960
Pop 116,000

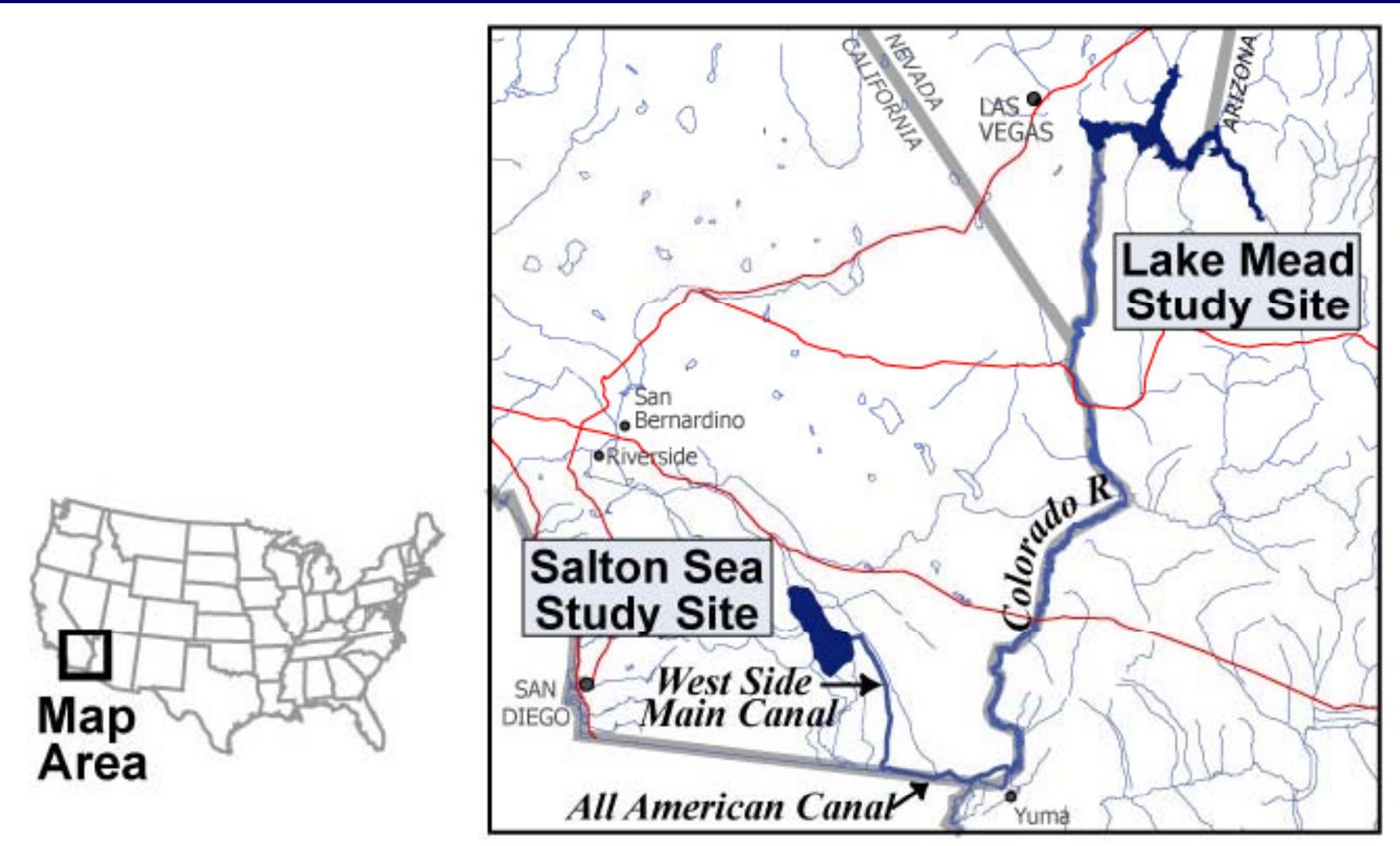
1970
Pop 262,000

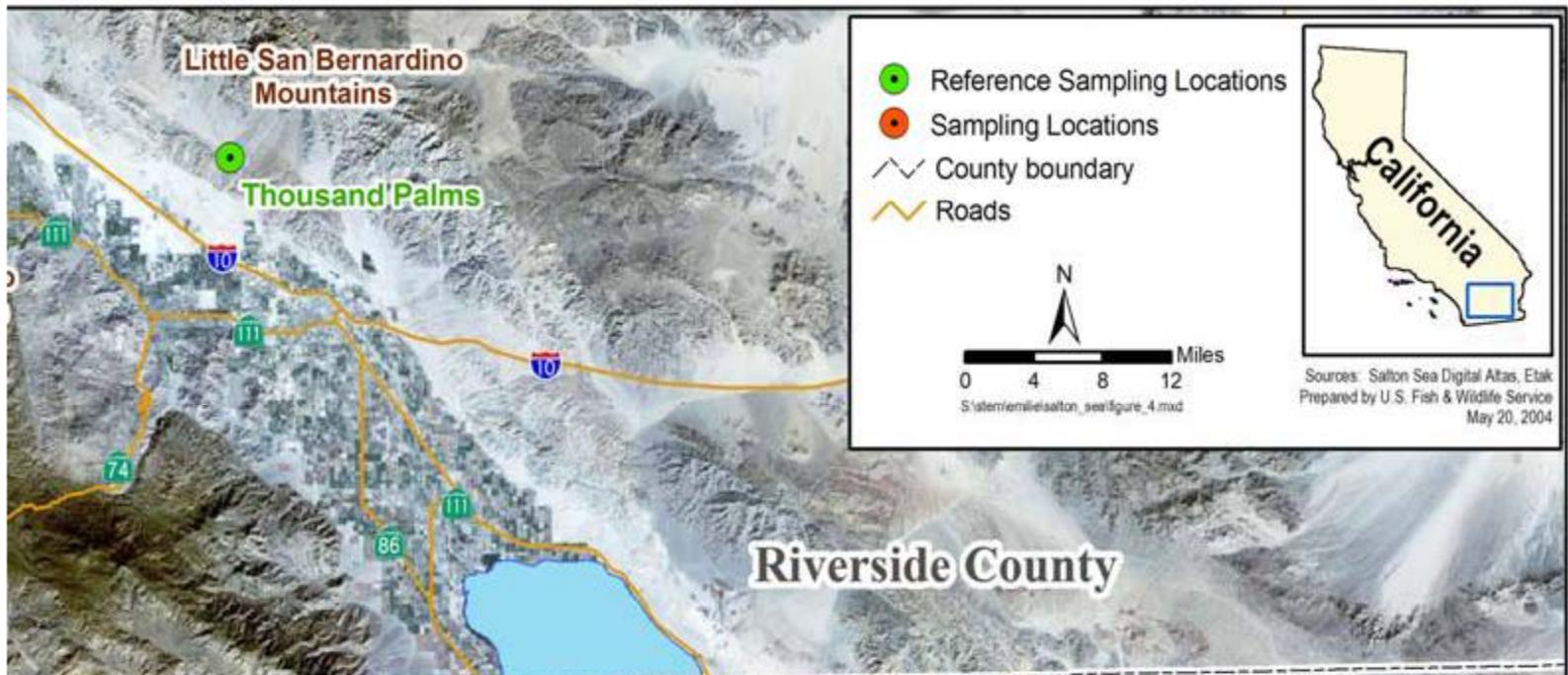
1980
Pop 444,000

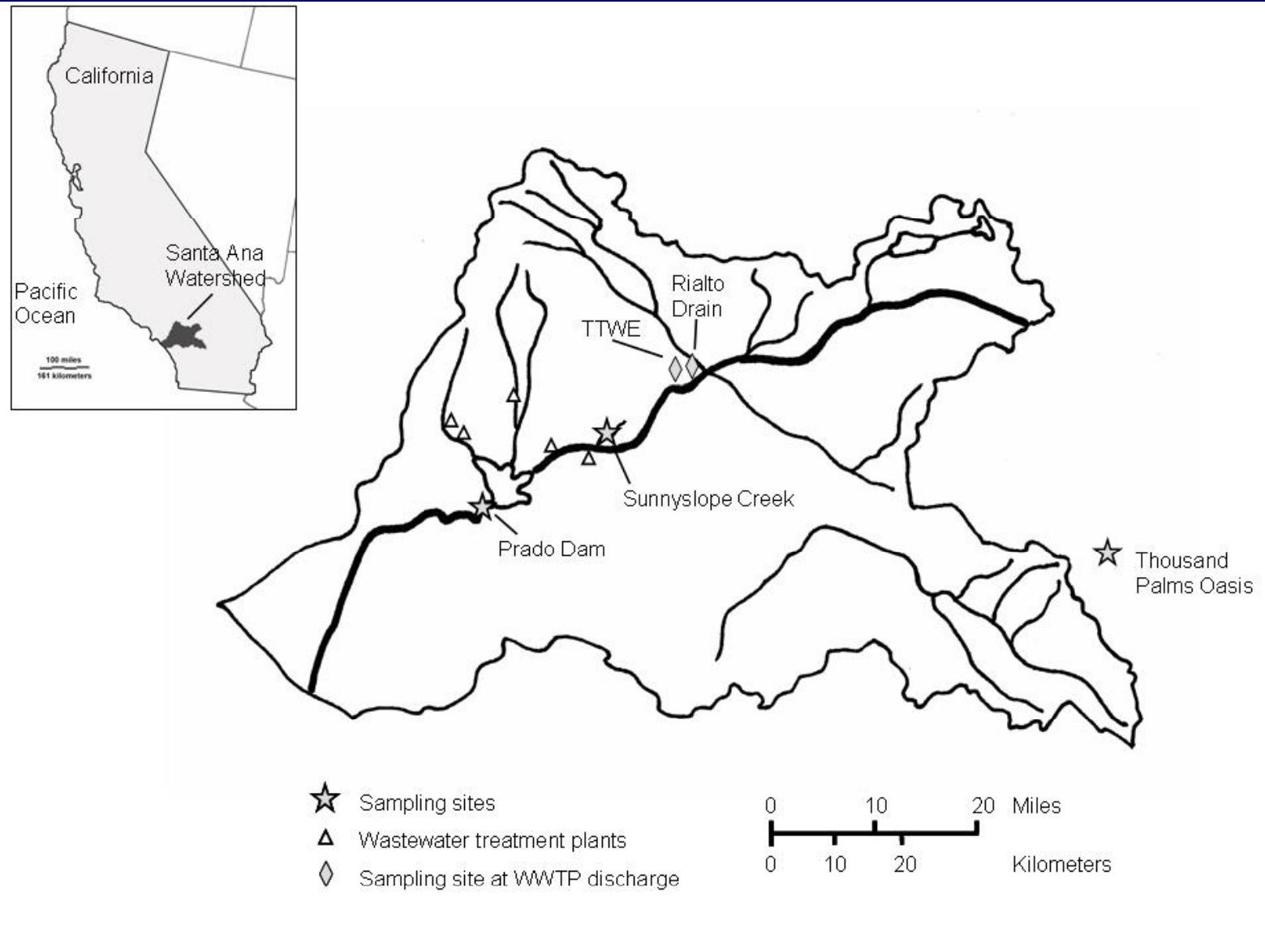
1990
Pop 708,000

2000
Pop 1,300,000

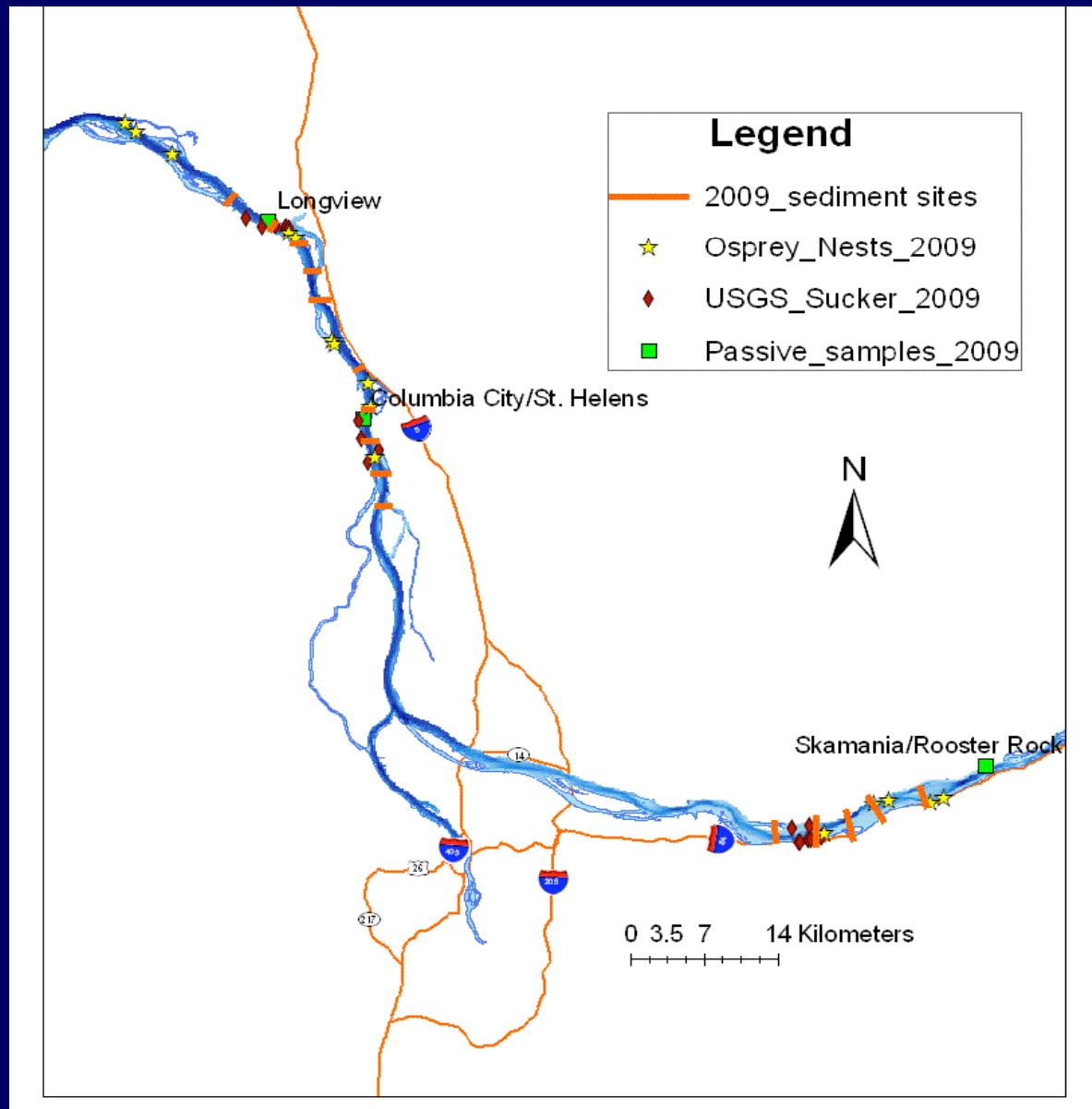
Lower Colorado R. Study Sites







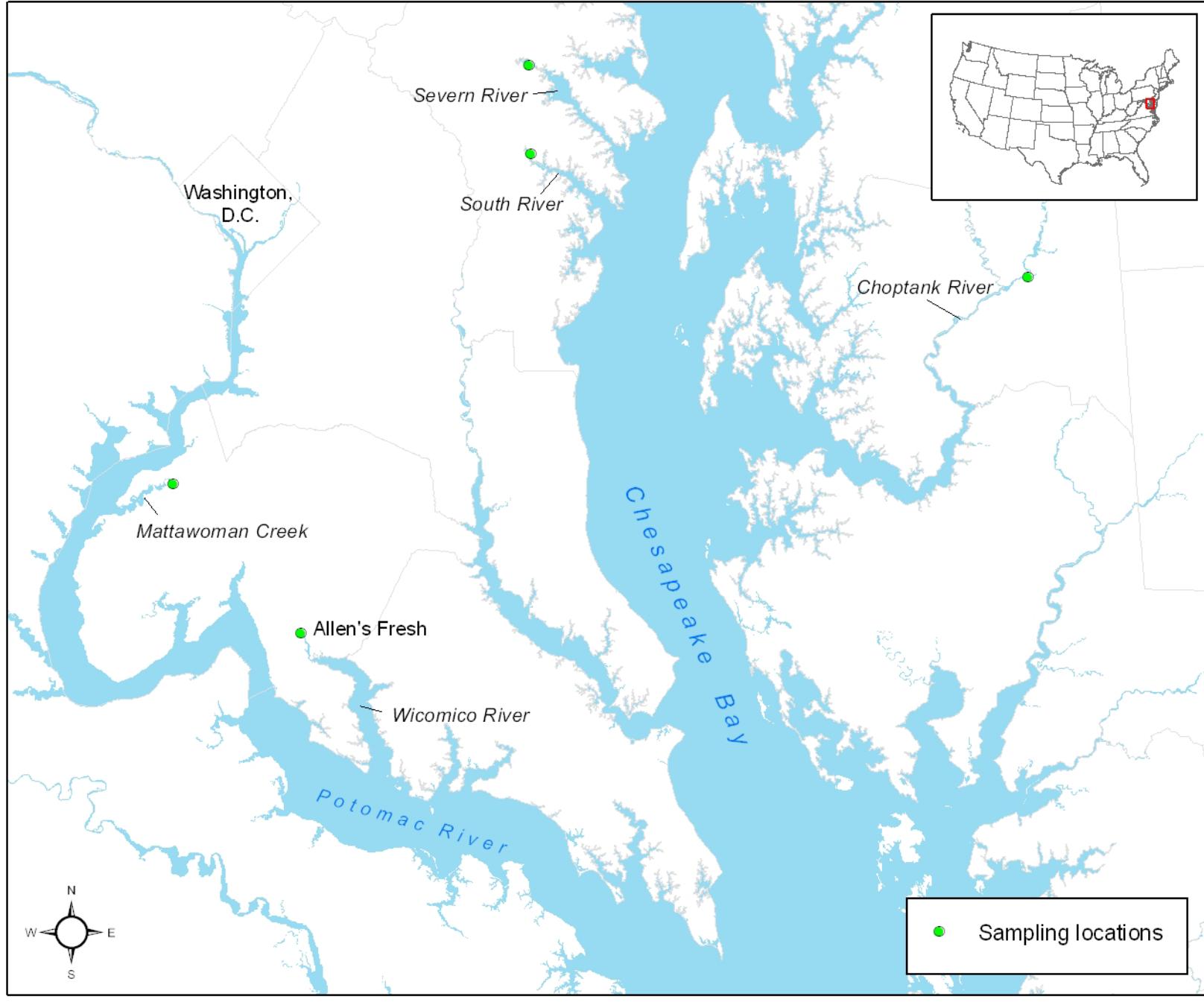


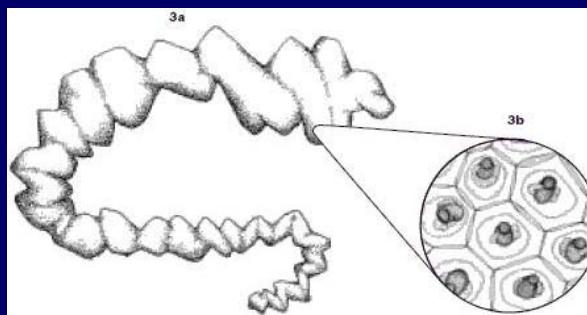


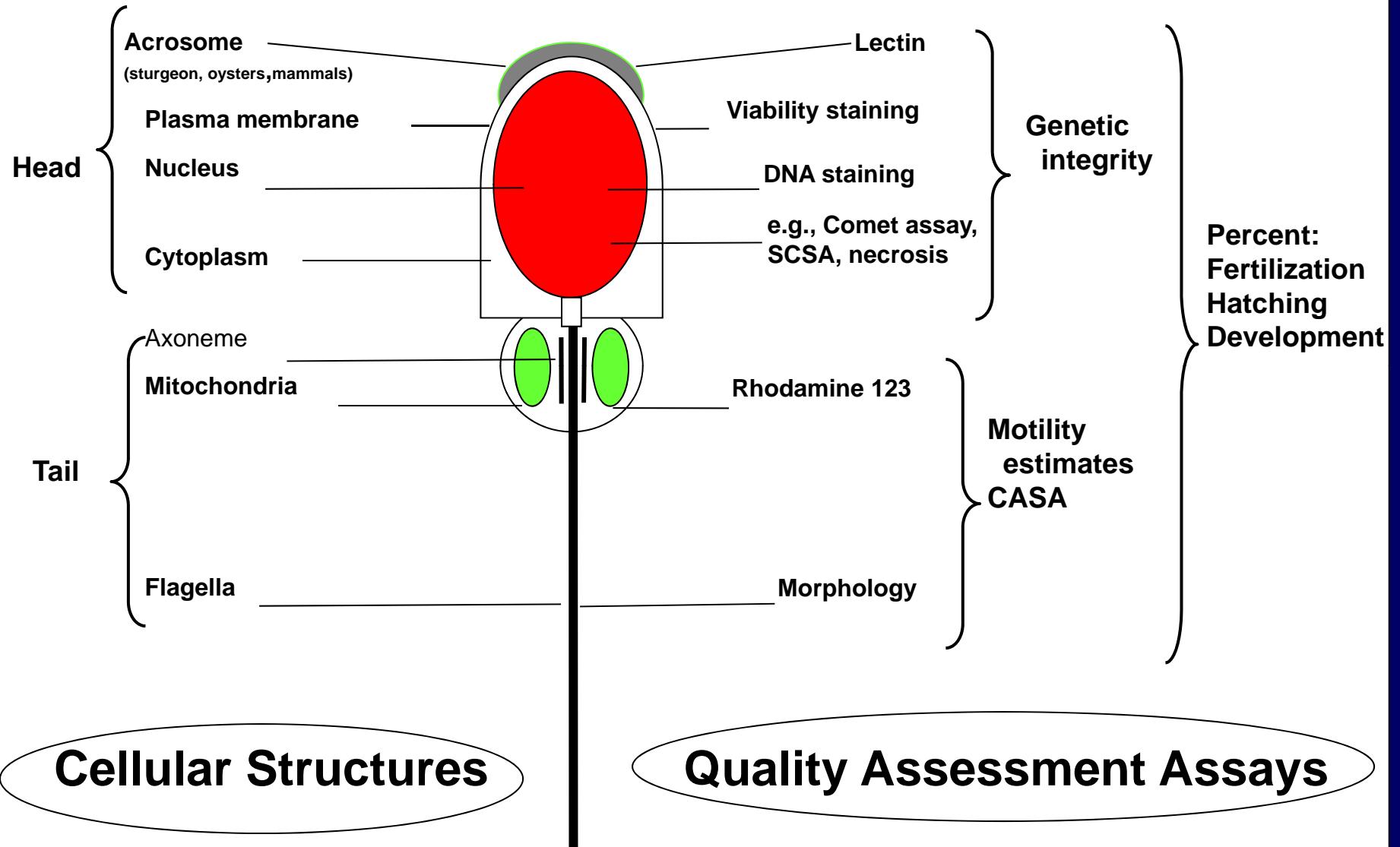
Task D 2009: Biomarker assessment of PBDE and EDC exposure and effects in resident fish: male gametes



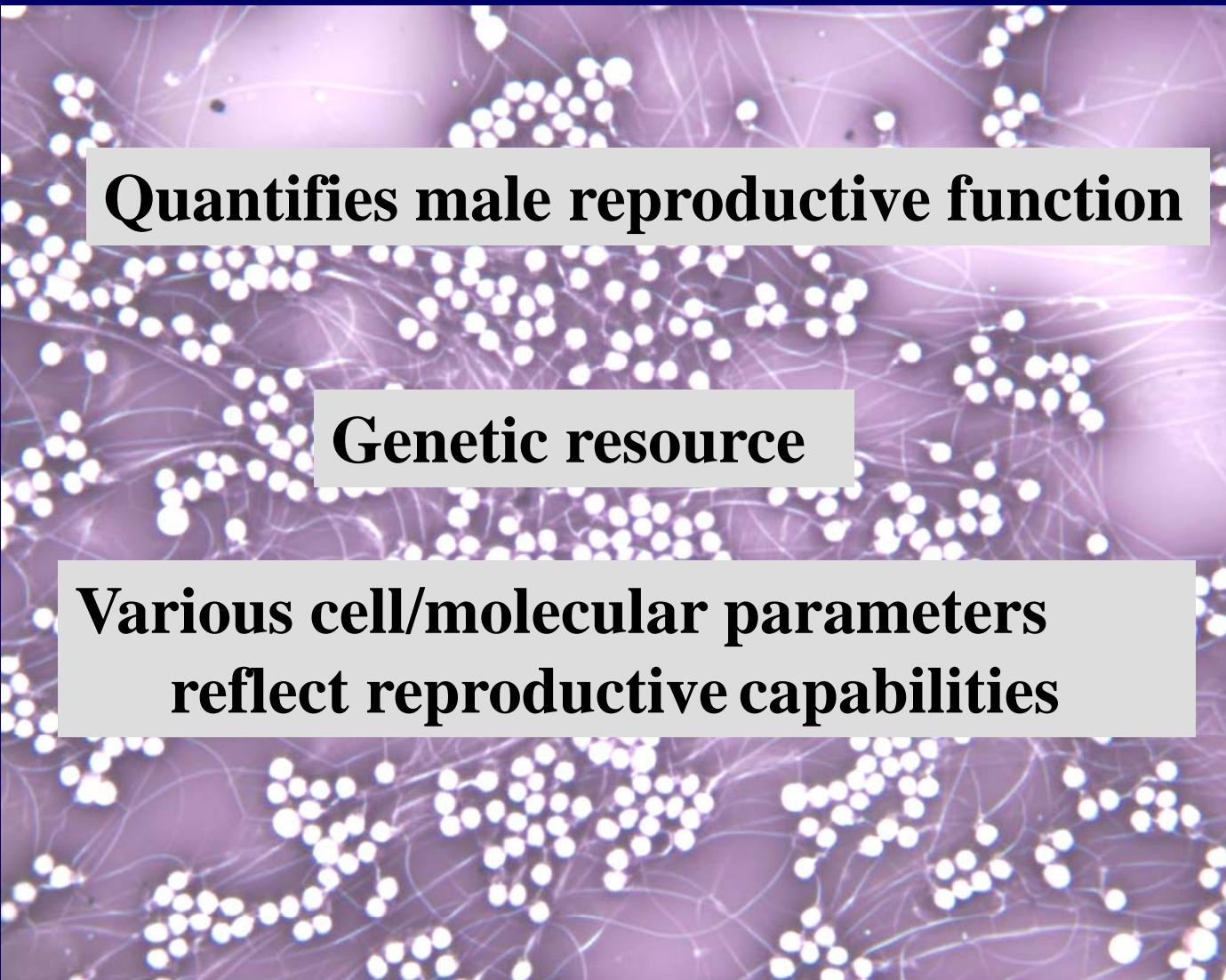
Largescale Suckers
(*Catostomus macrochelius*)







Studies on Spermatozoa



Quantifies male reproductive function

Genetic resource

**Various cell/molecular parameters
reflect reproductive capabilities**

Overview Flow Chart: Sperm Cell Quality

Collect Carp (March 06-08, n=13/site)

Collect Razorback suckers (n=11,'08; n=15,'09)

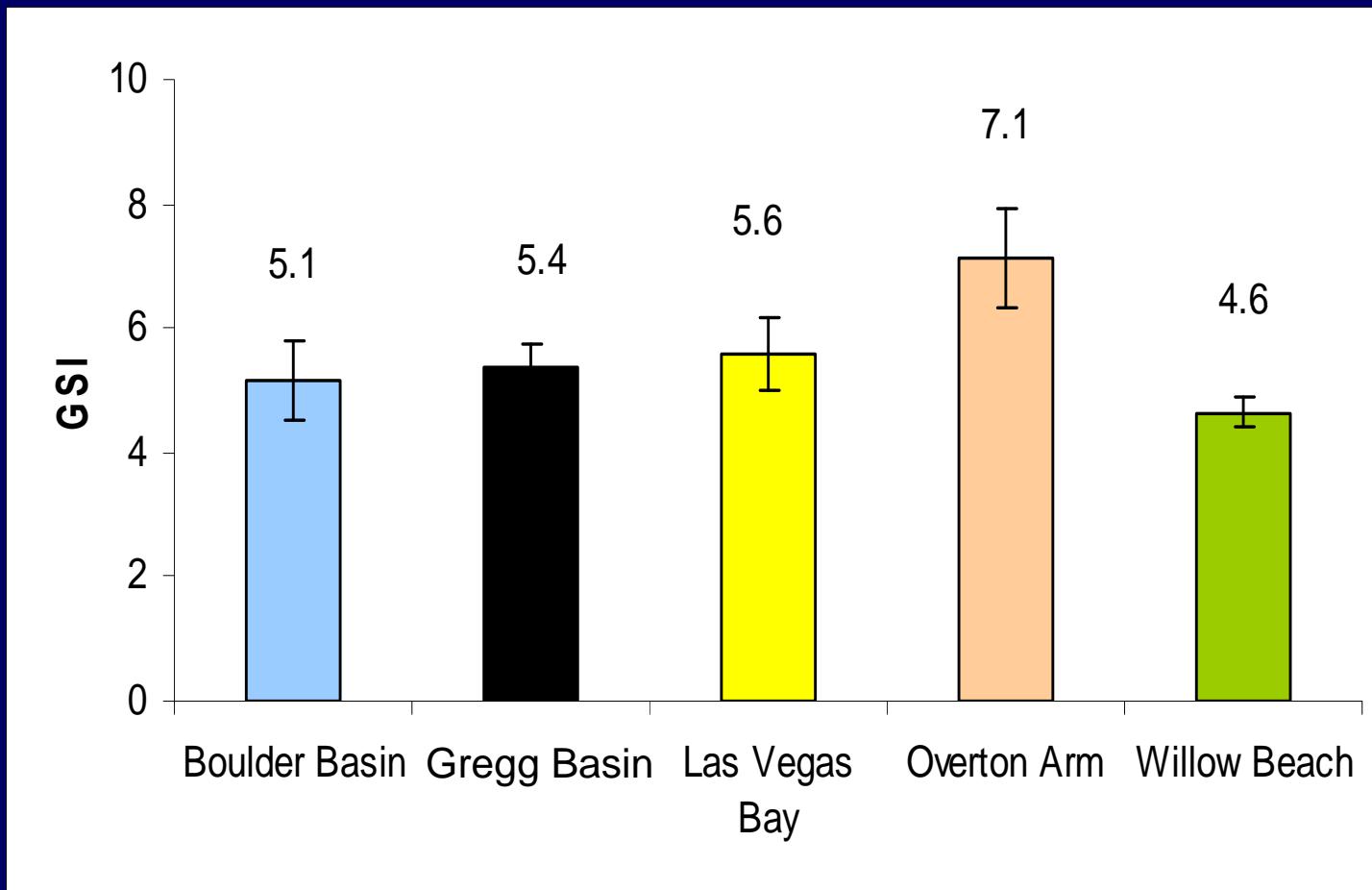


Overnight shipment to lab

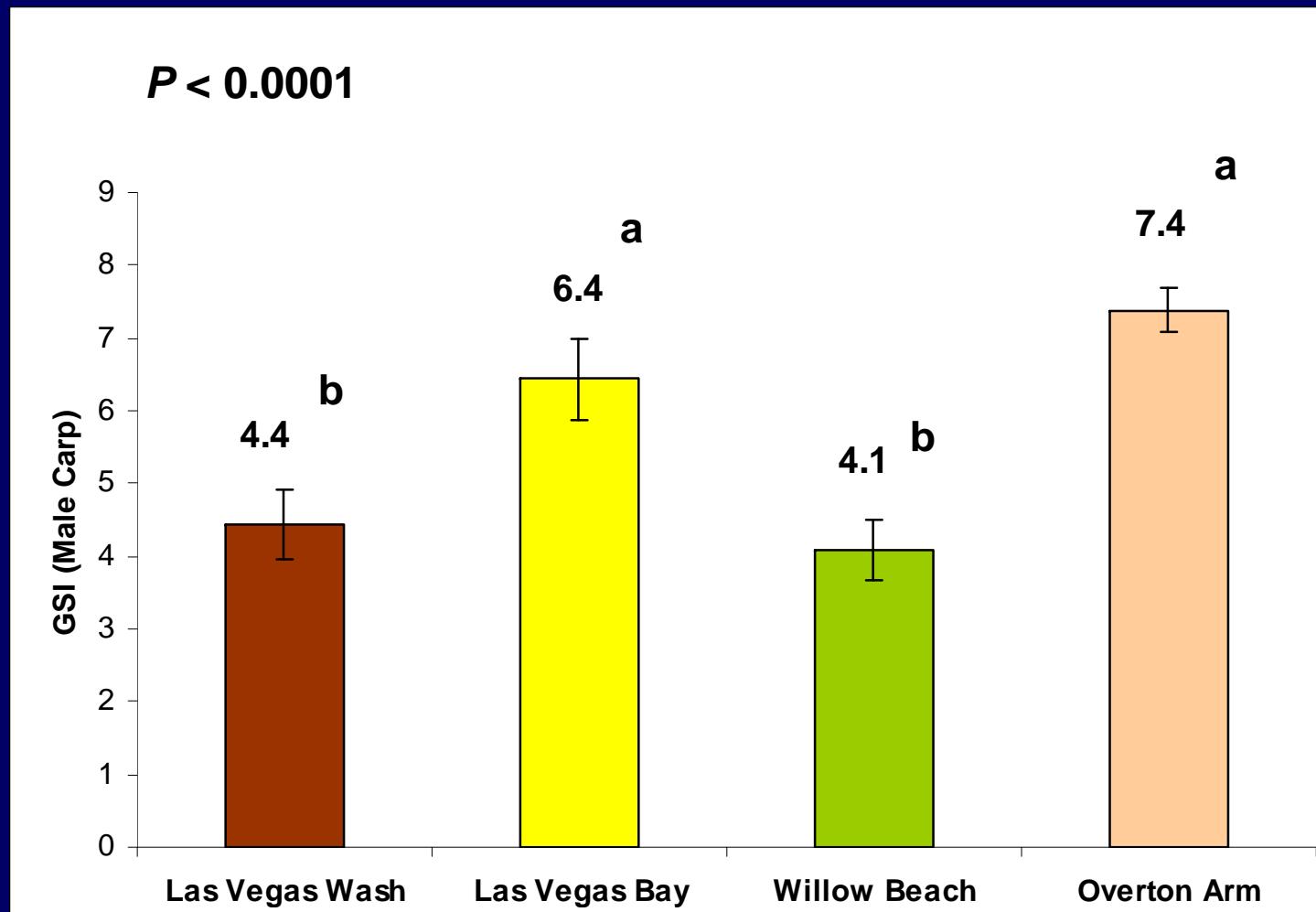


Study milt.....

Male Carp GSI: Lake Mead 2006



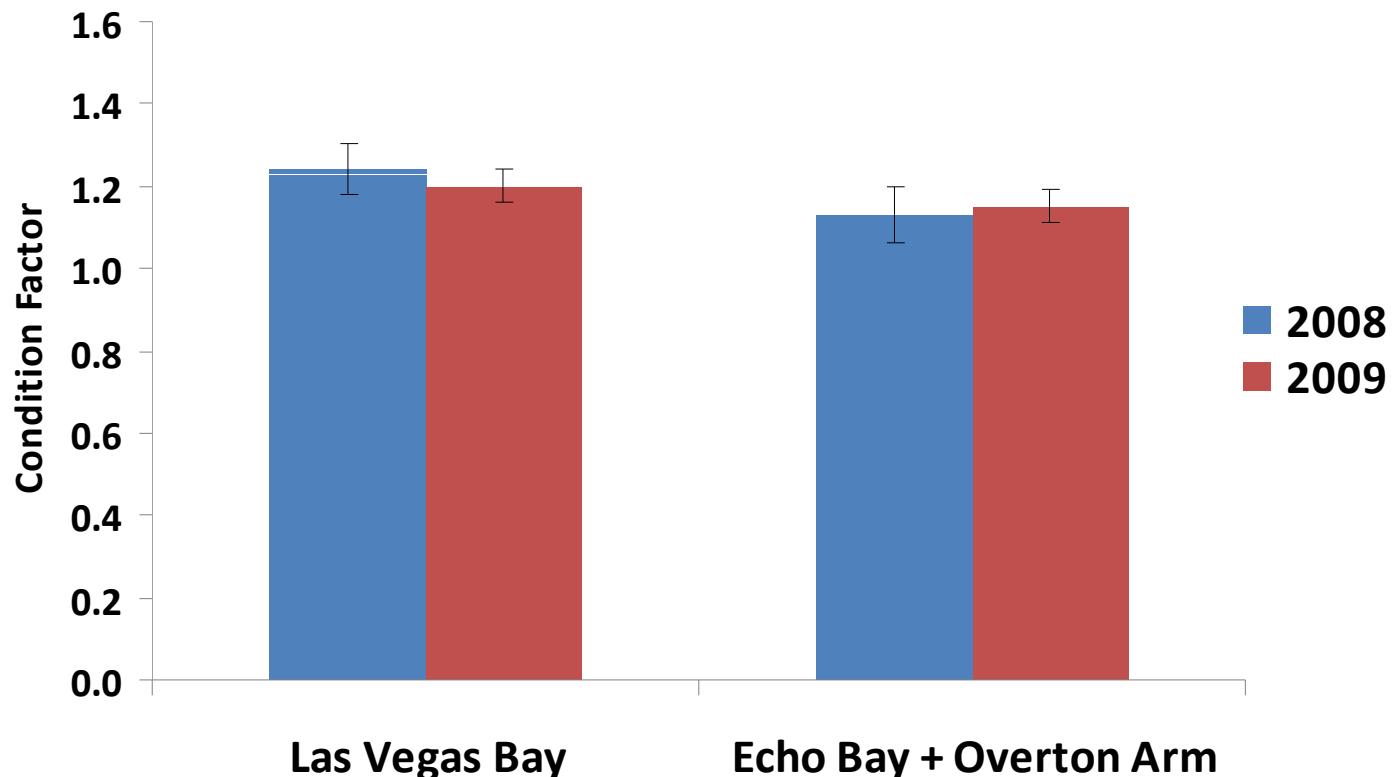
Male GSI: Lake Mead 2007



Razorback Sucker Condition Factor '08-09

NS

Razorback Condition factor



con't. Flow Chart: Sperm Cell Quality

Prepare slide for later morphology assessments by brightfield and image analysis



Perform visual motility assessments by darkfield microscopy; take aliquot for computer assisted sperm analysis (CASA)

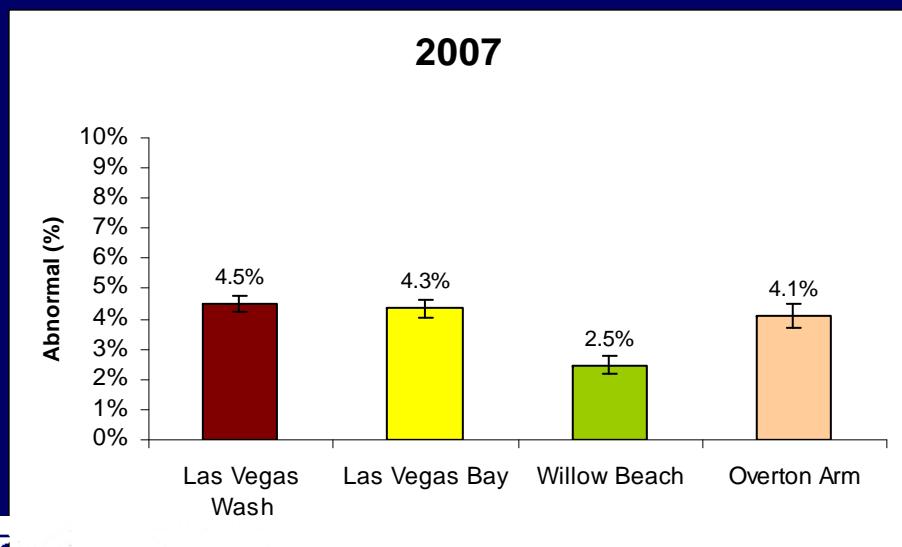
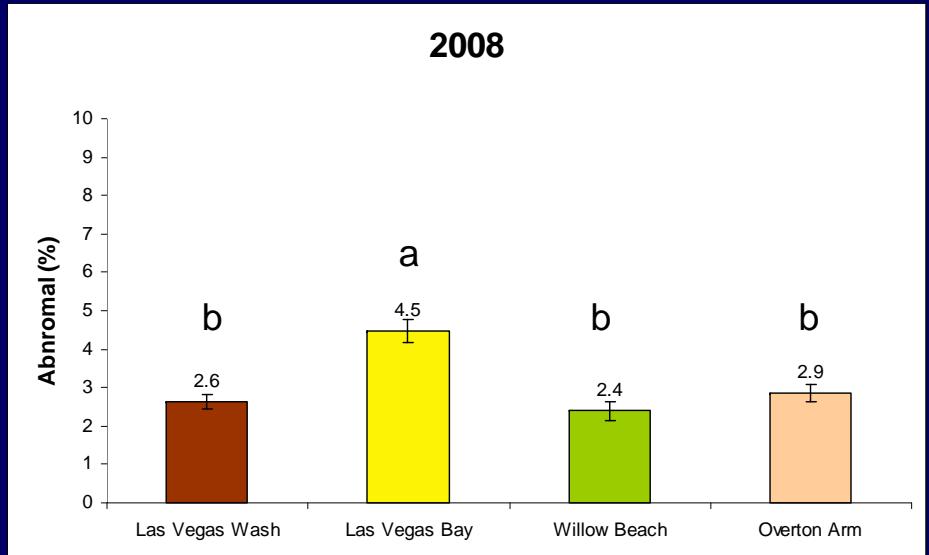
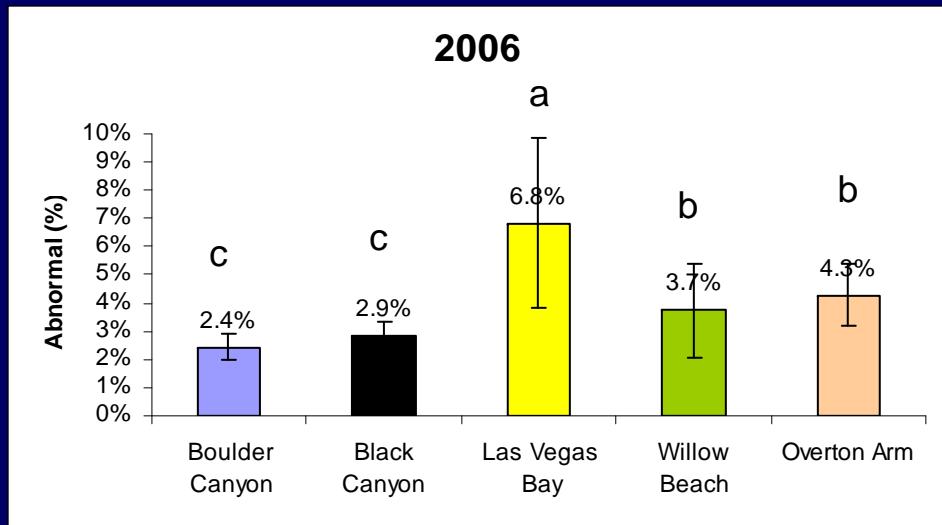


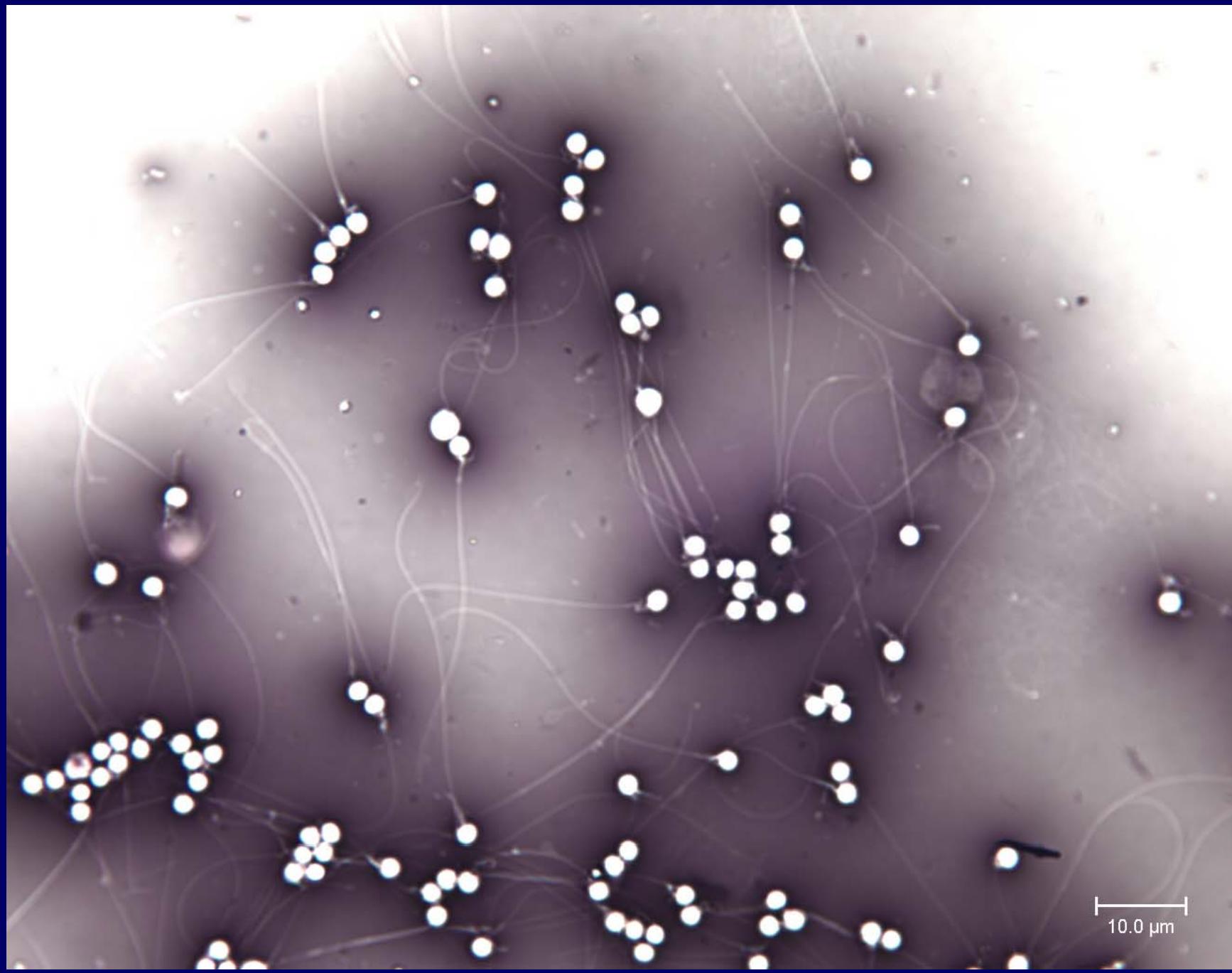
Flow cytometry for membrane integrity (viability), mitochondrial function (mito membrane potential), apoptosis (programmed cell death).

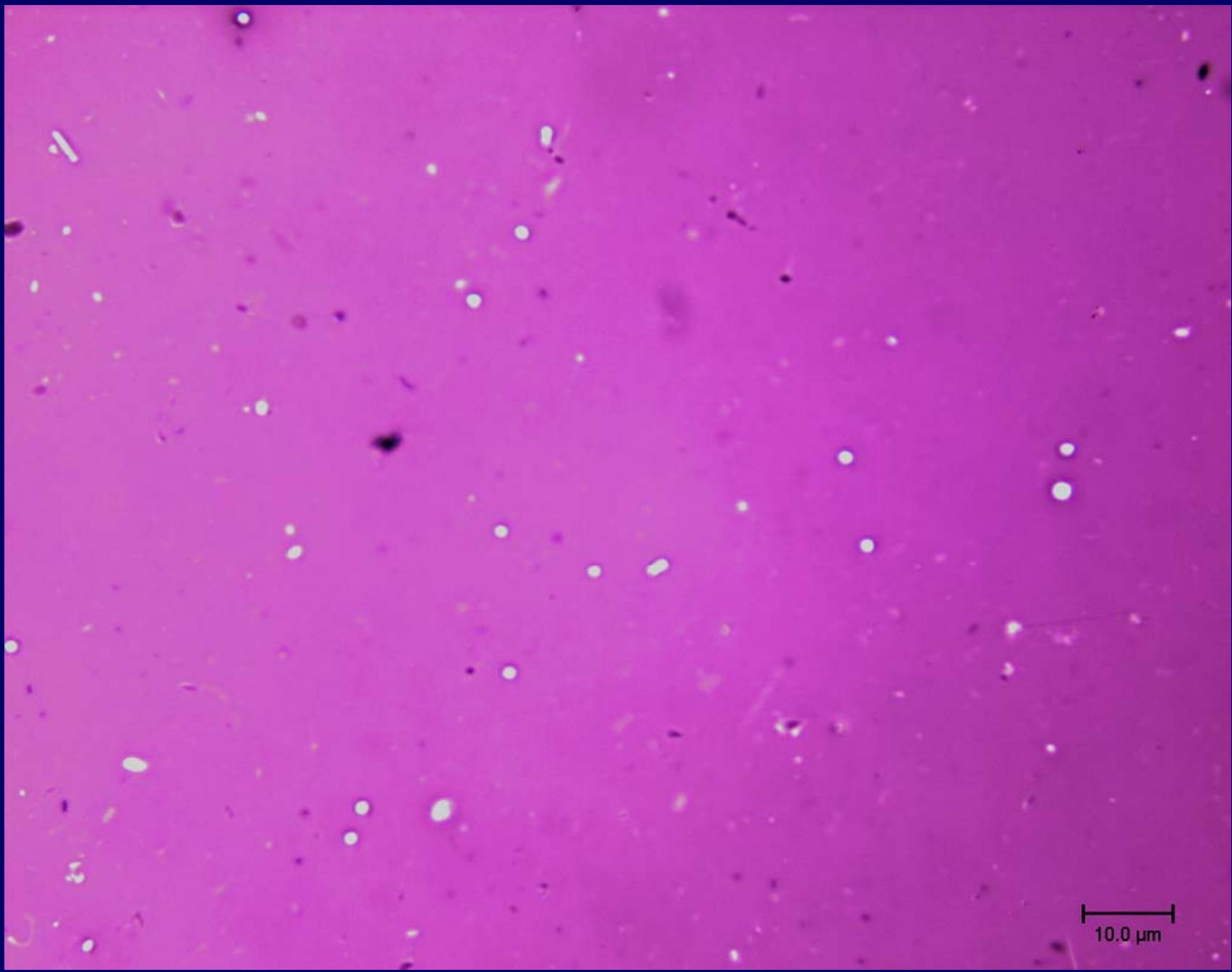


Fix cells for later, sperm counts per mL milt, and DNA integrity (fragmentation)

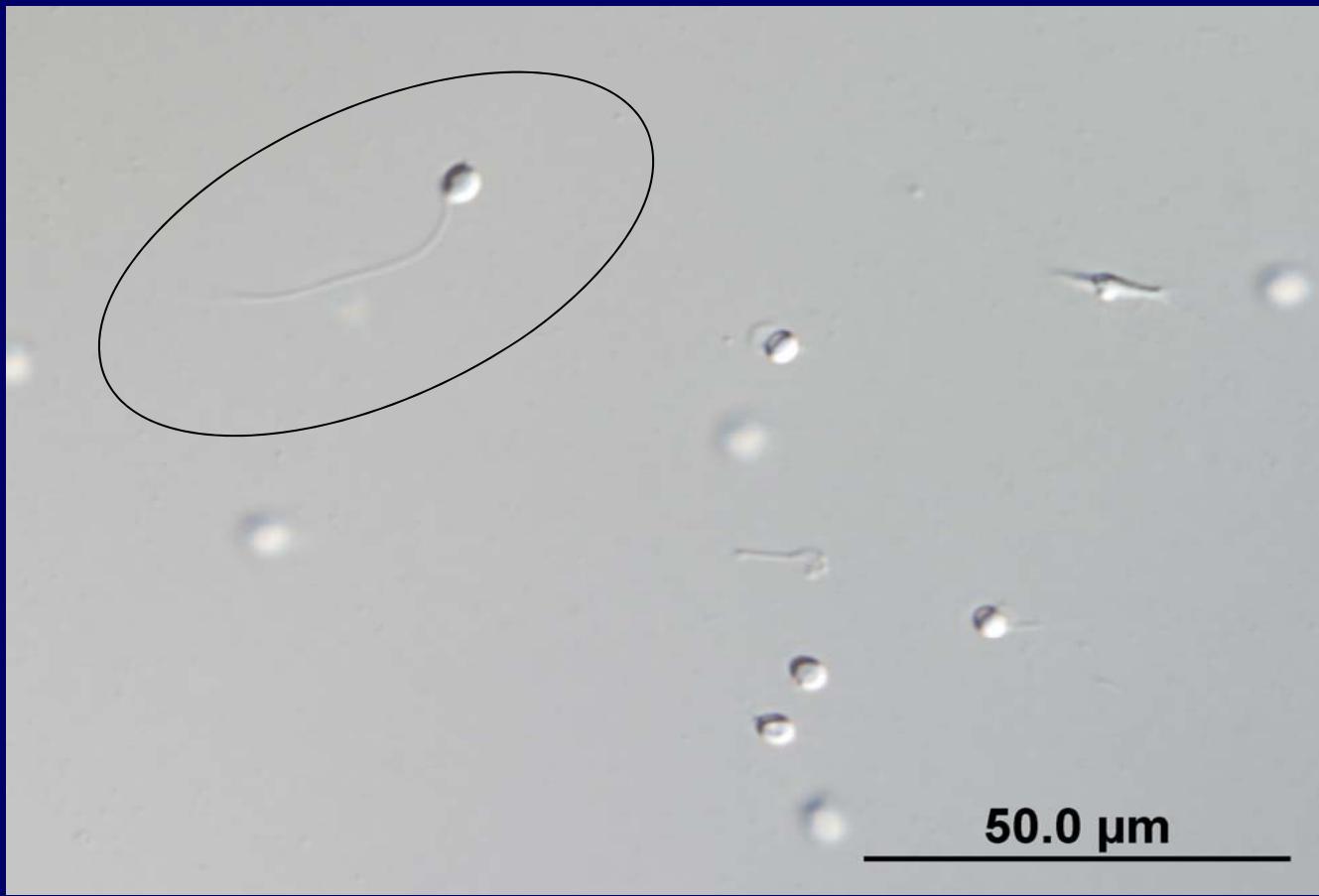
Cell Morphology



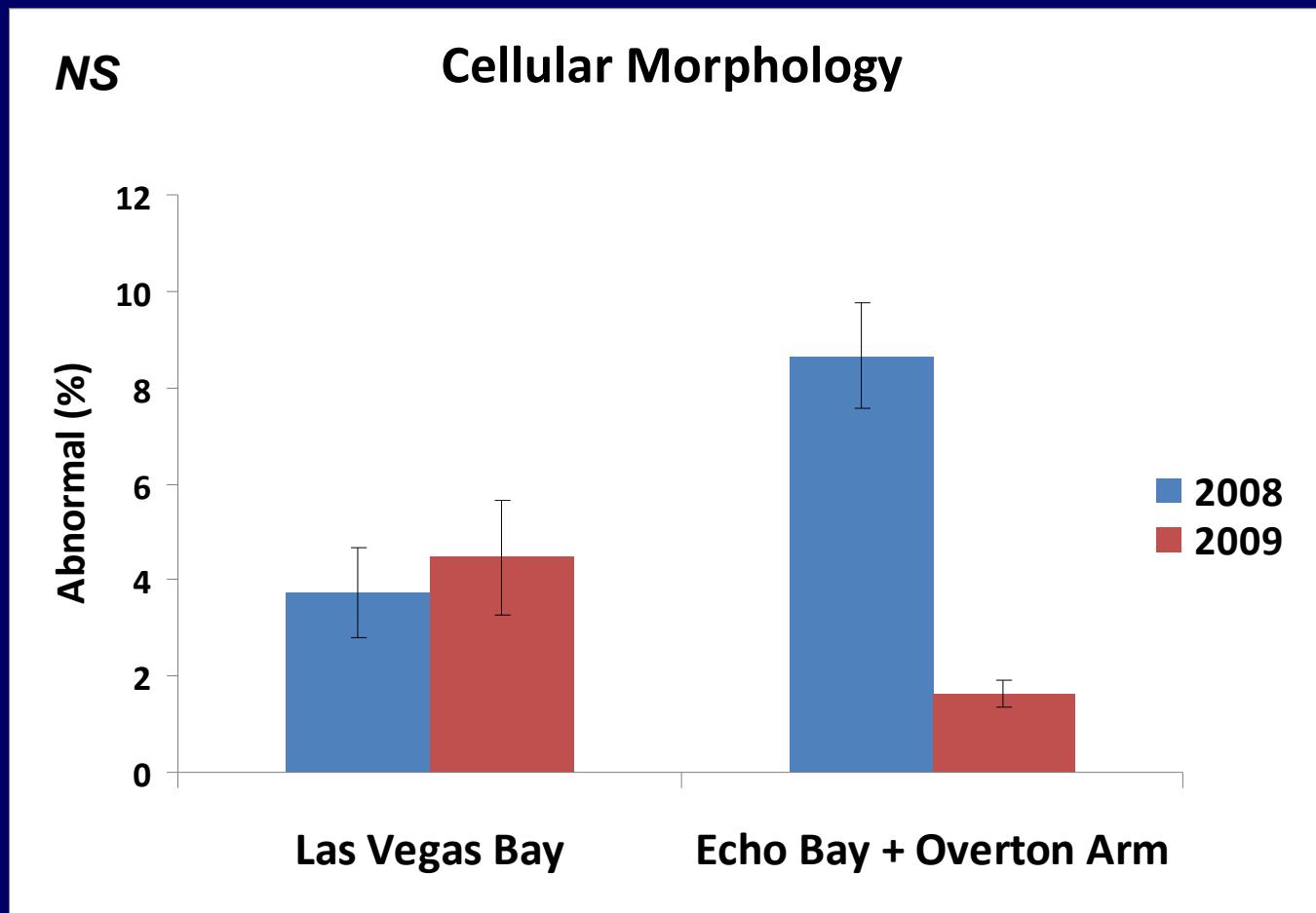




10.0 μm



Razorback Sucker: Sperm Morphology



Assessment of Motility

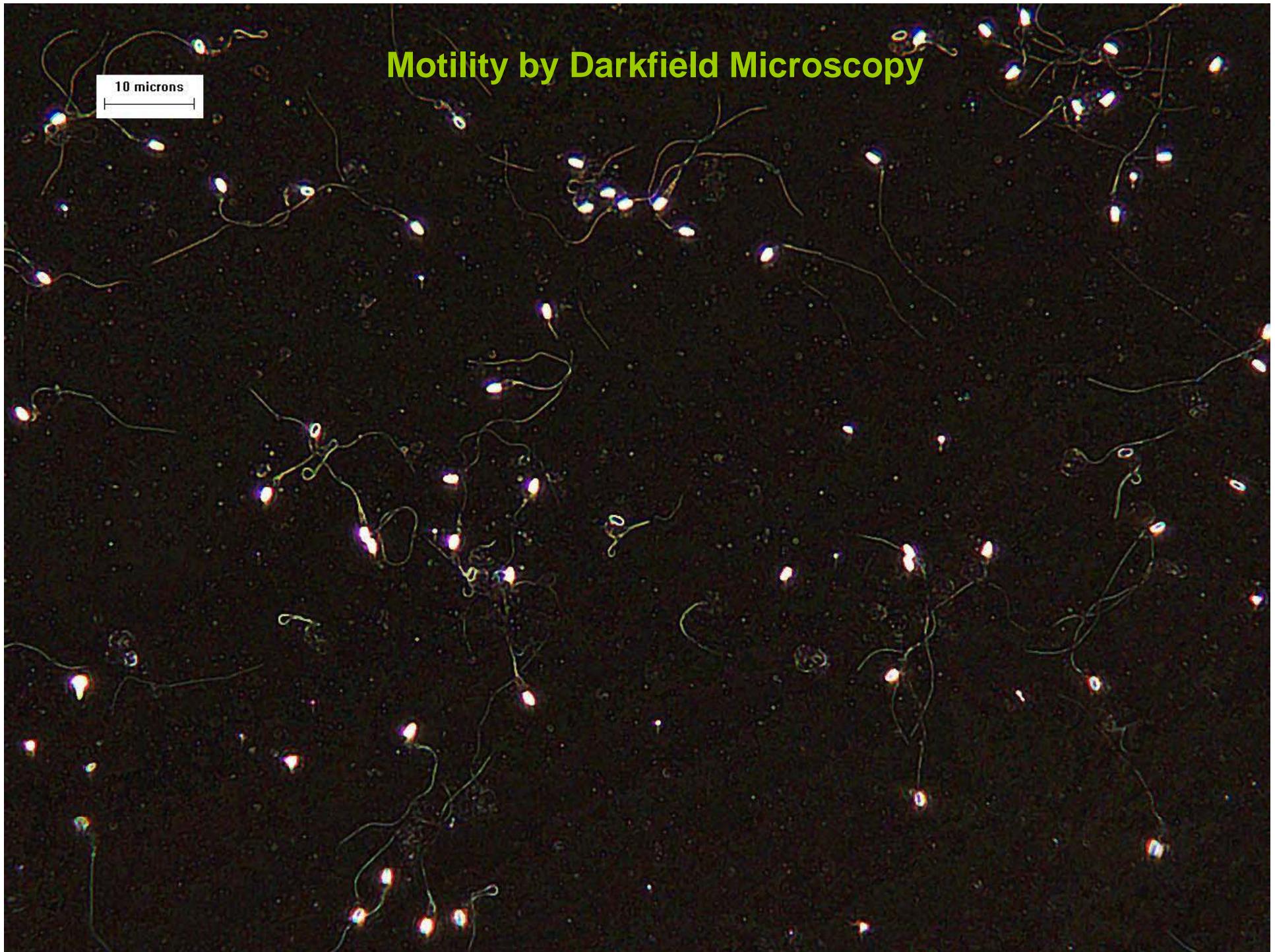
The most commonly used quality assessment.

Variable correlations with fertility in other species.

Fueled by mitochondria.

WHO: morphology, motility, counts

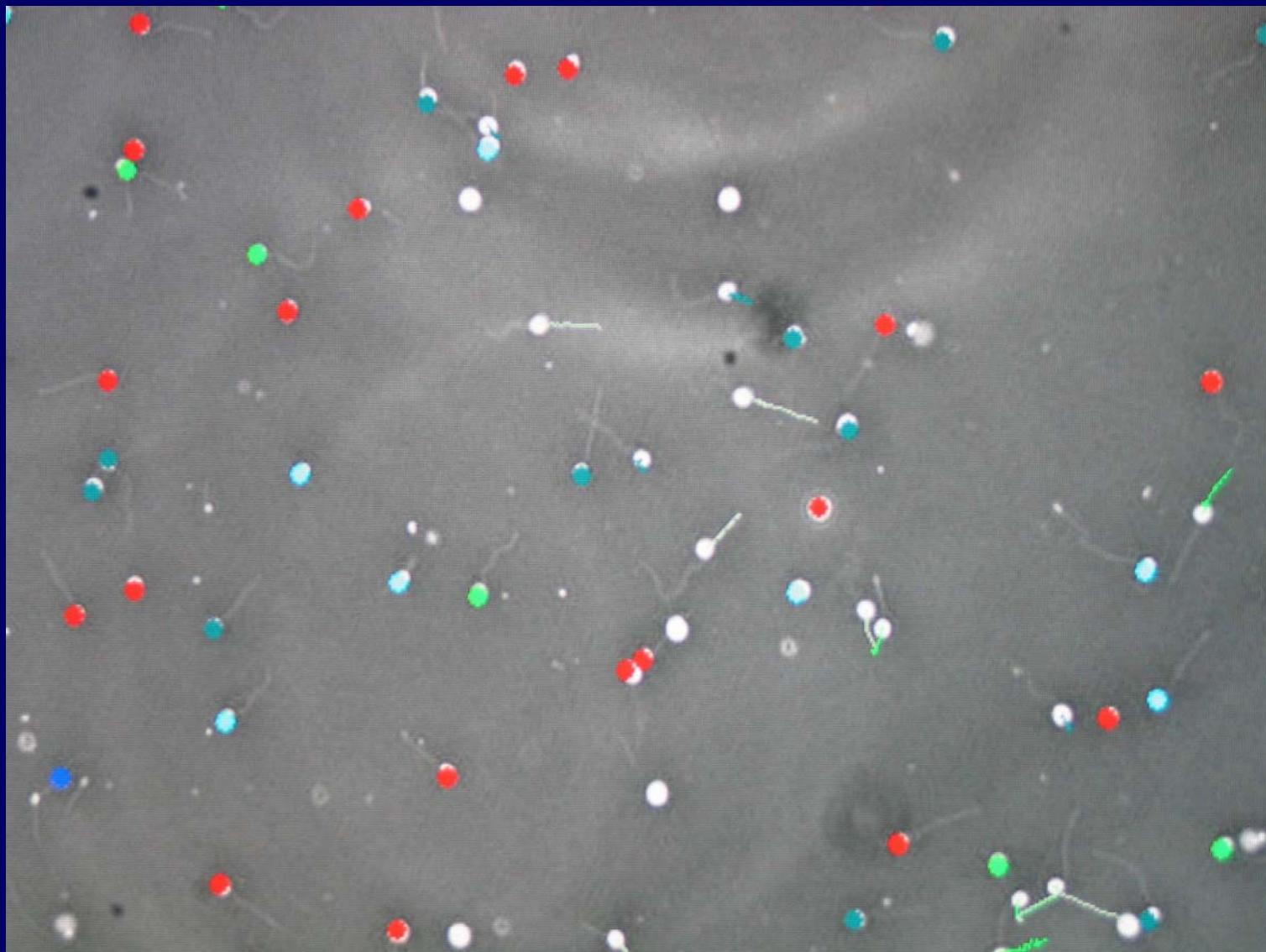
Motility by Darkfield Microscopy

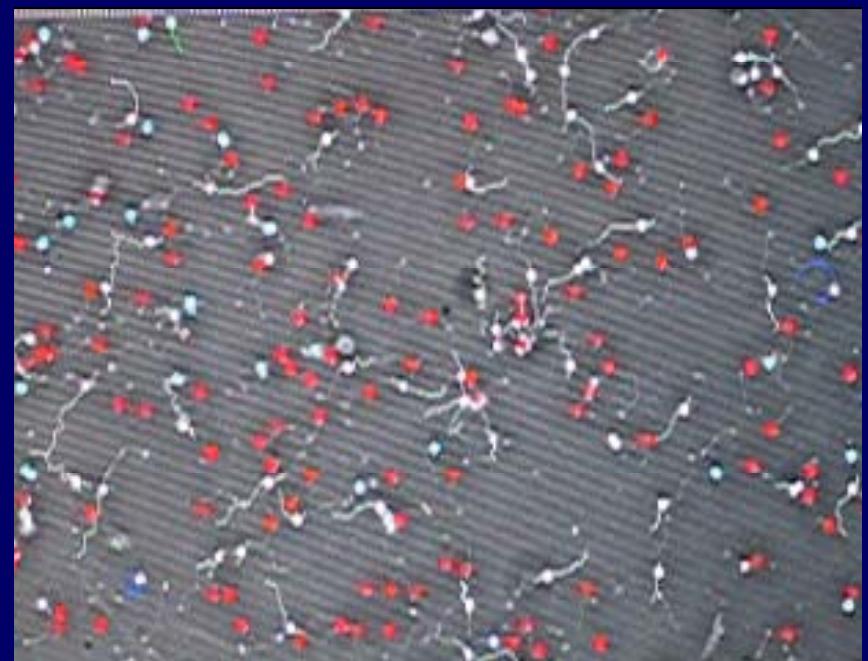
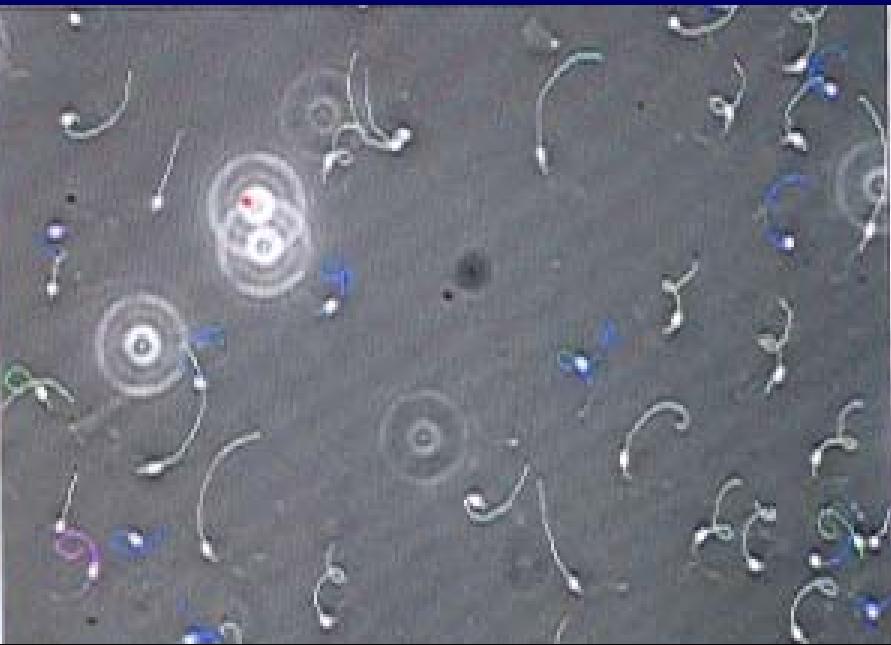


Motility by CASA: Computer Assisted Sperm Motion Analysis

curvilinear velocity
average path velocity
linear velocity
linearity
beat – cross frequency
amplitude lateral head displacement

total motility
progressive motility

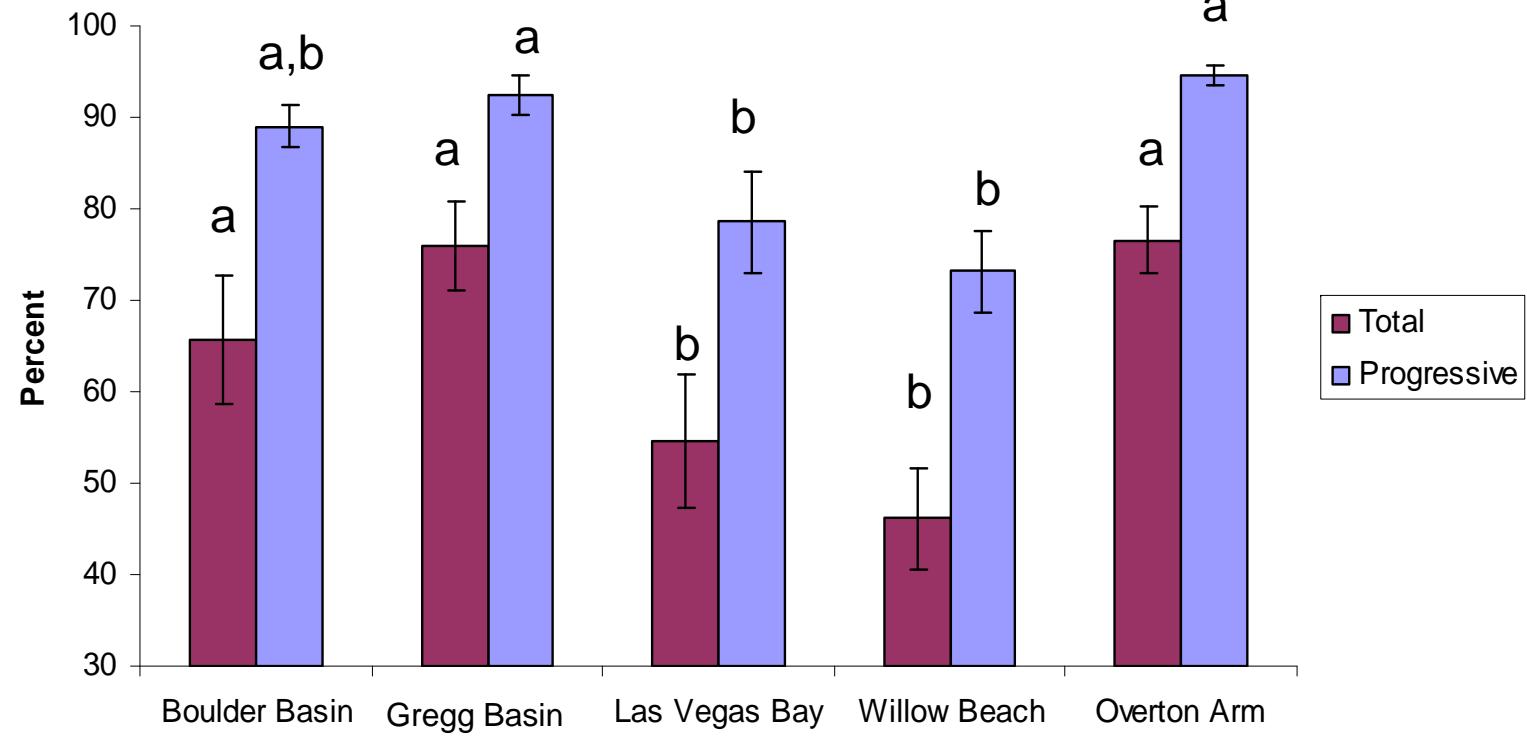




Motility: 2006

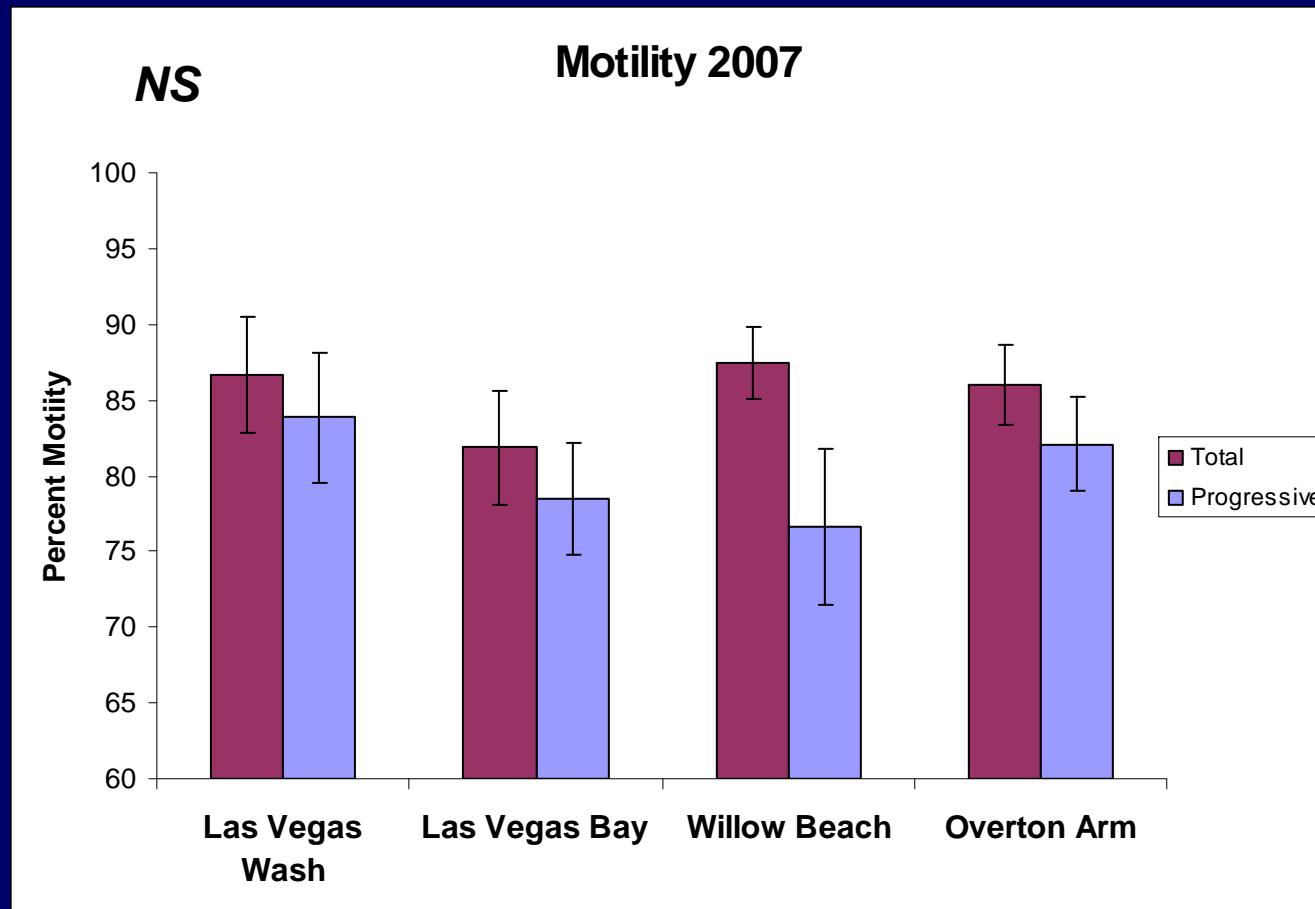
$P < 0.0001$ Total
 $P = 0.0080$ Prog.

Motility 2006



Total OA,GB, BB>LVB, WB
Prog. OA, GB > LVB, WB

Motility: 2007

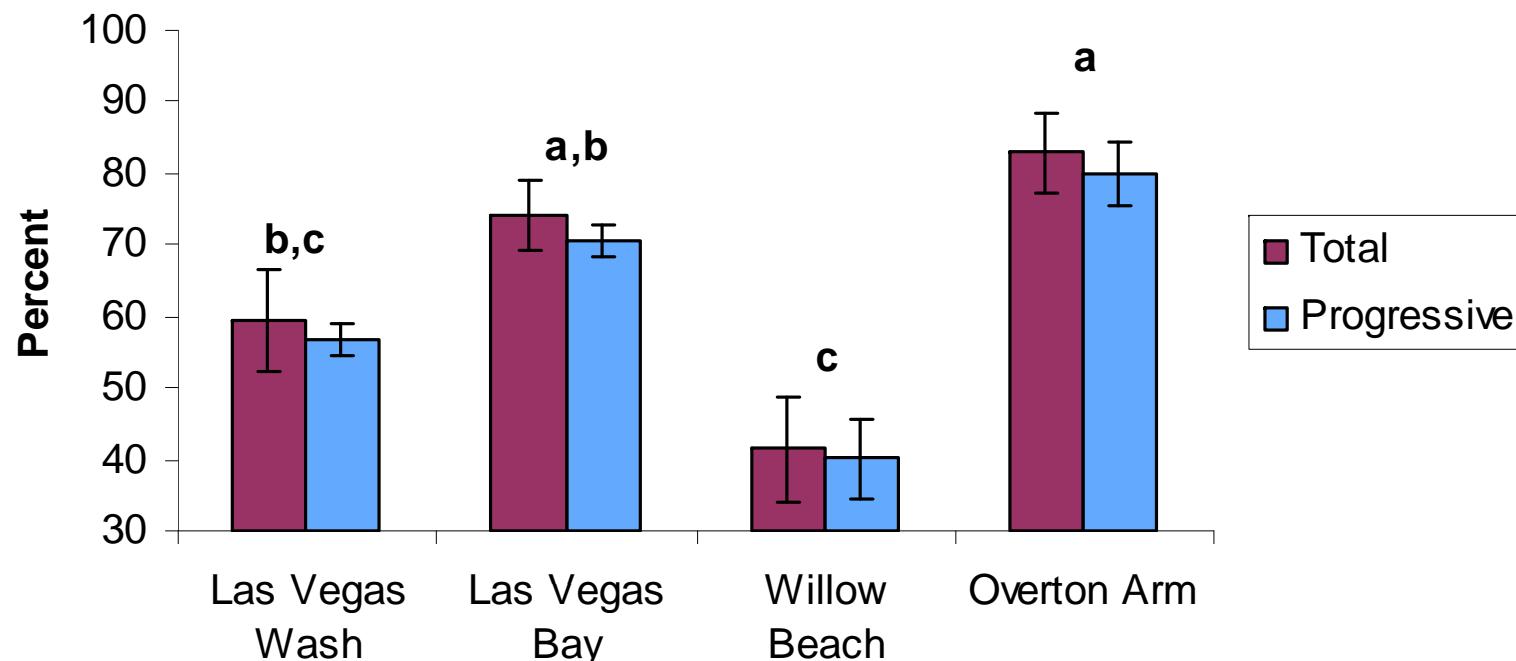


Total & Prog. LVB=LW=WB=OA

Motility: 2008

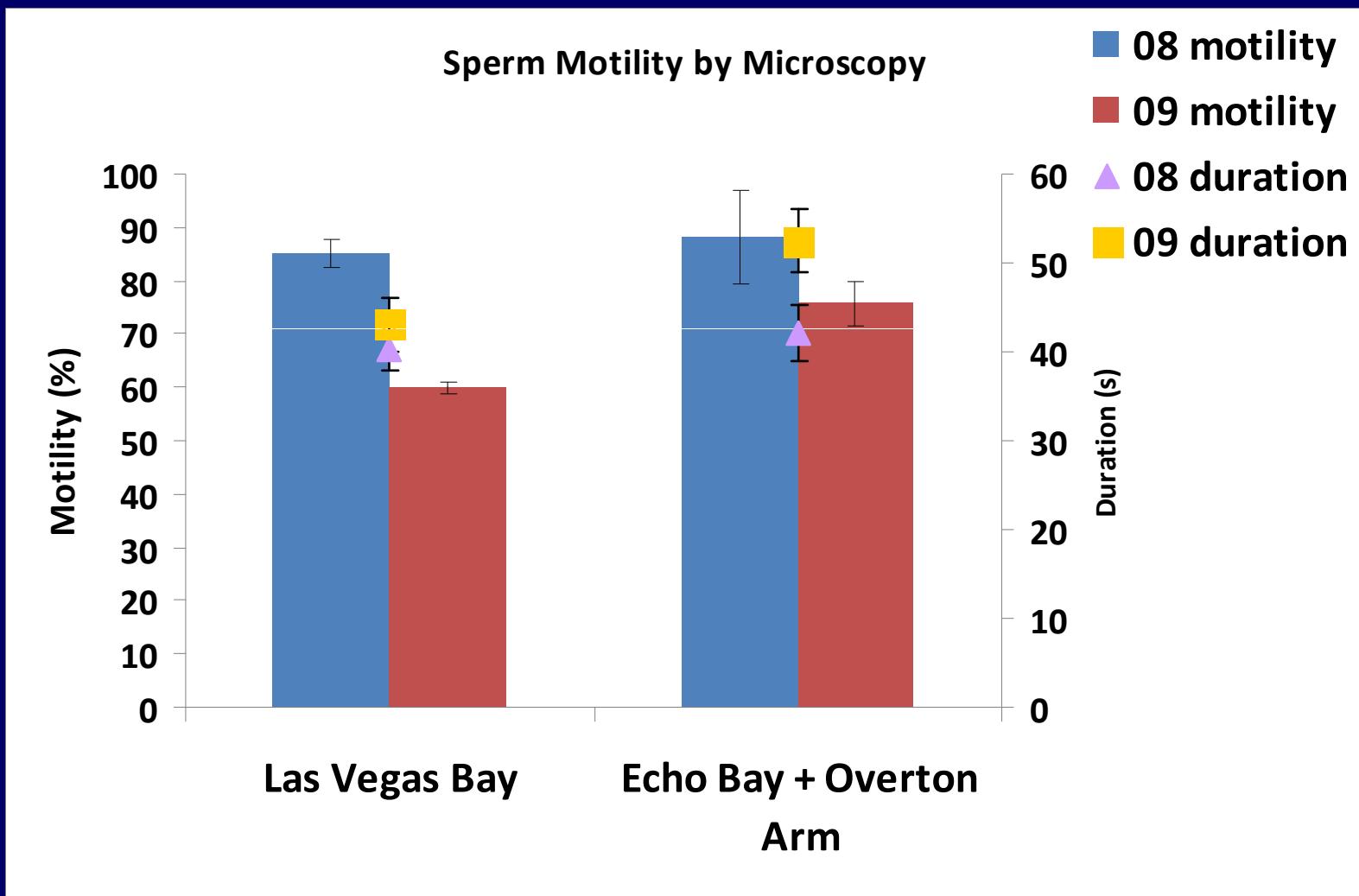
$P < 0.0001$ Total
 $P < 0.0001$ Prog.

Motility 2008

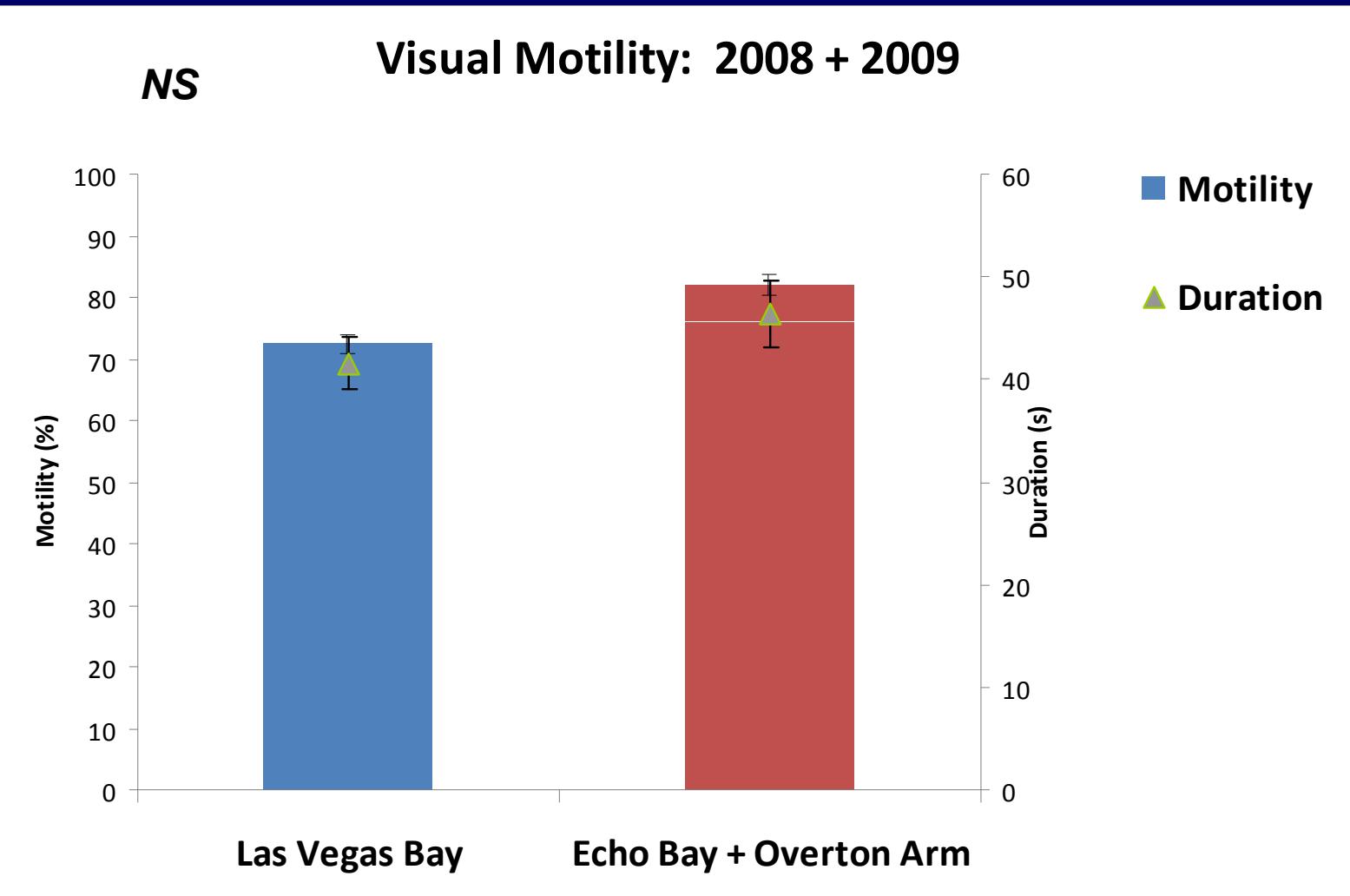


Total & Prog. OA>LVB>LVW>WB

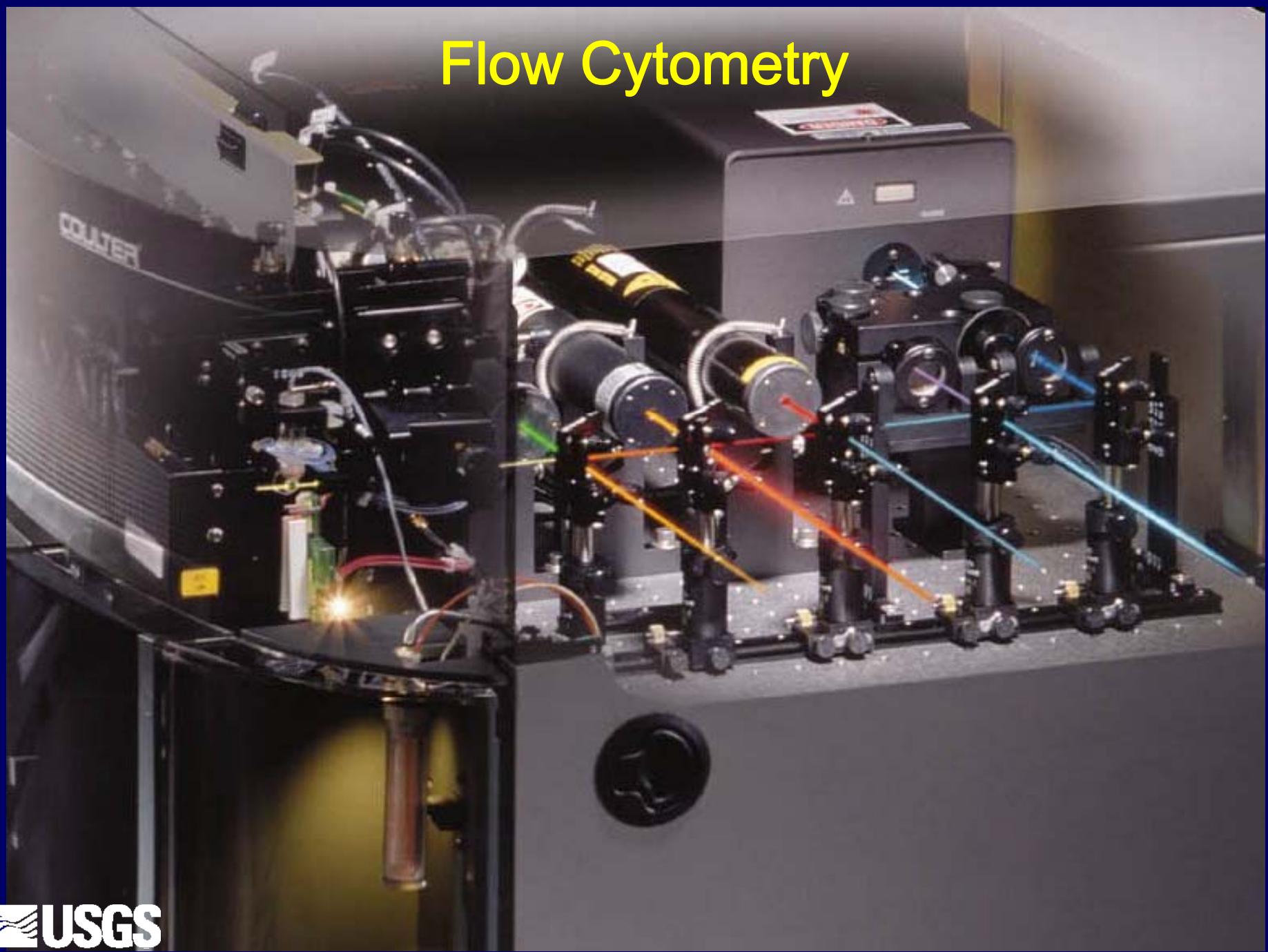
Razorback Sucker: “Visual” Motility



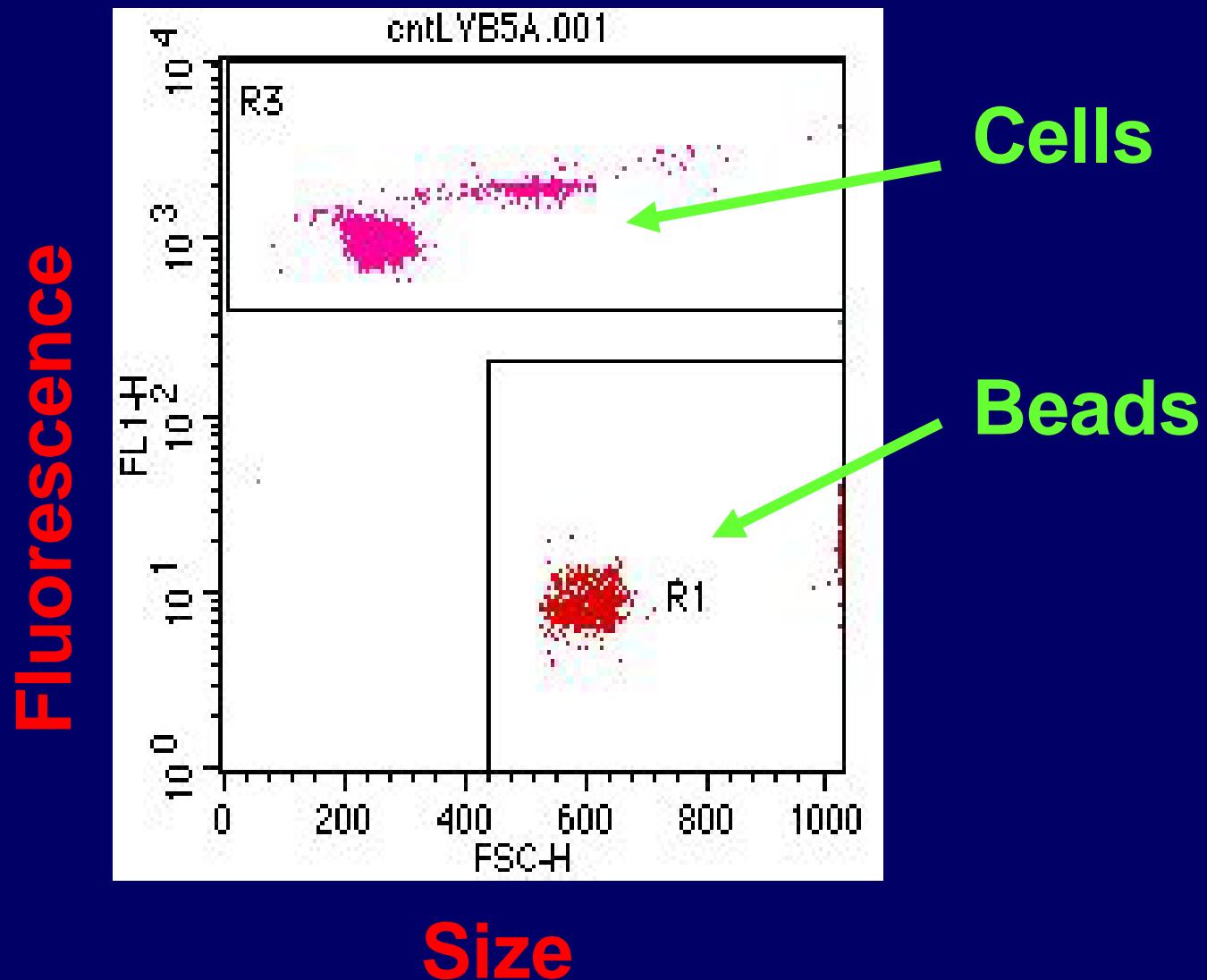
Razorback Sucker: “Visual” Motility



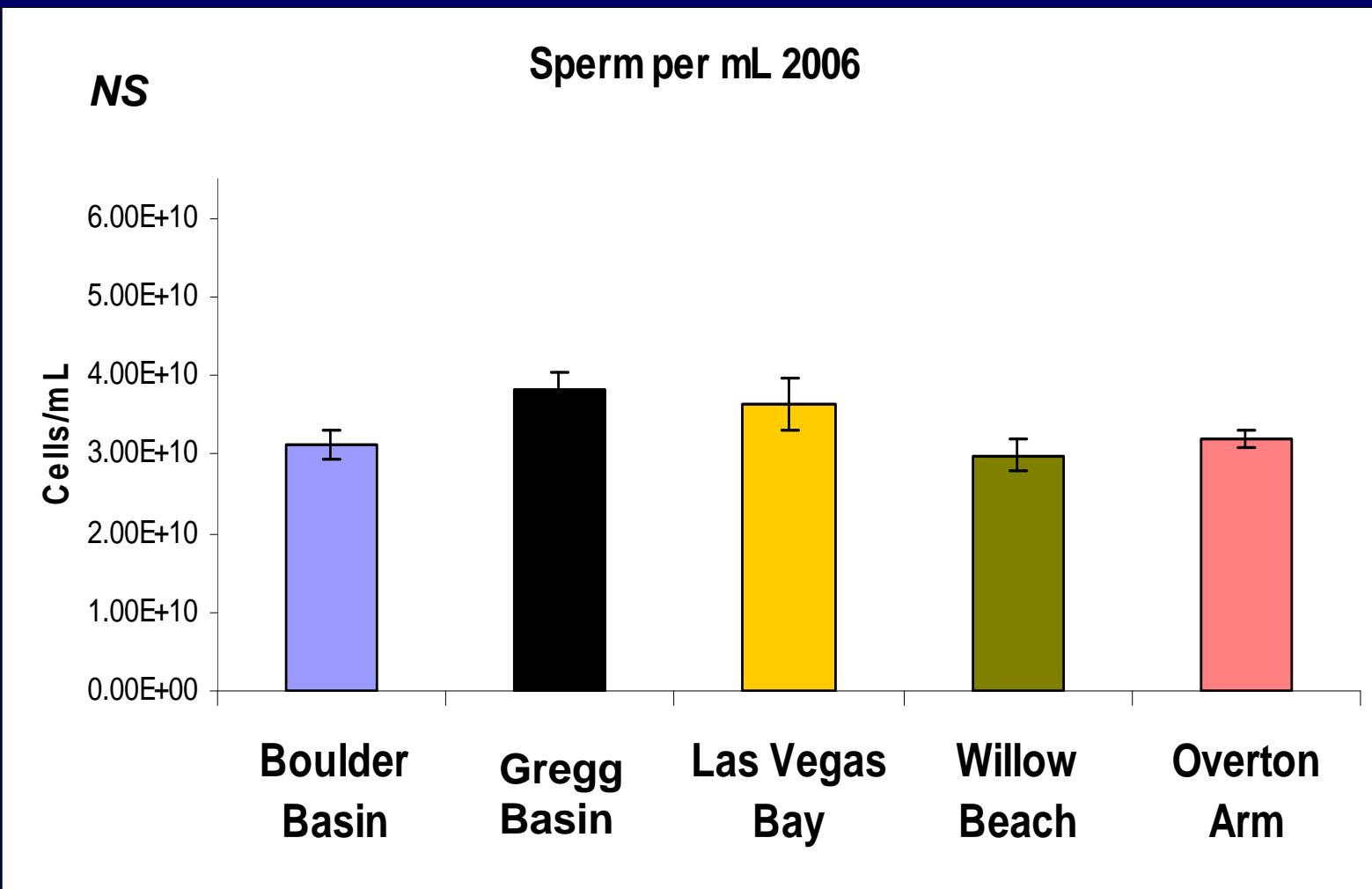
Flow Cytometry



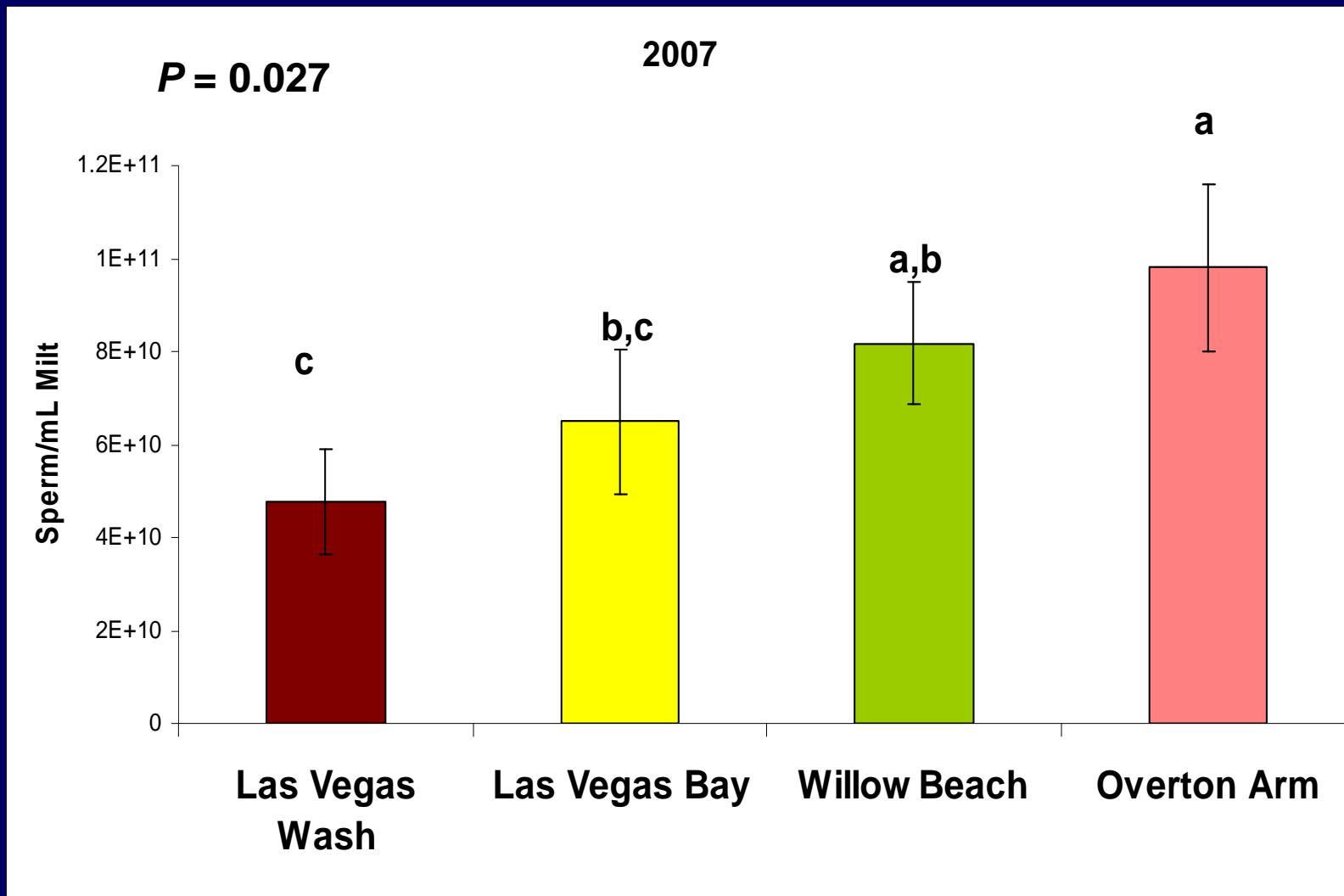
Sperm Counting by Flow Cytometry



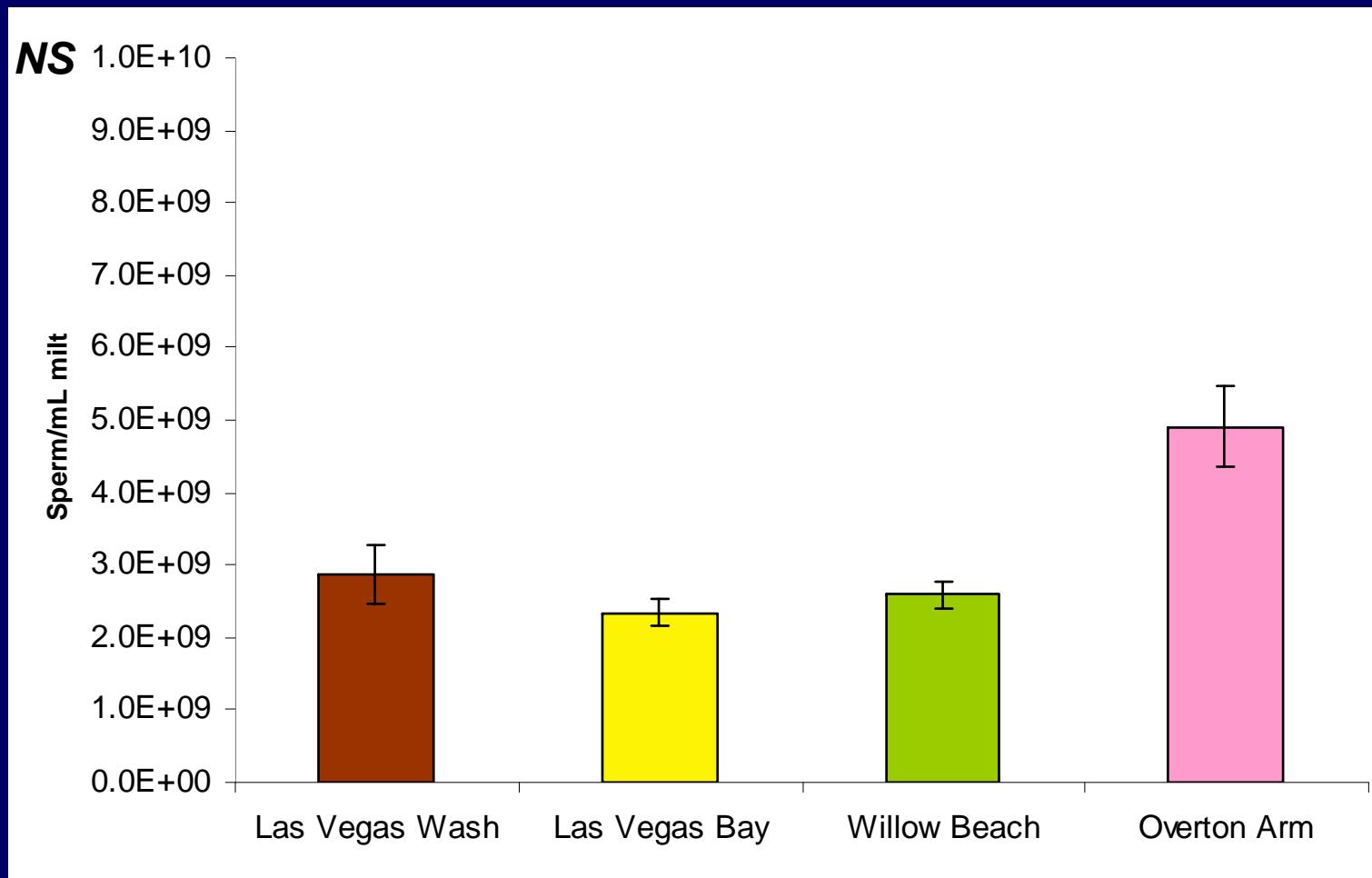
Carp Sperm Counts: 2006



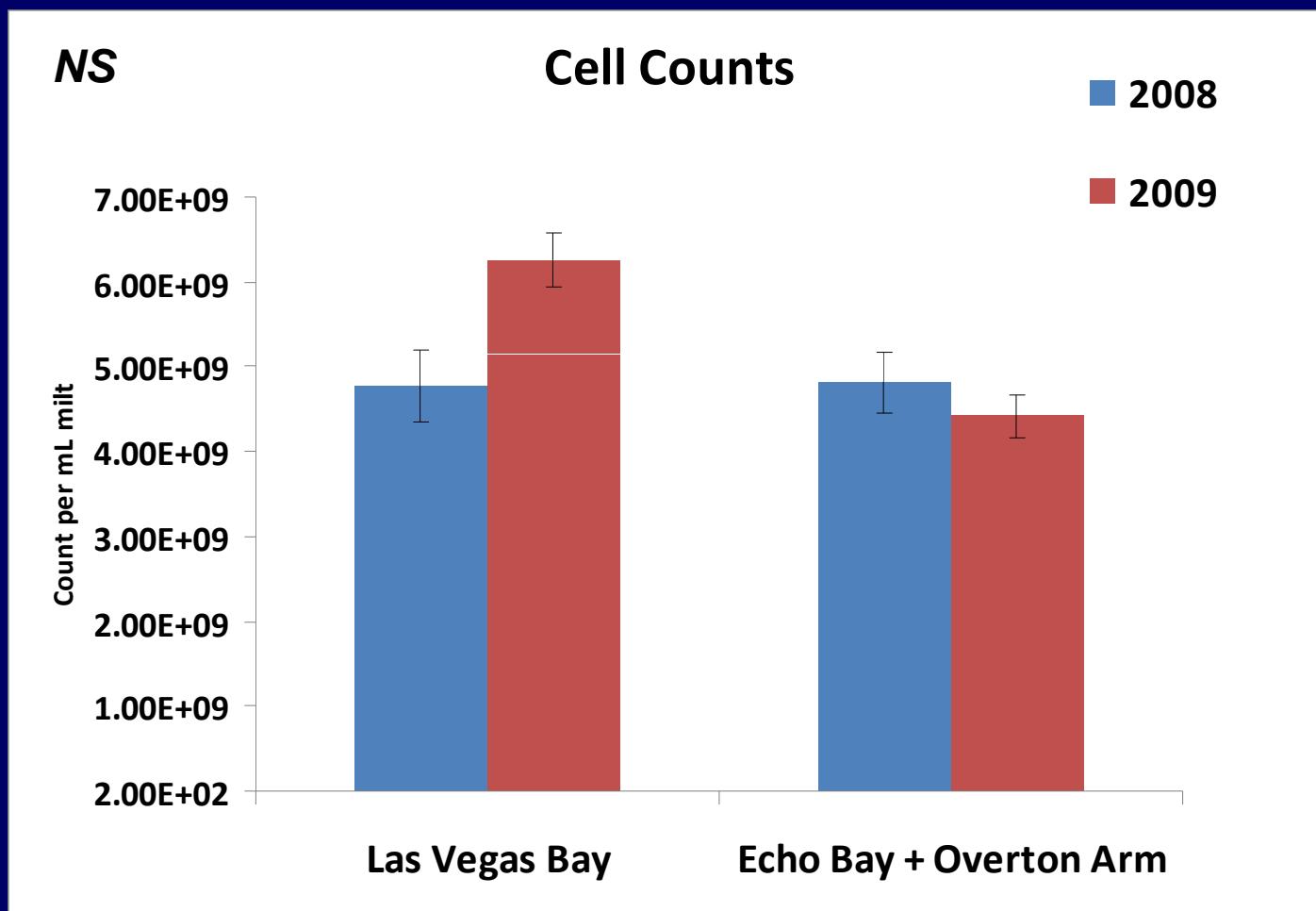
Carp Sperm Counts: 2007



Carp Sperm Counts: 2008



Razorback Sucker: Sperm Counts

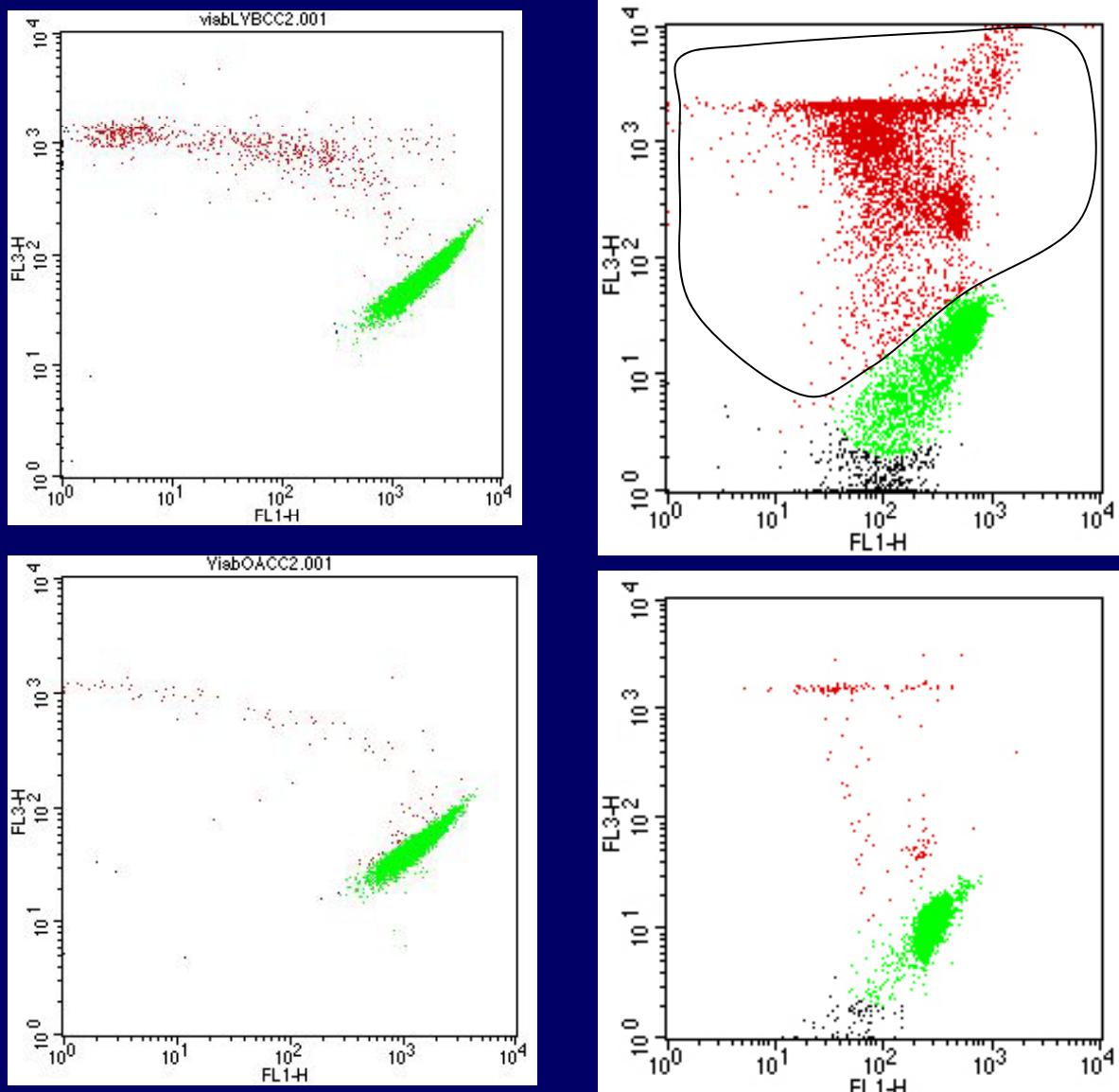


Membrane Integrity and Mitochondrial Membrane Potential

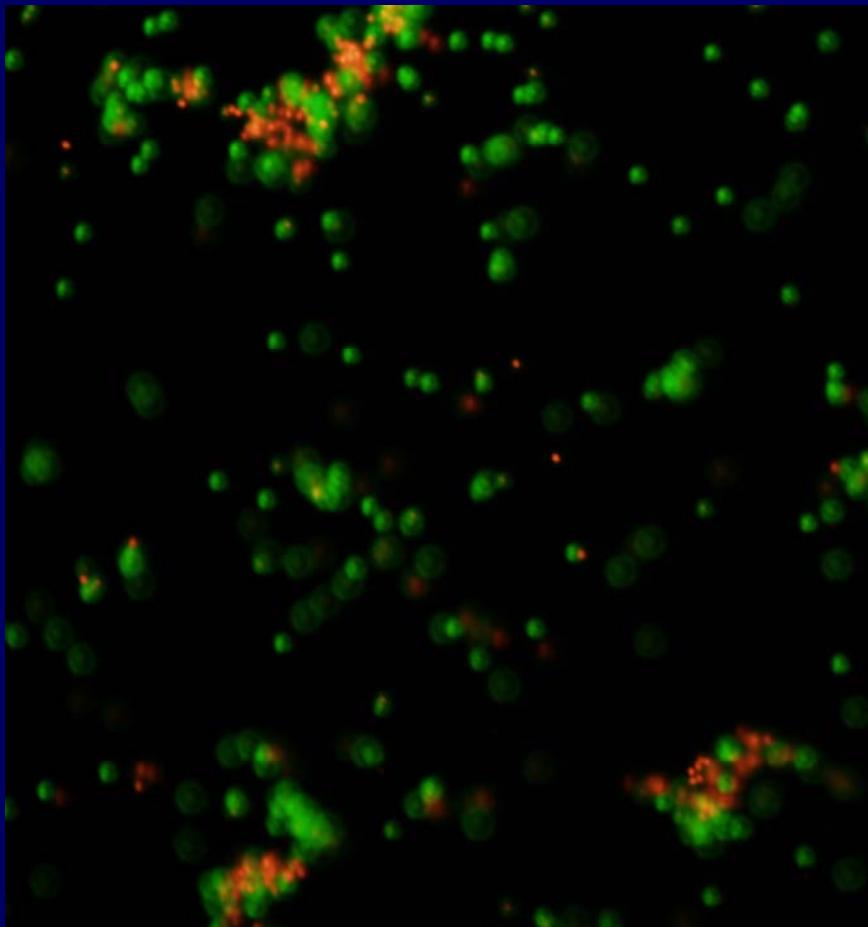
green = better

red = not so good

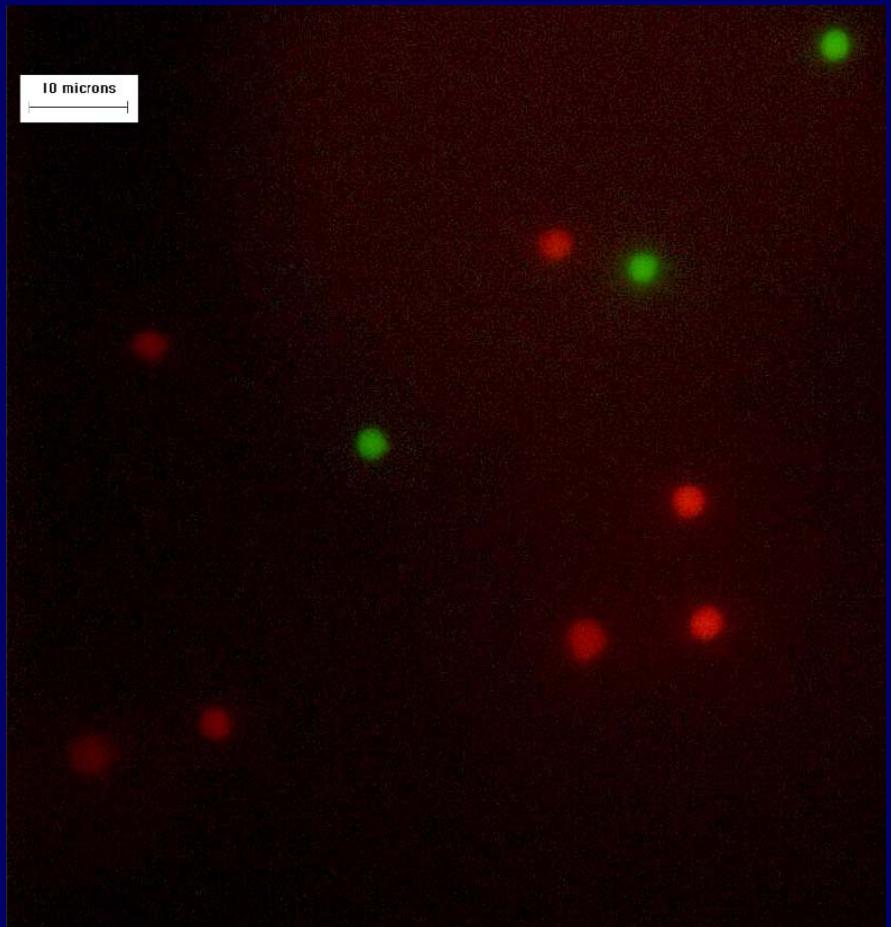
10K cells, 3x/fish



Membrane Integrity: Viability



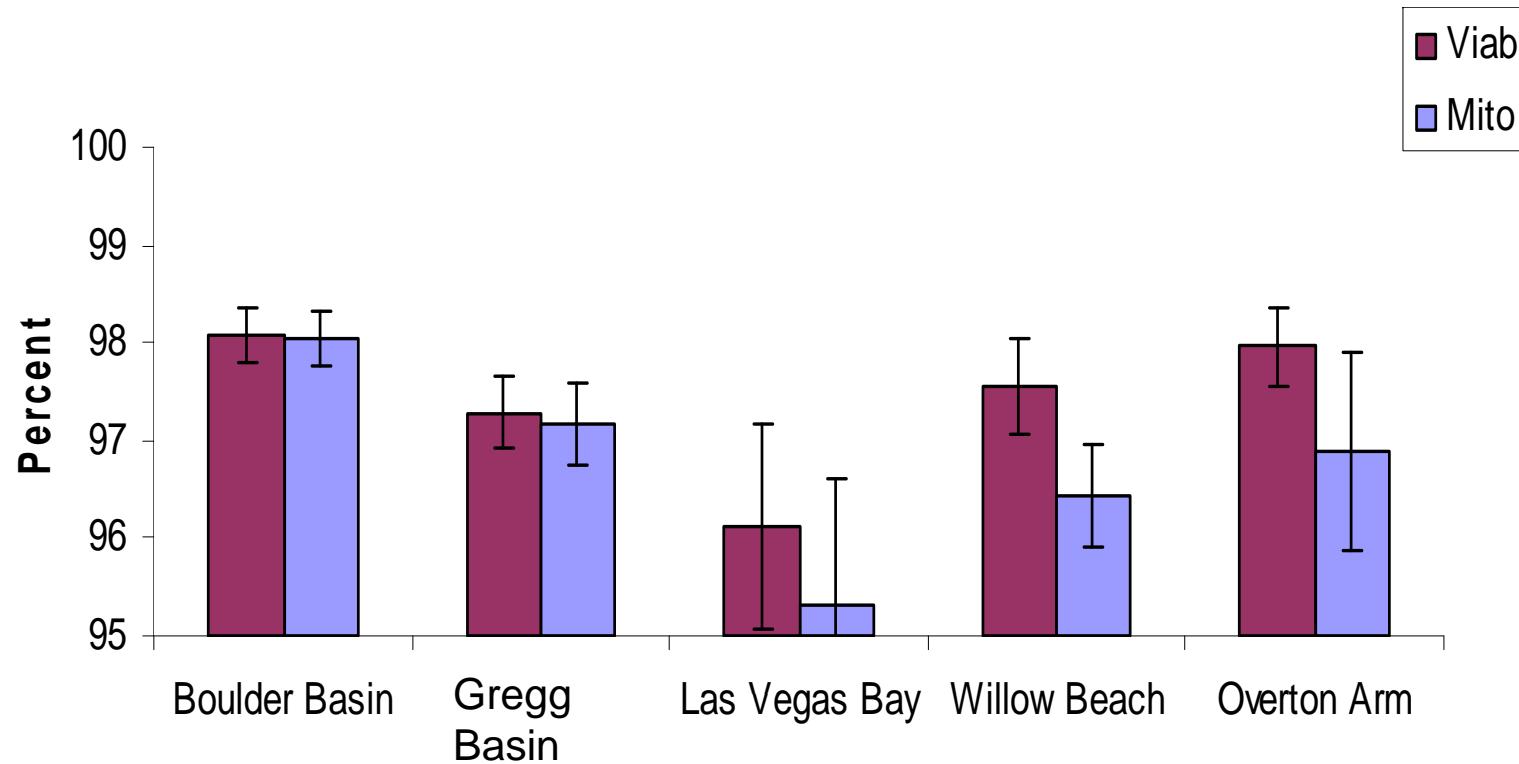
Razorback Sucker



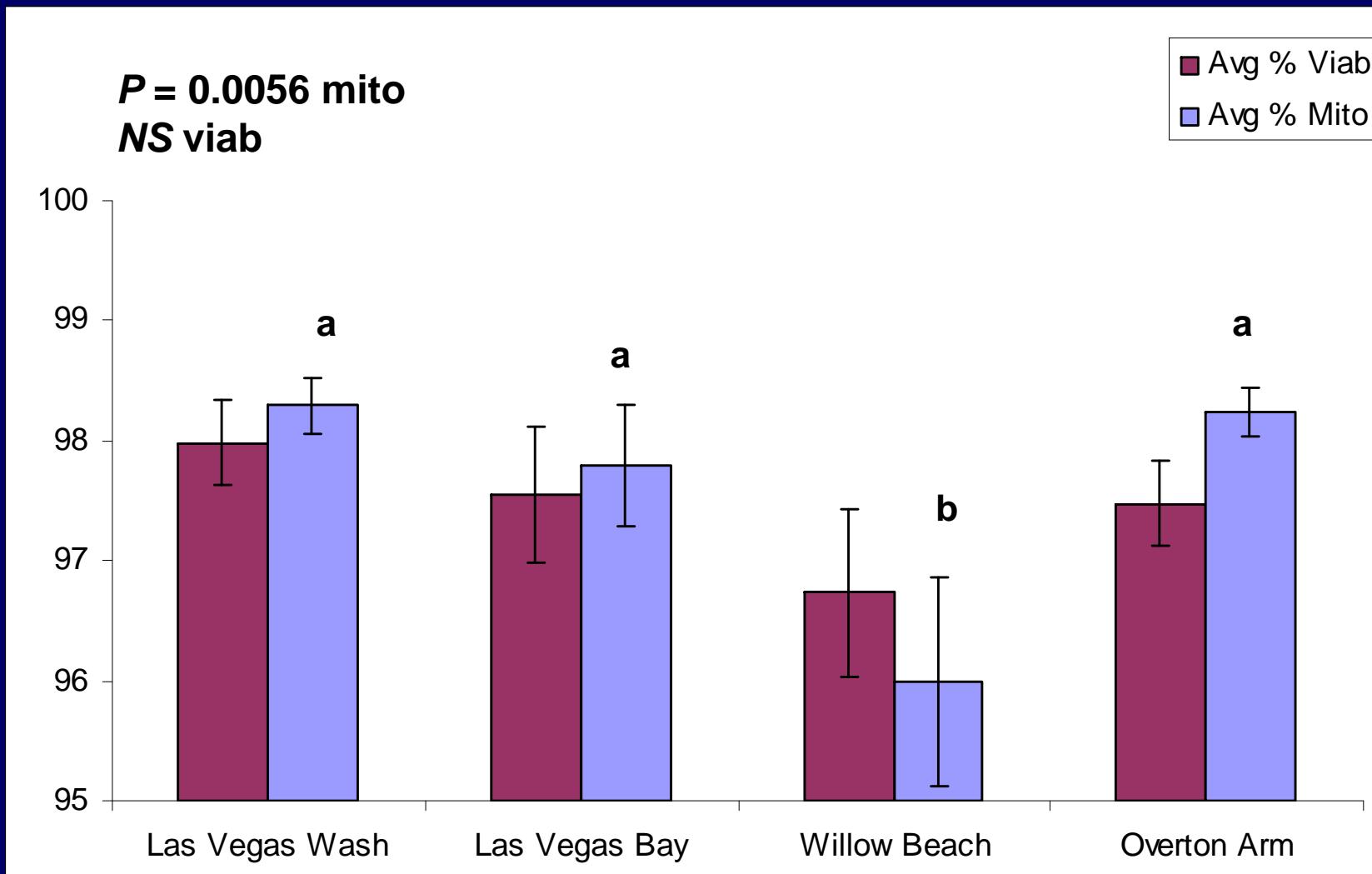
Carp

Carp Viability and Mitochondrial Function: 2006

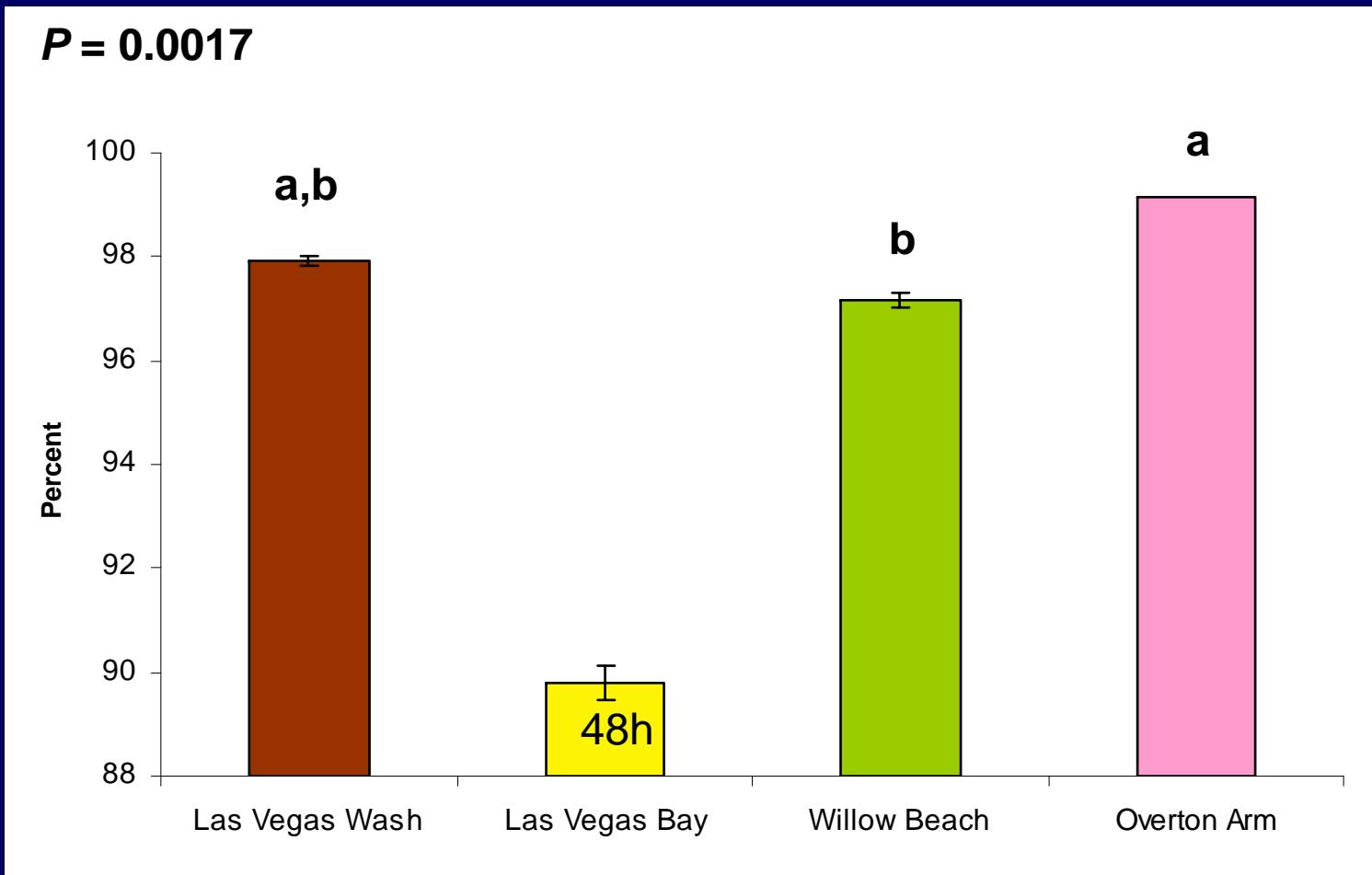
NS



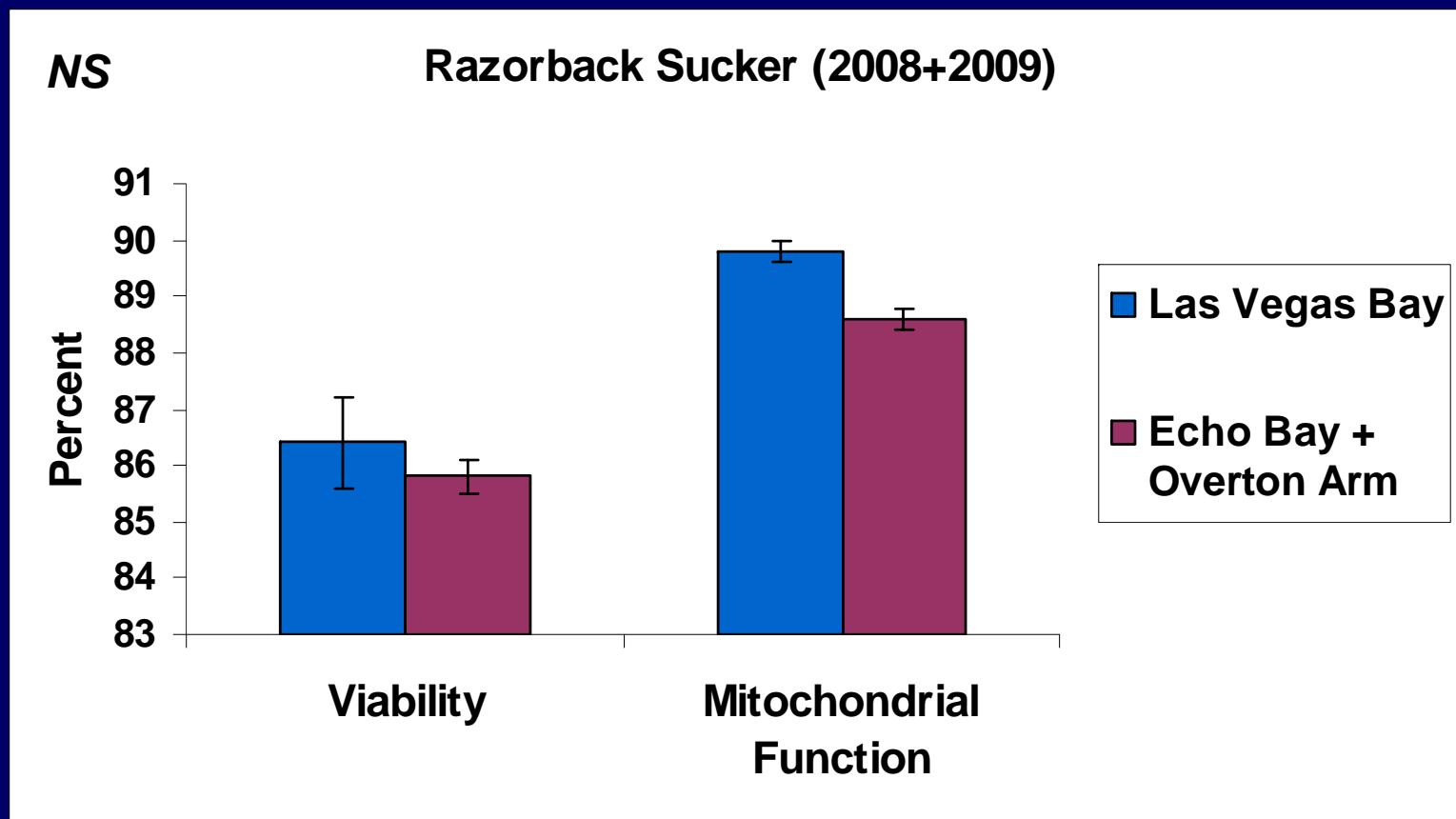
Carp Viability and Mitochondrial Function: 2007



Carp Mitochondrial Function: 2008

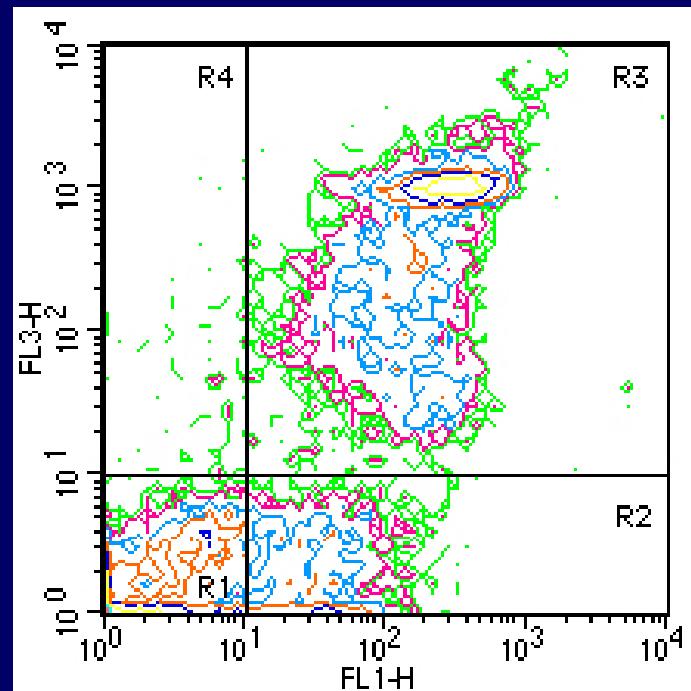
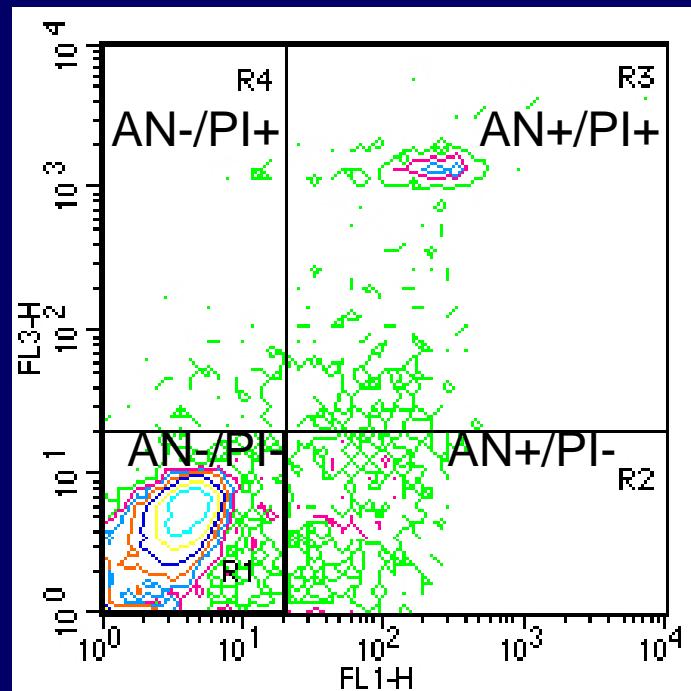


Razorback Sucker: Viability and Mitochondrial Function



Apoptosis

Occurs prior to mitochondrial dysfunction and cell death



Green

Annexin-V binding

Carp Apoptosis 2007, 2008

2007

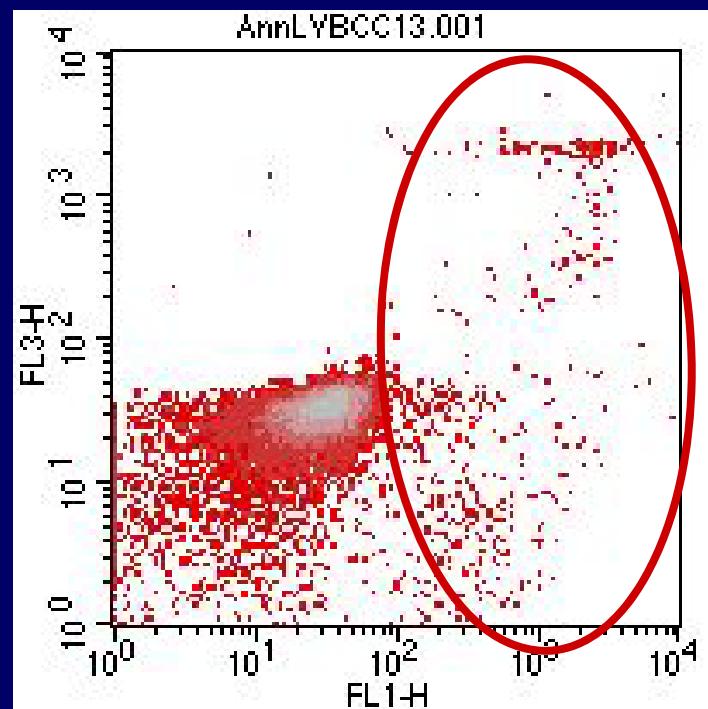
WB > OA = LVW = LVB

$P < 0.0001$

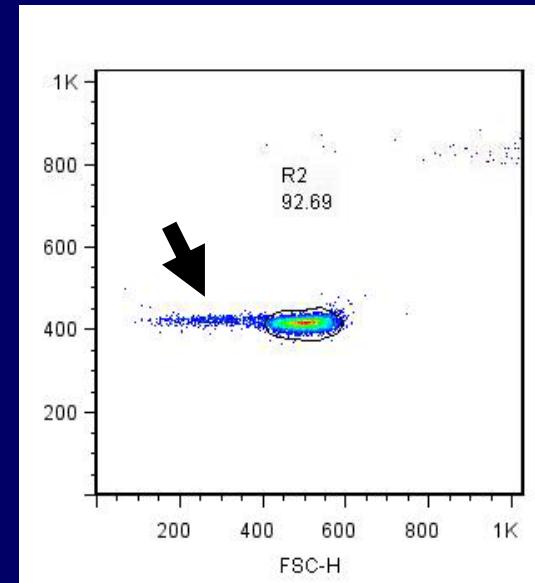
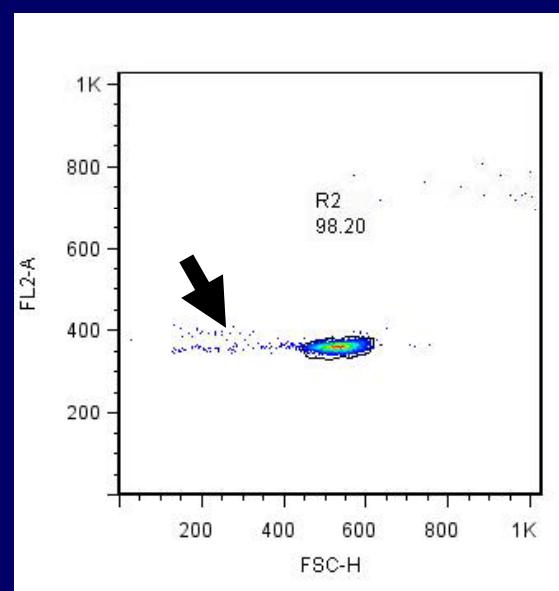
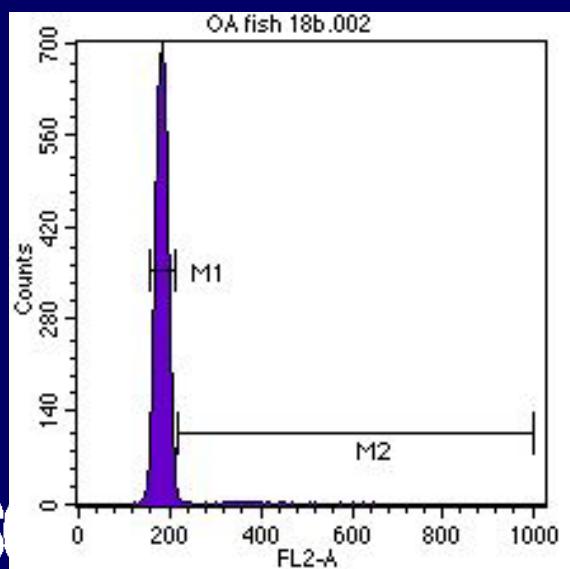
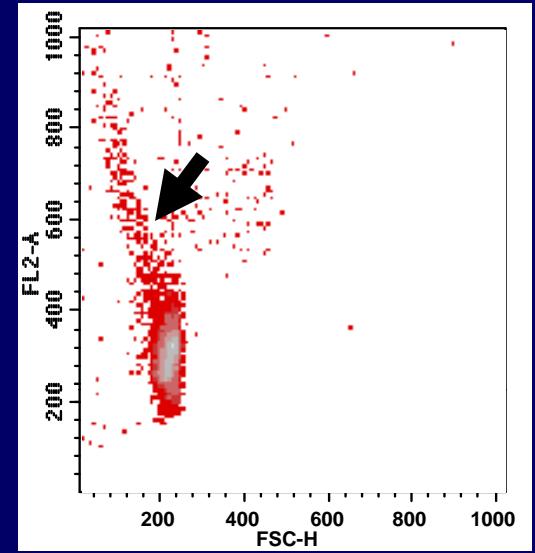
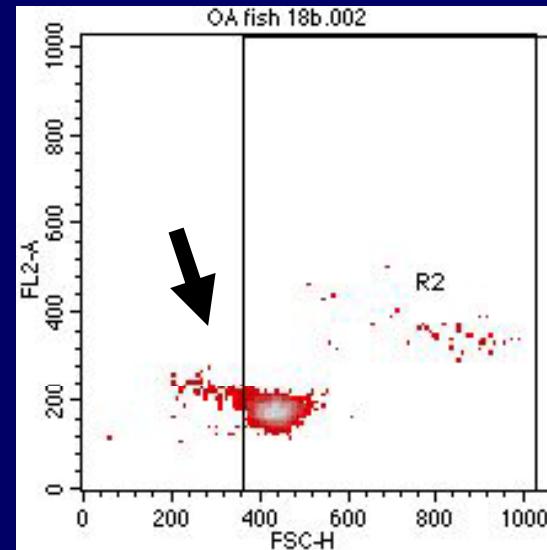
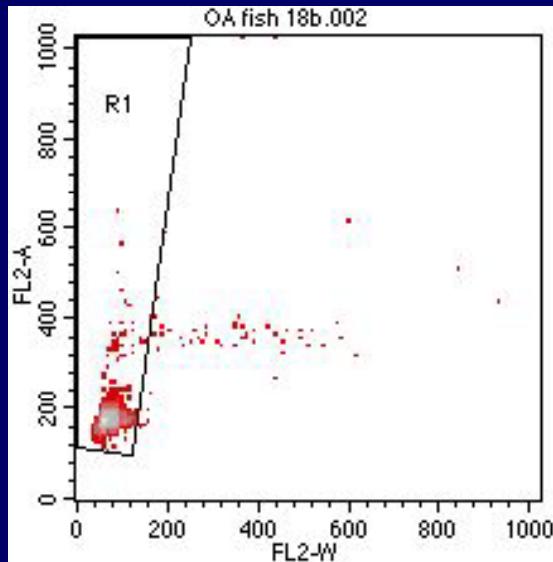
2008

WB \geq LVW \geq OA

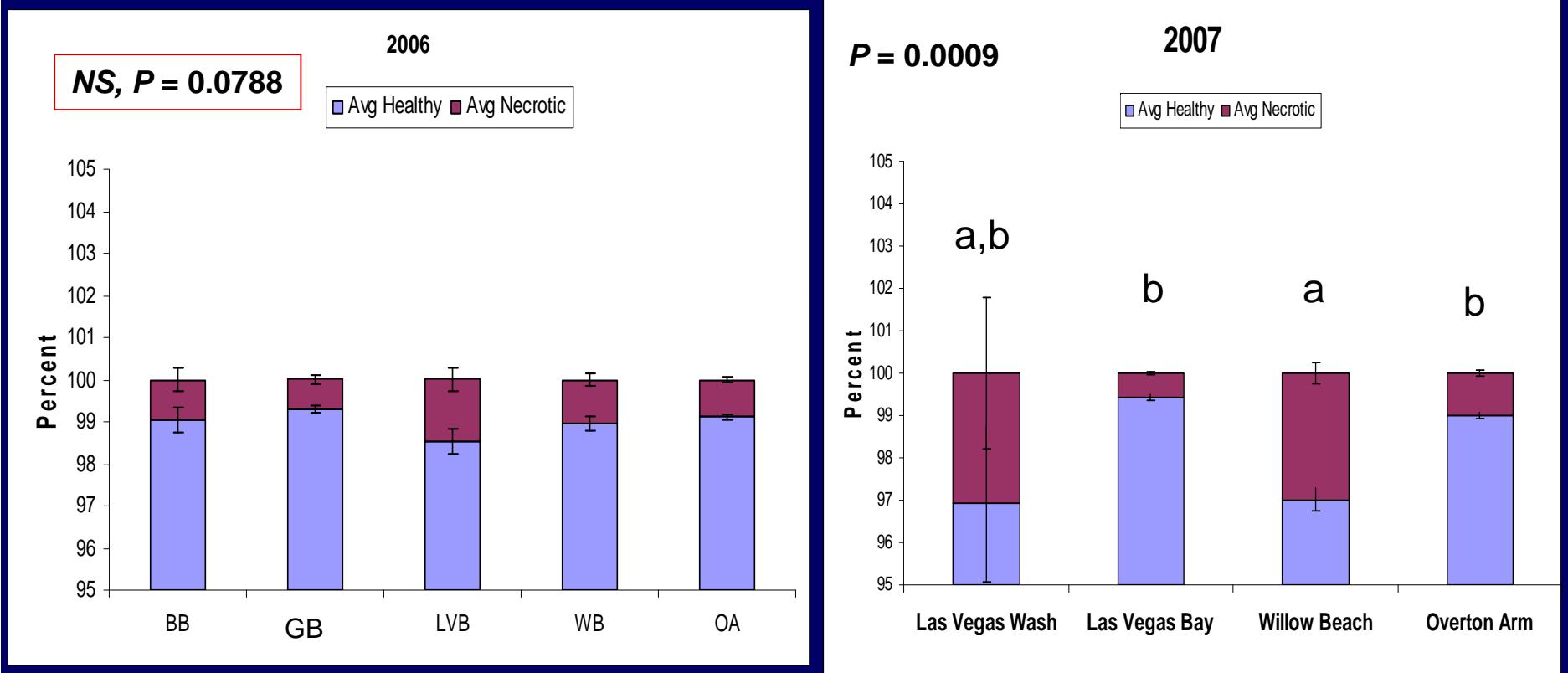
$P < 0.046$



Gating for Nuclear DNA Fragmentation



2006 and 2007: Percent Fragmented DNA



WB \geq LVW \geq LVB = OA

Site Differences in Carp Sperm Cell Quality

	2006	2007	2008
Morphology	*		*
Motility	*		*
Viability			
Mito function		*	*
Apoptosis		*	*
Sperm count		*	
DNA fragmentation		*	<i>in analysis</i>

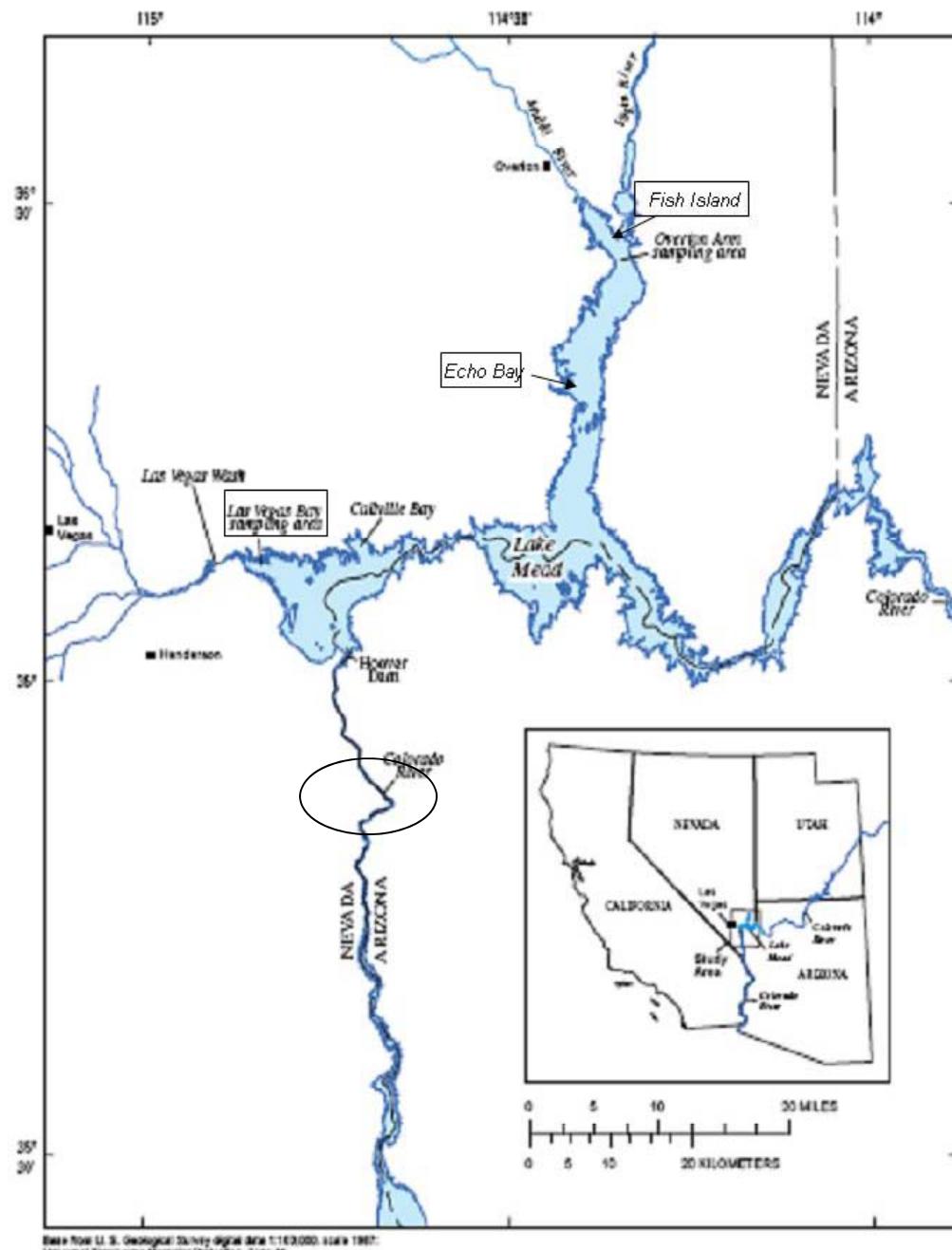
Carp Tissue 2006

Carp male composites contaminants (ng/g ww)

Site	Octachlor ostyrene	total PCBs	Hexa Chloro benzene	trans- Chlor dane	p,p'- DDE	p,p'- DDT	Methyl Triclo san	gamma- BHC (Lindane)	Total PBDE
LVB	3.30	94	0.49	1.59	90.7	3.0	18.5	2.32	118.80
WB	0.45	381	0.27	1.06	44.6	0.9	1.9	0.61	88.4
OA	0.30	16	.	0.42	19.2	0.6	0.30	0.05	21.1
GREGG	.	14	.	0.46	10.0	0.1	0.07	0.03	6.4

Kathy Echols, CERC, USGS



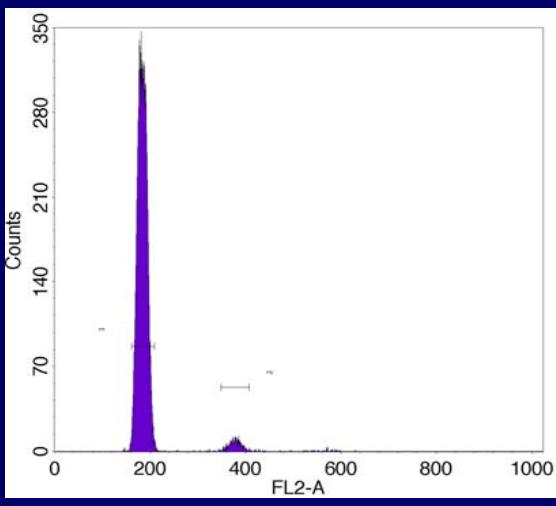
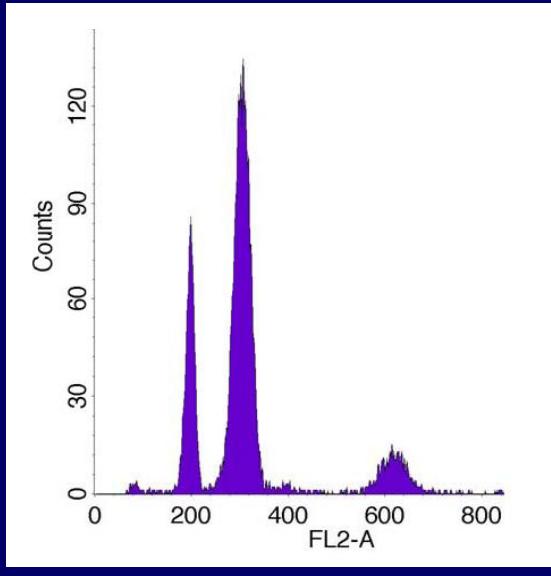
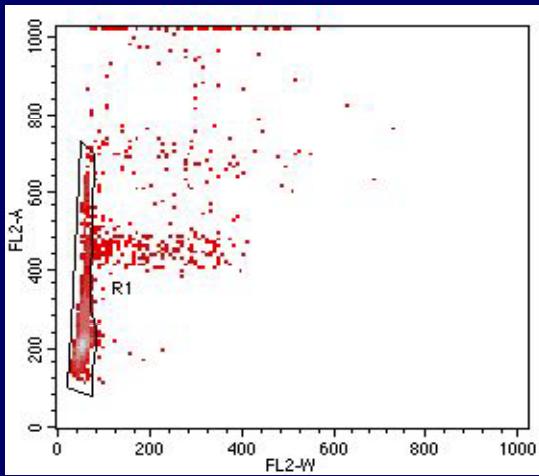
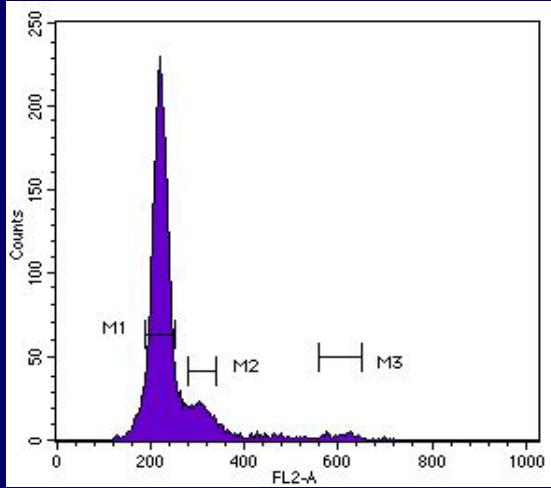


Willow Beach National Fish Hatchery



27 8:42AM

Spermatogenesis/Staging



Summary

Protocols for shipping, handling, and sperm quality analyses have been verified and standardized for carp and razorbacks.

Useful for biomonitoring and genetic conservation.

Multiple biomarkers and species, over more than one year provides biologically relevant information.

Male gamete quality differences exist among sites.

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