Exploring Synthetic Biology in the Great Lakes

Sponsored by the Great Lakes Protection Fund

Organized by the Woodrow Wilson International Center for Scholars, Science, Technology & Innovation Program

April 15, 2013

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Project Background

Synthetic biology has the potential to address and/or solve many of the world's most pressing issues (particularly those surrounding energy, food, and medicine) and potentially move us from a chemical- and petroleum-based economy towards a bio-based economy. As these products and their associated production processes evolve, questions need to be asked about what kinds of information is needed to support rigorous risk assessments, who should collect such data, and whether existing governance systems are adequate to address the risks. Addressing these concerns within the Great Lakes will be complicated given the ecological and political complexities. The Great Lakes share a border with Canada, eight states, including nearly 40 Tribal Nations, more than half a dozen major metropolitan areas, and numerous county and local governments. In addition, within the United States there are at least 10 federal agencies operating under roughly 100 different federal programs. A governance system which can identify potential risks early, address those risks, and adapt as the science of synthetic biology and its applications evolves is critical in order to harness all the potential benefits while minimizing any potential risks to the Great Lakes ecosystem and human communities.

In this first planning meeting held April 15, 2013 participants were presented with Asian carp as a case study for how synthetic biology may be used as an early intervention/control strategy for invasive species into the Great Lakes. The goal was to think strategically and critically about what a governance system could look like that addresses the ecological, political, and regulatory realities of the Great Lakes as it relates to synthetic biology.

Introduction to Synthetic Biology

Peter Carr from the Massachusetts Institute of Technology's Lincoln Laboratory began the day discussing the technical aspects of the safety components for synthetic biology (Appendix A). He divided those into six areas: physical containment, genetic containment, nutritional containment, orthogonal antibiotics (bringing in new chemistry beyond what is found in nature), training and scenario brainstorming and communication and transaction surveillance.

He discussed his definition of what synthetic biology is or could be in terms of bringing engineering rigor to the way we engineer living systems. And bringing other engineering disciplines and ideas into how we think about designing living system, i.e. electrical engineering. He explained that our ability to read and write DNA has outpaced our ability to design and rewire large pieces of DNA and this is where the work of his lab and others is focusing. Part of their research is to enable the complexity and sophistication of what we are able to engineer into living systems by imparting an alternate genetic code, so it no longer reads the same genetic language that it finds in its environment (rE.coli project). One question they are examining is whether they can re-map/restructure the genetic code (orthogonal) different enough so that if the organism picked up DNA from another source, the engineered organism would misinterpret that DNA and its functionality would be lost. This was explained in relation to how a virus is able to infect other cells/organisms based on the common genetic code. These design systems are attempting to address what capabilities an organism could acquire through horizontal gene flow.

Additional questions raised were:

What are the risks of the machinery (rE.coli) being transferred?

- Modest potential, organism would have more trouble if it picked up an additional function. Hard to imagine what new improved function an organism would get if they picked up some of the functionality of the machinery (rE.coli).

The Great Lakes Ecosystem

Alan Steinman from the Annis Water Resources Institute at Grand Valley State University gave an overview of the threats to the Great Lakes ecosystem (Appendix B). These were discussed in terms of the diversity of threats (habitat loss, nonpoint runoff, toxics, invasive species, and development), changing importance over time (diminishing, stable or increasing), differing over location (upper vs. lower lakes), and in terms of multiple stressors and their relative strength which can vary depending on location. He stressed that there is not one simple stressor that we need to focus on, they change over time, and differ over locations and that there needs to be a holistic approach.

Specific issues of concern discussed:

- Land use change
- Most of the pollution is coming from non-point sources,
    - Large or small-scale (homes).
    - Harmful algal blooms
- Over 180 aquatic invasive species (ones we can see)
    - Microbial communities?
    - Changes food webs and bioenergetics
    - Prey fish are declining as zebra mussels are increasing
- Profound changes in the way the ecosystem functions (benthification) and the economics (stocking, fishing etc.) surrounding those food webs.
- Areas of concern, toxic hotspots.
    - Hundreds of millions of dollars focused on AOCs.

Dr. Steinman also gave an overview of the Great Lakes Environmental Assessment & Mapping Project (GLEAM) which mapped multiple stressors (current) across the Great Lakes and developed ratings based on the particular stressor to create a cumulative stressor map. Alan suggested that if synthetic biology could be a tool he would support that.

Additional questions raised were:

- Synergistic effects, prediction models, how can we predict stressors and what are the food web disruptions?
    - Could synthetic biology be used to address these?
- What are the species we want to disrupt with synthetic biology?
- How can we predict these stressors at large are causing the loss of system resilience?
- Do you want a vibrant ecosystem or to go back to the natural?
- Scope and rate of dissemination of stressors, and what's the impact on resilience and likelihood of causing an outbreak?

Legal Frameworks

Noah Hall from Wayne State University provided a legal analysis of the regulatory and legal frameworks that may govern synthetic biology in the Great Lakes. He focused primarily on upfront approvals on the federal level, challenges to federal actions (states and citizens), and post-action liability.

Hall's Bottom-Lines were:

- Current regulations are not a barrier to the deployment of synthetic biology (federal actions), based on the coordinated framework
- Huge potential road blocks in terms of challenges to federal actions however
- Not a major concern for post-action liability

Coordinated Framework of 1986

- Genetically altered things are not presumed to be more dangerous than their natural counterparts, a presumption that synbio applications are not more dangerous
- USDA focuses on agriculture, EPA on pesticides, FDA on food safety/drugs
- 1991 supplemented with intentional releases, vast majority pose no additional risk

FIFRA

- Synbio solution to control a pest. Asian carp could meet the definition of a pest
- Focuses on private sectors use, not necessarily on regulating federal government actions
- Would have to list Asian carp as a pest (not done), and list synbio app as a pest (not done)
- USDA Plant pest protection act, could regulate if they wanted to. Same holds for FDA (adultered drug)
    - Glow fish as example, all agencies chose not to regulate
- If the government wants to regulate it has all the legal authority to do so if they choose

Challenges to Federal Actions

NEPA

- EIS, any federal deployment would trigger this, unless exempted by Congress for the solution (congress could direct an agency to do this)
- EIS could be used to stop or delay the action by providing information to be used

Clean Water Act

- Synbio regulated as a point source
    - Depends on the physical release
    - Fish released from fish ponds is considered a point source, fish introduced by a person is not
    - Carp are considered a pollutant?
    - Would require a CWA permit
- State 401 certification
    - Gives the state authority to certify federal action
    - Need to define uses of water bodies
    - Need water quality standards and criteria for biological pollution
    - Could consider ESA depending on the results of the EIS

Boundaries Water Treaty

- Synbio would be considered a pollutant
- Congress would have to create an exemption in order for States to use synthetic biology or create a governance system to regulate it.

Additional questions raised were:

Is synthetic biology considered the same as GM?

Is there a greater commerce clause risk on biologics (biological pollution) vs. others?

- Individual State bans on glow fish, and importation on biological pollution is a valid discrimination against interstate commerce. However it assumes a commercial application of synthetic biology.

Who owns the salmon?

- Not owned, it’s managed as a state resource (trustee)

What would a water quality standard look like for an invasive species?

- Invasive species are treated like a chemical pollutant not a pollutant that reproduces.

The bio-economy – economic potential of synthetic biology in the Great Lakes

Mark Bunger from Lux Research discussed the economic and investment landscape and how they are developing a technology road map for synthetic biology (Appendix C). Companies are interested in replacing networks of genes. Mark's data suggests that venture investment has peaked and is now declining, however he stressed that this is a natural cycle as the technology matures. Companies are moving towards getting away from petroleum based products. One reason suggested was because countries that produce oil are moving into producing their own chemicals and products based on petroleum.

He presented data using social network analysis, which showed the connection/collaborations between companies. The larger the connections between companies, the more mature the field. One question raised was, what's the impact to the great lakes region? By looking at the connections between firms (nodes) the data suggests that it won't be one thing but a series of issues linked together (nodes). By developing a technology road map for synthetic biology in the great lakes, what does it mean if you categorize an entire organism as a pesticide? It's an interesting question for a road map. It moves you towards observational science and the potential to analyze ecosystem level manipulation by incorporating the organism, stressor, and ecosystem.

The Asian Carp – Biology and Physiology

Kelly Baerwaldt from the U.S. Army Corps of Engineers discussed some of the biological traits and monitoring programs for Asian Carp. They have found that Asian carp have multiple spawning periods, up to four times a year, up to 400 million eggs (one female). It was suggested that vigilance is needed for a good monitoring program based on the vast coverage of the carp and the areas that are natural reproduction zones. Particularly the smaller sized fish. She discussed the various monitoring efforts underway: commercial netting, electrofishing, telemetry (tagged fish), environmental DNA (eDNA). Based on their monitoring program no big head or silver carp have been captured above the electric barrier (Appendix D) in 2011 and 2012.

Environmental DNA Testing Program

What does the eDNA data mean?

- Where did it come from
- How long has it been there
- Did more than one carp contribute to the sample
- It provides a yes/no answer
- Used as a data source, not a response mechanism
- Takes two weeks to get results
- Are new testing methods/equipment needed
- Could you crowd source the program if better tests were developed

    - eDNA kits
    - possible iGEM team project
- Need correlations of eDNA "hits" to whether or not there are actual fish in the location

What selection factors to go after?

- 30 sub samples, they are analyzing for selection factors
- Are there weak spots we can go after?

Additional questions raised were:

Are there geographically distinct sub-populations?

What is the predation rates in Asian carps native environments?

- Not a lot, humans; mostly based on size
- How many fish do you need to fish to see an effect?
    - Need to fish all size classes
- What is the level of predation?
    - Eggs vs. fry

What are the spawning requirements for the eggs?

- Need flowing water
- Spawn in flowing water
- Swim vertically after hatch
- Area of research they are exploring

What are the Plankton requirements?

- Phytoplankton productivity
    - Is there a point where they can no longer be sustained based on carp?
    - Carp may be moving north because of lack of phytoplankton?
    - Carp's versatility is one worry if they get to Great Lakes
    - Can you target areas based on phytoplankton?

What are the Habitat Issues?

- Why are carp not moving up the Mississippi river?

Michael Jawson (Appendix E) from the U.S. Geological Survey discussed research being conducted on control strategies in relation to the biology of Asian carp. He started by explaining that Asian carp are lake fish that need flowing water to reproduce. One important point that arose from the discussion was that Asian carp have not been fully sequenced. Another major issue that was raised is the apparent hybridization of Asian carp with Silver carp, concerns over the purity of the species and whether a phylogenetic tree may be needed. He also presented data on the micro-biome of carp identifying bacterial species unique to silver, bighead and grass carp. They are looking into developing markers to identify these unique bacteria and found 773 unique species of bacteria that live on silver carp.

Oral delivery technologies

- Selective control agents (synbio could potentially help here)
    - Scientists have developed agreements to access pharmaceutical or agrochemical company chemical libraries to identify potential candidate toxicants in order to target compounds that are currently in use to reduce costs and time required to obtain full registration
    - A literature review of known fish toxicants is in progress to develop structure-activity relationships (SAR) to understand what characteristics of fish toxicant molecules cause the toxicity. This will be an ongoing effort through the spring and summer.
    - A salt formulation of GD-174 (a chemical fish toxicant) has been synthesized by collaborators at Viterbo University
    - Tests to confirm the toxicity and specificity of the chemical to carp will be conducted by USGS in mid- to late spring
    - Doesn't need to be a chemical, could be a bacteria for example
- Selective uptake
- Selective release within species
    - Carp have higher levels of trypsin
    - Could the delivery be triggered by trypsin
- Does the delivery method matter in terms of approval or is it based solely on the delivered component (chemical, drug, etc.)
- Attractants

Databases

A searchable database of >200 compounds has been developed and is being analyzed to identify common structural components and correlations with activity

- Having the database will allow us to determine structural characteristics of current piscicides to screen other chemical databases to identify potential piscicides
- Having access to silver carp and bighead carp cell lines will allow us to more rapidly screen chemicals to look for cytotoxic effects
- If candidates are identified they must be registered with USEPA before they will be broadly available
- The tool is intended to allow other scientists to analyze the database for chemical characteristics that might predict chemical toxicity

- The intent of this project is to work with industry to identify new candidate chemicals to evaluate as piscicides. The next phase of this project would be to transition to the identification of cellular communication proteins that could be developed as targets for vaccine development

Additional questions raised were:

What is the growth rate of carp?

Could you develop a system to slow the metabolism down?

Synthetic Biology Solutions – Realities and Potentials

The timelines of what's needed for carp don't match with the maturity of synthetic biology at the moment. Need to close the gap between our understandings of the organism vs. the biology of the carp, i.e. eggs, behavior, growth rate etc. Whole genome sequencing of the Asian carp is an immediate need, along with comparative genomics of native carp species and invasive carp species to develop unique target receptors in the invasive species. Do you need a living delivery system? Could you engineer a bacteria or virus vs. engineering the carp? If you were to use a virus, what are the pathogen host interactions?

The question of what is the ideal situation with Asian carp was a theme that closed out the meeting? Are we talking about complete elimination or a manageable population of Asian carp? Are we past the point of natural systems? Do we need to think about the invasive species problem from a more managed systems perspective? Where do these organisms (invasive or synthetic) fit into the managed system? It was suggested that we think of these issues from an ecosystem management perspective and not species management. The focus should be on ecosystem services, not necessarily just based on native species.

Potential Areas of Research

1. Explore the micro-biome of the carp and how this might be used to develop bio-control strategies.
2. Further develop eDNA program to include better testing kits, possible distribution for a crowd sourced collection program, encourage iGEM teams to tackle this problem.
3. Legal frameworks
4. Development of a risk assessment framework
5. Public perception of the issue, who participates in this type of bio-control decision?
6. Technology road map for synthetic biology in the Great Lakes region

Participant List

Allen	John	Michigan Governor's Office
Amaral	Luis	Northwestern University
Baerwaldt	Kelly	US Army Corps of Engineers
Buchsbaum	Andy	National Wildlife Federation
Bunger	Mark	Lux Research
Carr	Peter	MIT
Hall	Noah	Wayne State University
Jawson	Michael	USGS
Jewett	Michael	Northwestern University
Kuiken	Todd	Woodrow Wilson Center
Kuzma	Jennifer	University of Minnesota
Leonard	Joshua	Northwestern University
Pfleger	Brian	University of Wisconsin-Madison (GLBRC)
Rankin	David	Great Lakes Protection Fund
Rejeski	Dave	Woodrow Wilson Center
Sharpe	Leah	NOAA
Steinman	Alan	Grand Valley State University
Swackhamer	Deborah	University of Minnesota
Tyo	Keith	Northwestern University
VanHerik	Russell	Great Lakes Protection Fund

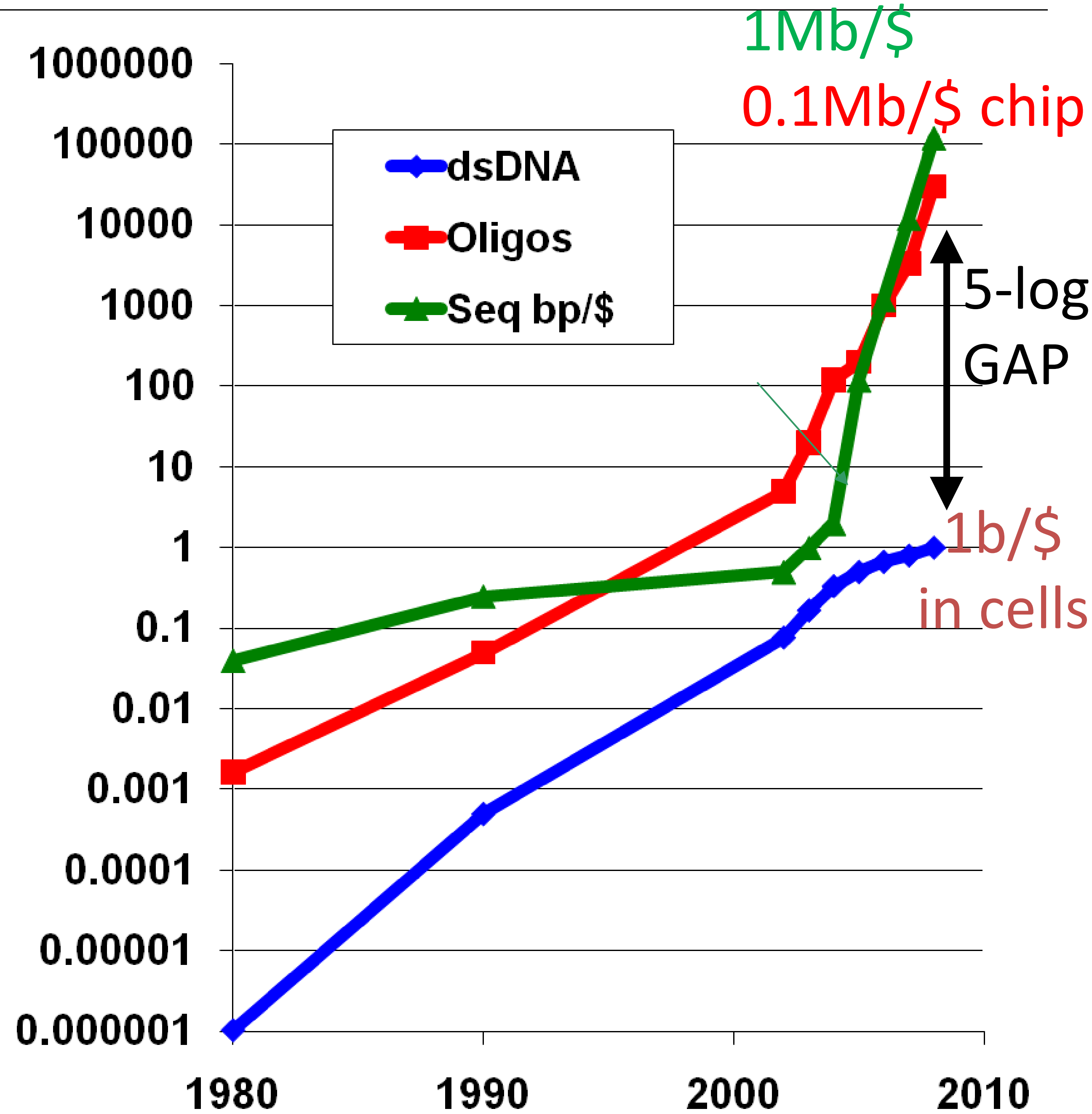
Appendix A

Peter Carr Presentation

Genomes Reading & Writing

Moore's law =1.5x/yr vs 10x/yr

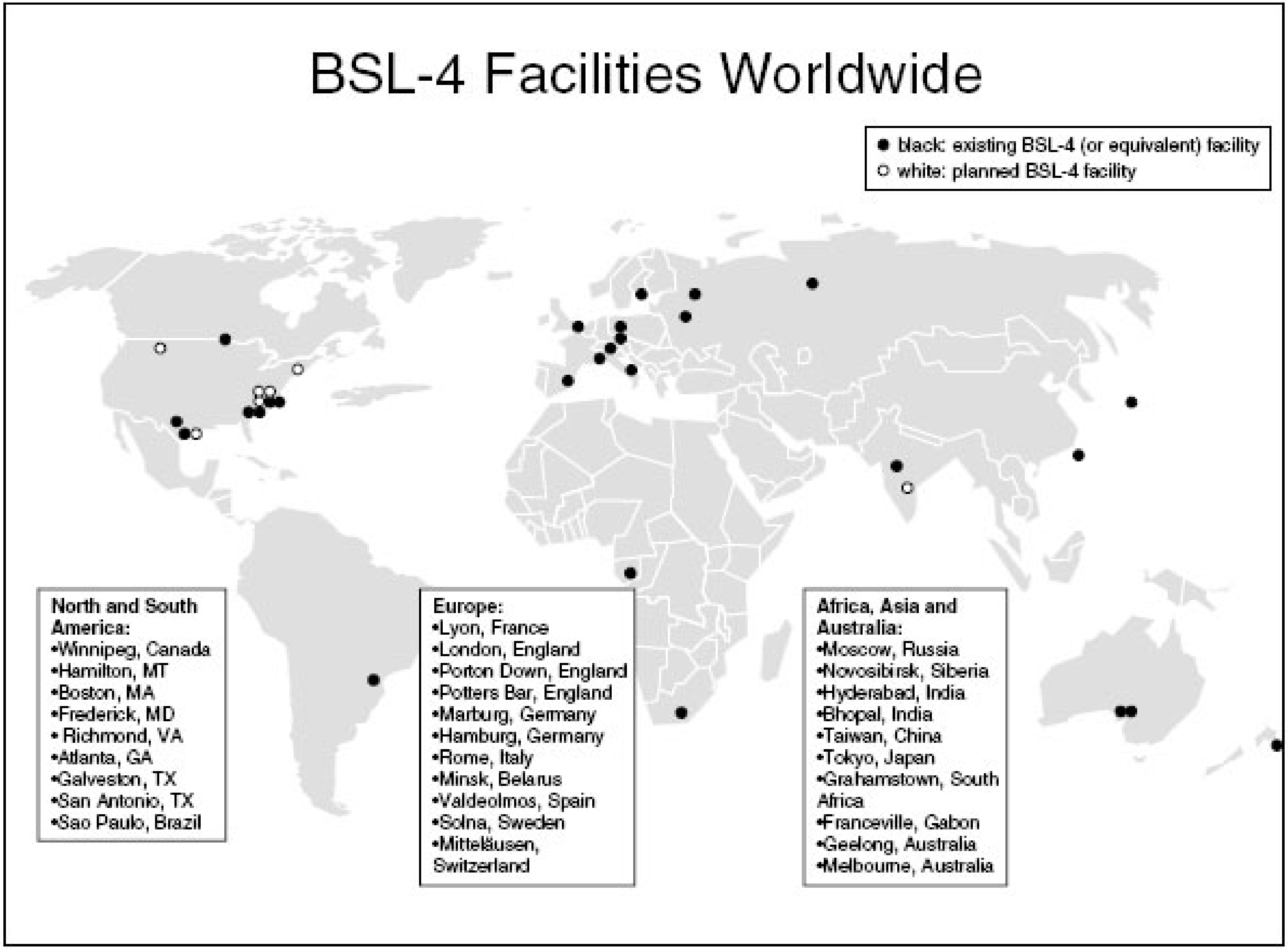
Accelerating exponentials



Safety Components for Synthetic Biology

1. Physical containment (e.g. BSL1-4)
2. Genetic containment
3. Nutritional containment
4. Orthogonal antibiotics
5. Training & scenario brainstorming
6. Communication & transaction surveillance

1. Physical Containment:



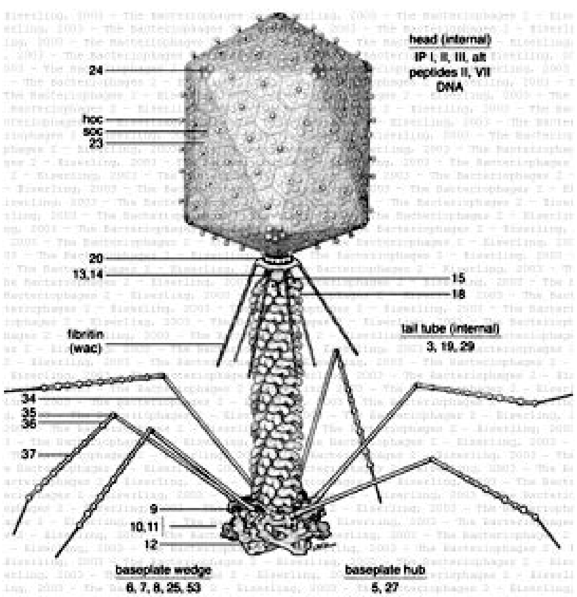
Marburg, Ebola, Lassa, Crimean-Congo hemorrhagic fever, Smallpox

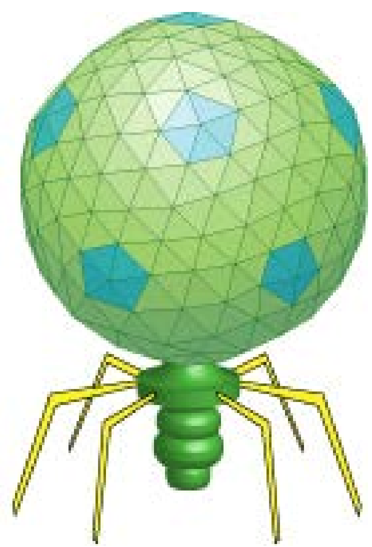
2. Genetic Containment

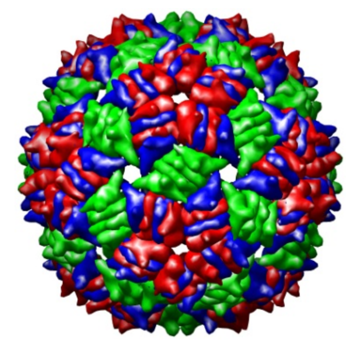
Resistance to all viruses & other horizontal transfers

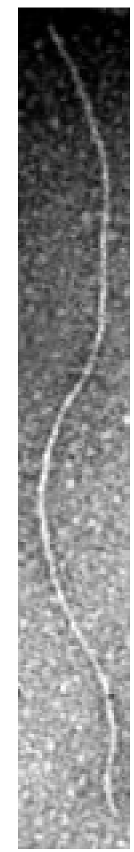
by changing genetic code of host

Freeing 10 of 33 tRNAs & 1 of 3 release factors
= 13 of 64 codons = 157,307 bp changes
= 3.4 % of the genome.

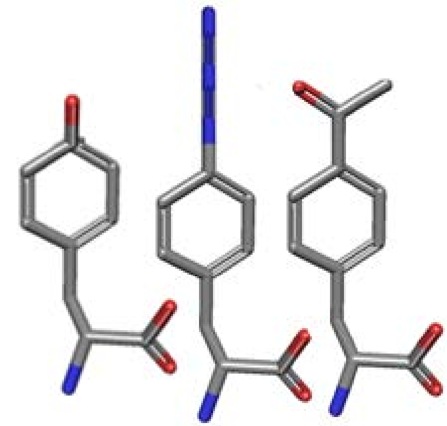








3. Nutritional Containment



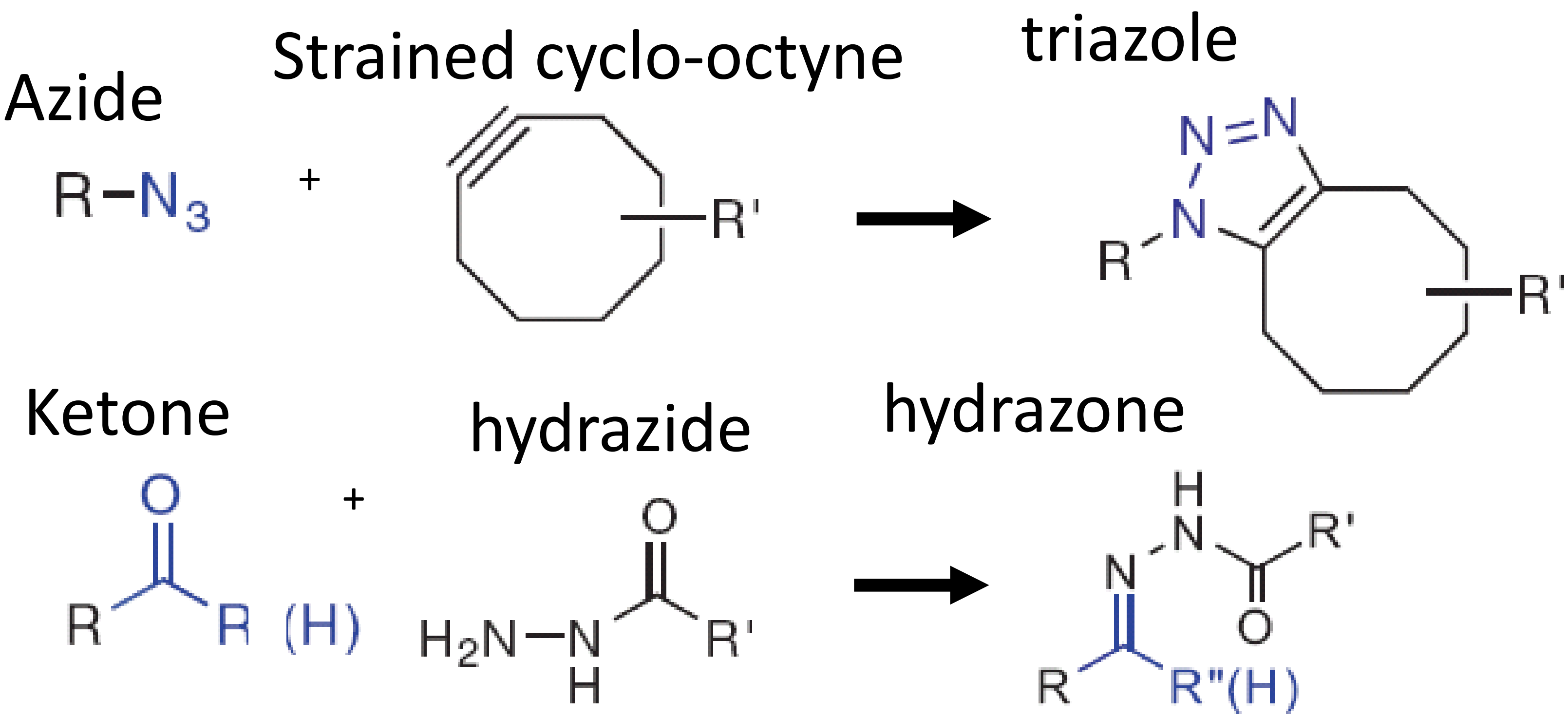
DAP (Curtiss 1976)
(rare, but present in the environment)

Phenylalanine, Tyrosine (F, Y)
p-Azido-phenylalanine (pAzF)
p-Acetyl-phenylalanine (pAcF)

Select for essential genes ‘addicted’ to novel amino acids.

4. Orthogonal Antibiotics

(ideally inexpensive)



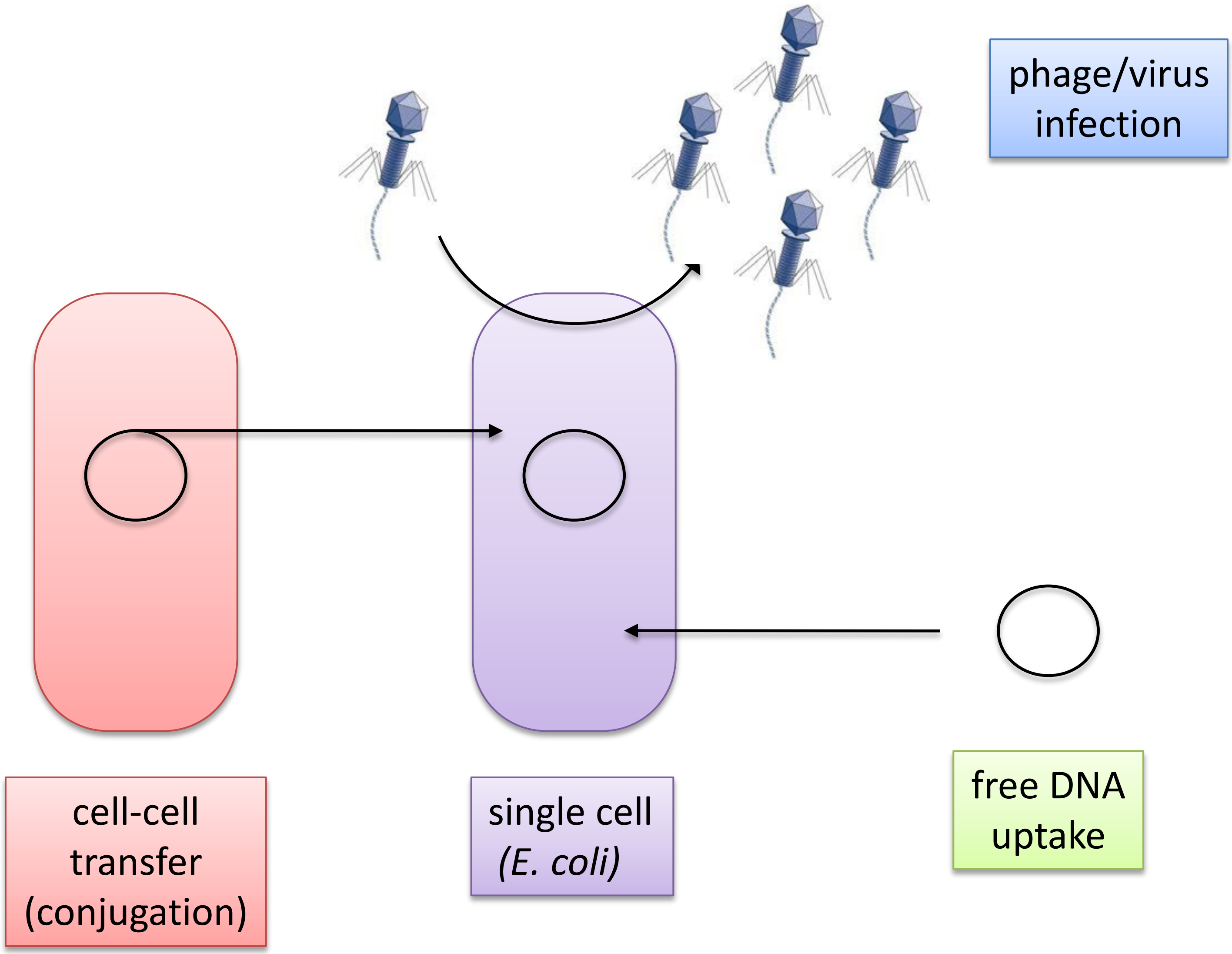
e.g. PEG-pAcPhe-hGH (Ambrx, Schultz) higher serum stability

Prescher, JA & CR Bertozzi (2005) Nature Chem Biol. Chemistry in living systems

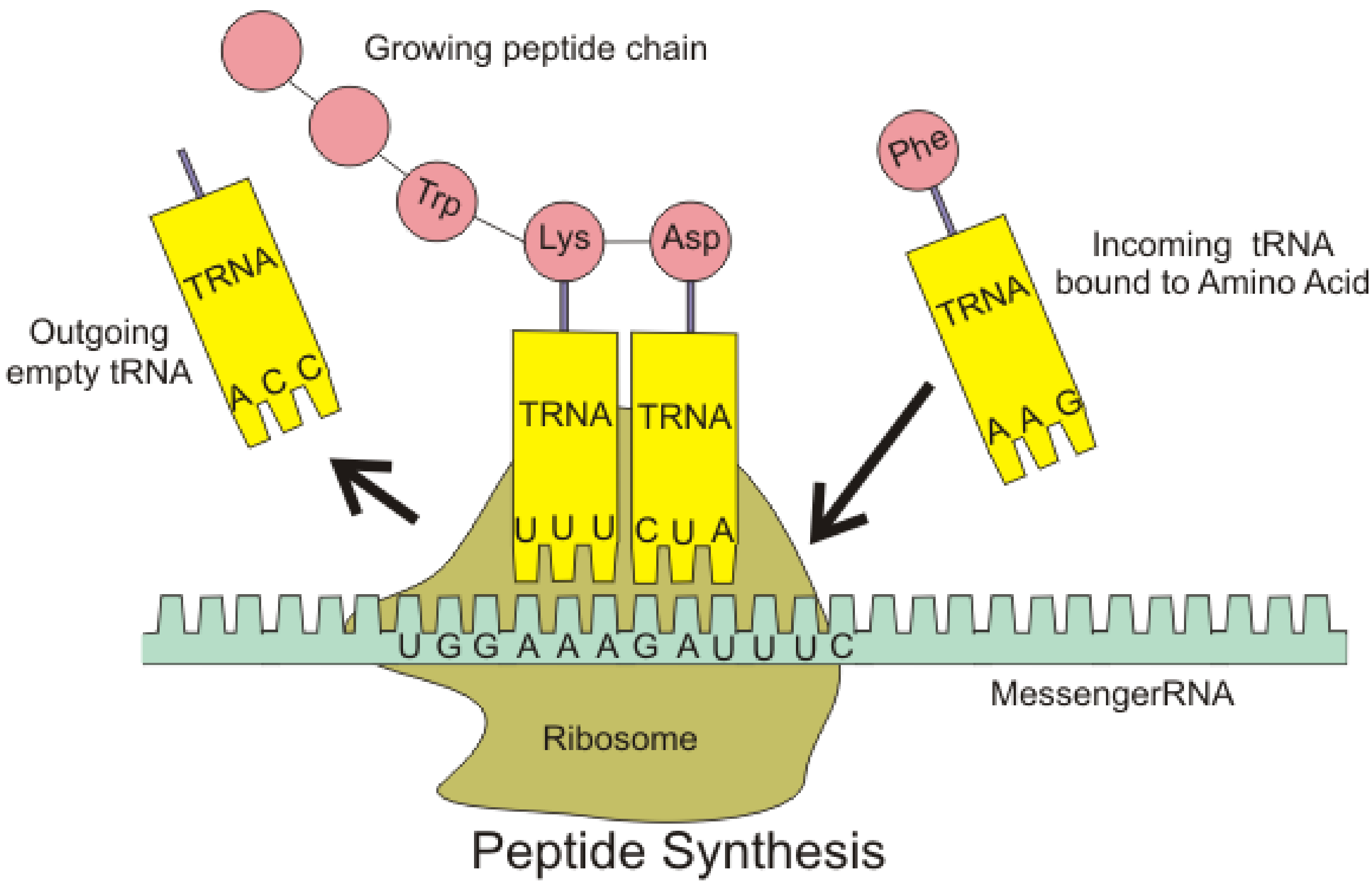
Safety Components for Synthetic Biology

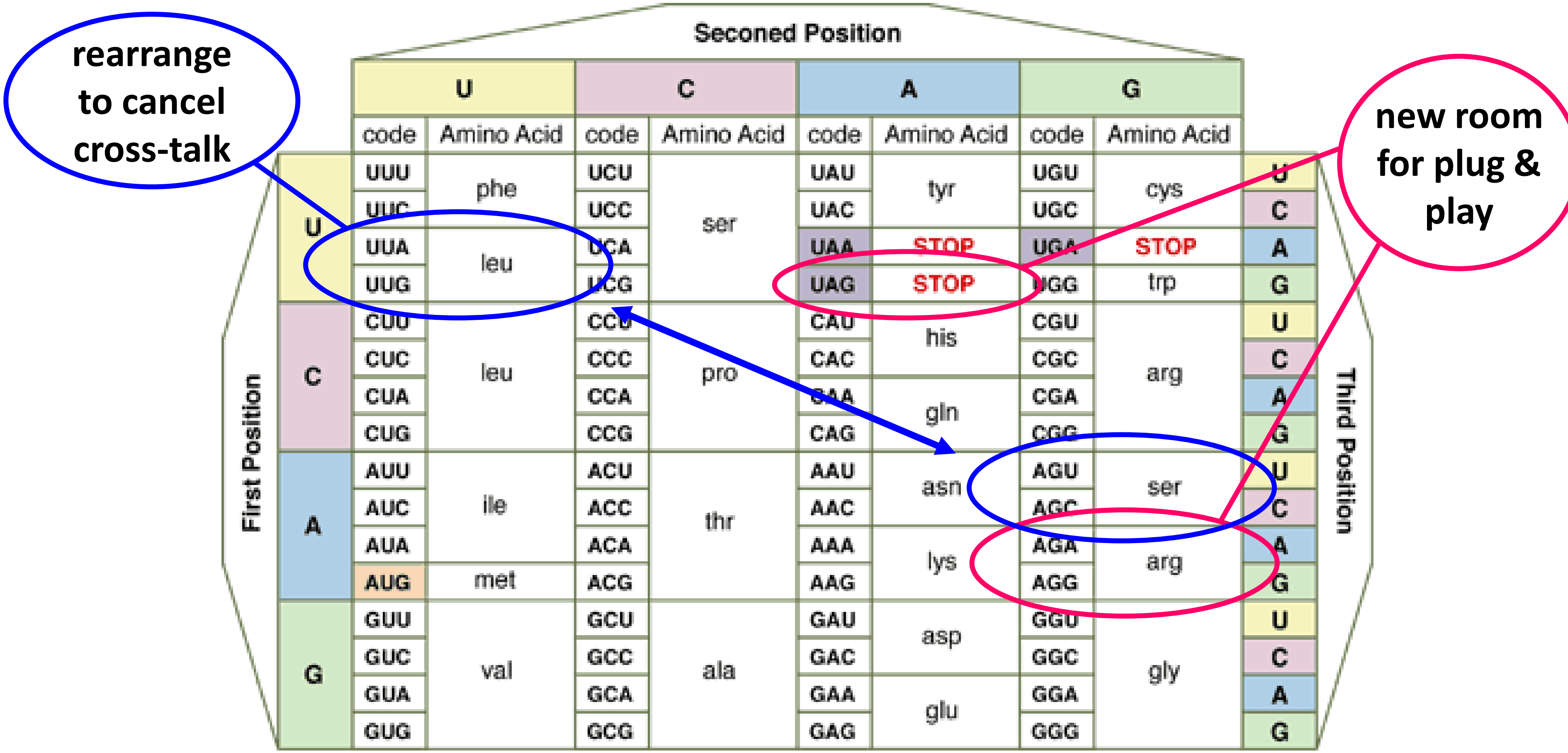
1. Physical containment (e.g. BSL1-4)
2. Genetic containment
3. Nutritional containment
4. Orthogonal antibiotics
5. Training & scenario brainstorming
6. Communication & transaction surveillance

Horizontal Gene Transfer



Basic Translation

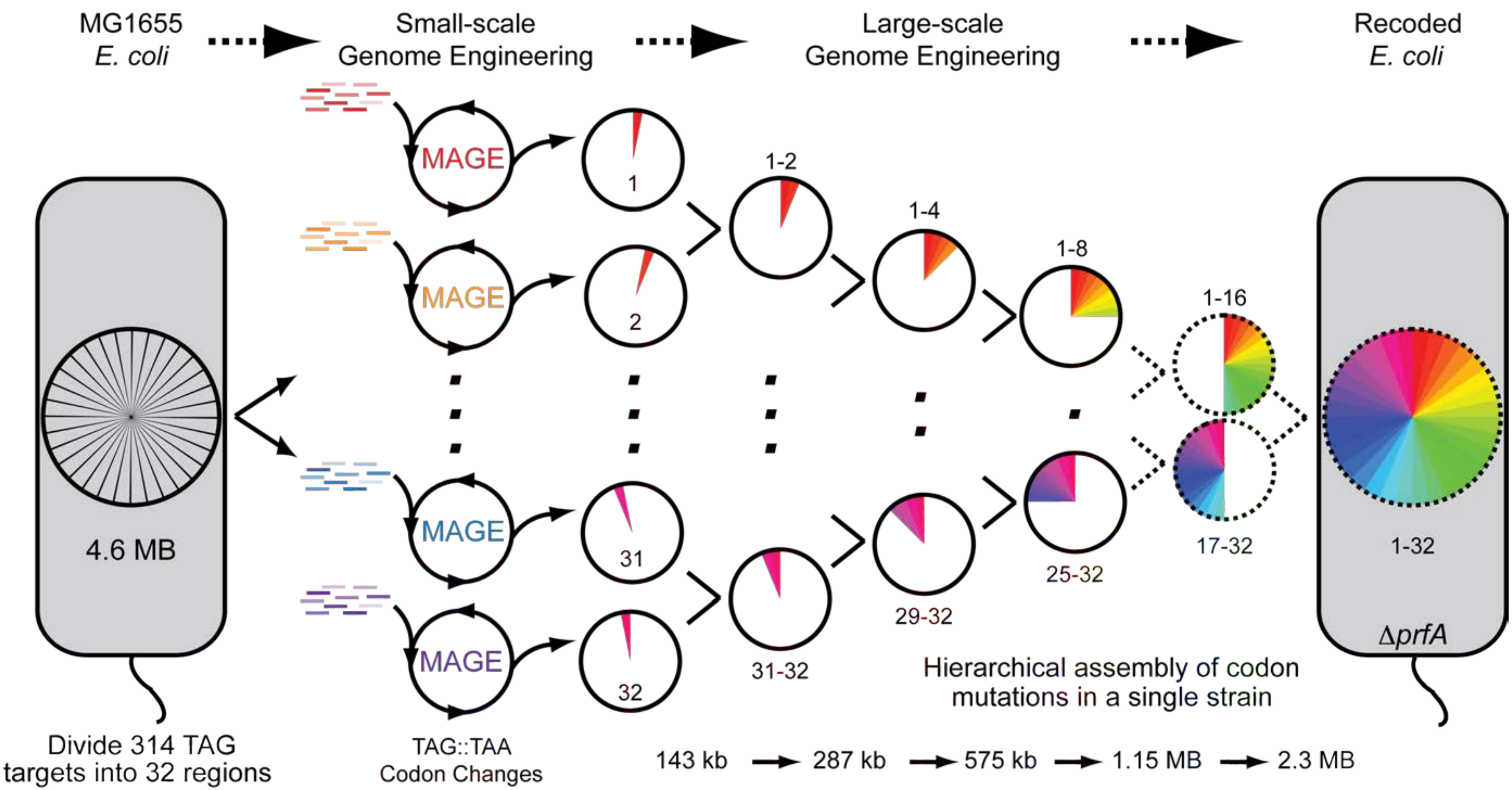




The genetic code is:
- fixed
- tolerant to mutation
- shared by “all” life

Can we make it...
- flexible, easy to engineer?
- more controlled?
- unique to one organism?

rE. coli 1.0 Construction and Status



Isaacs et al. (2011) *Science*

Appendix B

Alan Steinman Presentation

The Great Lakes Ecosystem

Alan Steinman, Ph.D.

Annis Water Resources Institute, GVSU

Great Lakes

- ~ 90% of US surface fresh water
- ~ 40 million people reside in the Great Lakes basin
- Source of drinking water, transportation, recreation, manufacturing, aesthetics, wildlife habitat

Threats to the Lakes: An Overview

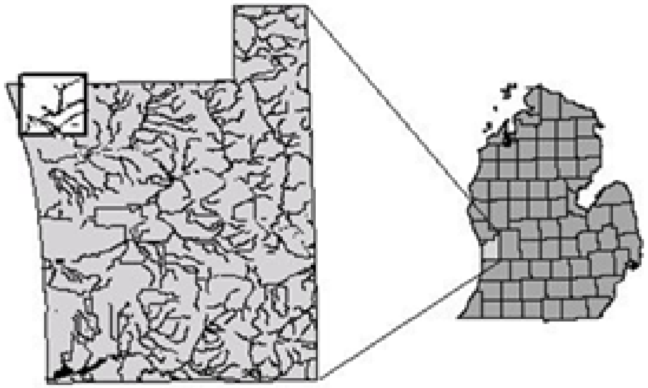
- Diverse
  - Nonpoint runoff, toxics, invasives, development
- Changing in importance over time
  - May be diminishing, stable, or increasing
- Differ by location
  - E.g., upper vs. lower lakes
- **Multiple stressors are at work, and their relative strength varies from place to place**

Modified from Dave Allan, UM

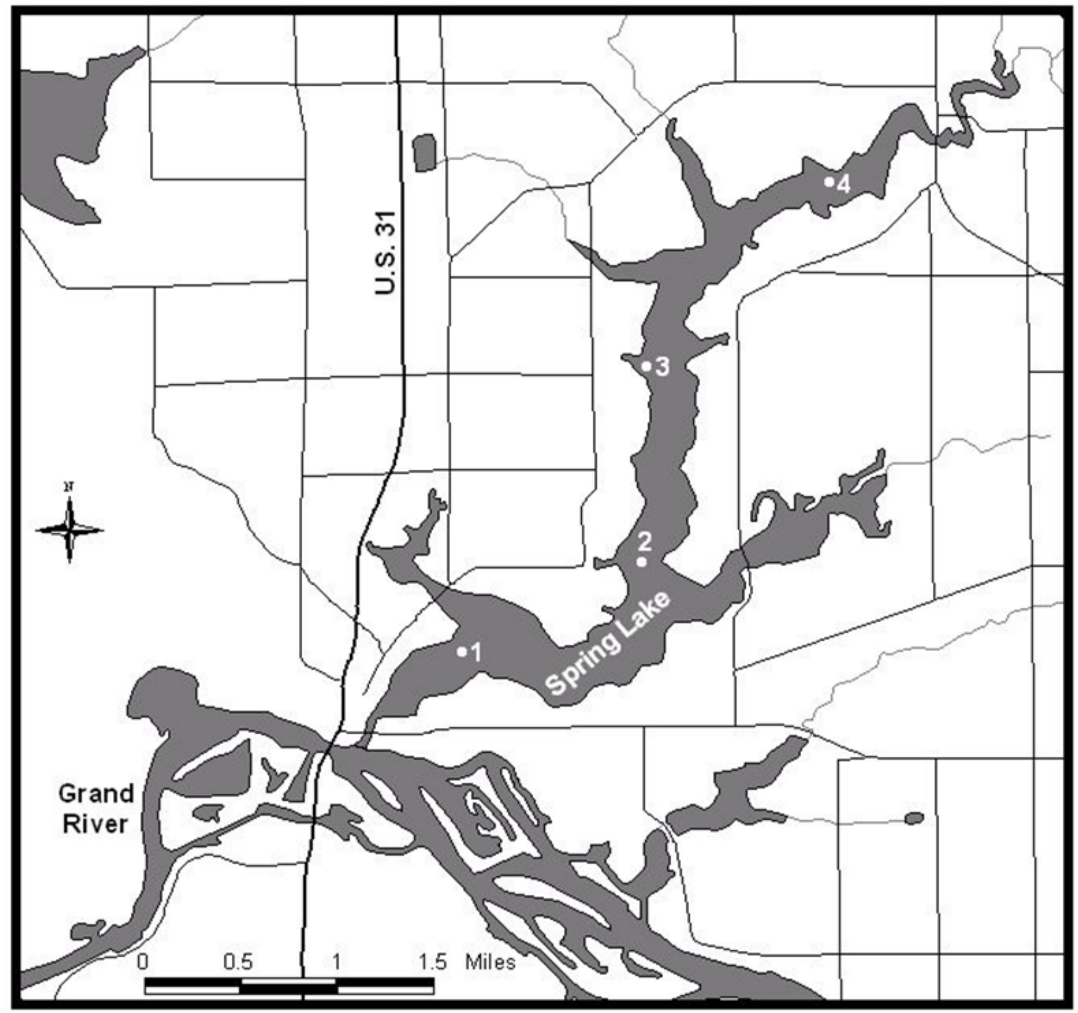
Today's Overview

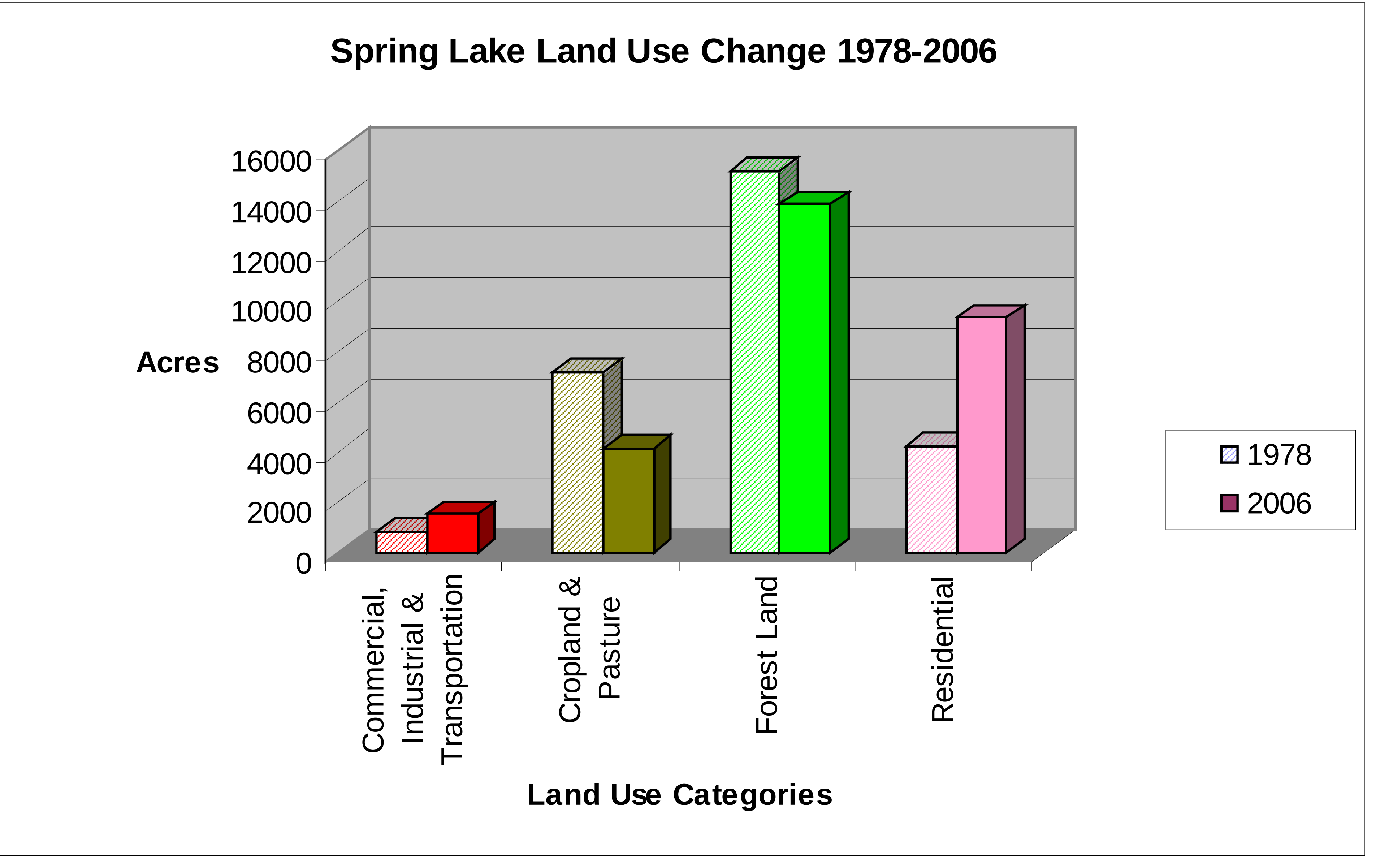
Aquatic Habitat Loss

- Changing land use
- Impacts to surface and ground water quality and quantity
- Loss of critical habitat (wetlands, riparian areas) and biodiversity



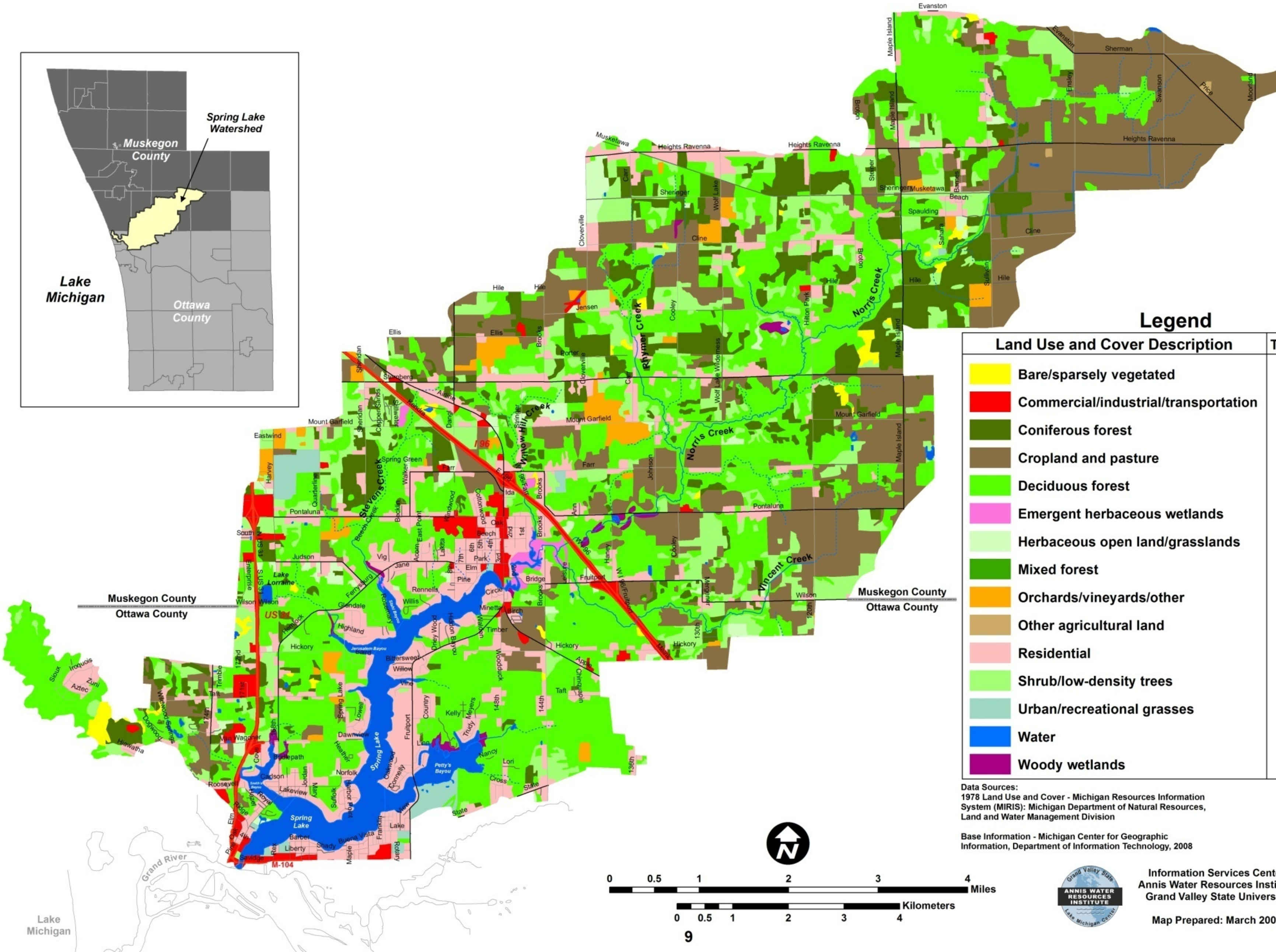
Spring Lake, Michigan





Spring Lake Land Use Change 1978-2006
Acres
16000
14000
12000
10000
8000
6000
4000
2000
0
Commercial, Industrial & Transportation
Cropland & Pasture
Forest Land
Residential
Land Use Categories
1978
2006

1978 Land Use and Cover



Legend

Land Use and Cover Description	Total Acres	%
Bare/sparsely vegetated	237	0.7
Commercial/industrial/transportation	838	2.5
Coniferous forest	3,267	9.7
Cropland and pasture	7,176	21.2
Deciduous forest	11,887	35.1
Emergent herbaceous wetlands	127	0.4
Herbaceous open land/grasslands	479	1.4
Mixed forest	0	0.0
Orchards/vineyards/other	598	1.8
Other agricultural land	13	0.0
Residential	4,221	12.5
Shrub/low-density trees	3,414	10.1
Urban/recreational grasses	286	0.8
Water	1,144	3.4
Woody wetlands	131	0.4

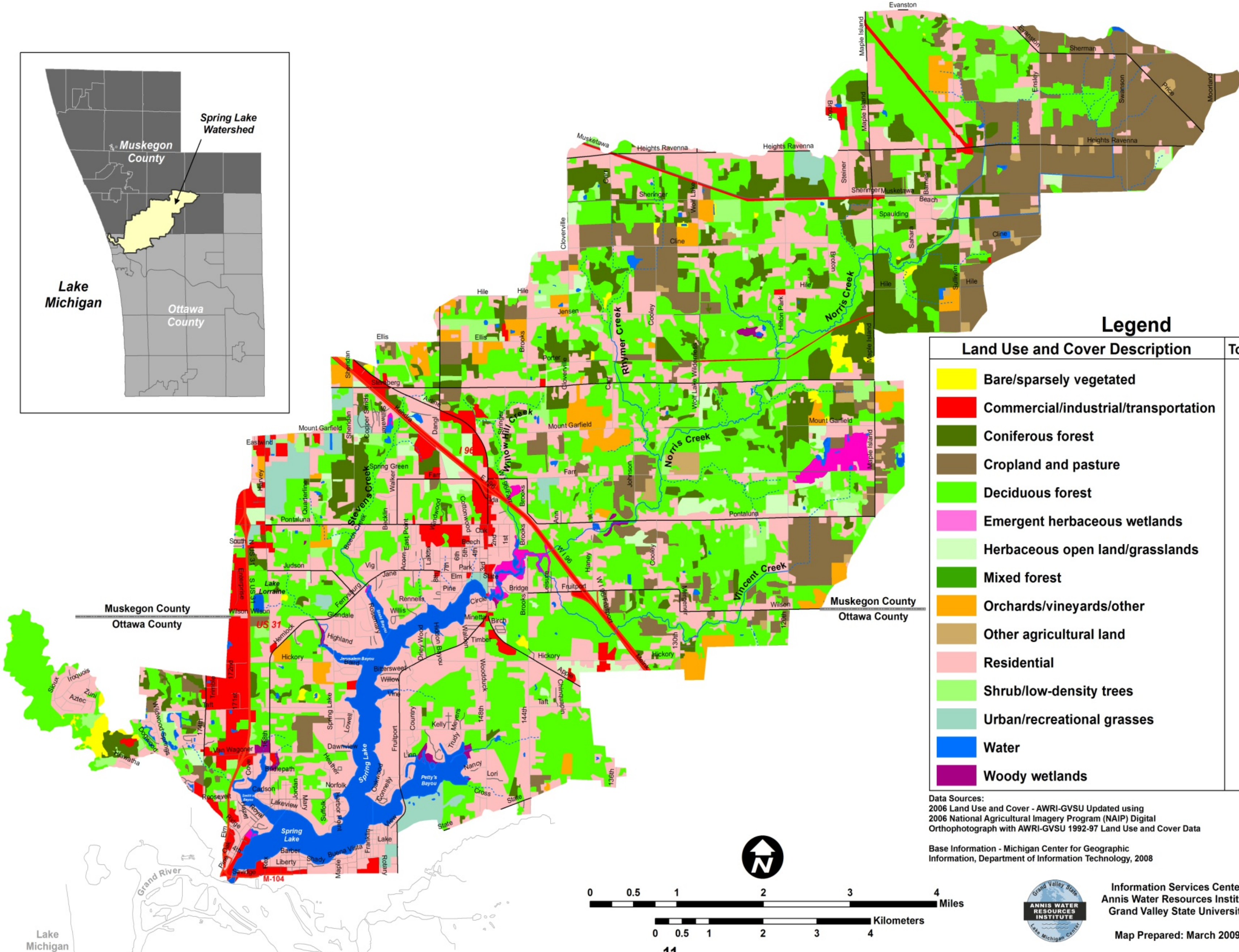
Data Sources:
1978 Land Use and Cover - Michigan Resources Information System (MIRIS): Michigan Department of Natural Resources, Land and Water Management Division

Base Information - Michigan Center for Geographic Information, Department of Information Technology, 2008

Information Services Center
Annis Water Resources Institute
Grand Valley State University

Map Prepared: March 2009

2006 Land Use and Cover



Legend

Land Use and Cover Description	Total Acres	%
Bare/sparsely vegetated	184	0.5
Commercial/industrial/transportation	1,504	4.4
Coniferous forest	2,643	7.8
Cropland and pasture	4,140	12.2
Deciduous forest	11,186	33.1
Emergent herbaceous wetlands	247	0.7
Herbaceous open land/grasslands	755	2.2
Mixed forest	5	0.0
Orchards/vineyards/other	834	2.5
Other agricultural land	214	0.6
Residential	9,343	27.6
Shrub/low-density trees	963	2.8
Urban/recreational grasses	523	1.5
Water	1,187	3.5
Woody wetlands	92	0.3

Data Sources:
2006 Land Use and Cover - AWRI-GVSU Updated using
2006 National Agricultural Imagery Program (NAIP) Digital
Orthophotograph with AWRI-GVSU 1992-97 Land Use and Cover Data

Base Information - Michigan Center for Geographic
Information, Department of Information Technology, 2008

0 0.5 1 2 3 4 Miles
0 0.5 1 2 3 4 Kilometers

Information Services Center
Annis Water Resources Institute
Grand Valley State University

Map Prepared: March 2009

Nonpoint Sources of Pollution

- Excess fertilizers, herbicides, and insecticides from agricultural lands and residential areas
- Sediment from improperly managed construction sites, crop and forest lands, and eroding streambanks
- Livestock, pet wastes, faulty septic systems, atmosphere
- Oil, grease, and toxic chemicals from stormwater runoff


06 . 17 . 2005

Harmful Algal Blooms (HABs)

- Microcystin is the most common cyanotoxin → hepatoxin and tumor promotor.

- The U.S. currently has no national standards for exposure to microcystin or other algal toxins.

- <u>WHO standards:</u>

- drinking water: 1 μg/L
- recreational: 20 μg/L

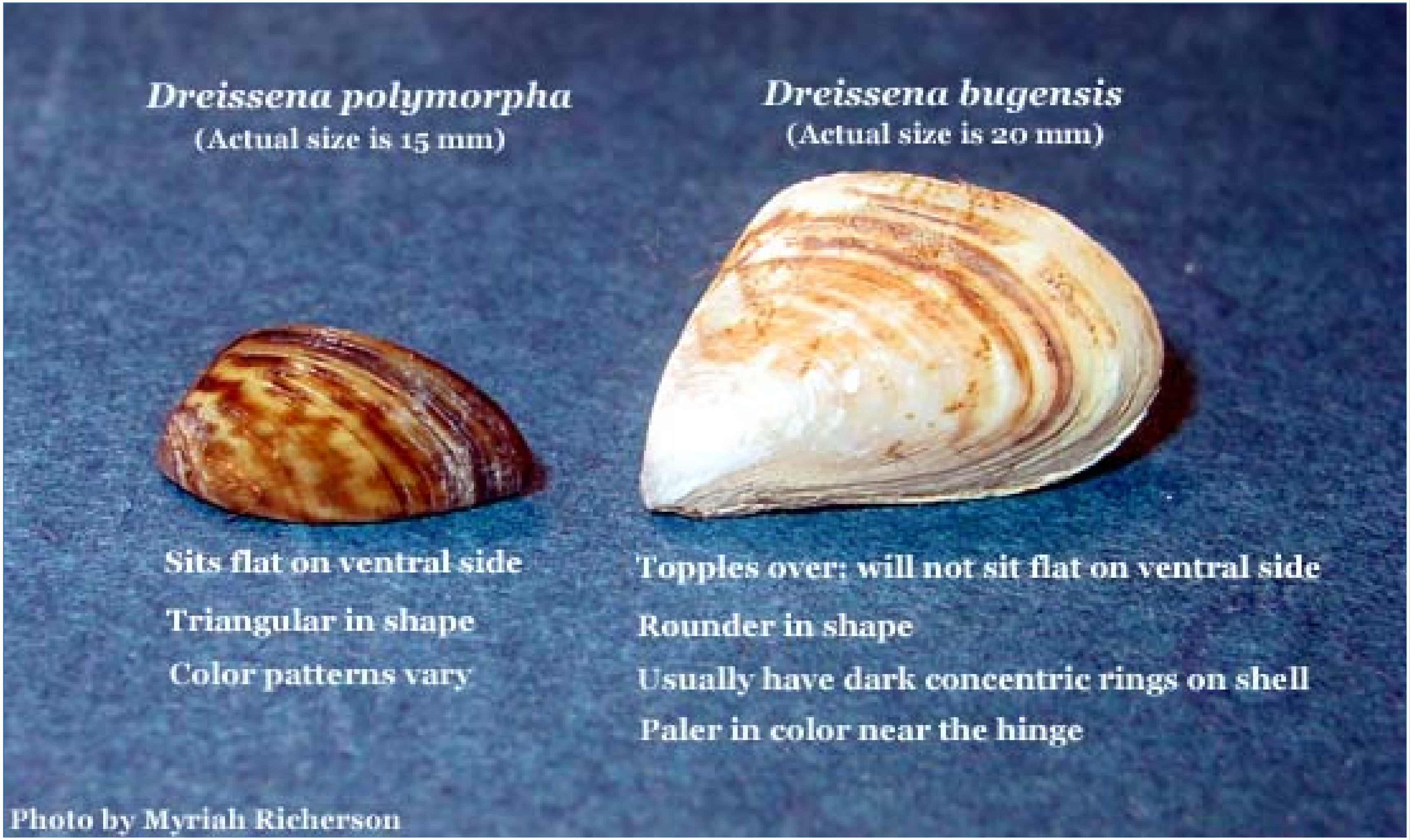
Western Lake Erie, October 2011

Photo Credit: NOAA

Invasive Species

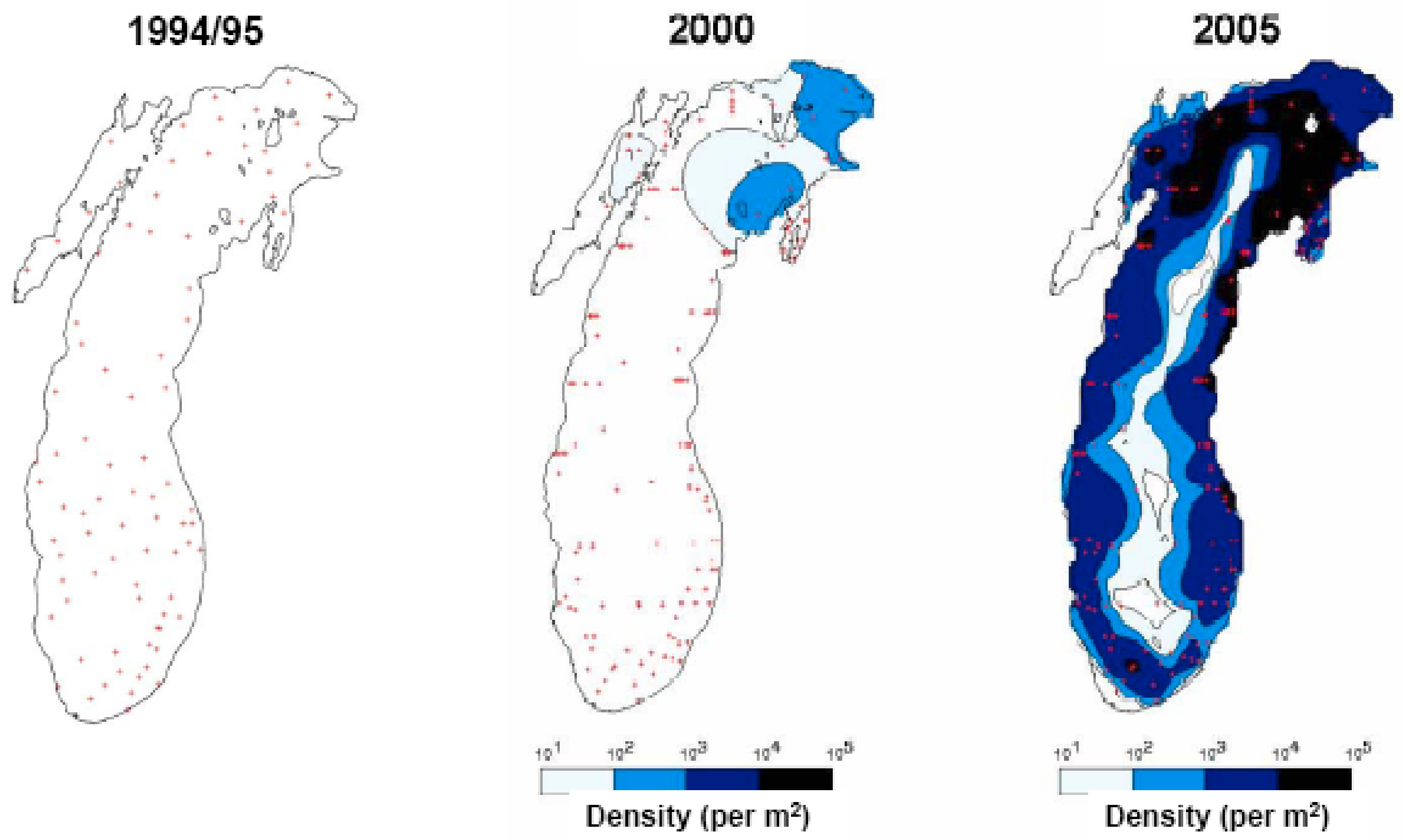
- > 180 aquatic invasive species to Great Lakes
- changes in food webs and bioenergetics
- economic costs

Zebra mussels Quagga mussels

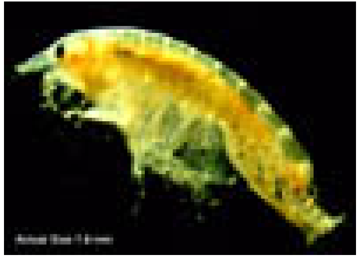


Credit: USGS

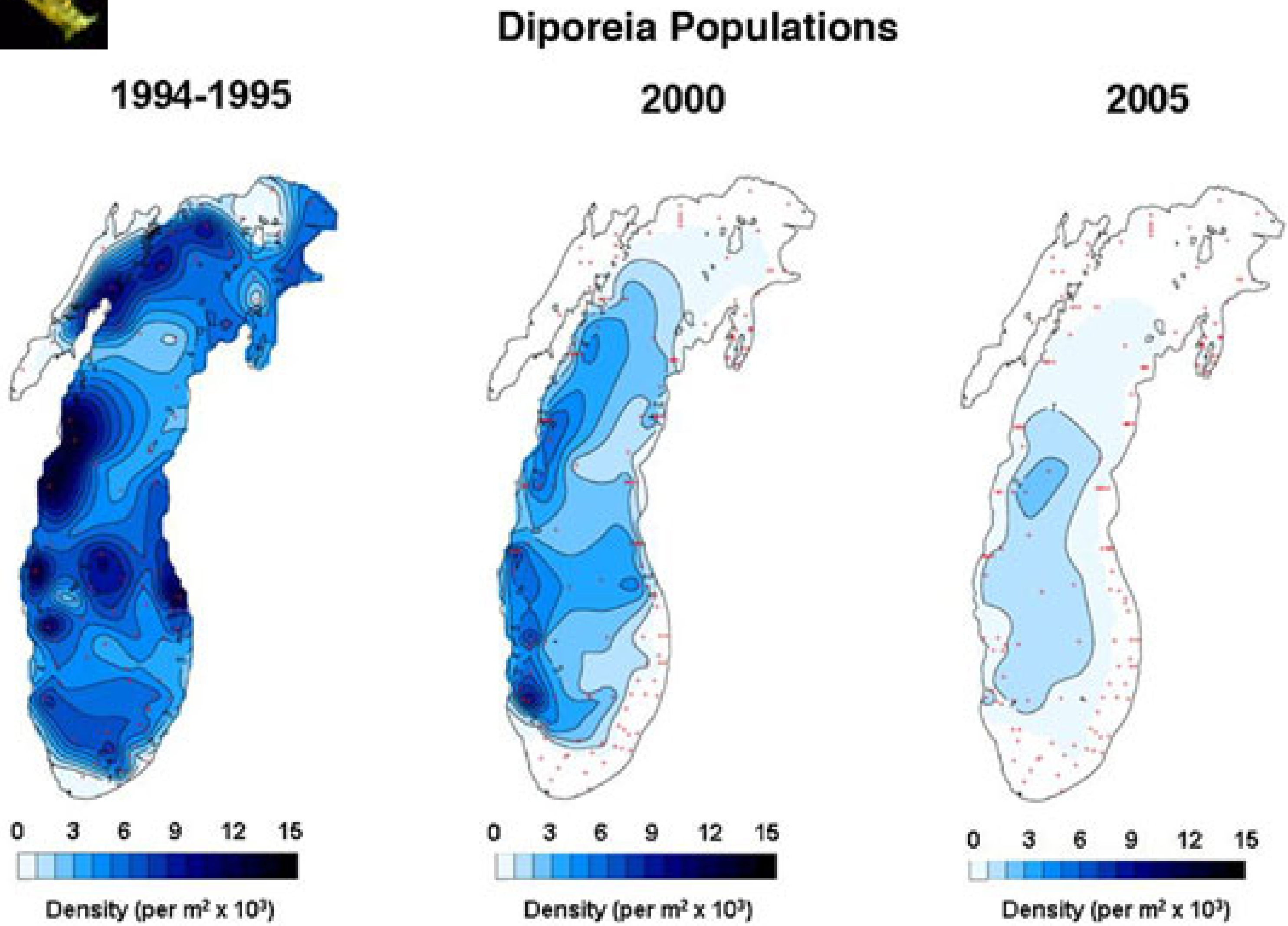
Dreissena rostriformis bugensis (quagga mussel)



Data: Tom Nalepa, GLERL



Diporeia



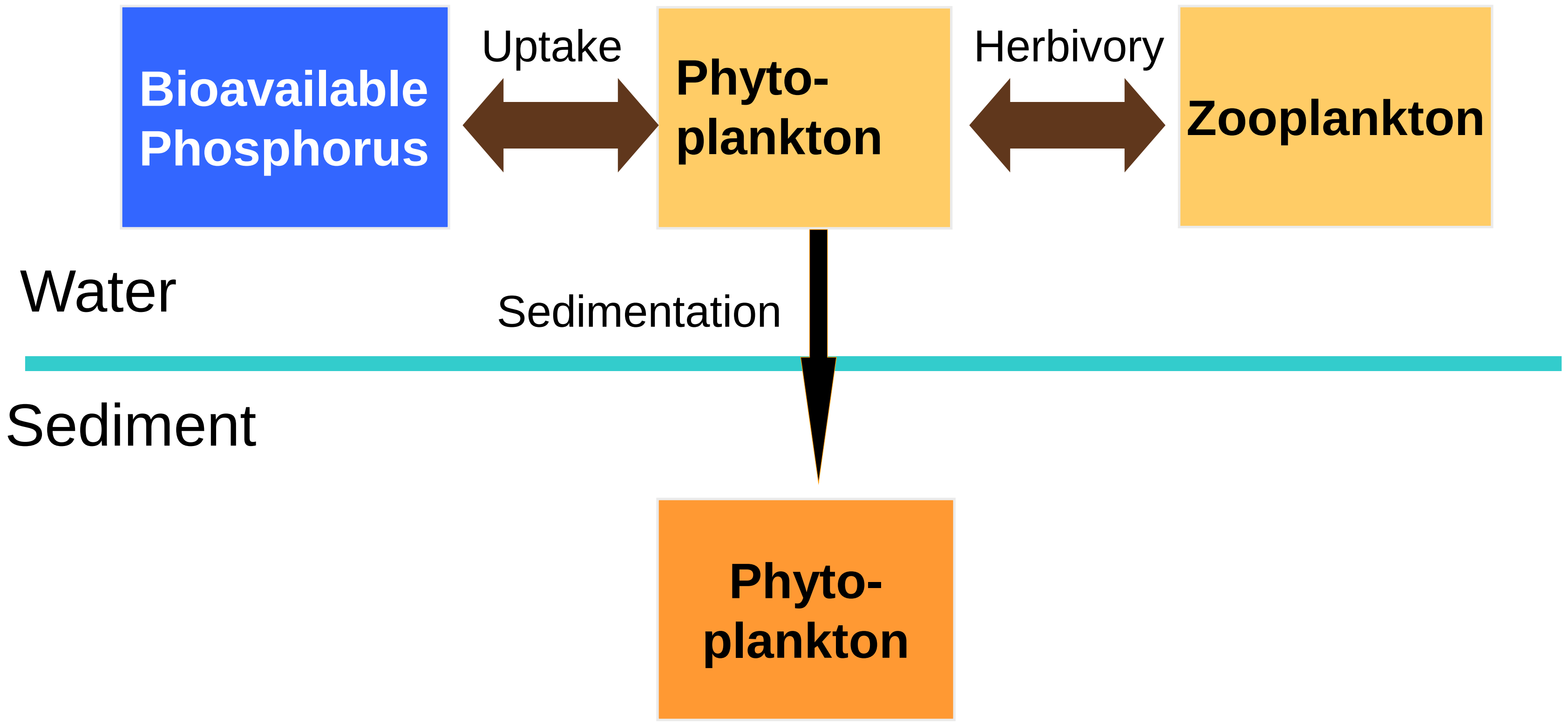
Data: Tom Nalepa, GLERL

Food Web Changes: Lake Michigan

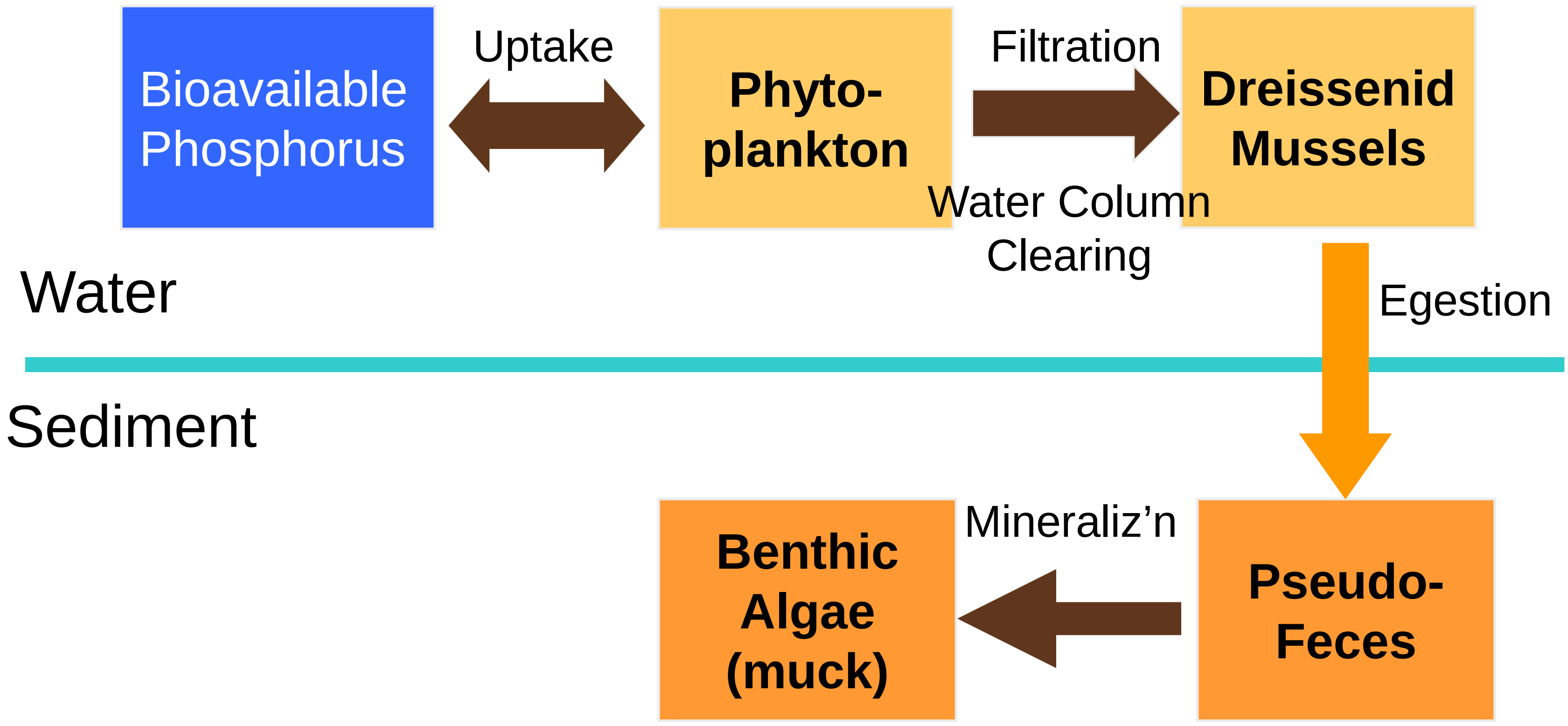
Biomass (Kilotons)	1989	2006	2007
Prey Fish (alewife; bloater)	400	61	30
Quagga/Zebra mussels	0	212	245

Data: USGS, Great Lakes Science Center

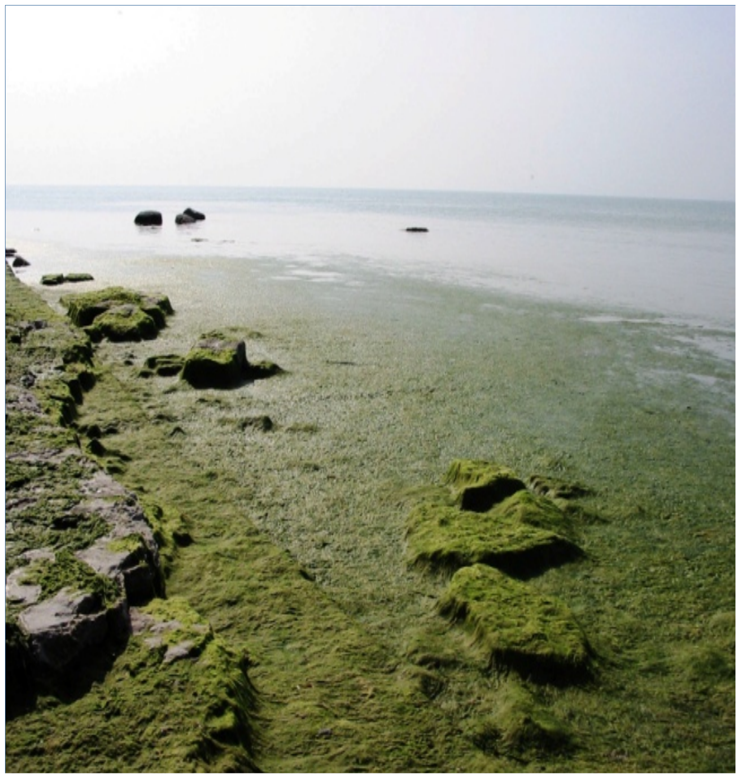
Benthification of Great Lakes: pre-dreissenids



Benthification of Lake Michigan: post-dreissenids



Cladophora: a recurring problem



Cladophora bloom – Lake Erie

Photo courtesy of S. Higgins – U. of Waterloo

Dying *Cladophora* mat

Photo courtesy of U. of Wisconsin - GLWI

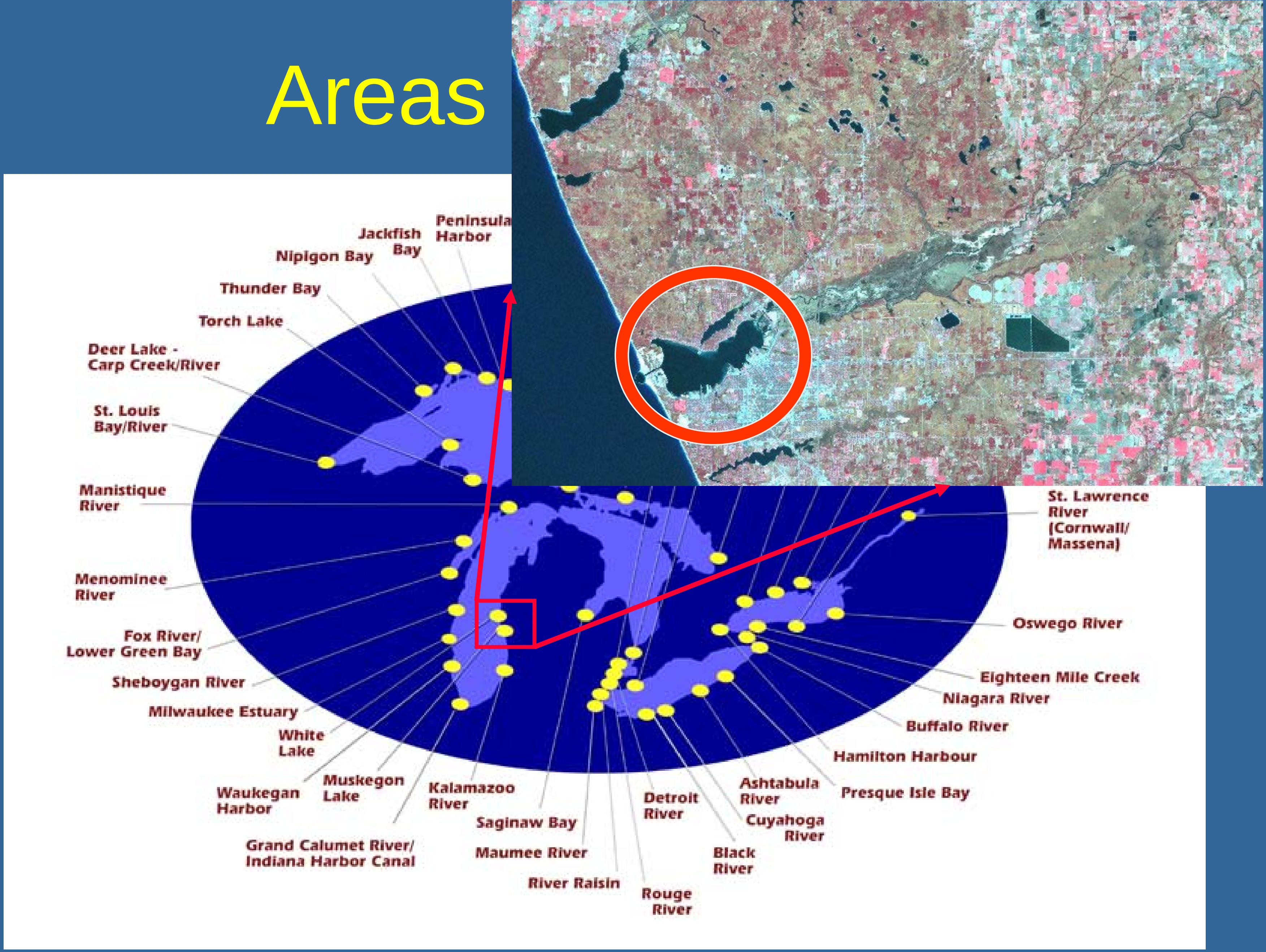
Photo courtesy of S. Higgins – U. of Waterloo

Economic Impacts

- Annual US control costs:
  - Sea Lamprey: ~ $10 million (barriers, lampricides, etc.)
  - Dreissenids - $100 million (pipe cleaning, hull fouling, etc.)
- Total estimated costs at hundreds of millions/yr (Anderson Economic Group 2012)

Photo credit: Great Lakes Commission

Areas

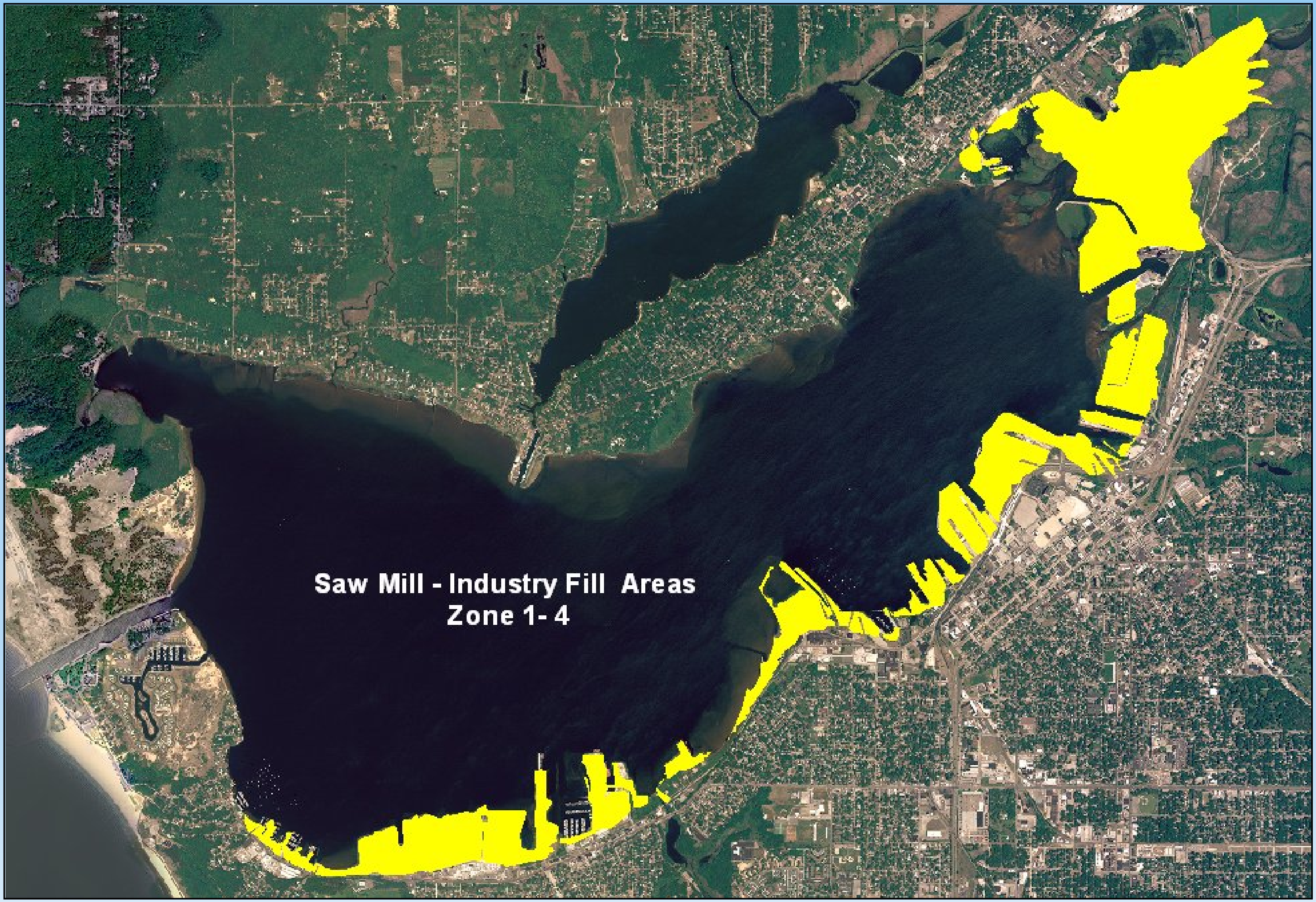


Peninsula Harbor
Jackfish Bay
Nipigon Bay
Thunder Bay
Torch Lake
Deer Lake - Carp Creek/River
St. Louis Bay/River
Manistique River
Menominee River
Fox River/ Lower Green Bay
Sheboygan River
Milwaukee Estuary
White Lake
Waukegan Harbor
Muskegon Lake
Kalamazoo River
Grand Calumet River/ Indiana Harbor Canal
Saginaw Bay
Maumee River
River Raisin
Detroit River
Rouge River
Black River
Cuyahoga River
Ashtabula River
Presque Isle Bay
Hamilton Harbour
Buffalo River
Niagara River
Eighteen Mile Creek
Oswego River
St. Lawrence River (Cornwall/ Massena)


Location of AWRI
Muskegon Lake
Lake Michigan

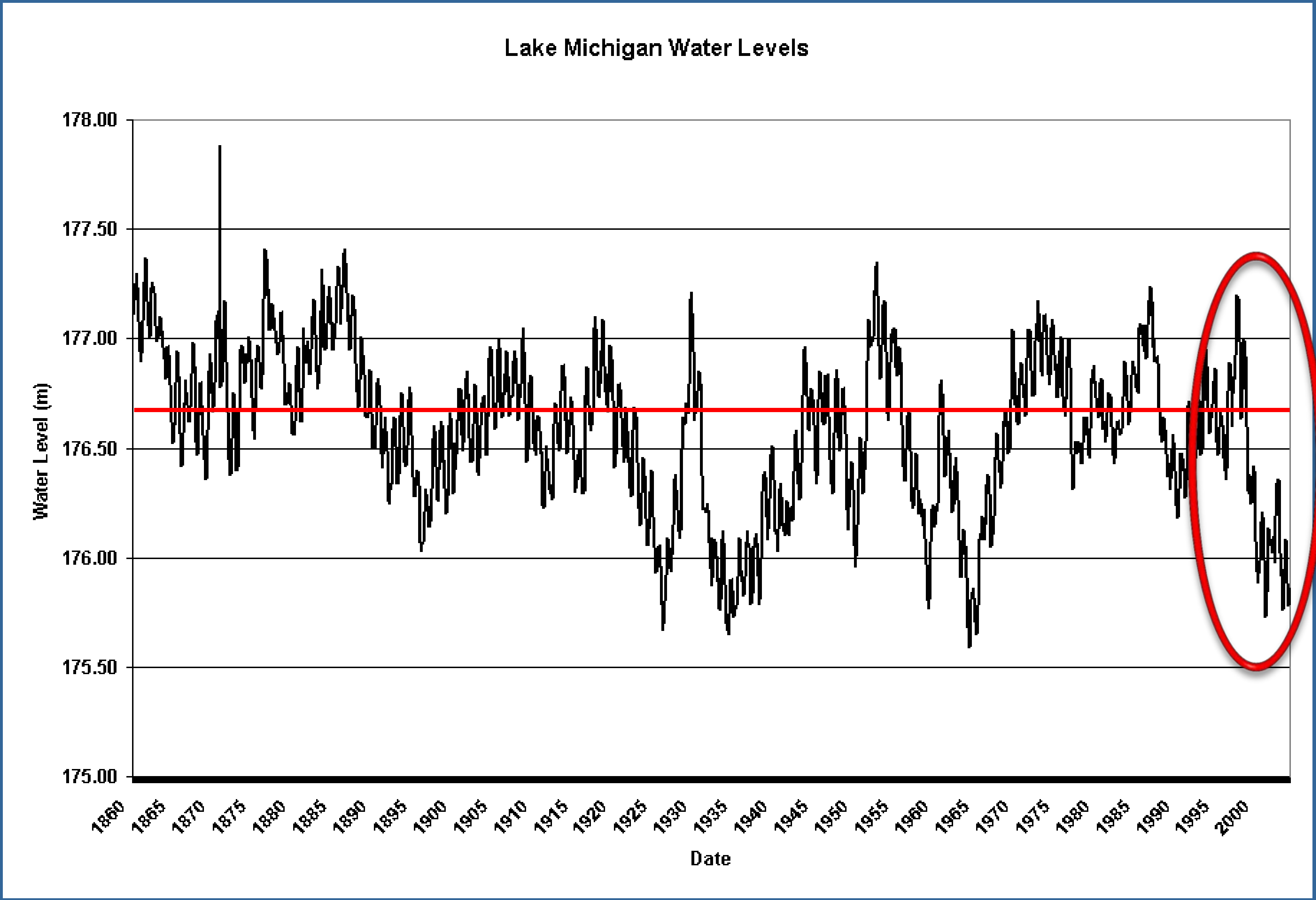
Muskegon 1889

- Over 16% of open water filled in
- 66% of the shoreline has been hardened



Muskegon Lake, MI: 1900-1960

Water Levels


Lake Michigan Water Levels
Water Level (m)
178.00
177.50
177.00
176.50
176.00
175.50
175.00
1860
1865
1870
1875
1880
1885
1890
1895
1900
1905
1910
1915
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1935
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1945
1950
1955
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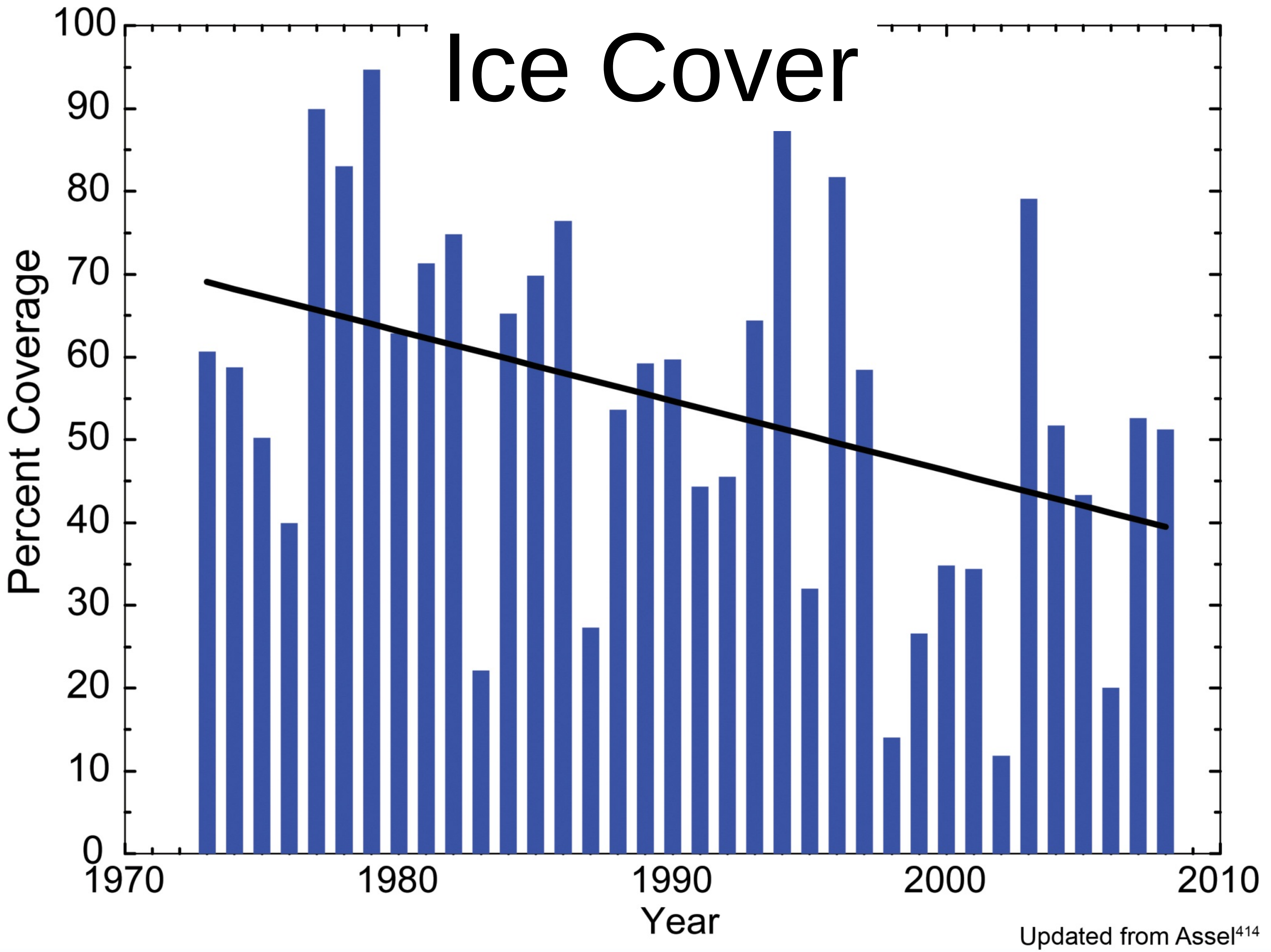
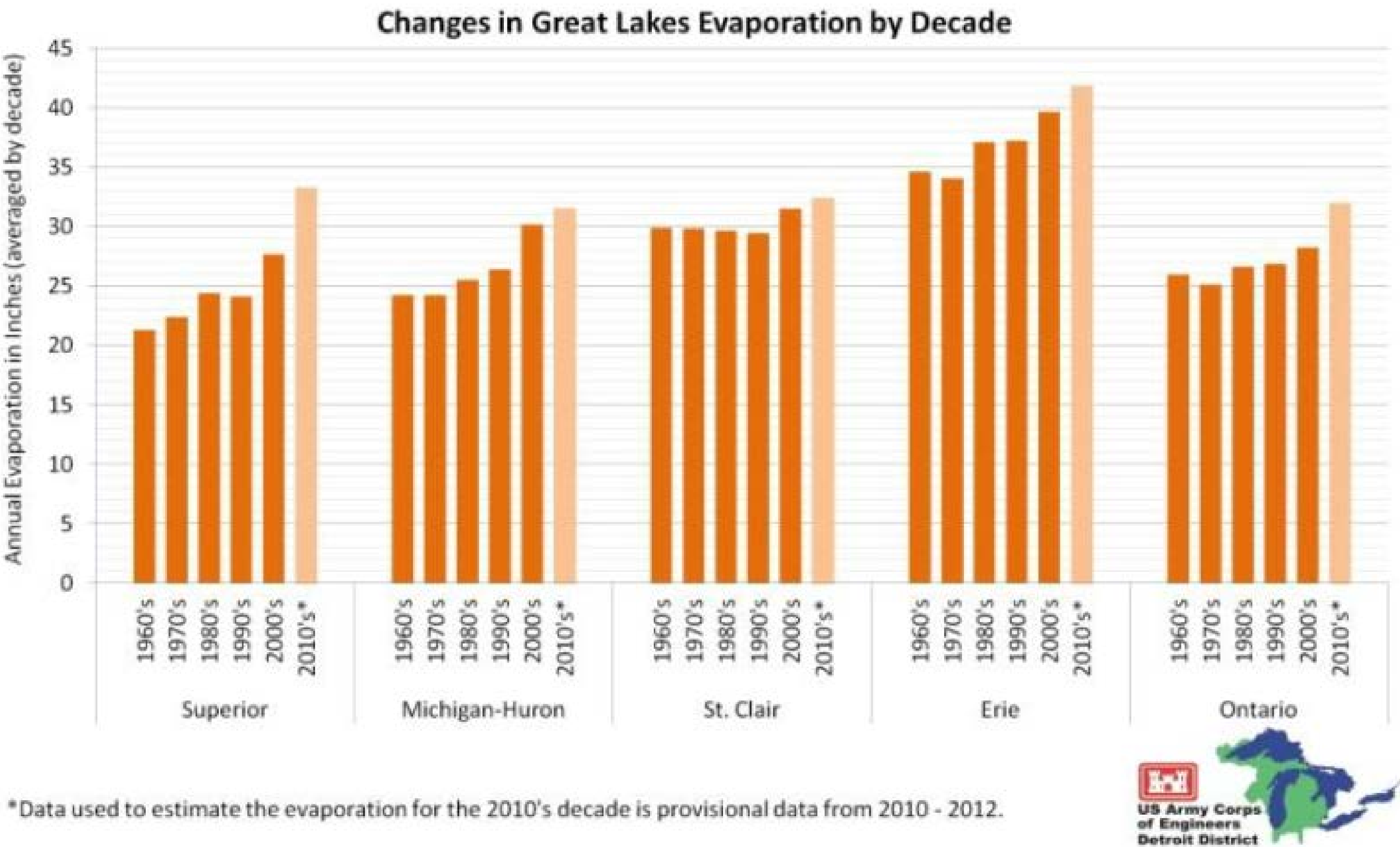
1975
1980
1985
1990
1995
2000
Date


Ice Cover
Percent Coverage
100
90
80
70
60
50
40
30
20
10
0
1970
1980
1990
2000
2010
Year
Updated from Assel414



Data Source: US Army Corps of Engineers

GLEAM Overview

Great **L**akes **E**nvironmental **A**ssessment & **M**apping project

- Map the intensity of multiple stressors across the Great Lakes (1-km^2 resolution)
- Develop weightings of relative impact of each stressor by habitat type, based on expert judgment
- Derive a cumulative stress map summing all individual stressors

Project GLEAM: Mapping Individual Stressors Across the Great Lakes

ALLAN, J.D., SMITH, S.D.P., MCINTYRE, P.B., HALPERN, B., BOYER, G., BUCHSBAUM, A., BURTON, A., CAMPBELL, L., CHADDERTON, L., CIBOROWSKI, J., DORAN, P., EDER, T., INFANTE, D., JOHNSON, L., LODGE, D., READ, J., RUTHERFORD, E., SOWA, S., STEINMAN, A., JOSEPH, C. And MARINO, A.

Fred A. and Barbara M. Erb Family Foundation

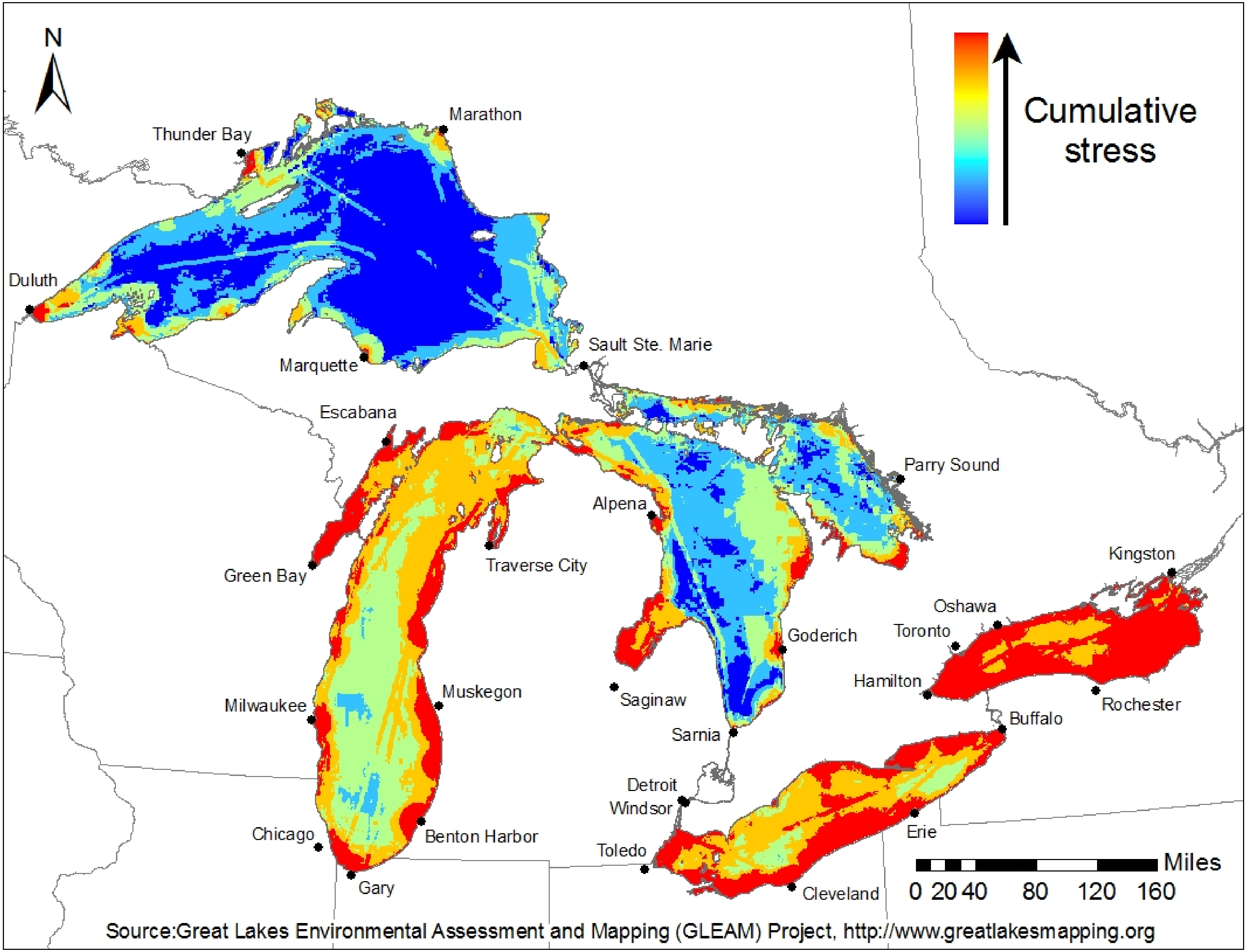
Stressors Affecting the Great Lakes

Category	Number of Stressors
Aquatic Habitat Alterations	11
Climate Change	3
Coastal Development	5
Fisheries Management	7
Invasive/Nuisance Species	10
NPS/Runoff	5
AOC/Toxics	7
Water Levels	2

Survey: Relative impact of stressors

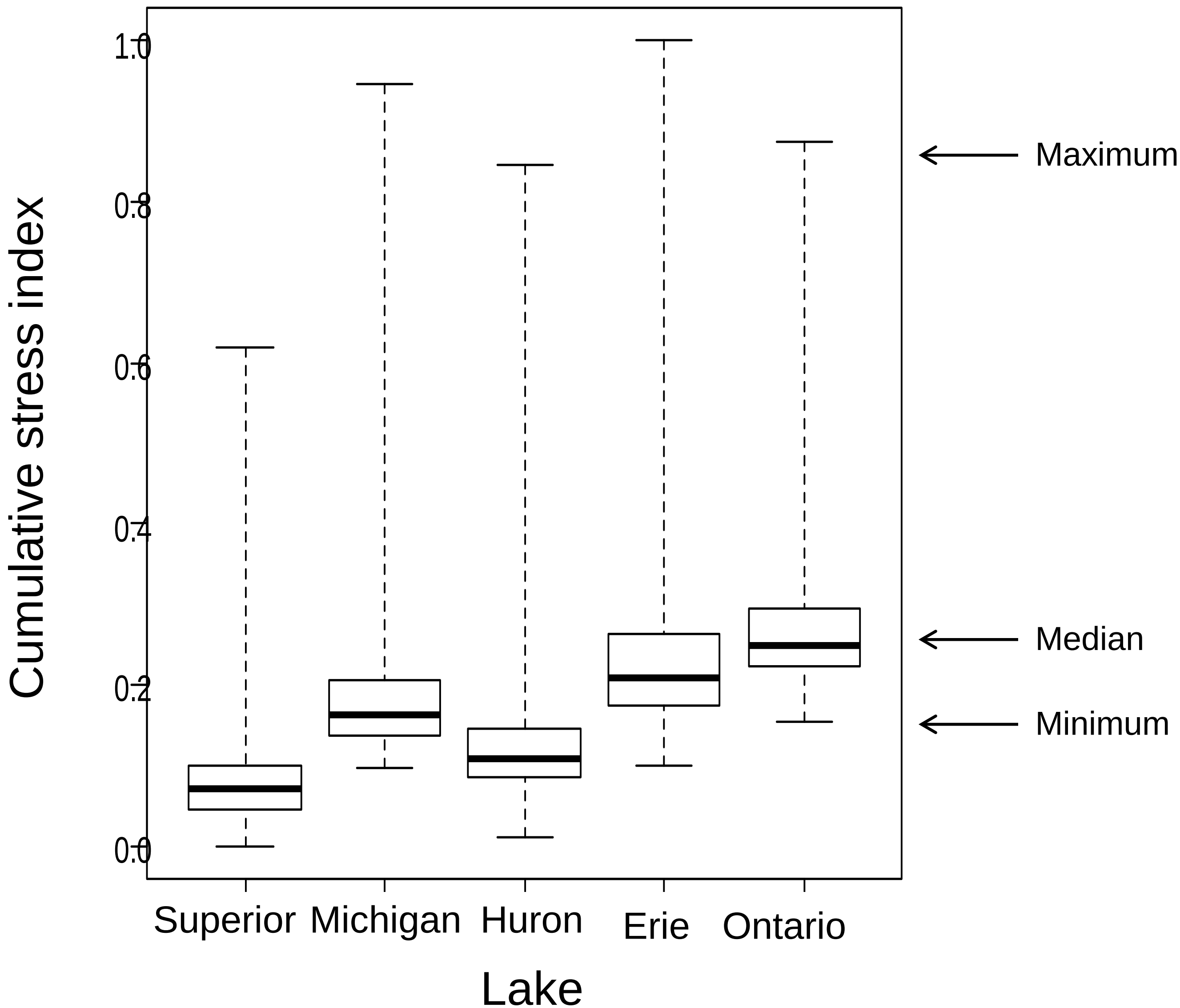
Experts surveyed to weight individual stressors:

- Stressor specific
  - e.g., Hg is twice as harmful as nitrogen
- Habitat specific
  - e.g., Hg in wetlands is twice as harmful as in open water
- Quantitative
  - "Ecosystem impact" is quantified for 5 criteria: temporal frequency, spatial extent, ecological scope, magnitude of change, recovery time
  - Survey uses scenario comparisons to elicit how to combine these criteria for overall stressor influence
  - Current stressors only

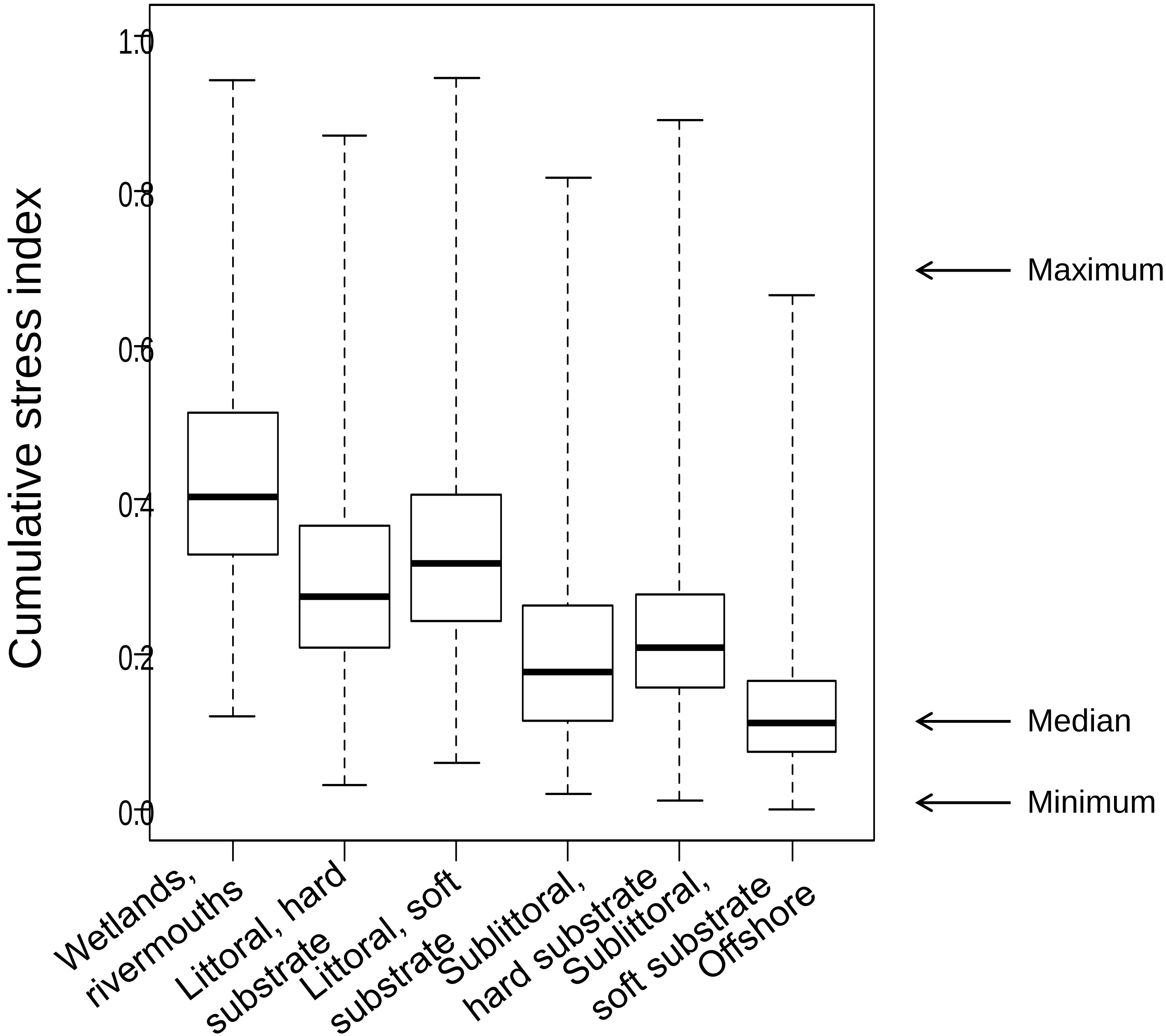


Allan et al. 2013 PNAS

Differences among Lakes



Differences across Habitats



Some Take-away Messages

- Cumulative stress is widespread but variable across the Great Lakes
  - Unsurprisingly, nearshore regions, LE and LO experience relatively greater stress
- Locations of greatest benefit to people generally experience greater stress
- Locations receiving restoration dollars generally experience high stress

THE GREAT LAKES

Image courtesy Michigan Sea Grant

Produced by Michigan Sea Grant. www.miseagrant.umich.edu
MICHU-08-717

Appendix C

Mark Bunger Presentation

The bio-economy – economic potential of synthetic biology in the Great Lakes

Mark Bünger, Research Director

Lux Research, Inc.

About Lux Research

- Helps clients find **new business opportunities** from emerging technologies in physical and life sciences
- Offers ongoing **technology and market intelligence**, as well as market data and consulting services
- Over **250 clients on six continents** – multinational corporations, investors, governments, and SMEs
- **Global reach,** with over 80 employees in Boston, New York, Amsterdam, Singapore, Shanghai, Seoul, and Tokyo
- Combines deep **technical expertise** with **business analysis** to support strategic decisions

Technology coverage

- Solar Components
- Solar Systems
- Grid Storage
- Mobile Energy
- Alternative Fuels
- Bio-based Materials & Chemicals
- Formulation and Delivery
- China BioPharma
- BioElectronics
- Water
- Exploration and Production
- Advanced Materials
- Printed, Flexible, and Organic Electronics
- Energy Electronics
- Sustainable Building Materials
- Efficient Building Systems
- China Innovation

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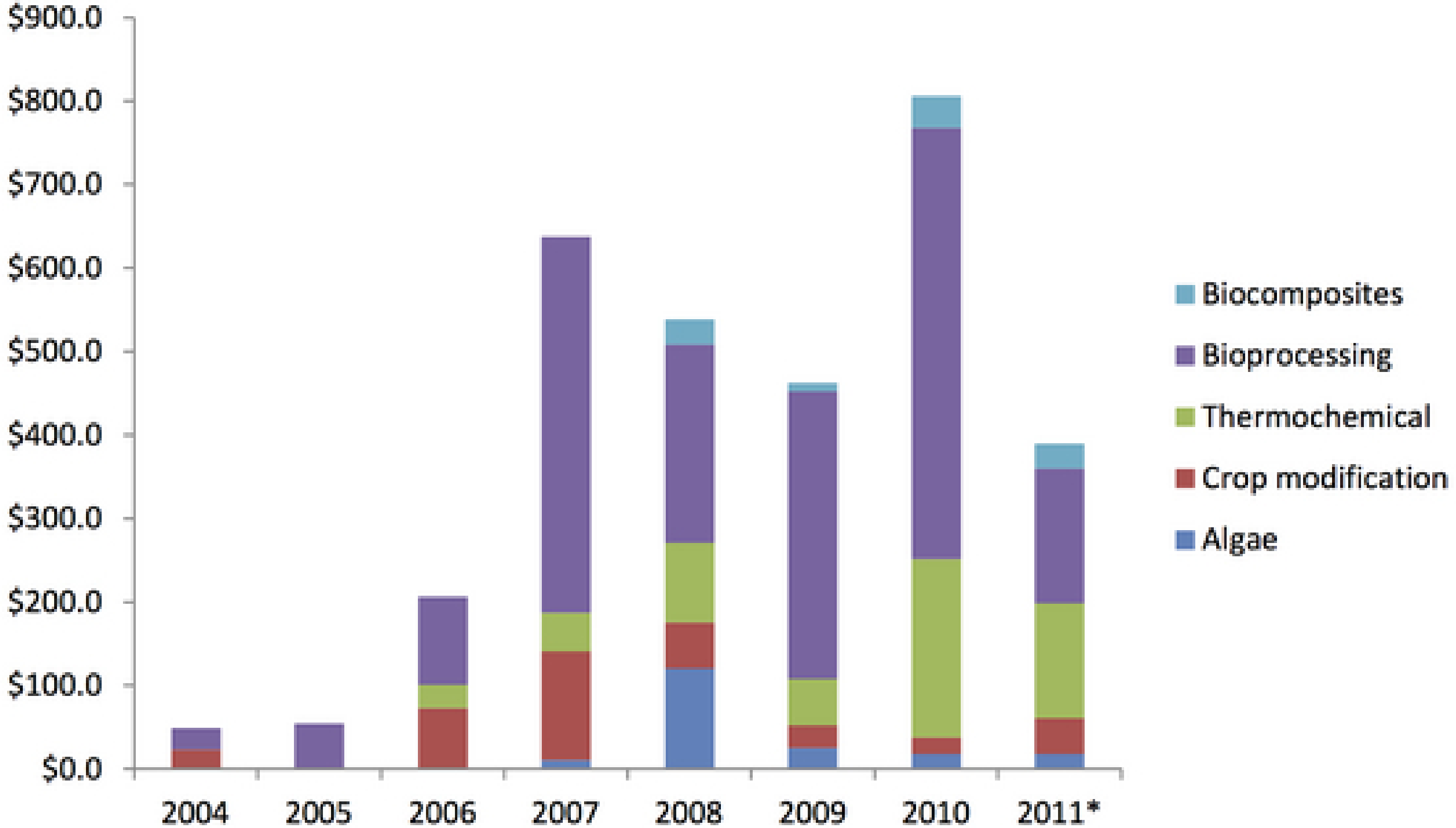
Venture investment in industrial biotech, 2004-2011



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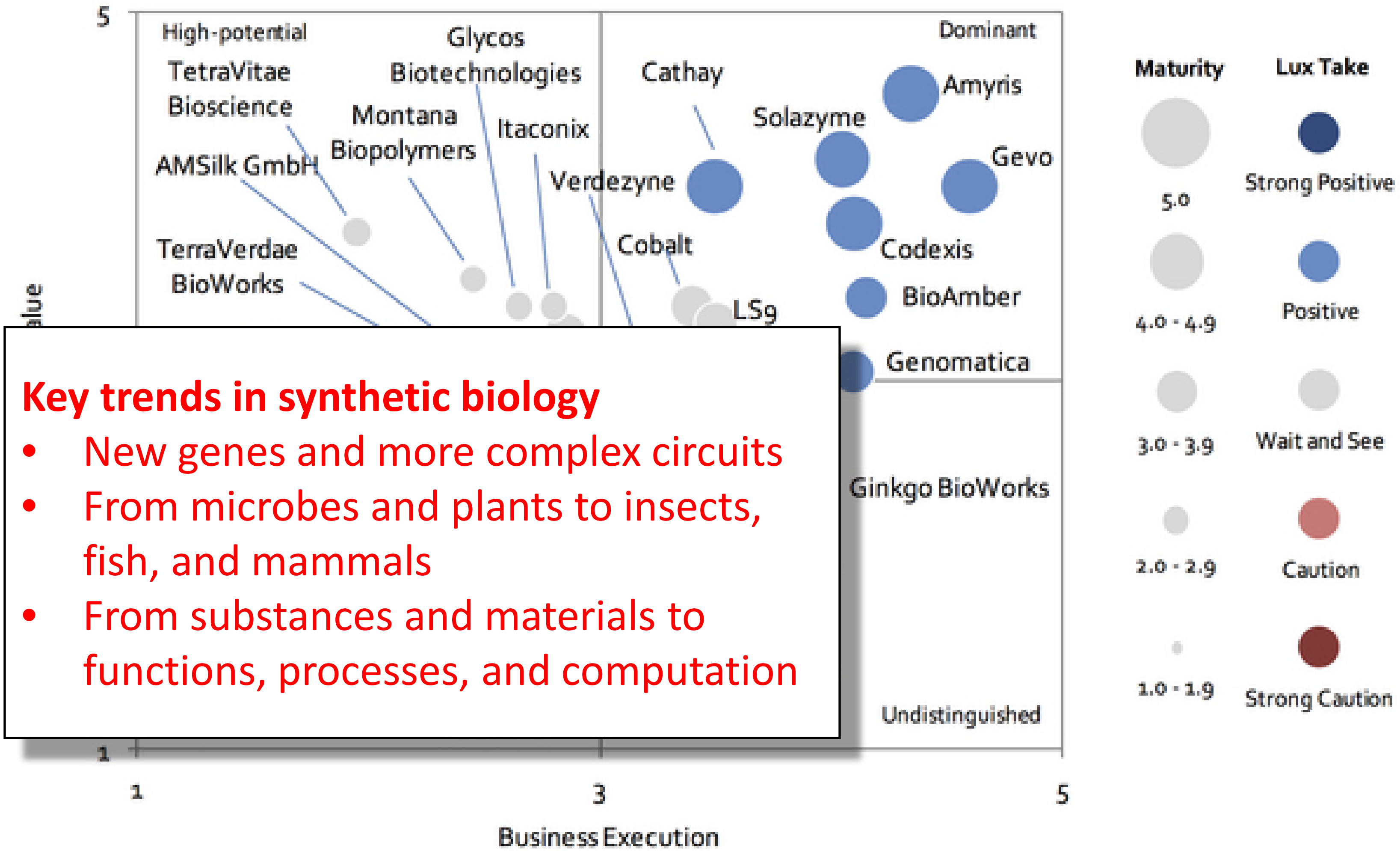
Key players in synthetic biology (Bio-based Chemicals and Materials, Alternative Fuels)



Key trends in synthetic biology

- New genes and more complex circuits
- From microbes and plants to insects, fish, and mammals
- From substances and materials to functions, processes, and computation

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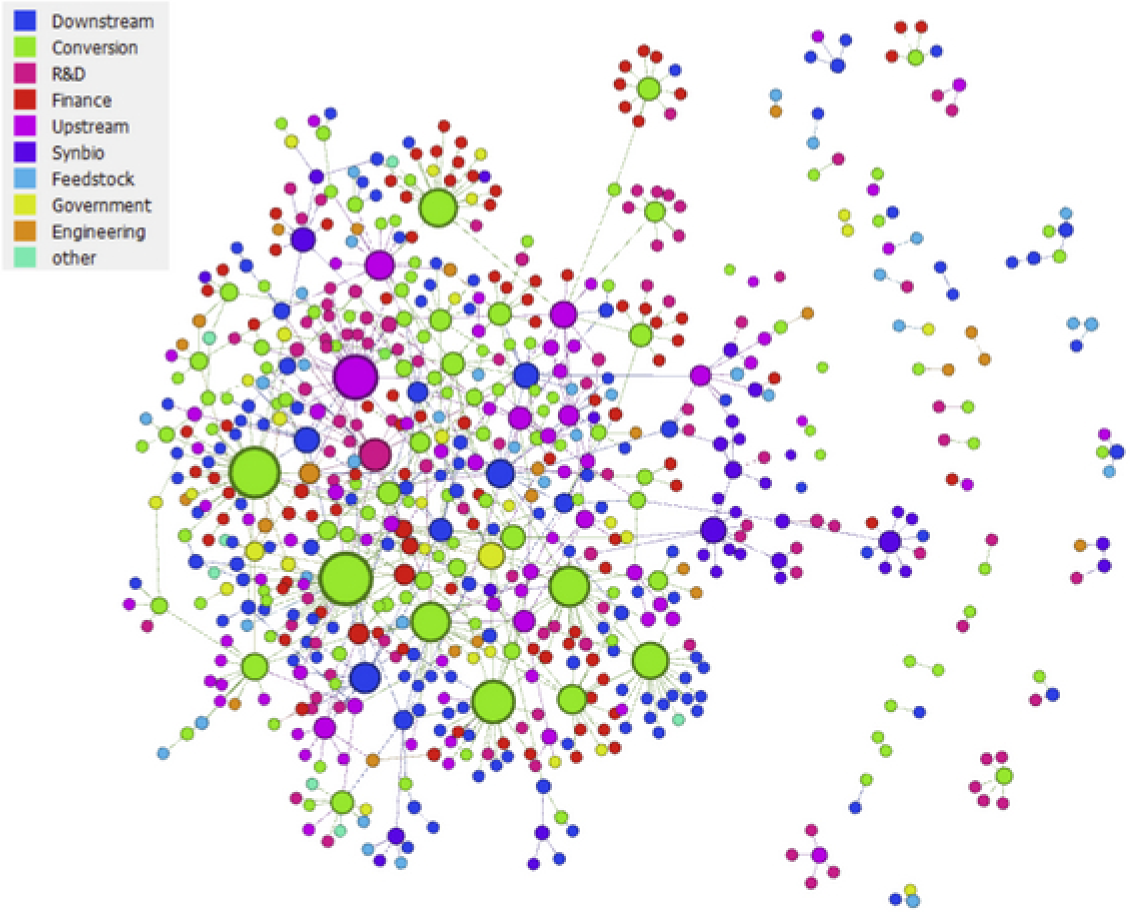
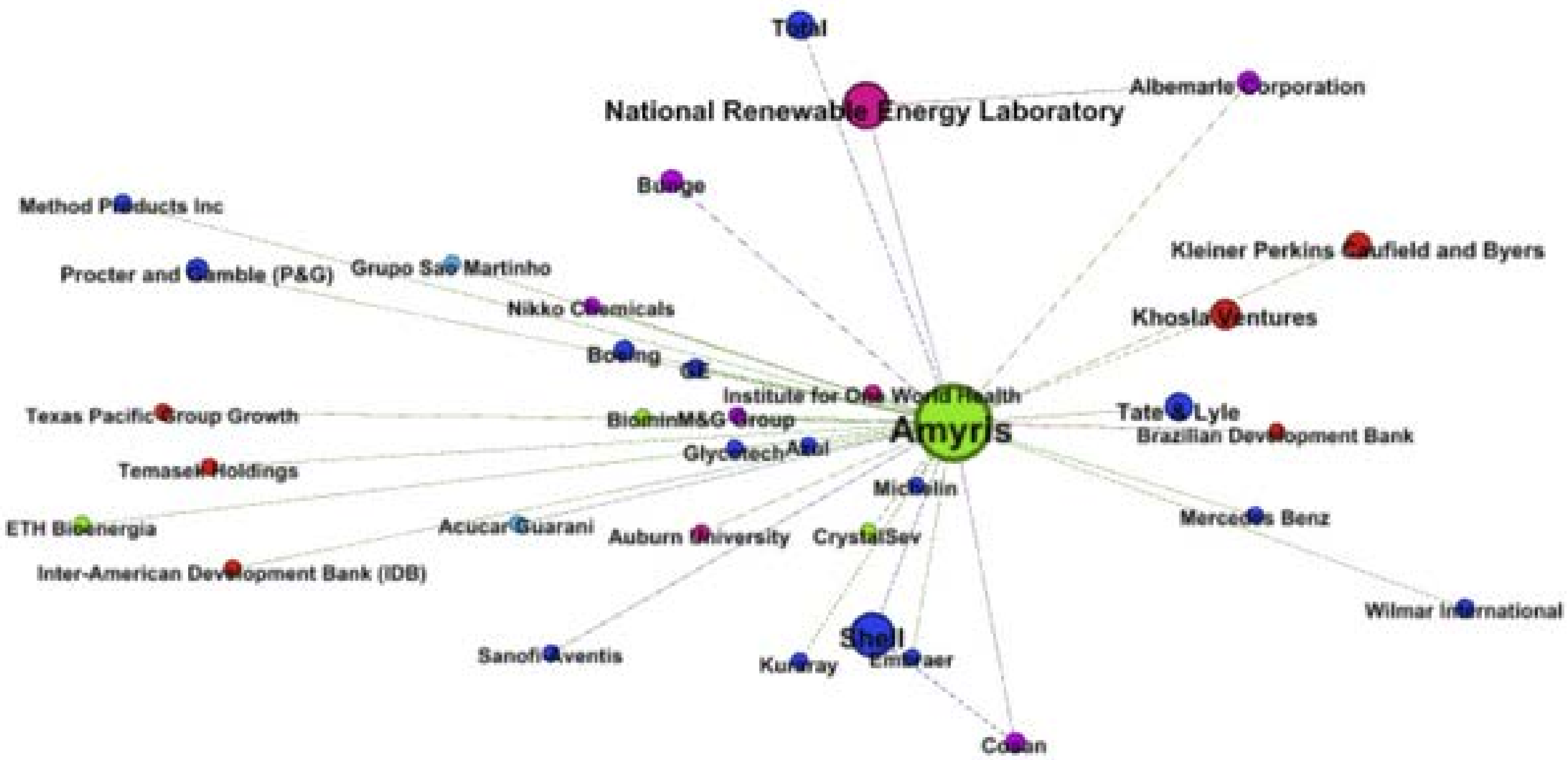
The BBMC Social Graph Colored by Value Chain Position; Larger Size Reflects More Connections

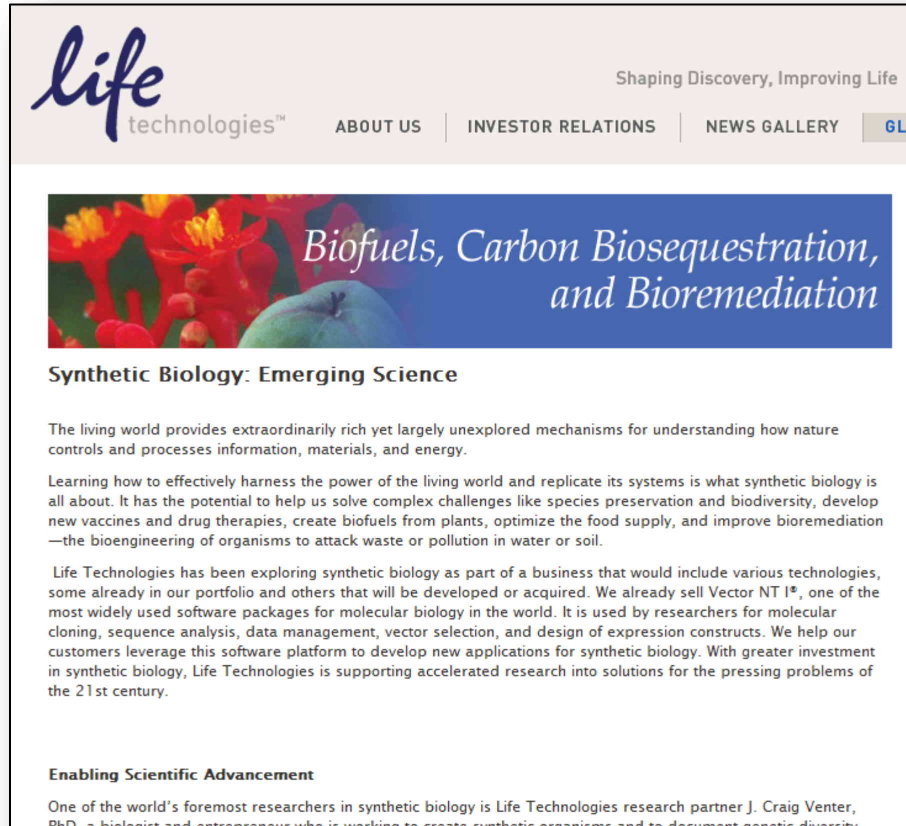


Amyris's partnership web



What opportunities does Life Technologies see? Will they be your partner or competitor in 10 years?

- Biofuels, Carbon Biosequestration, and Bioremediation
- Spending "$100 million"
  - $47M for Geneart
  - $10 M in Synthetic Genomics
  - <$10M in SG Biofuels, sequencing Jatropha genome
- "Sell access to synthetic gene sequences, sell the cells into which those genes are put, and the chemicals used to put the genes in the cells" (-Forbes)



Synthetic Biology: Emerging Science

The living world provides extraordinarily rich yet largely unexplored mechanisms for understanding how nature controls and processes information, materials, and energy.

Learning how to effectively harness the power of the living world and replicate its systems is what synthetic biology is all about. It has the potential to help us solve complex challenges like species preservation and biodiversity, develop new vaccines and drug therapies, create biofuels from plants, optimize the food supply, and improve bioremediation—the bioengineering of organisms to attack waste or pollution in water or soil.

Life Technologies has been exploring synthetic biology as part of a business that would include various technologies, some already in our portfolio and others that will be developed or acquired. We already sell Vector NT I®, one of the most widely used software packages for molecular biology in the world. It is used by researchers for molecular cloning, sequence analysis, data management, vector selection, and design of expression constructs. We help our customers leverage this software platform to develop new applications for synthetic biology. With greater investment in synthetic biology, Life Technologies is supporting accelerated research into solutions for the pressing problems of the 21st century.

Enabling Scientific Advancement

One of the world's foremost researchers in synthetic biology is Life Technologies research partner J. Craig Venter, PhD, a biologist and entrepreneur who is working to create synthetic organisms and to document genetic diversity

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Going far-out: tracking science fiction's predictions

- Motorola's Star-Tac phone was a near-perfect replica of Star Trek's tricorder; Captain Picard reads his memos on an iPad

- Veggie burgers and in-vitro meat were predicted in the novel The Space Merchants (1952).
- "…there wasn't a man around the table who didn't shudder at the thought of soyaburgers and regenerated steak…"

Modern Meadow

We track predictions made in fiction and popular science – with a skeptical, but open eye – to inspire unconventional thinking, envision long-range scenarios, and get a sense of the broader public's expectations about the future

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Node-by-node detailed analysis

We gather detailed information about each node in the roadmap, synthesizing disparate sources to aid in forecasting future developments:

- Products and developers
- Scientific papers
- Patents and IP issues
- Venture investments
- Lead user/hacker projects and hackerspaces
- Other stakeholders/ opinionmakers (e.g. NGOs)

For example, in the field of synthetic biology, we track:

- 120+ Developers
- 300+ Scientific papers and 200+ labs
- 1000+ Patents
- 100+ Venture investments
- 450+ Lead user/hacker projects and 40+ hackerspaces/DIY programs (examples below)

BioCurious http://biocurious.org/
NYC Resistor www.nycresistor.com/
Genspace http://genspace.org/
BioArt Laboratories http://bioartlab.com/
La Paillasse http://www.lapaillasse.org/about/?lang=fr
LA Biohackers http://www.meetup.com/LAbiohackers/events/86592242/
Dutch DIY Bio Group http://www.meetup.com/Dutch-DIY-Bio/
DIYBio Singapore http://diybiosingapore.wordpress.com/
Biomodd http://www.biomodd.net

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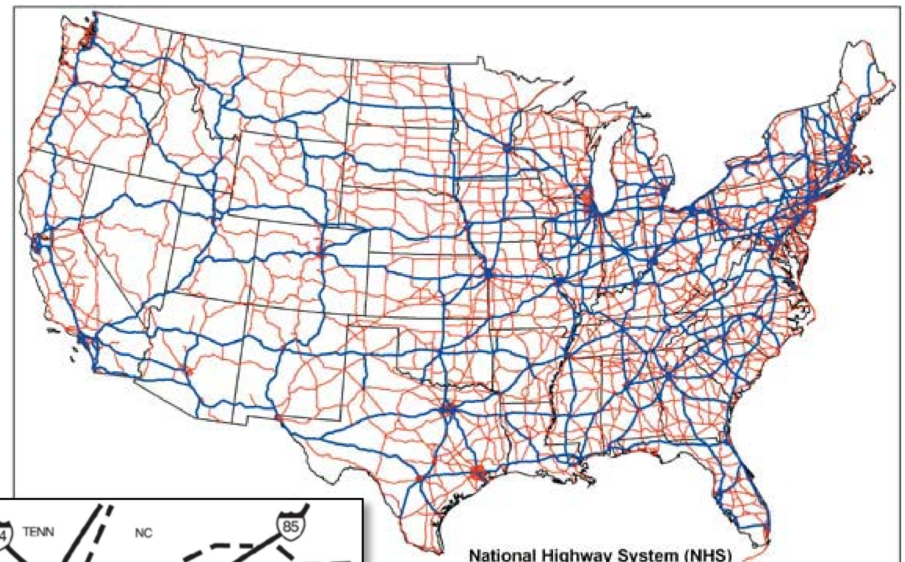
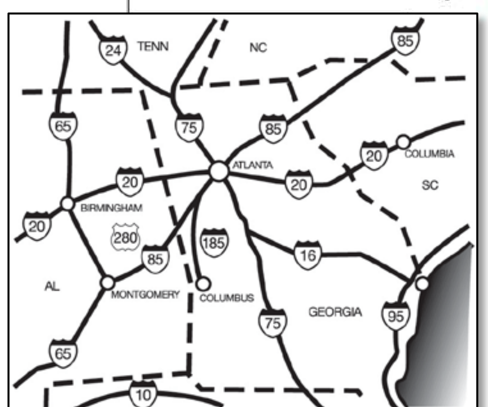
What's a roadmap?

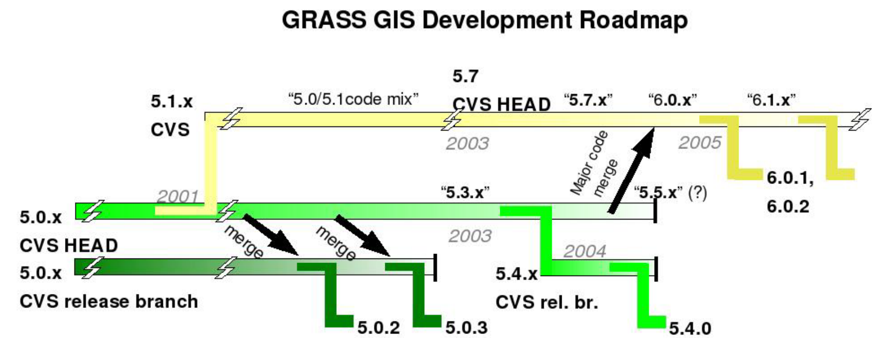
- Shows nodes (destinations, cities, intersections)
- Shows route(s) from node to node – including multiple options
- Dimensions correspond to physical reality (vertical = North-South, horizontal = East-West etc)
- Arrangement is not arbitrary (distance, boundaries, sequence, white space, and paths are important)
- Shows one level of detail, but integrates with higher and lower levels
- Purpose: a tool for planning

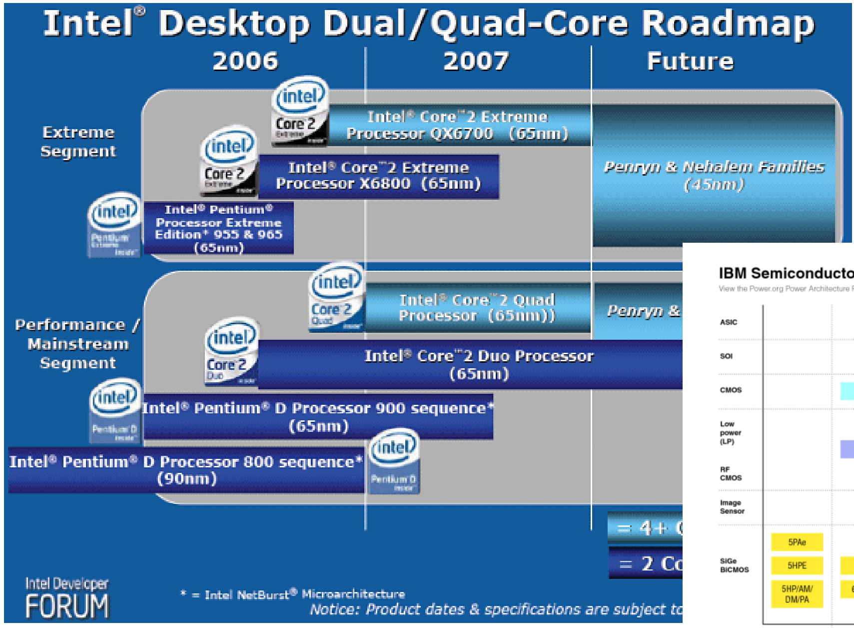


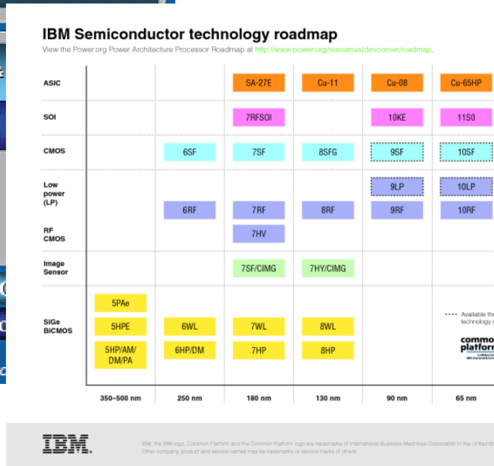


What's a technology roadmap?

- Shows nodes (products, applications, merges/forks)
- Shows route(s) from node to node – including multiple options
- Dimensions correspond to physical reality (vertical = component layer, horizontal = time etc)
- Arrangement is not arbitrary (distance, boundaries, sequence, white space, and paths are important)
- Shows one level of detail, but integrates with higher and lower levels
- Purpose: a tool for planning







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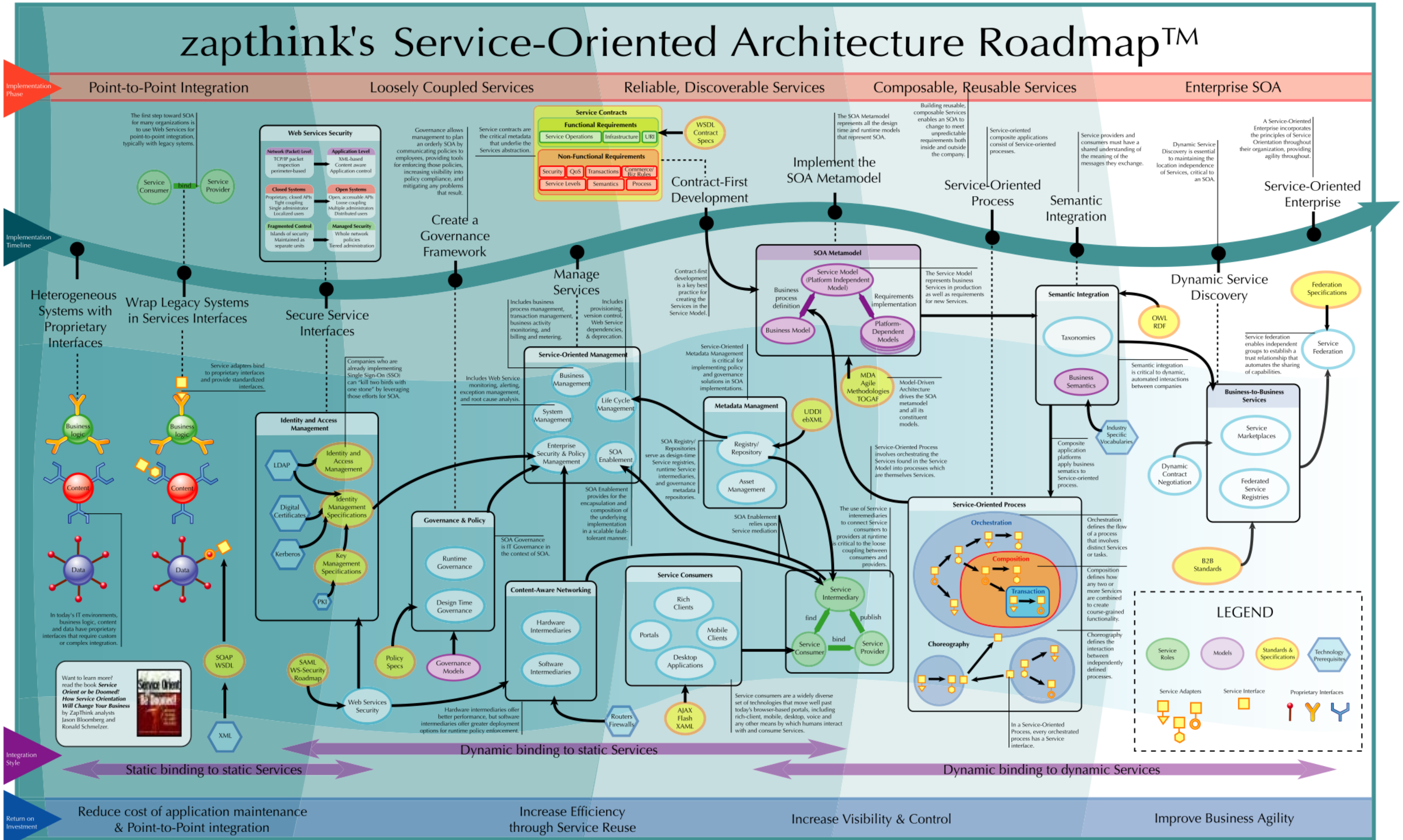
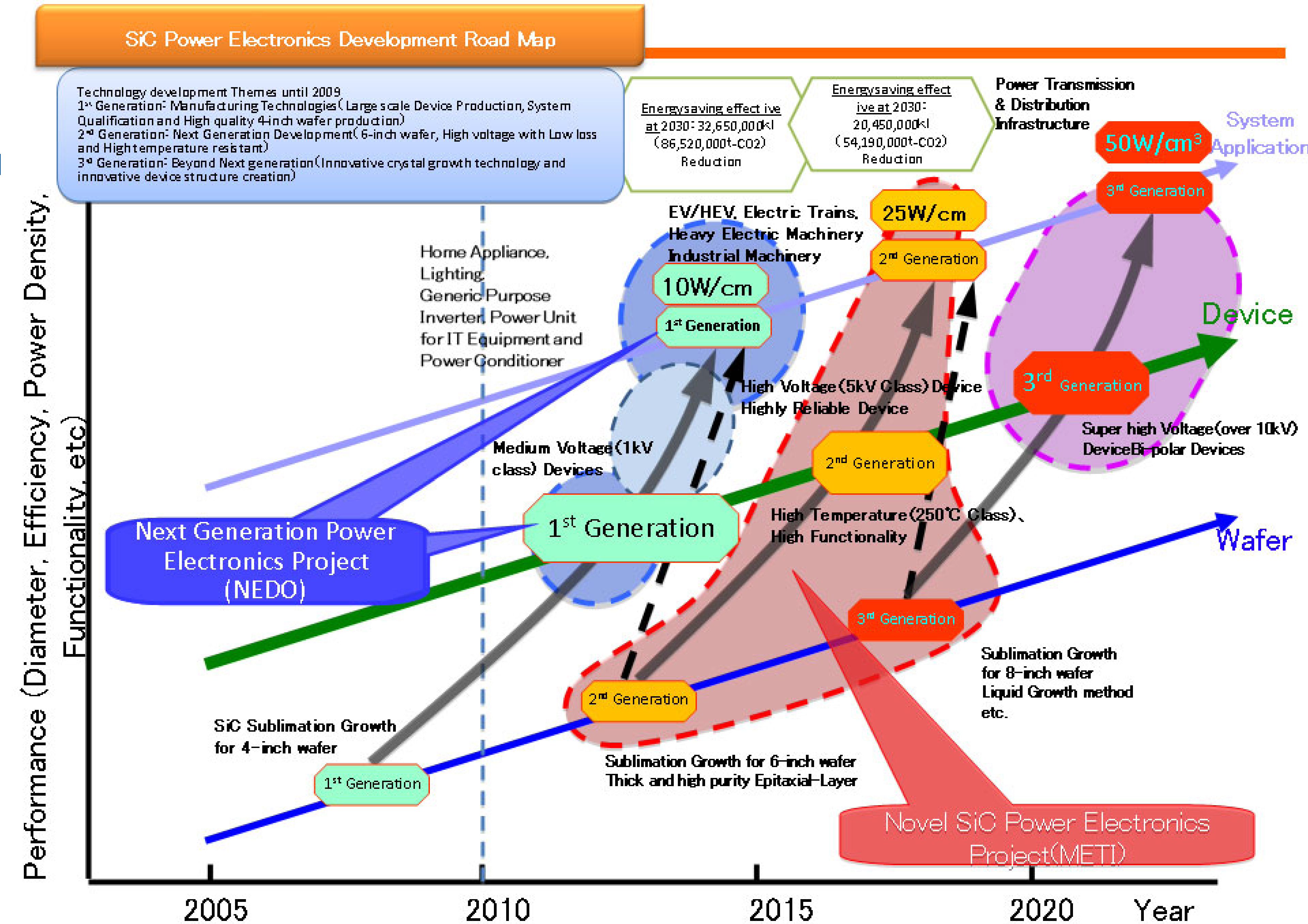
Produced by: zapthink



Last modified: October 2005
ZapThink Document ID: ZTS-GI103

ABOUT THIS POSTER:
Service-Oriented Architecture (SOA) is a set of architectural and organizational best practices that help organizations leverage technology resources in an agile manner. The ZapThink SOA Roadmap™ represents a path that companies can follow to lower the risk of their SOA initiatives to increase their ROI.

Take Credit for reading this poster by going to **http://www.zapthink.com/takecredit.html** and enter the code ROADPOST. Earn ZapCredits you can exchange for free research and more.


SiC Power Electronics Development Road Map
Technology development Themes until 2009
1st Generation: Manufacturing Technologies (Large scale Device Production, System Qualification and High quality 4-inch wafer production)
2nd Generation: Next Generation Development (6-inch wafer, High voltage with Low loss and High temperature resistant)
3rd Generation: Beyond Next generation (Innovative crystal growth technology and innovative device structure creation)
Energysaving effect ive at 2030: 32,650,000kl (86,520,000t-CO2) Reduction
Energysaving effect ive at 2030: 20,450,000kl (54,190,000t-CO2) Reduction
Power Transmission & Distribution Infrastructure
System Application
50W/cm3
3rd Generation
EV/HEV, Electric Trains, Heavy Electric Machinery Industrial Machinery
25W/cm
2nd Generation
Home Appliance, Lighting, Generic Purpose Inverter, Power Unit for IT Equipment and Power Conditioner
10W/cm
1st Generation
Device
High Voltage (5kV Class) Device
Highly Reliable Device
3rd Generation
Super high Voltage (over 10kV) Device Bi-polar Devices
Medium Voltage (1kV class) Devices
2nd Generation
1st Generation
High Temperature (250℃ Class), High Functionality
Next Generation Power Electronics Project (NEDO)
Wafer
3rd Generation
Sublimation Growth for 8-inch wafer
Liquid Growth method
etc.
2nd Generation
SiC Sublimation Growth for 4-inch wafer
1st Generation
Sublimation Growth for 6-inch wafer
Thick and high purity Epitaxial-Layer
Novel SiC Power Electronics Project (METI)
Performance (Diameter, Efficiency, Power Density, Functionality, etc)
2005
2010
2015
2020
Year

A roadmap for synthetic biology: a general sketch

Biological scale

Ecosystem

Multicelled organism

Tissue / organ

Cell community

Cell

Genome (plasmid, chromosome)

Biomolecule

Atom/small molecule

- Some people define synthetic biology as an engineering mindset applied to biology.
- Specifically, "engineering mindset" in this context means simplifying technology/”parts” at one level (through e.g. standards and modularity) in order to innovate at a higher level.
- These levels are sometimes called "layers of abstraction." For example, standardized DNA sequences ("biobricks") encode enzymes that a synthetic biologist can arrange to make logic circuits (e.g. a "repressilator"); the synthetic biologist can concentrate on the properties and interactions of the enzymes without needing to consider their DNA sequence.
- In this chart, terms to below are generally components of terms to above. So as lower-level terms become well-characterized and standardized, synthetic biologists will be able to use them as parts to create new designs at the levels above.

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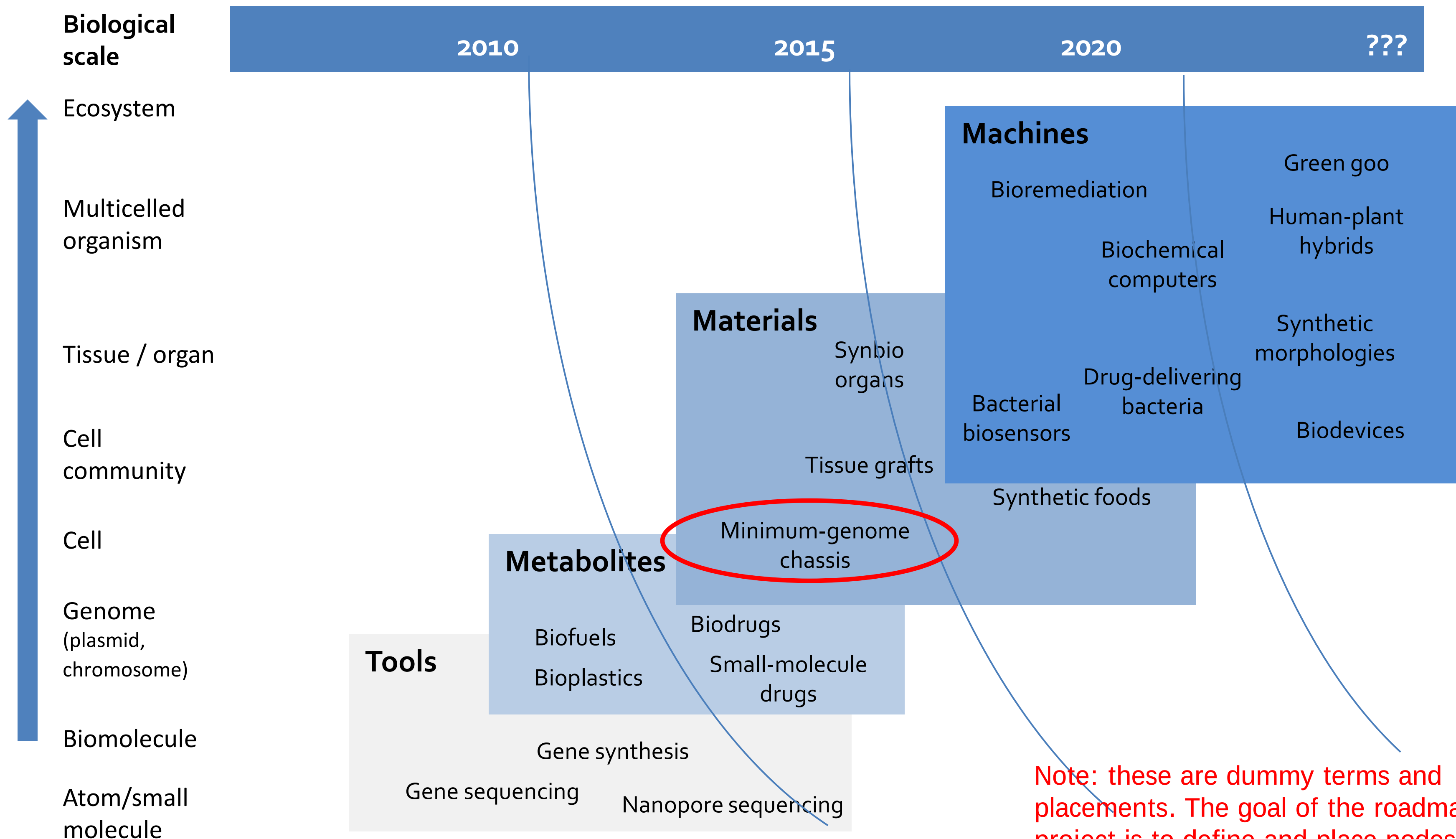
A roadmap for synthetic biology: a general sketch



Note: these are dummy terms and placements. The goal of the roadmap project is to define and place nodes and routes between them, based on actual data.

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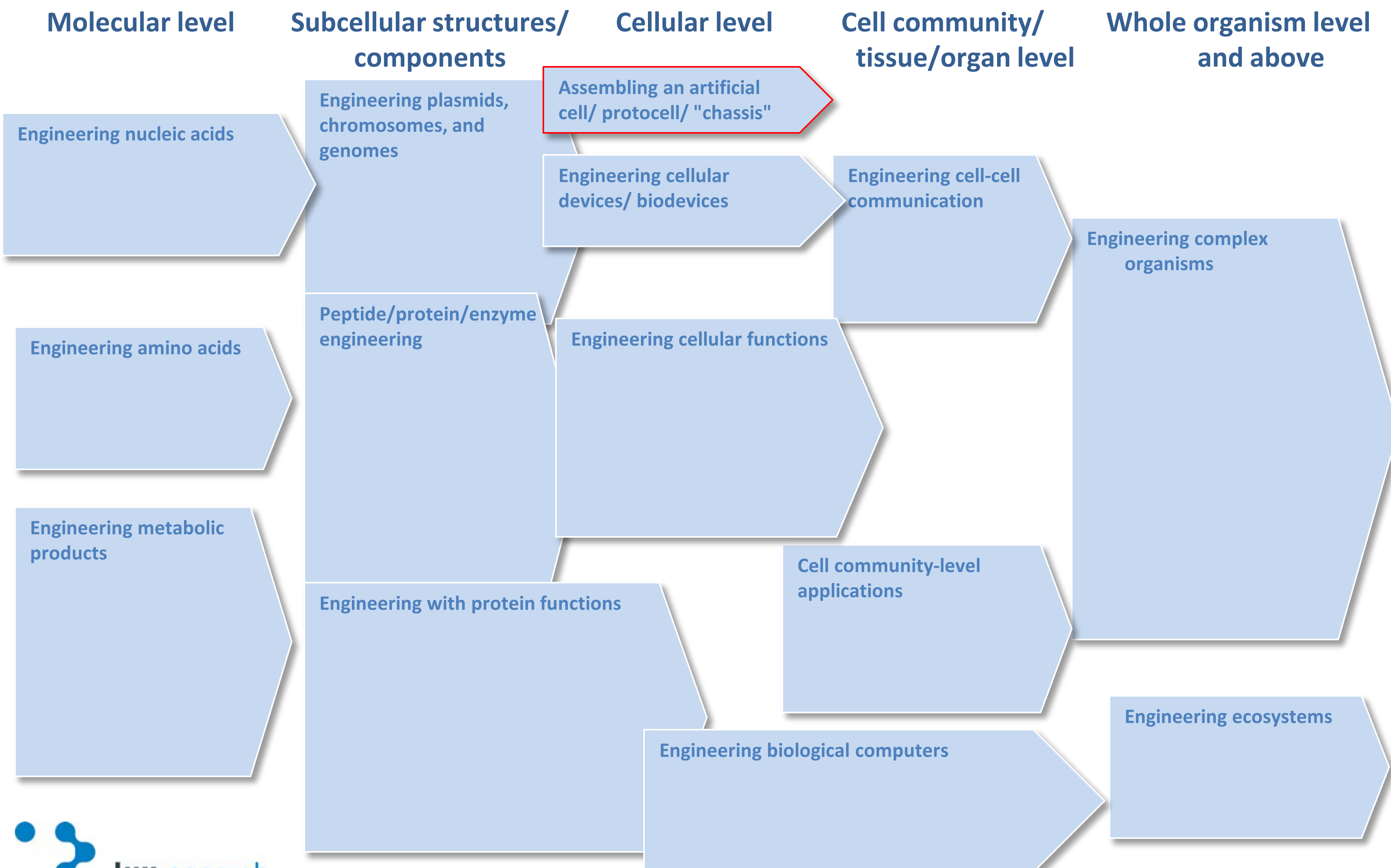
The Minimum-genome chassis is one of 54 "cities" in 13 "states" on our roadmap



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Synthetic Biology Technology Roadmap draft – detailed level

Molecular level | **Subcellular structures/ components** | **Cellular level** | **Cell community/ tissue/organ level** | **Whole organism level and above**

Nodes in gray italics are general tools in genetic engineering, and not as specific to synthetic biology as the other sections are.

Engineering nucleic acids
1. DNA sequencing
2. DNA synthesis
3. Novel nucleic acids (NAs)
4. Engineered gene cassettes

Engineering amino acids
1. Biological drugs and monoclonal antibodies (mAbs)
2. Novel amino acids (AAs)

Engineering metabolic products
1. Small-molecule drugs/medicines
2. Industrial alcohols
3. Advanced biofuels and renewable chemicals
4. Flavors, scents, and other complex molecules

Engineering plasmids, chromosomes, and genomes
Nuclear transfer
DNA splicing
induced polyploidy
mutagenesis
plasmid
copy-number variation
Induced pluripotency
gene therapy

Peptide/protein/enzyme engineering
Rational protein design
directed evolution
site-directed mutagenesis
codon optimization
folding
phage display
–omics (e.g. proteomics)

Engineering with protein functions
1. catalysts and metabolic pathways
2. signaling (peptides, receptors, fluorescence)
3. mechanical (flagella, pumps)
4. structural (hair, spider silk, nacre, catalyst support…)
5. logical (promoters, operons, transcription factors)
6. DNA analogues

Assembling an artificial cell/ protocell/ "chassis"

Engineering cellular devices/ biodevices
1. Drug delivery bacteria

Engineering cellular functions
1. Internal regulation and signaling
2. Metabolism and catabolism
3. Movement, mobility, and transport (e.g. chemotaxis)
4. Division/replication
5. Cell death/apoptosis

Engineering biological computers
1. Data storage/encoding
2. Communication
3. Computational circuits (Boolean logic, Oscillator/repressilator, Imaging)

Engineering cell-cell communication
1. Physical/juxtacrine signals
2. Chemical signals
3. Light signals

Cell community-level applications
1. Preventing biofilms
2. Producing in-vitro meat
3. Repairing, replacing, and regenerating organs

Engineering complex organisms
1. Cloning livestock and pets
2. Bringing back extinct species, and the "modern mammoth"
3. Controlling body plan/hox genes/ontology
4. Biohacking, "radical chimerism," and the winged mouse
5. Growing a home – the "gourd house"
6. Human lifespan extension
7. Transhumanism

Engineering ecosystems
1. Bioremediation
2. CO2 sequestration
3. Bioterrorism
4. Apocalypse/green goo

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Q: What's a "Minimum-genome chassis"?
A: the simplest possible living cell

- A widespread goal of synthetic biology is to create a minimal cell chassis – a biological "breadboard" upon which to test new genetic circuitry. This would be a living cell, genetically **engineered to have only the minimal genes required to survive and reproduce**. The point of such a cell is that, without any extraneous functions, it would be easier to understand the workings of new genetic functions introduced into the cell. The minimal genes and their interactions would be easier to discover, and they would cause less inadvertent interference with the novel genes. (http://www.synberc.org/chassis)
- We can now begin working on our ultimate objective of synthesizing a **minimal cell containing only the genes necessary to sustain life** in its simplest form. This will help us better understand how cells work. … With this successful proof of principle, the group will now work on creating a minimal genome, which has been a goal since 1995. … This minimal cell will be a platform for analyzing the function of every essential gene in a cell. (http://www.jcvi.org, May 2010)

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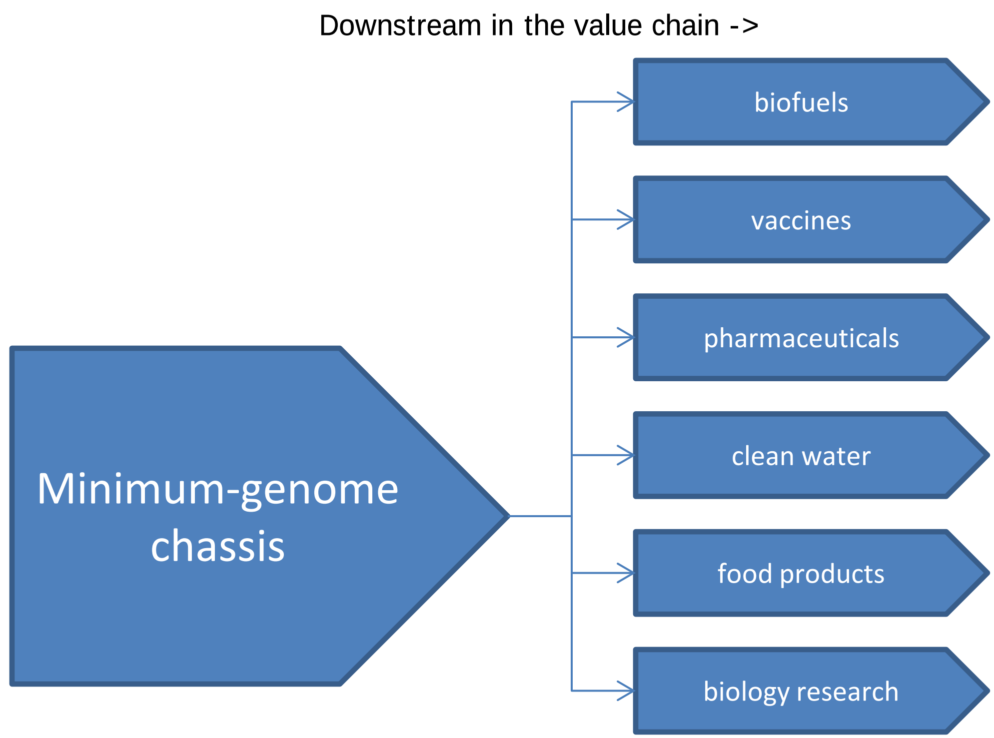
Putting the Minimum-genome chassis on the Roadmap -- where can you go from there?

20-May-2010
PRESS RELEASE
FOR IMMEDIATE RELEASE

First Self-Replicating Synthetic Bacterial Cell

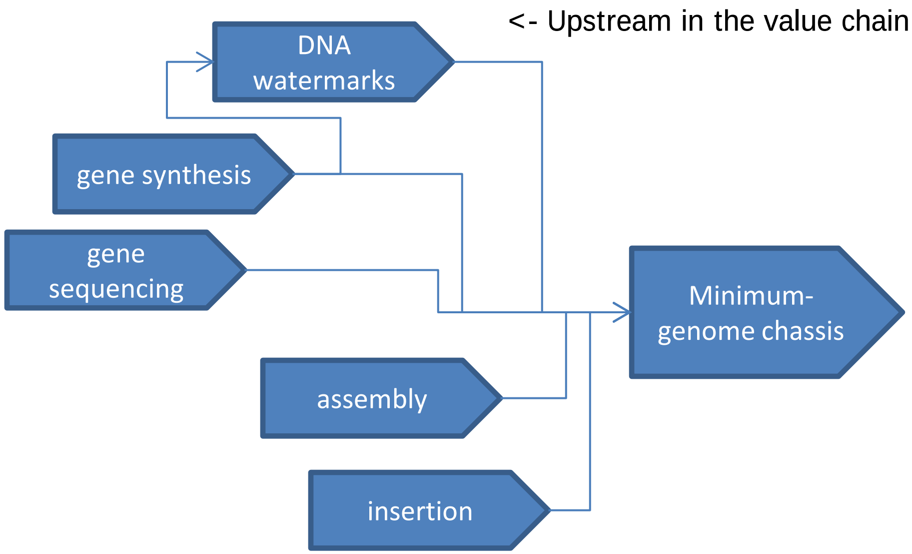
First Self-Replicating, Synthetic Bacterial Cell Constructed by J. Craig Venter Institute Researchers

ROCKVILLE, MD and San Diego, CA (May 20, 2010) — Researchers at the J. Craig Venter Institute (JCVI), a not-for-profit genomic research organization, published results today describing the successful construction of the first self-replicating, synthetic bacterial cell. The team synthesized the 1.08 million base pair chromosome of a modified *Mycoplasma mycoides* genome. The synthetic cell is called *Mycoplasma mycoides* JCVI-syn1.0 and is the proof of principle that genomes can be designed in the computer, chemically made in the laboratory and transplanted into a recipient cell to produce a new self-replicating cell controlled only by the synthetic genome. This research will be published by Daniel Gibson et al in the May 20th edition of *Science Express* and will appear in an upcoming print issue of *Science*.



… The JCVI scientists envision that the knowledge gained by constructing this first self-replicating synthetic cell, **coupled with decreasing costs for DNA synthesis**, will give rise to wider use of this powerful technology. This will undoubtedly lead to the development of many important applications and products including **biofuels, vaccines, pharmaceuticals, clean water and food products**. The group continues to drive and support ethical discussion and review to ensure a positive outcome for society. Funding for this research came from Synthetic Genomics Inc., a company co-founded by Drs. Venter and Smith.

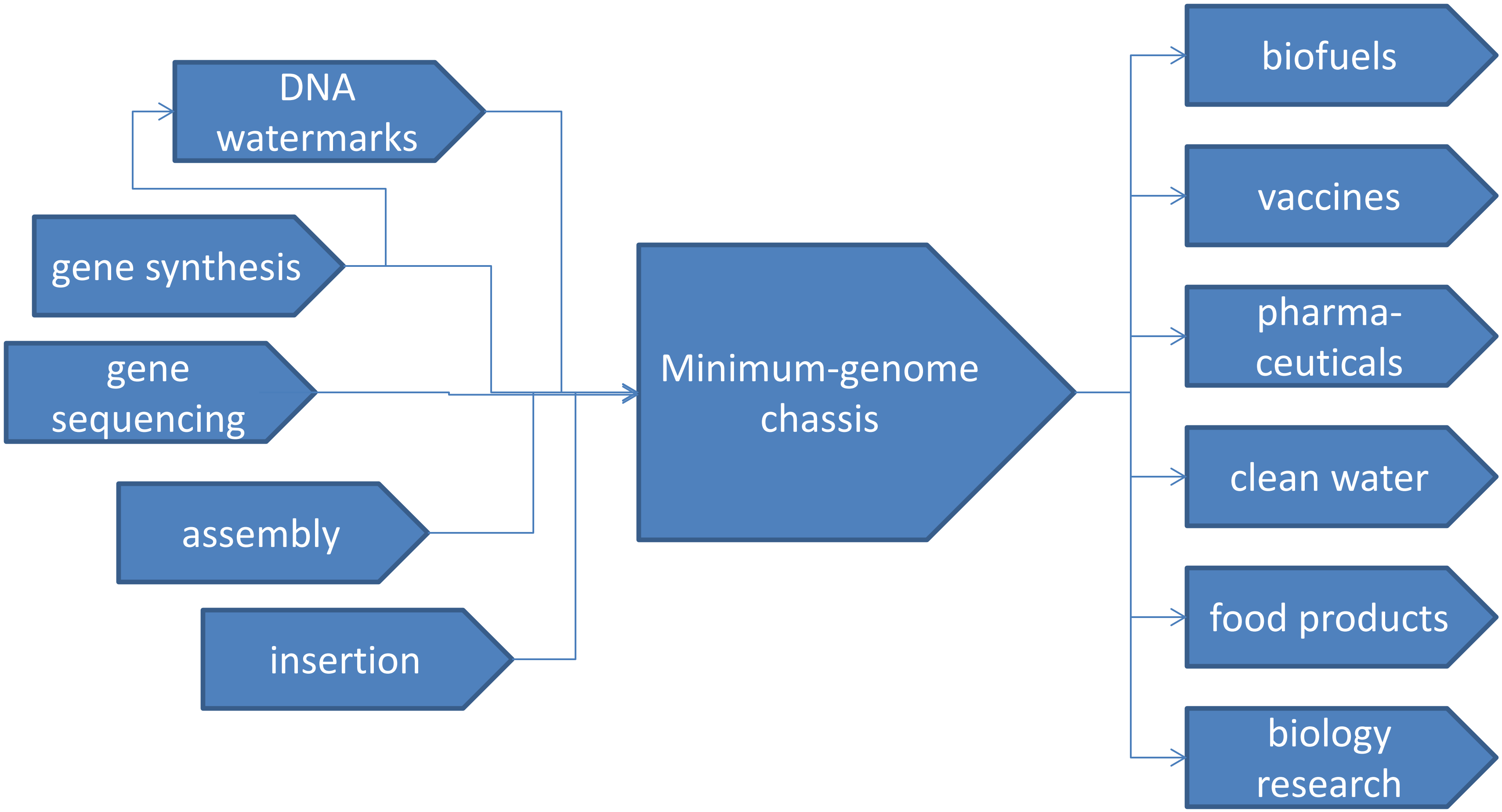
Putting the Minimum-genome chassis on the Roadmap – what do you need to get there?



- **Gene sequencing:** JCVI scientists began with the accurate, digitized genome of the bacterium, *M. mycoides*.
- **Gene synthesis:** The team designed 1,078 specific cassettes of DNA that were 1,080 base pairs long… The cassettes were made according to JCVI's specifications by the DNA synthesis company, Blue Heron Biotechnology.
- **DNA watermarks:** (The team) designed and inserted into the genome what they called watermarks. These are specifically designed segments of DNA that use the "alphabet" of genes and proteins that enable the researcher to spell out words and phrases. The watermarks are an essential means to prove that the genome is synthetic and not native, and to identify the laboratory of origin.
- **Assembly:** The JCVI team employed a three stage process using their previously described yeast assembly system …. all 11, 100 kb segments were assembled into the complete synthetic genome in yeast cells and grown as a yeast artificial chromosome.
- **Insertion:** The complete synthetic M. mycoides genome was isolated from the yeast cell and transplanted into Mycoplasma capricolum recipient cells that have had the genes for its restriction enzyme removed. …
- **Minimum-genome chassis:** "We can now begin working on our ultimate objective of synthesizing a minimal cell containing only the genes necessary to sustain life in its simplest form. This will help us better understand how cells work." … The group will now work on creating a minimal genome, which has been a goal since 1995.

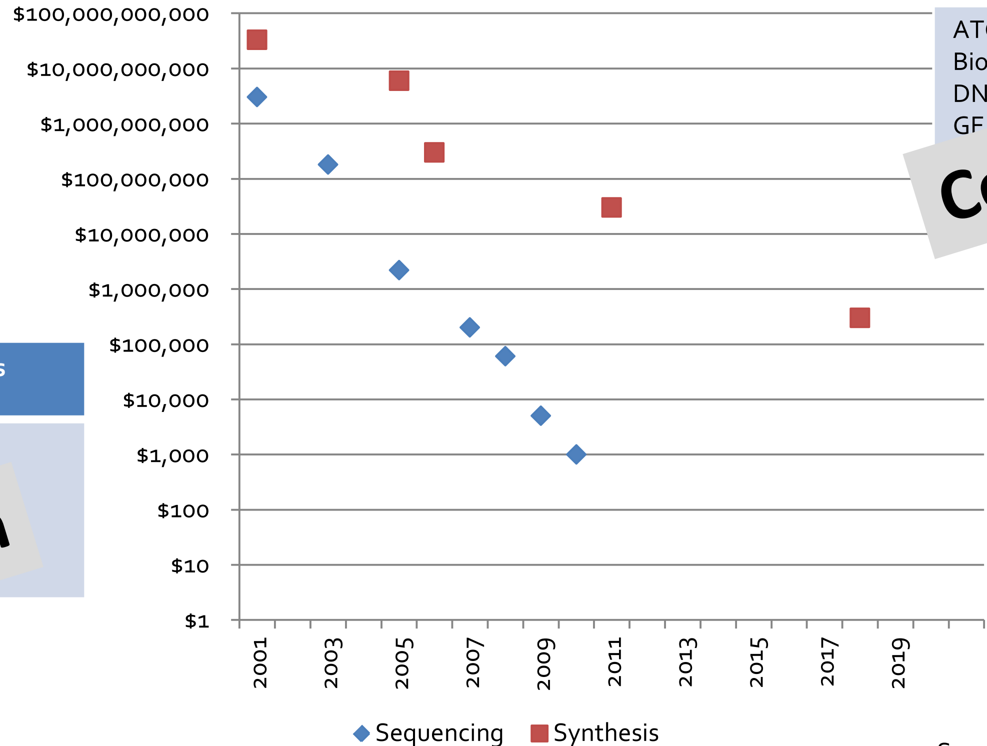
All text quoted directly from JCVI press release, "First Self-Replicating Synthetic Bacterial Cell", May 20, 2010

Putting the Minimum-genome chassis on the Roadmap – Where it fits in the value chain



DNA sequencing and synthesis
costs are rapidly declining

Cost to sequence or synthesize a human genome (3 billion bases)



Gene Synthesis Companies

ATG:biosynthetics, Blue Heron Biotechnology, Codon D... DNA 2.0, febit... GENE...

Consolidation

Gene Sequencing Companies

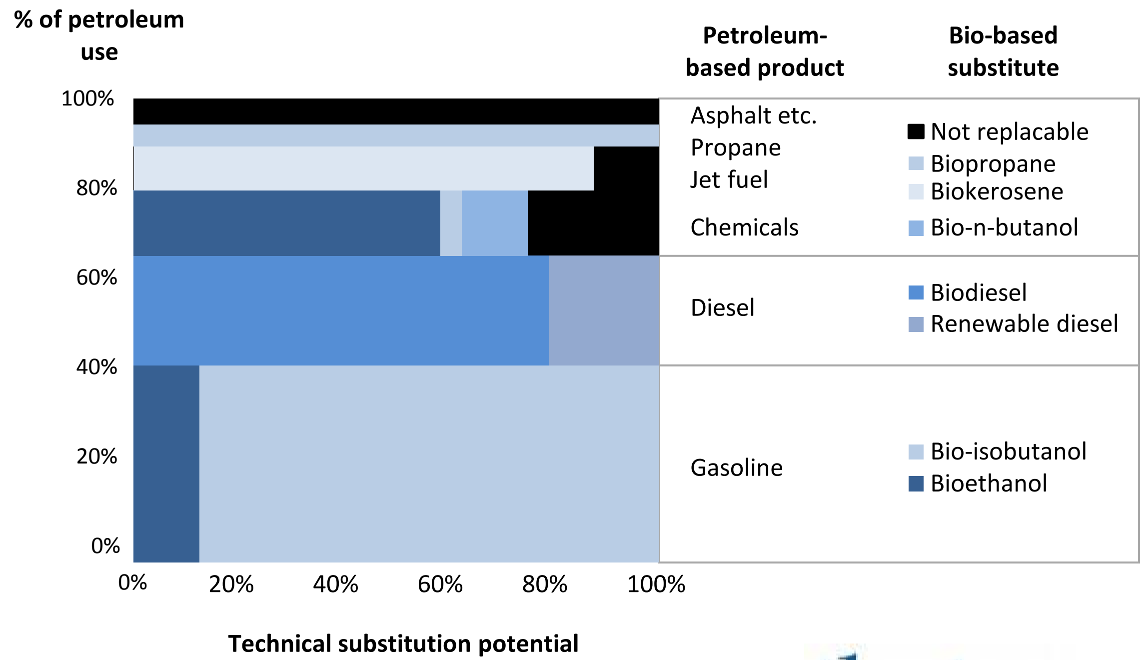
454 Life Sciences, Helicos Bio, Oxford Nanopore, Illumin... RainDance, Life T... Pacific B...

Innovation

Source: Lux Research. See also Rob Carlson

luxresearch

Bio-based fuels and materials can **replace 92% of petroleum products**



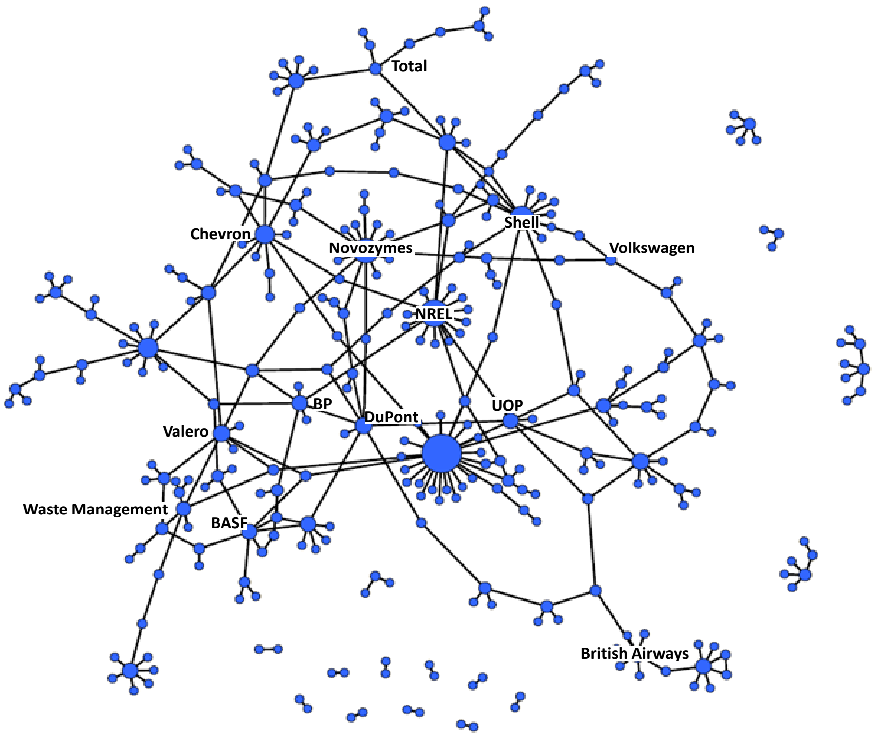
- Ethanol is a partial substitute for gasoline, but drop-in replacements (4- and 5-carbon alcohols) exist
- Ditto for diesel, jet fuel, and most petrochemicals
- Only heavy, tarry petrochemicals lack bio-replacements

jbei Joint BioEnergy Institute

LS9, INC.

BASF

Synthetic biology is shifting **from R&D to scale-up**



AMYRIS

solazyme

TERRABON

VIRENT

luxresearch

What we see:

- Corporate-startup partnerships are maturing the space
- Leading developers are shifting focus from science to scaling up capacity
- IPOs are succeeding: Amyris, Codexis, Gevo, Solazyme…
- …but stocks have suffered afterwards

What it means:

- Very difficult to enter with technology innovation today; partnerships are a must
- Drop-in fuels will leapfrog cellulosic ethanol

Tomorrow's innovators are already **playing with biotech toys**

Apple IIe (1980)

Discovery's
DNA Explorer Kit

DNA Depot's
Bioremediation by Oil-Eating Bacteria Kit

Biohacker clubs

Glowing Bacteria:
Transformation with a
Firefly Gene Kit

luxresearch

luxresearch

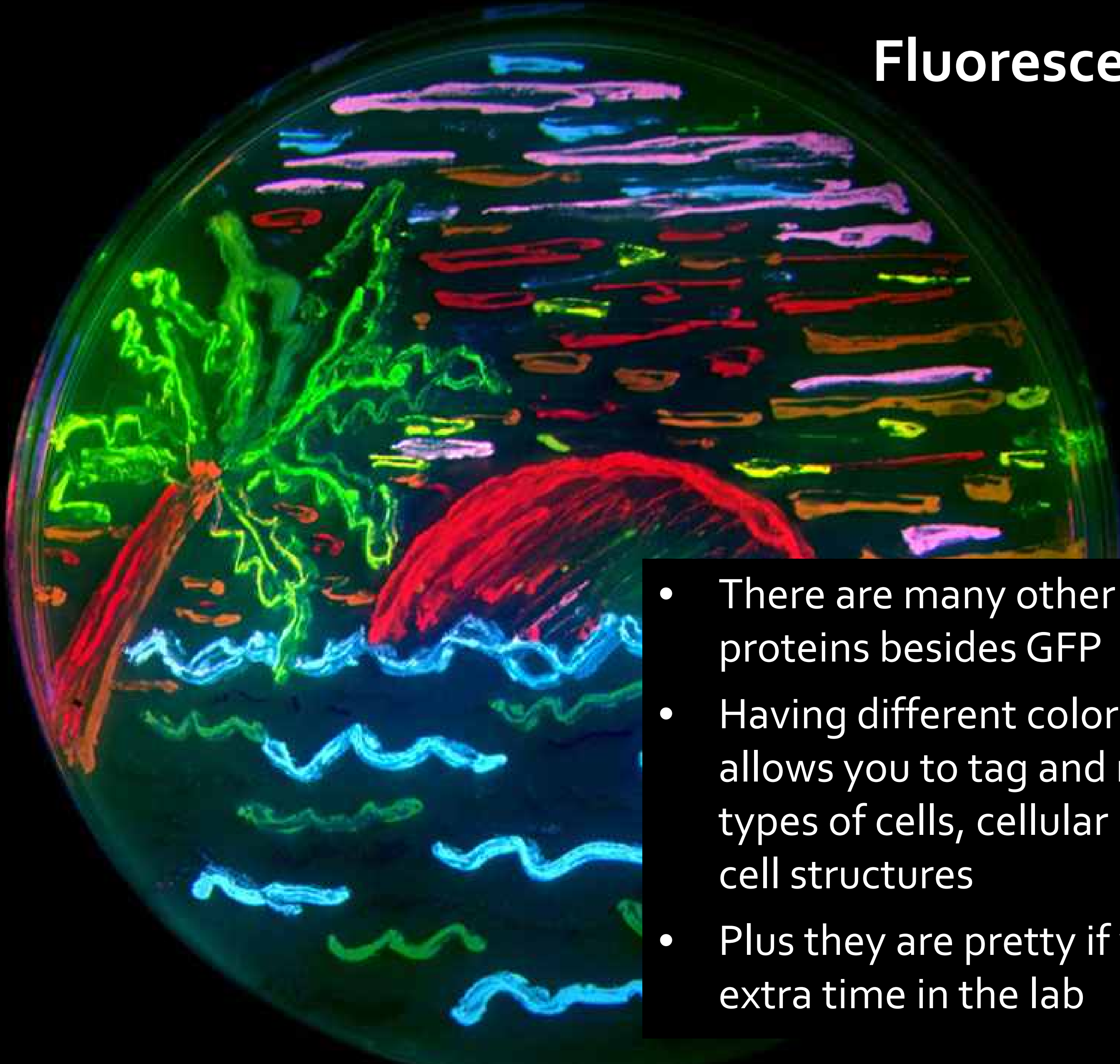
Thank you

Mark Bünger, Research Director

Lux Research, Inc.

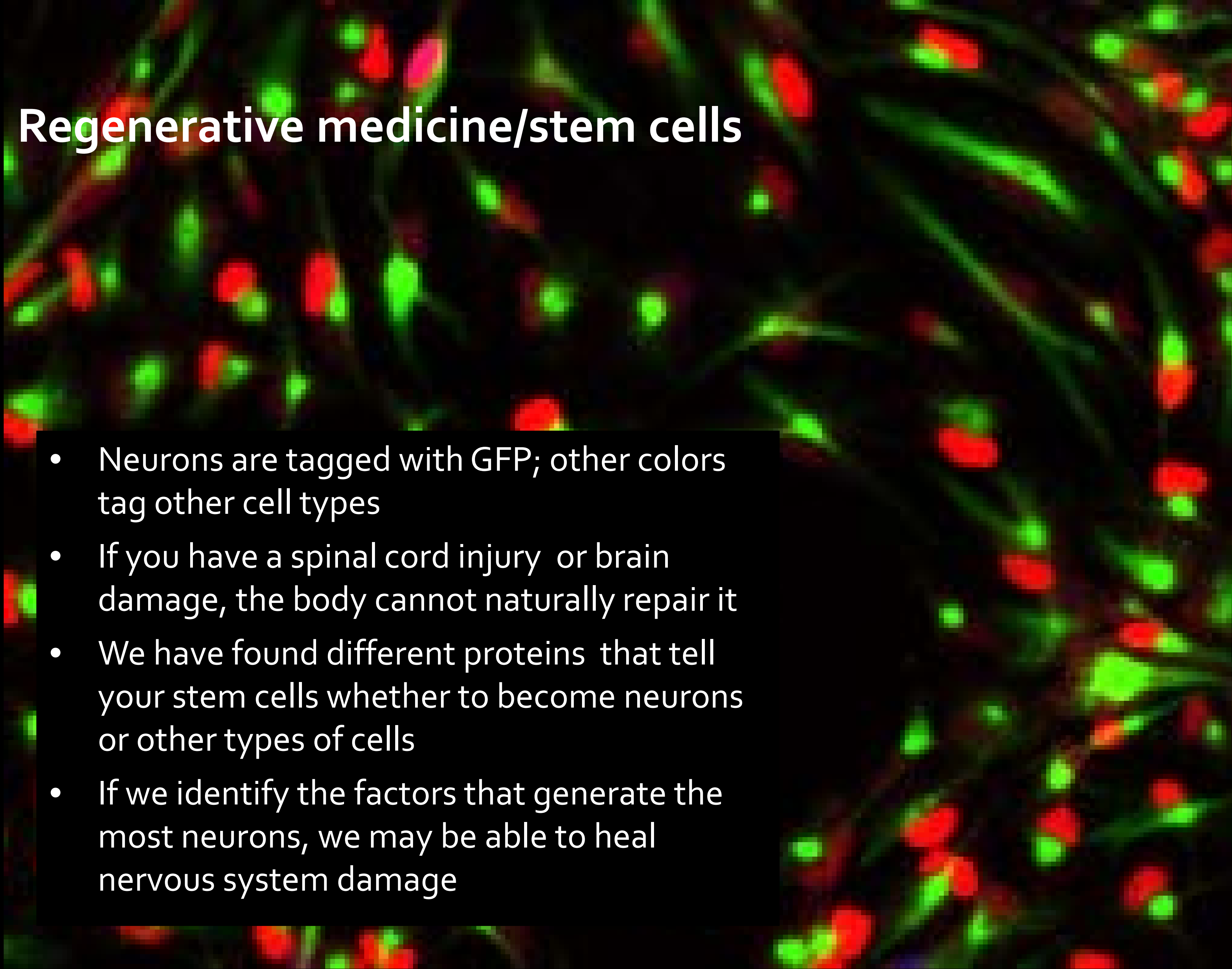
mark.bunger@luxresearchinc.com

Fluorescent tagging



- There are many other fluorescent proteins besides GFP
- Having different colored proteins allows you to tag and monitor many types of cells, cellular processes, and cell structures
- Plus they are pretty if you have a little extra time in the lab

Regenerative medicine/stem cells



- Neurons are tagged with GFP; other colors tag other cell types
- If you have a spinal cord injury or brain damage, the body cannot naturally repair it
- We have found different proteins that tell your stem cells whether to become neurons or other types of cells
- If we identify the factors that generate the most neurons, we may be able to heal nervous system damage

GloFish® www.glofish.com

- These zebrafish were originally engineered with GFP to glow green in the presence of pollutants
- The researchers used other color genes to add red and yellow fish
- For sale in Taiwan, US (except California), other markets since 2003

comments on this story

Stories by subject

- Biotechnology
- Cell and molecular biology
- Chemistry

Stories by keywords

- Lasers
- Green fluorescent protein
- GFP
- Biological lasers

This article elsewhere

Published online 12 June 2011 | Nature | doi:10.1038/news.2011.365

News

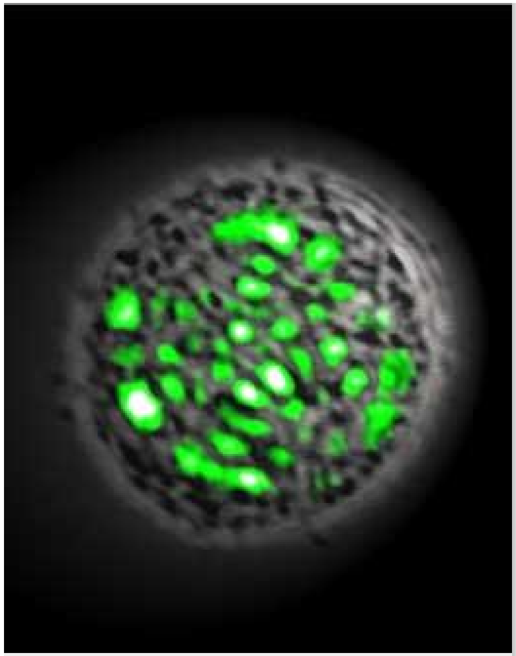
Human cell becomes living laser

Jellyfish protein amplifies light in first biological laser.

Zoë Corbyn

Scientists have for the first time created laser light using living biological material: a single human cell and some jellyfish protein.

"Lasers started from physics and are viewed as engineering devices," says Seok-Hyun Yun, an optical physicist at Harvard Medical School and Massachusetts General Hospital in Boston, who created the 'living laser' with his colleague Malte Gather. "This is the first time that we have used biological materials to build a laser and generate light from something that is living." The finding is reported today in *Nature Photonics* [1].



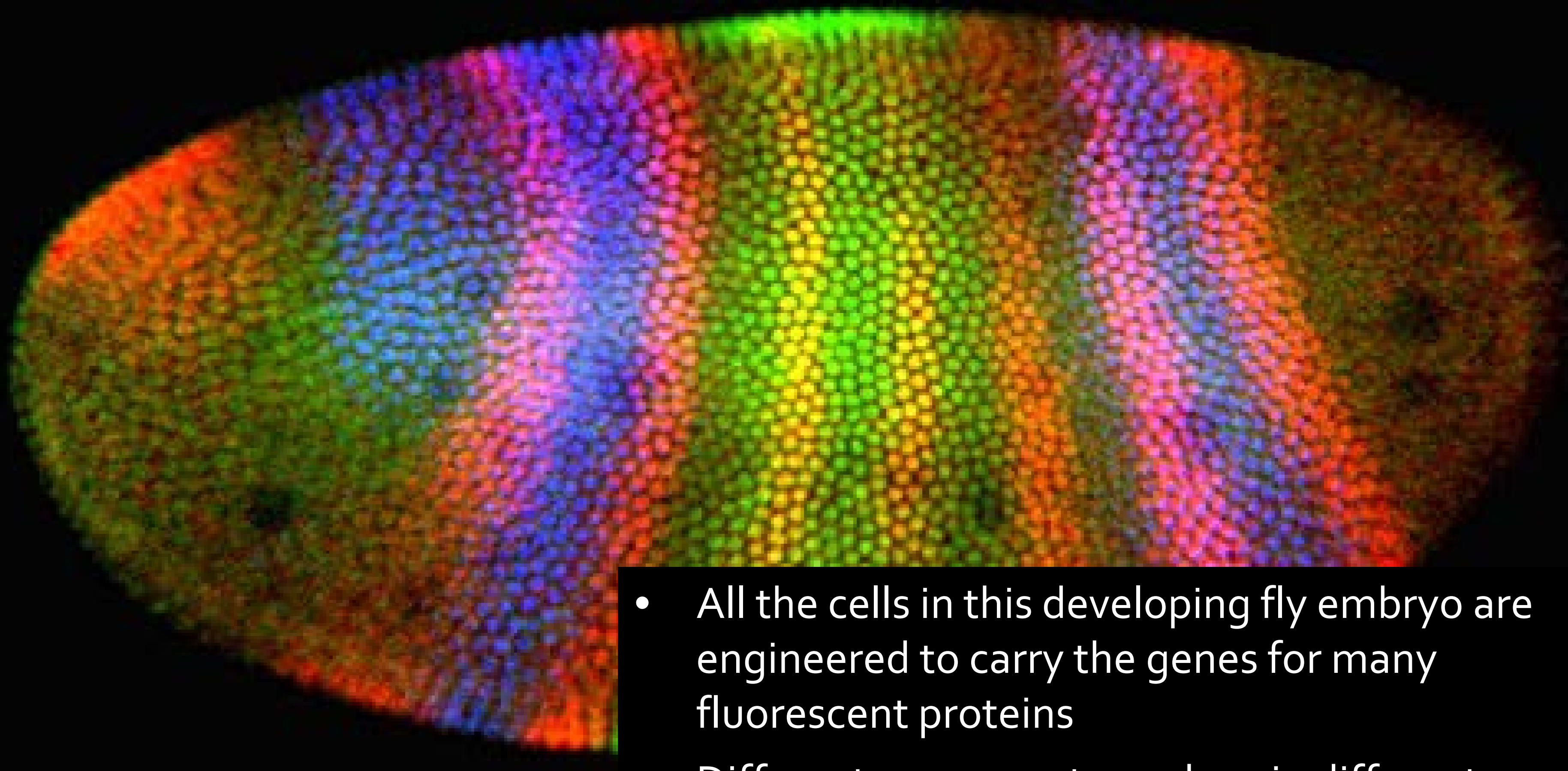
Microscope image of a living laser in action. Due to the irregular internal structure of the cell, the laser spot has an apparently random pattern.

Malte Gather

Laser cells!

Wow!

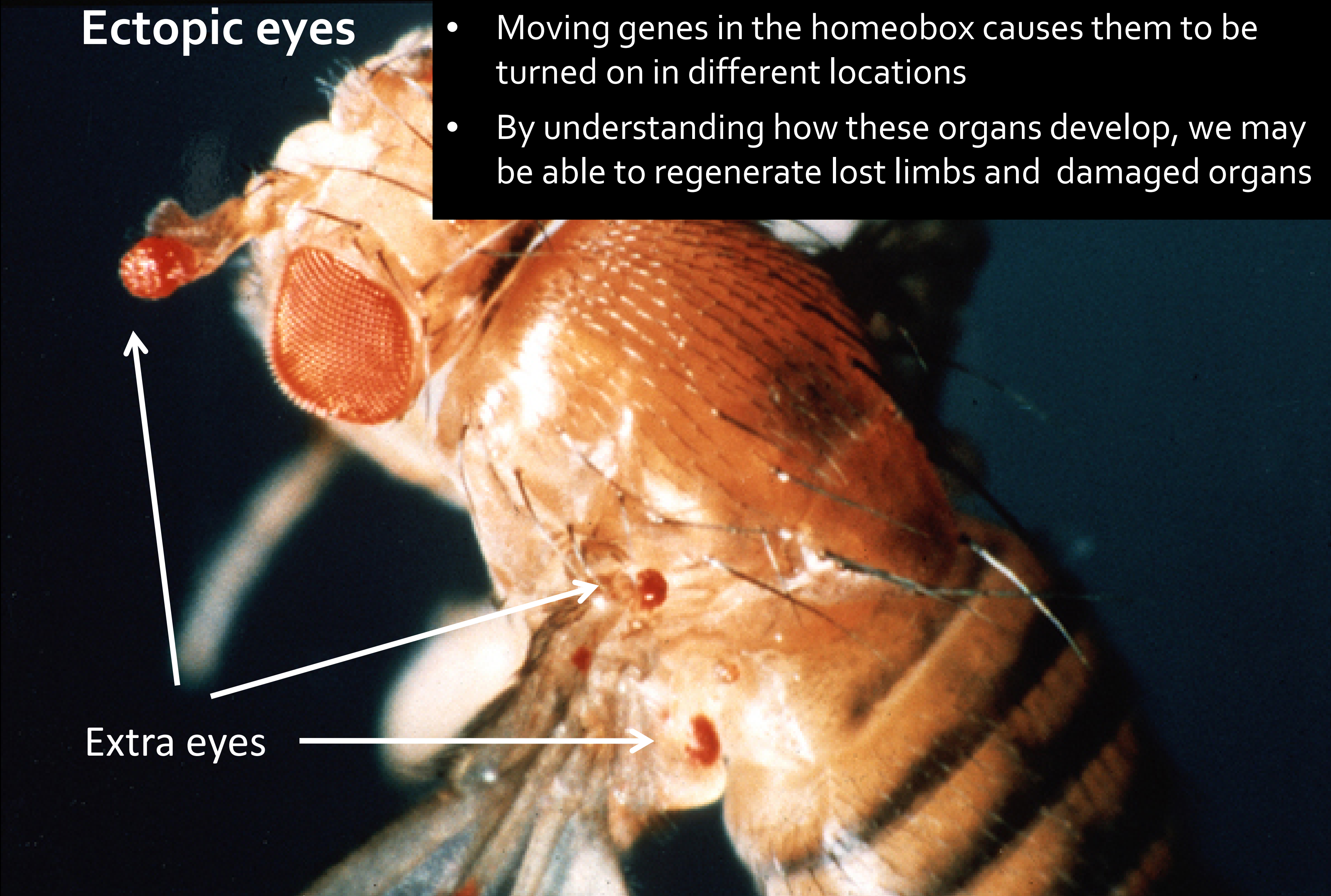
Developmental biology/embryology



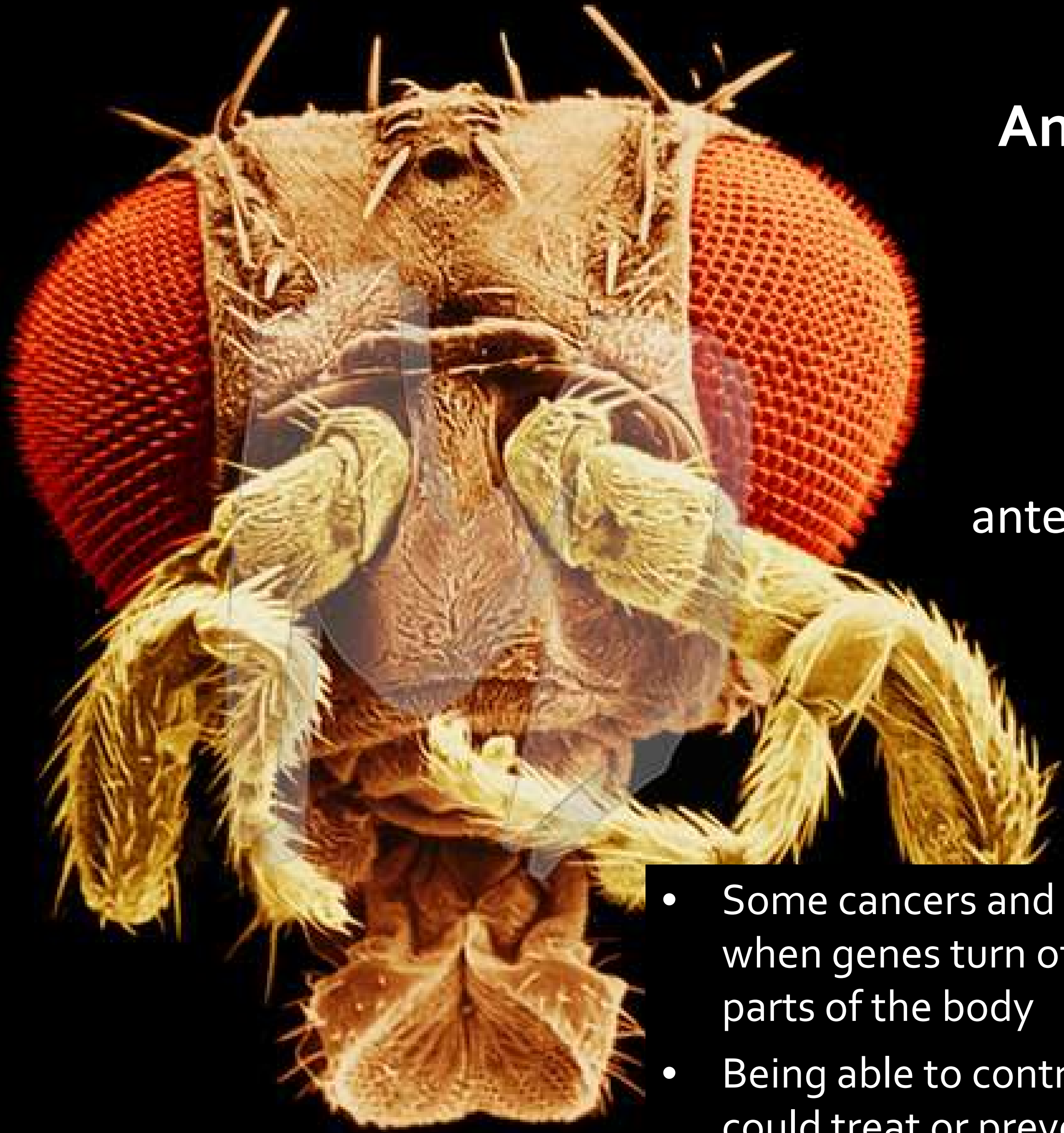
- All the cells in this developing fly embryo are engineered to carry the genes for many fluorescent proteins
- Different genes are turned on in different parts of the body, and will become head, thorax, abdomen…
- By understanding how other animals develop, we may be able to treat birth defects

Ectopic eyes

- The “homeobox” is a segment of DNA that functions like a physical model of the overall organism
- Moving genes in the homeobox causes them to be turned on in different locations
- By understanding how these organs develop, we may be able to regenerate lost limbs and damaged organs



Antennapedia



Legs where antennae should be

- Some cancers and other diseases arise when genes turn off or on in the wrong parts of the body
- Being able to control genes locally could treat or prevent these diseases

Optogenetics

- This rat's neurons express channel rhodopsin, a light-sensitive protein from algae
- The researcher can elicit specific responses (fear, happiness, hunger…) without having to actually scare/cuddle/starve the animal
- Understanding how the brain responds to various stimuli teaches us about trauma, emotion, addiction, and other mental/physical functions
- Could lead to fiber-optic artificial nerves

Appendix D

Kelly Baerwaldt Presentation

Asian Carp: Population Monitoring and Prevention of Great Lakes Invasion

Kelly Baerwaldt
US Army Corps of Engineers
eDNA Program Manager
15 April 2013

ACRCC

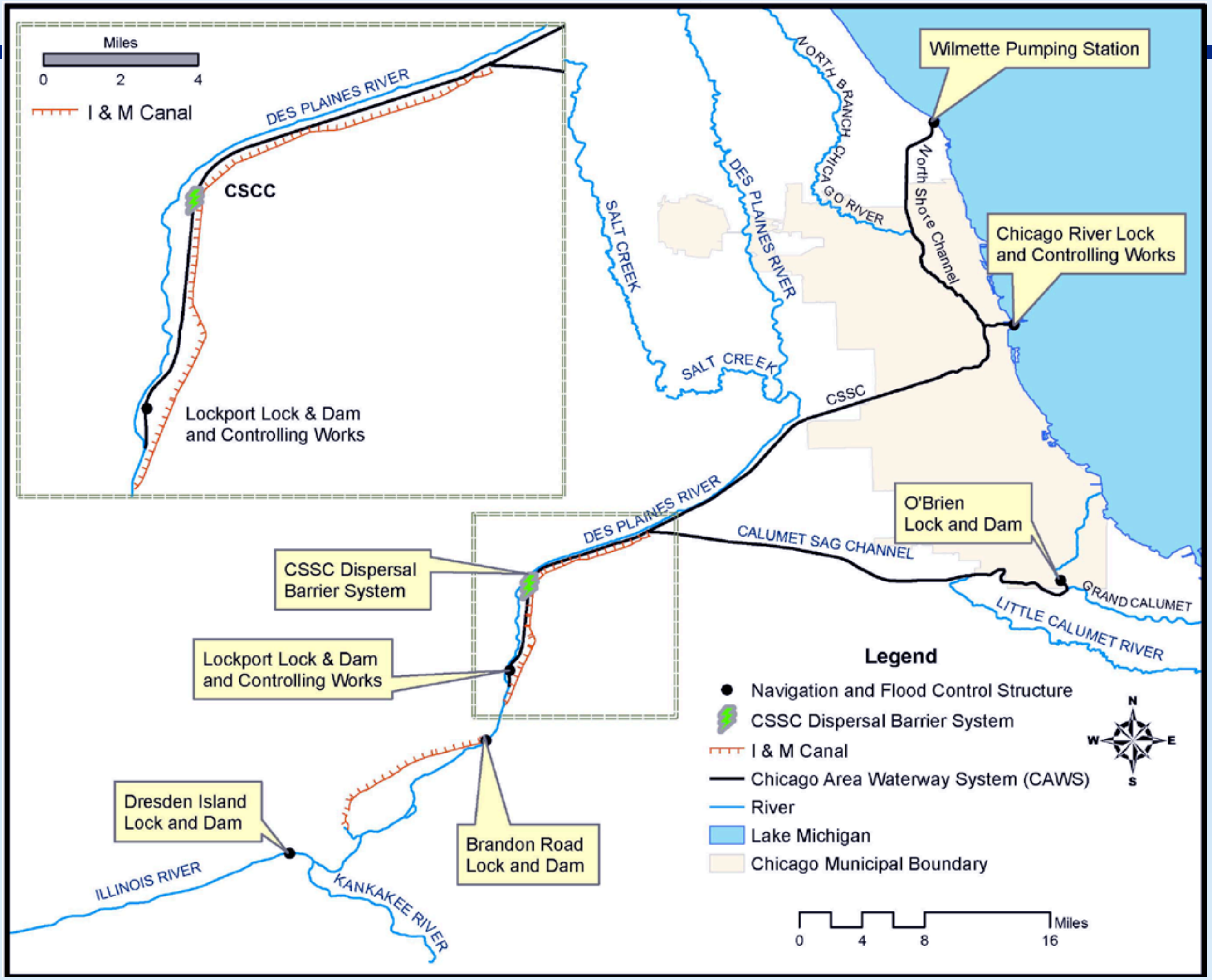
Asian Carp: Why Are We Worried?

- Silver carp tend to jump
- Large bodied
  - Bighead: 5 feet, >100 pounds
  - Silver: 3 feet, 60 pounds
- Reproduce, develop quickly
  - 1 female = up to 400 million eggs
  - Can spawn multiple times/year
  - Grow to >12” in first year, making them less vulnerable to predators
- Eating machines
  - 40% (adults) to 120% (juveniles) of body weight/day
  - Planktivores; compete directly and indirectly
  - Eat same food as native and commercially important species
- Illinois Waterway downstream population = high abundance


COMING SOON (MAYBE)
INVASION OF THE FLYING FISH
PRODUCED BY ARKANSAS FISH FARMERS • DISTRIBUTED BY MISSISSIPPI RIVER • RELEASE DATE EARLY 1980s
STARRING: ASIAN CARP • FEATURING: FLYING SILVER CARP • CO-STARRING: BIGHEAD CARP, BLACK CARP AND GRASS CARP

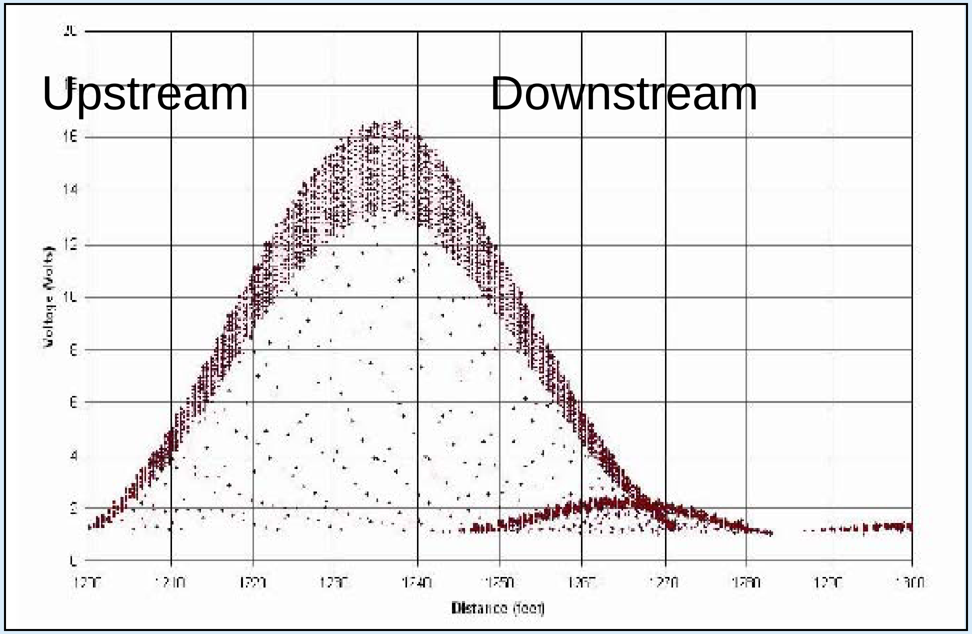
Chicago Area Waterway System (CAWS)

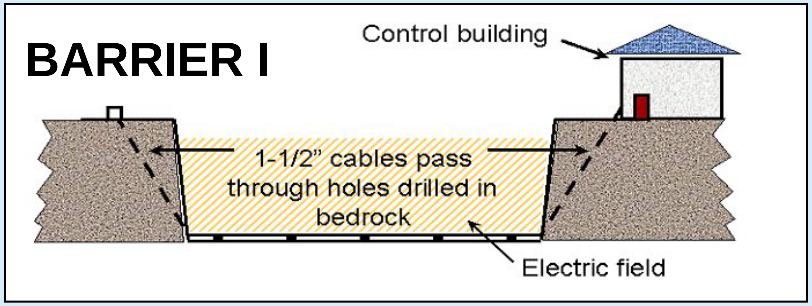
- Only continuous pathway connecting the Great Lakes and Mississippi River basins
- Consists of 78 miles of canals and modified streams
- Five outlets to Lake Michigan within the CAWS



How Do The Dispersal Barriers Work?

- Electrodes are Secured to the Bottom of the Canal.
- Electrical Cables Connect the Electrodes to the Control Building.
- A Pulsing DC-Current is Generated in the Control Building & Sent to the Electrodes Creating an Electric Field in the Water.
- The Electric Field is Uncomfortable for Fish & They Do Not Swim Across It





Asian Carp Regional Coordinating Committee (ACRCC)

Federal Executive Committee
John Goss, Chair
Cameron Davis, Co-Chair

Jim Bredin
Bill Bolen | Margaret Burcham
Michael Parks | Fred Drummond
Charles Wooley | Leon Carl

↓

Regional Coordinating Committee
John Goss, Chair
Cameron Davis, Co-Chair

USEPA	Illinois
USFWS	Indiana
USGS	Michigan
USCG	Minnesota
GLFC	New York
USACE	Ohio
NOAA	Pennsylvania
USDOT	Wisconsin

City of Chicago
MWRD

Monitoring and Rapid Response Workgroup
John Dettmers
John Rogner

Communication and Outreach Workgroup
Katie Steiger-Meister
Chris McCloud

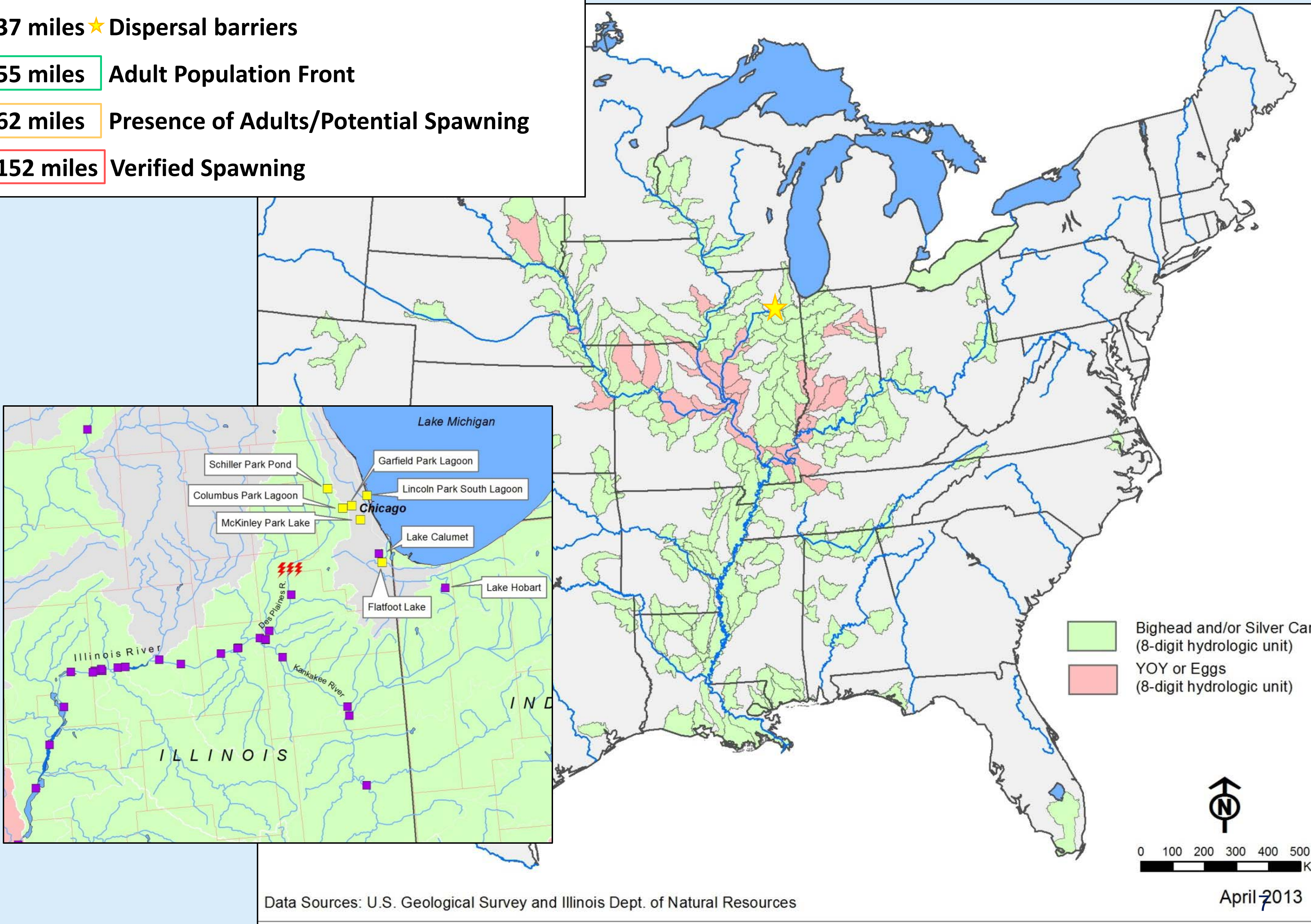
Distances from Lake Michigan in IWW

37 miles ★ **Dispersal barriers**

55 miles **Adult Population Front**

62 miles **Presence of Adults/Potential Spawning**

152 miles **Verified Spawning**



Asian Carp Monitoring Tools of The Trade
COMMERCIAL NETTING

Description: Physical removal of adult bighead and silver carp from Dresden Island and Marseilles Pools

Requirement: ILDNR contract with commercial fishers

ASIAN CARP Regional Coordinating Committee

Asian Carp Monitoring Tools of The Trade
ELECTROFISHING

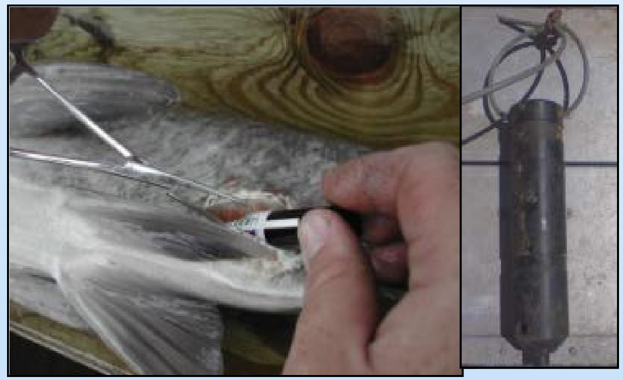
Description: Surveillance of fixed sites and reaches above the Barrier for early detection monitoring using fleet of interagency electrofishing boats

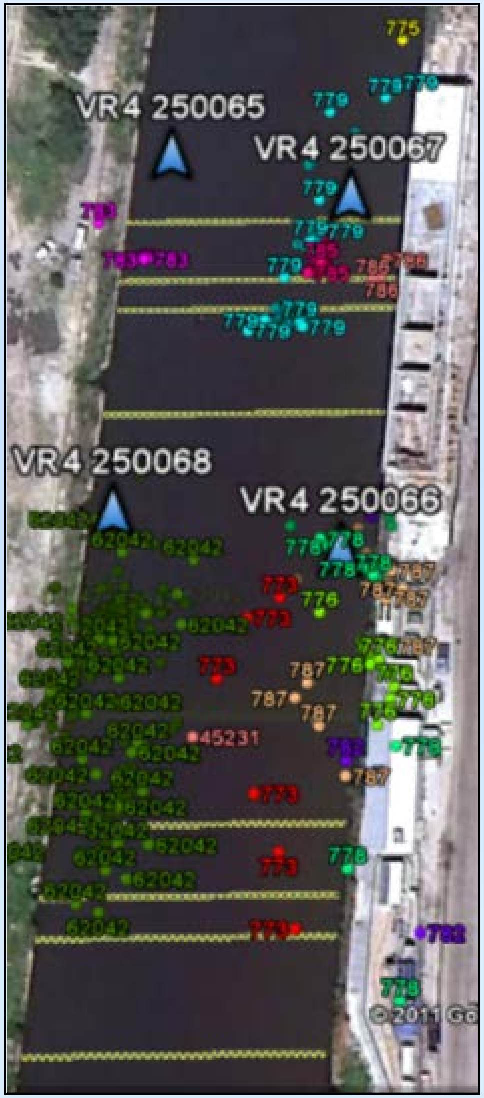
Requirement: USACE/ILDNR/USFWS crews every other week

Asian Carp Monitoring Tools of The Trade
TELEMETRY (Tagged fish)

Description: Tagging Asian carp w/ultrasonic tags & monitoring for tag signals from stationary and mobile receivers. Also observe tagged surrogate fish behavior as they approach Barrier.

Requirement: 240 tags; 30 receivers; 1 mobile unit (USACE lead)



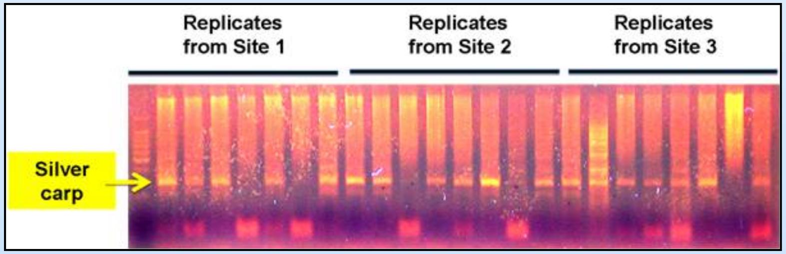


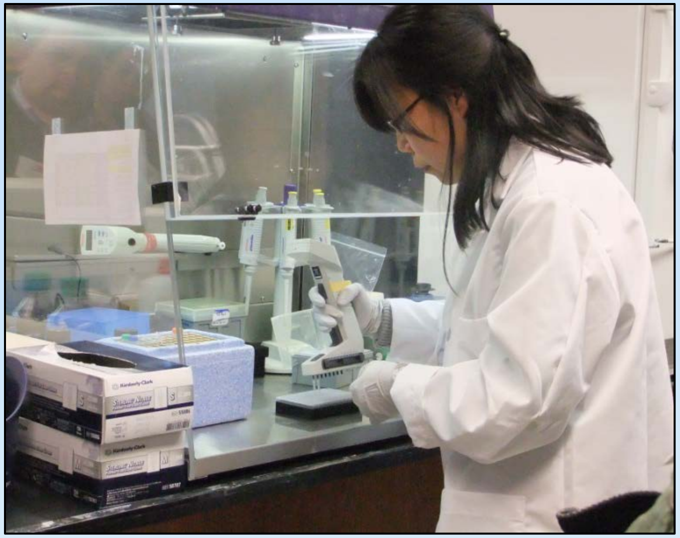
ACRCC

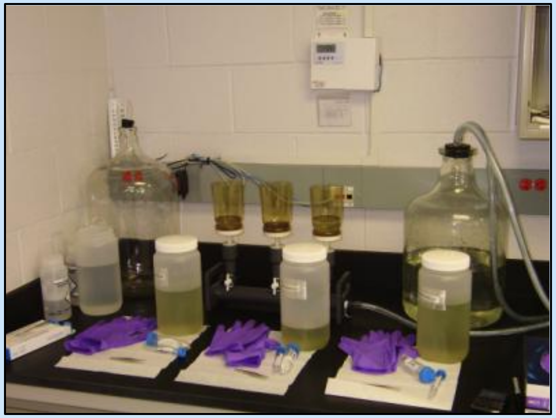
Asian Carp Monitoring Tools of The Trade Environmental DNA (eDNA)

Description: Genetic surveillance method for detecting bighead and silver carp DNA above Barrier

Requirement: Bi-Weekly sampling from priority sites, USFWS/ILDNR collect, USACE filters and processes







Population Monitoring Upstream of the Dispersal Barrier

No Bighead Carp or Silver Carp captured or observed in 2011 or 2012

- Thousands of hours of effort in electrofishing and netting; hundreds of thousands of fish collected (demonstrates through sampling of fish populations and low to zero abundance of Asian carp)

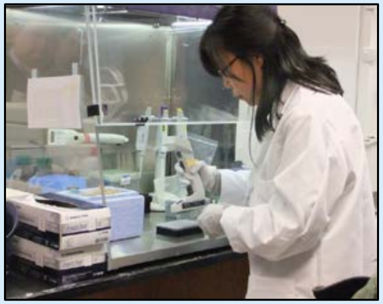
- Nearly 250 tagged fish in system, over 6 Million detections of those fish, no upstream movement through Fish Barrier (demonstrates effectiveness of barrier)

- Genetic surveillance (eDNA) has shown positive detections for both species in areas where monitoring has failed to detect Asian carp.

- New research on eDNA suggests that alternate sources of DNA may be the cause (such as birds, boats and barges, sewage outfalls); we are working with federal agency partners to further refine this tool for its application and interpretation

eDNA Calibration Study (ECALS)

ECALS is an interagency study (USACE-USGS-USFWS) that will improve our understanding and interpretation of eDNA positive detections



- What does a positive eDNA detection in the CAWS mean?
  - Where did it come from?
  - How long has it been there?
  - Did more than one Asian carp contribute to the sample?
- Improve sampling and analytical efficiency
  - Reduce the TIME and COST
- Provide context and improve confidence in conclusions based on eDNA monitoring as an effective tool for resource managers in decision making

ECALS is funded through the ACRCC Framework, with three major tasks:

1. Vectors:
   – Alternative sources of eDNA? (birds, barges, sewers, sediments, fishing gear)
2. Markers:
   – Develop new markers: estimate minimum numbers of individual Asian carp
3. Calibration:
   – Increase efficiency (sampling, lab analysis)
   – Determine eDNA degradation and influence of environmental factors (temperature, pH)
   – Hydrodynamic model: how eDNA moves through system
   – Assign probabilities to eDNA vectors based on all ECALS results

- Actively assessed in ECALS:
  - Sewers ✓
  - Barges ✓
  - Birds ✓
  - Fertilizers ⃠
  - Gear contamination ✓
  - Sediments ✓

Photo by Kevin Irons, ILDNR

Appendix E

Mike Jawson Presentation

Asian Carp
Exploring Synthetic Biology in the Great Lakes

Dr. Michael Jawson

USGS

Director, Upper Midwest Environmental Sciences Center

April 15, 2013

USGS Focus

(UMESC, CERC, IL WSC, IN WSC, GLSC, WFRC plus IL DNR, USACE, FWS and many university and other partners and collaborators)

- **Initially**
  - → **Can Asian carp get to, survive and thrive in the Great Lakes (Hydrology, Habitat and Life-cycle)**
  - → **Deterrence and Control**
  - → **Detection (eDNA/ECALS, microbial source tracking)**
- **Now**
  - → **Deterrence and Control**
  - → **which requires additional hydrological, habitat and life-cycle research**
  - → **Detection (eDNA/ECALS, MST, etc)**
  - → **Great Lakes and Mississippi River basin**

USGS

Initial Hydrology, Habitat and Life-Cycle Project Results

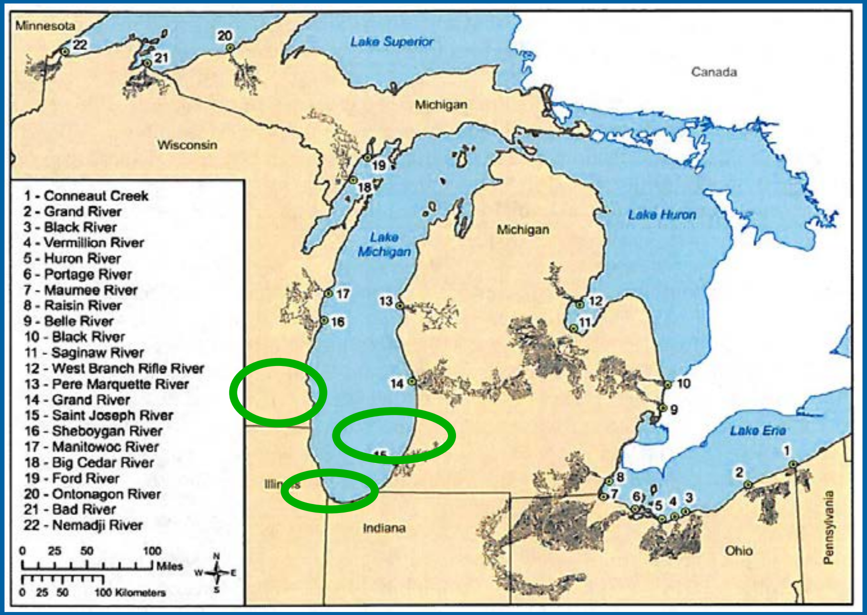
Asian carp – lake fish that need flowing water to reproduce.

(Duane Chapman, USGS, CERC)

USGS

Biological Risk Assessment

- Can Asian carp Survive and Develop Reproducing Populations in the Great Lakes?
- Assess suitability of tributaries as spawning habitat for Asian carp
- Determining conditions needed for successful spawning



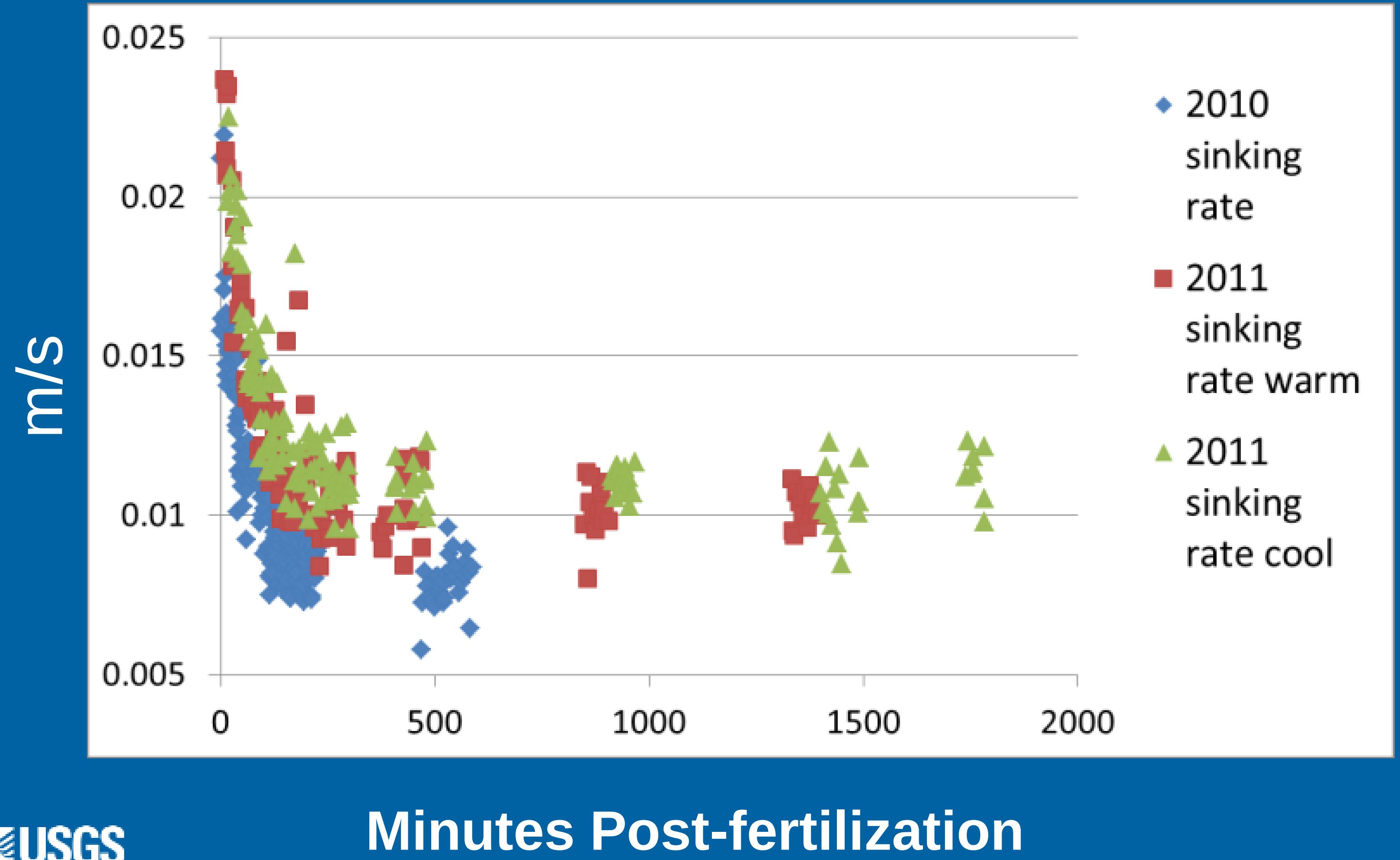
USGS

Trib Assessment Tool: Asian Carp Early Life History: Developmental Rate and Behavior

- Developed models of the developmental rate of bighead and silver carps at different temperatures
- Behavior of larvae described – swimming ability, speed, types of swimming, response to stimulus, location in water column
- Sinking rate and specific gravity of eggs at all developmental stages described
- Assessed survival of eggs on a variety of substrates

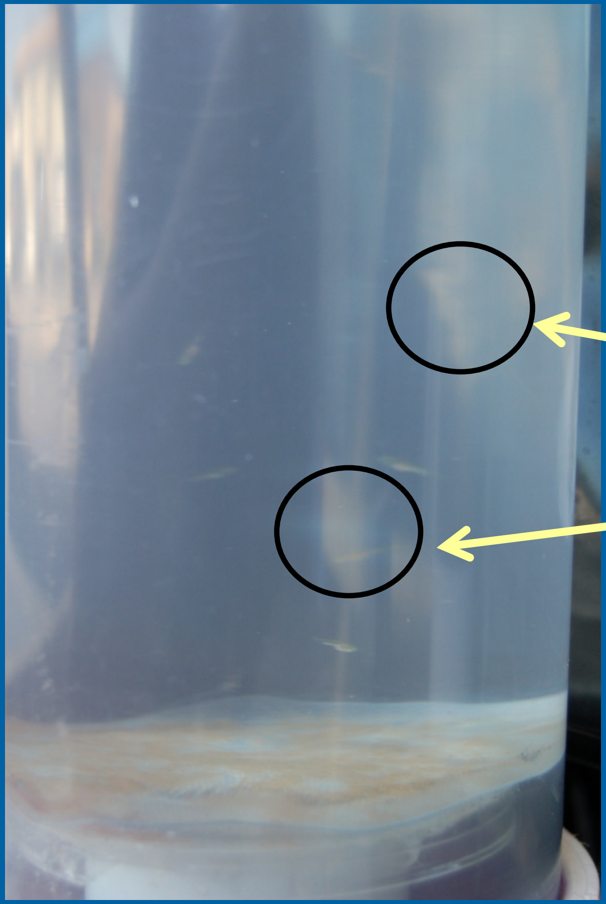
USGS

Sinking rate of bighead carp eggs



USGS

Fish Behavior Studies:
Early Life History Findings



Larval Asian carp

Vertical swimming occurs much sooner than previously thought

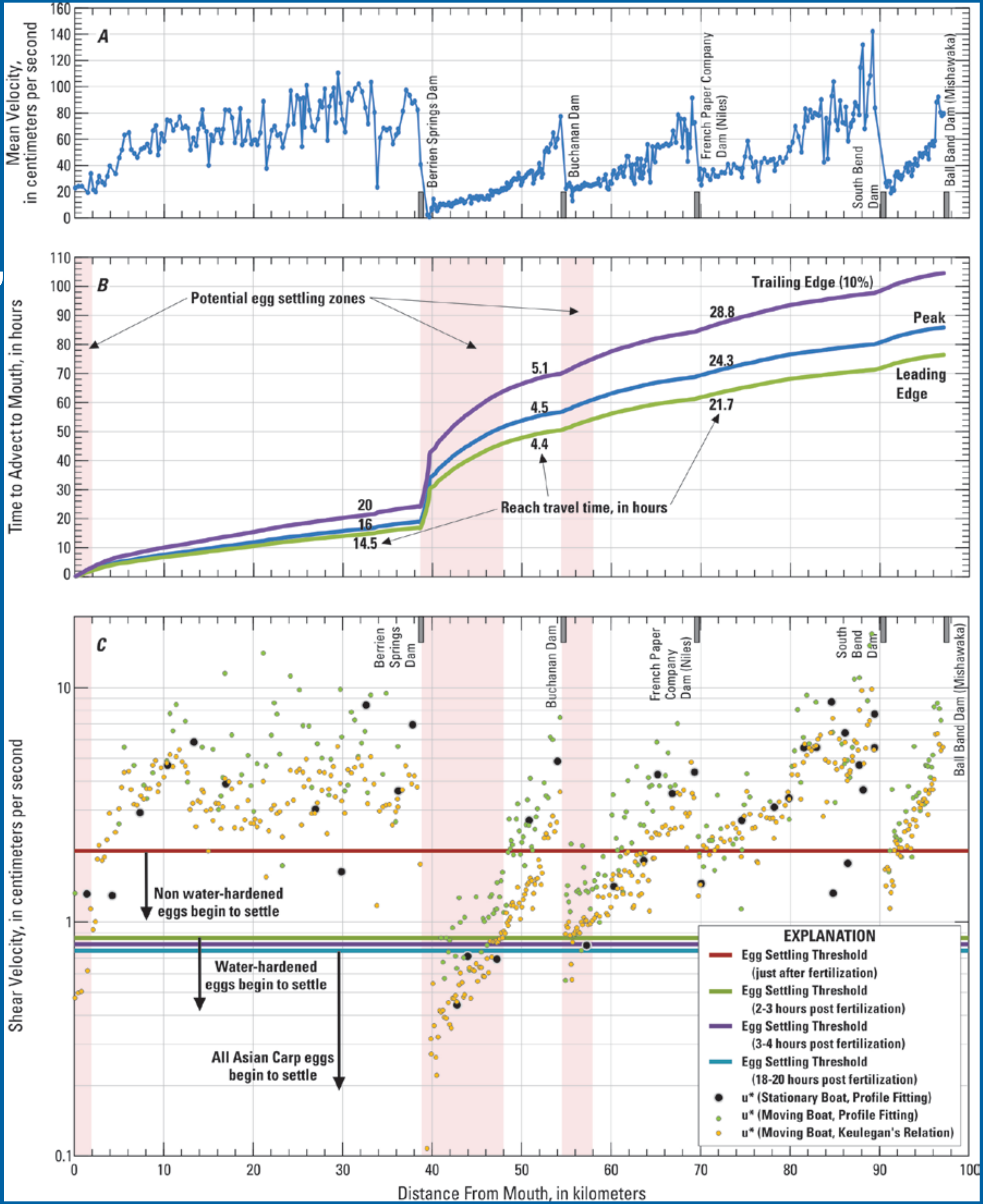
Tributary Assessment Tool

A model to ID suitable AC spawning rivers and inform potential management actions

- A prototype tool has been developed that incorporates biological and hydraulic data into a model (the “tool”) to assess risk of successful AC spawning in a river.
  - Study included Milwaukee, St Joseph, Maumee, and Sandusky Rivers
- The tool will help managers target locations in GL tributaries where eggs may settle to the river bottom and die. This model will also inform control efforts.

USGS

Hydraulic data analysis, St. Joseph River



Tributary Assessment Tool

A model to ID suitable AC spawning rivers and inform potential management actions – *Next Steps*

- The tool could be applied to assess other rivers both in and out of the GL basin.
  - Specific hydraulic data would need to be collected
- The first phase of this project will be completed by FY13 with some publications in FY14.
- Dissemination plans
  - Publication on Tool in review. Once published, we will help managers utilize the tool and information
  - Tool demonstration – April 2013 at Asian Carp meeting
  - Webex and workshops for managers

USGS

Bioenergetics Model

- A published theoretical bioenergetics model has been evaluated, had errors eliminated, and improved.
- Bioenergetics model testing indicates the current model:
  - over-predicts weight gain and growth where food resources are plentiful and
  - over-predicts weight loss and growth with low or no food availability.

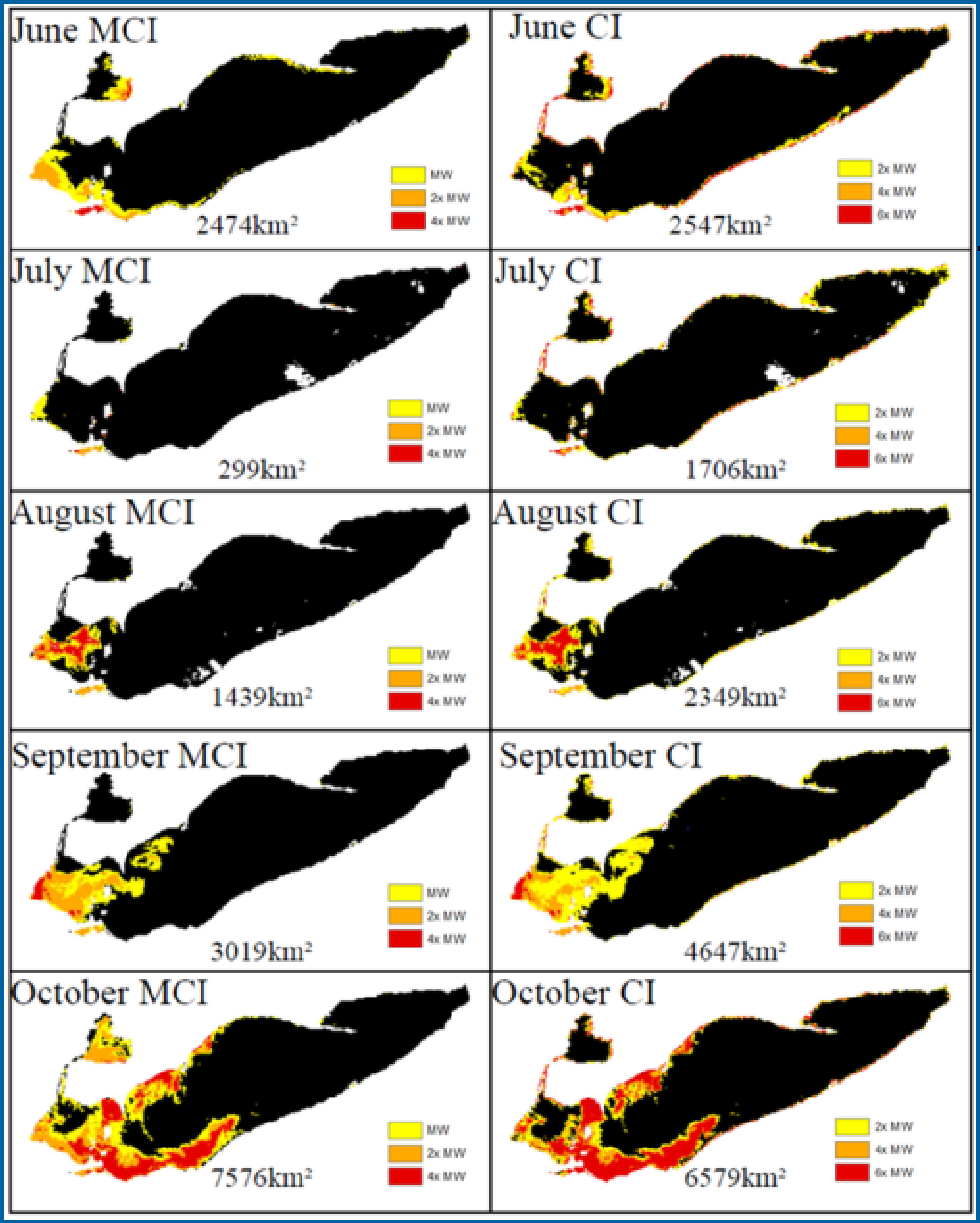
USGS

Asian carp use of Alternative Foods in the Great lakes

- **Two non-planktonic potential foods that are highly abundant in the Great Lakes (*Cladophora* algae and dreissenid pseudofecies) were evaluated as alternative food sources for bighead and silver carps**
- **These alternative foods can provide additional nutrition for Asian carp in areas and times of low planktonic food availability.**
- **Results of this project also indicate that bluegreen algae blooms could be very important food sources for bigheaded carps if they invade the lakes.**

USGS

Max chlorophyll index Cyanobacteria index



Lake Erie modeled with multiples of minimum algae to maintain wt for adult silver carp. Note that for CI yellow = 2X minimum.

USGS

Bioenergetics and Alternative Foods
Next Steps

- Results of study of Asian carp potential enhancing effect on bluegreen blooms currently under analysis.
- Bioenergetics model has been used to map areas with suitable concentrations of green and bluegreen algae in Lake Erie
- Similar maps for Lake Michigan under development
- Information will be made available in journal publications and through presentations in scientific meetings.

USGS

Chicago Area Waterway System Monitoring Network Evaluation

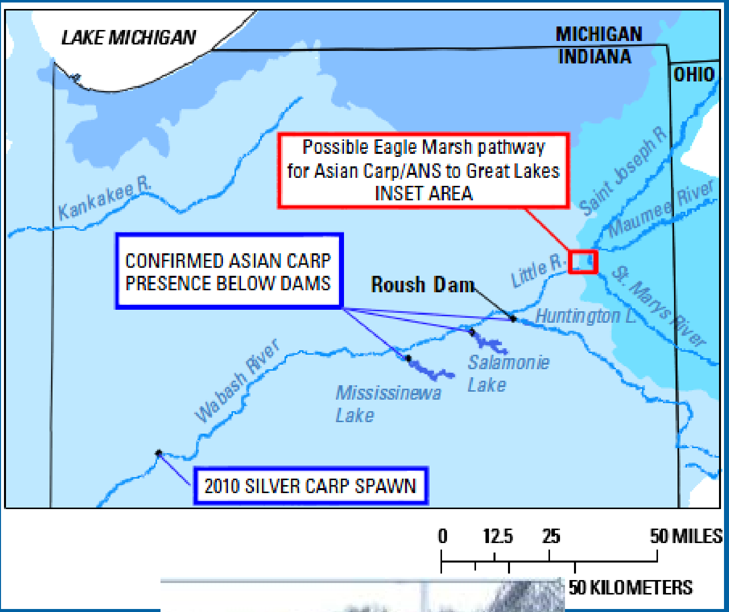
- The CAWS is a complex hydraulic system. USGS developed the CAWS database (CWO) to:
  - Understand how best to monitor and interpret sampling results and guide rapid response efforts
  - Facilitate efficient use of historic and current data for model development and analysis of waterway separation scenarios.
- Also collected new waterway data to fill data gaps and supplement fixed long-term monitoring stations

Chicago Waterway Observatory (CWO)
http://il.water.usgs.gov/data/cwo/

USGS

Wabash-Maumee Interbasin Connection:
Preventing adult Asian Carp transfer to Great Lakes

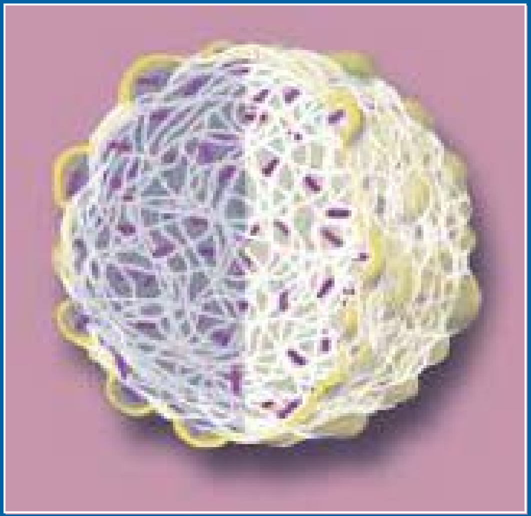
- **USGS streamflow gages improve separation design**
- **USGS radar gages identify high water internet alerts**
- **Similar strategies could be used in other high priority invasion pathways**
- **In cooperation with Purdue, expanding telemetry network in Wabash**



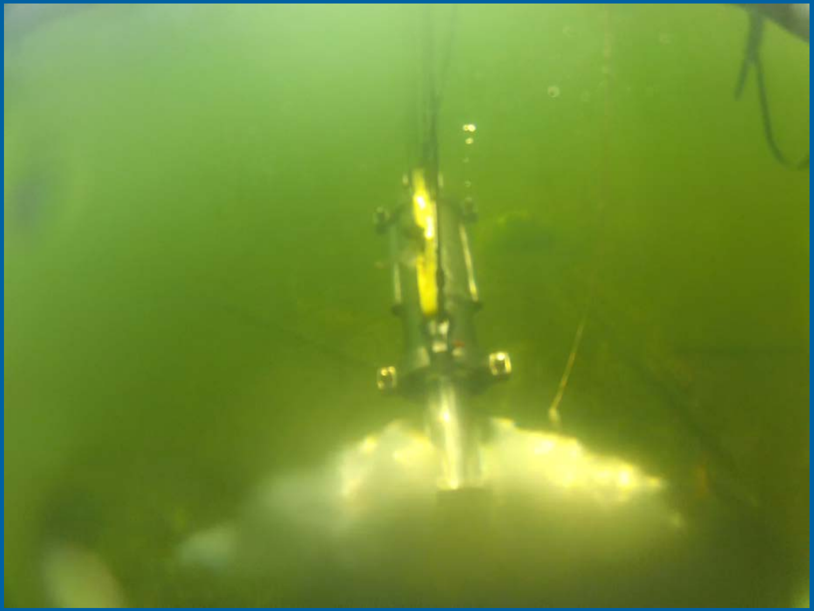
USGS

Deterrence and Control: Initial Project Results and Next Steps

- **Oral Delivery Technologies**



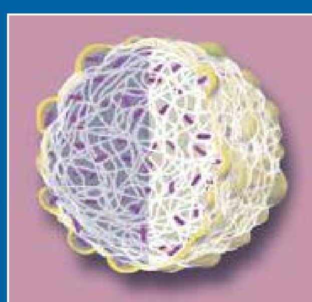
- **Seismic Pressure (Waterguns), *S*ound, CO_2, etc**

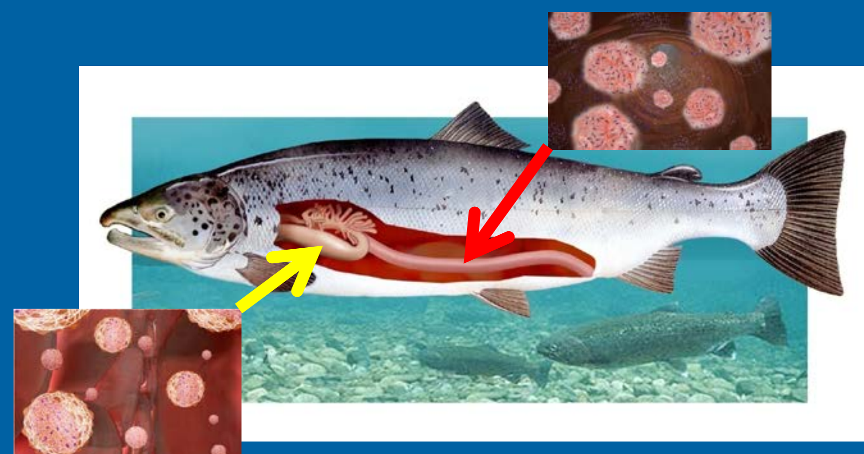


Oral Delivery
Biocide Control Strategy

Target invasive while minimizing non-target organism effects through:

- Selective agent (biological or chemical)
- Selective uptake by invasive
- Selective release within invasive species



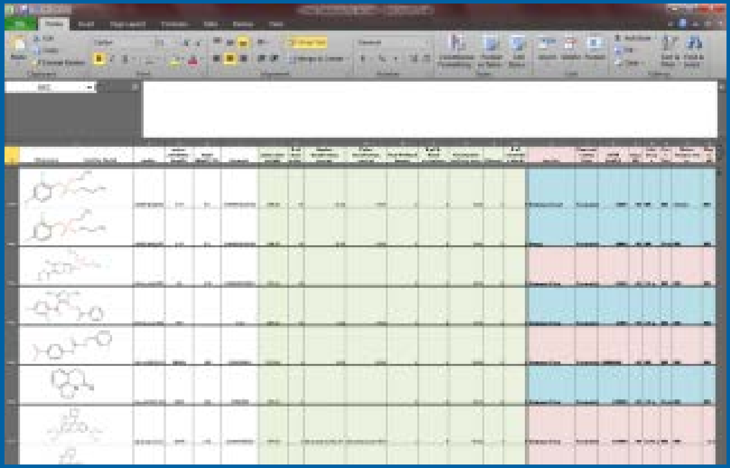


USGS

USGS

Identify Potential Compounds for Incorporation into Oral Delivery Technologies for Control of Asian Carp

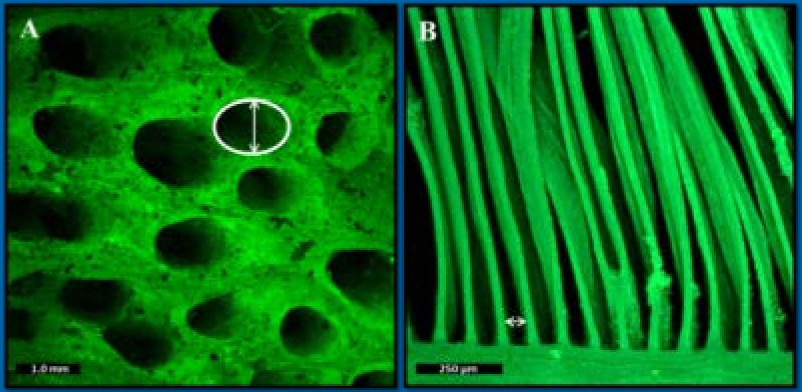
- Developed a searchable database of >200 chemicals – analyzing database to identify important chemical structures that predict toxicity
- Developed bighead carp and silver carp cell lines (US and Chinese) and selected native fish cell lines for use in cytotoxicity testing
- Synthesized two structural analogs of a potential piscicide for cell assay



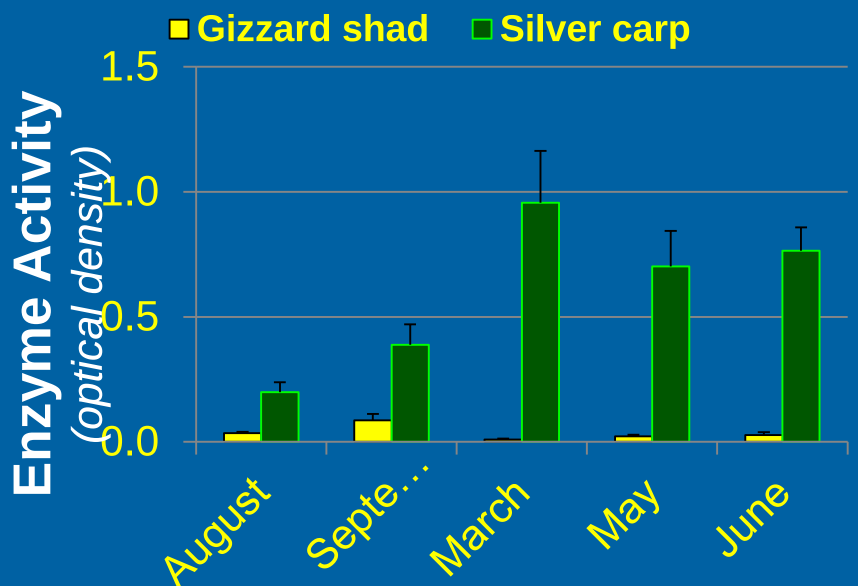
USGS

Technologies Using Oral Delivery Platforms for Species-Specific Control-Selective Microparticles

- Gizzard shad (GS) and silver carp have similar feeding habits
- Gill raker comparisons used for particle design: shape/size for least effect on GS
- Trypsin activity greater in silver carp than GS, there are also seasonal variances: this guides microparticle composition & application



Silver carp Gizzard shad



USGS

Technologies Using Oral Delivery Platforms for Species-Specific Control *Next Steps*

- Contracts established with Aquabiotics to provide antimycin and with Advanced BioNutrition to provide antimycin-incorporated microparticles.
- Antimycin delivered to USGS in Febuary 2013 then incoporated by ABN into microparticles with delivery to USGS expected in April 2013.
- Registration of antimycin in microparticles – workIng with FWS and EPA to move forward
- 2013 Field Season: Obtaining assistance from other agencies for test sites, obtain state/local permits
- Demonstration projects in our mobile lab will likely be completed in summer/fall 2013

Technologies Using Oral Delivery Platforms for Species-Specific Control
Next Steps

- USGS will work with the registrant of the microparticles to develop an application standard operating procedure and provide technical support to resource agencies.

Once project is complete:

- Likely need for technical and research support to maintain microparticle registration
- Incorporate alternative agents (pathogens, sedatives)

USGS

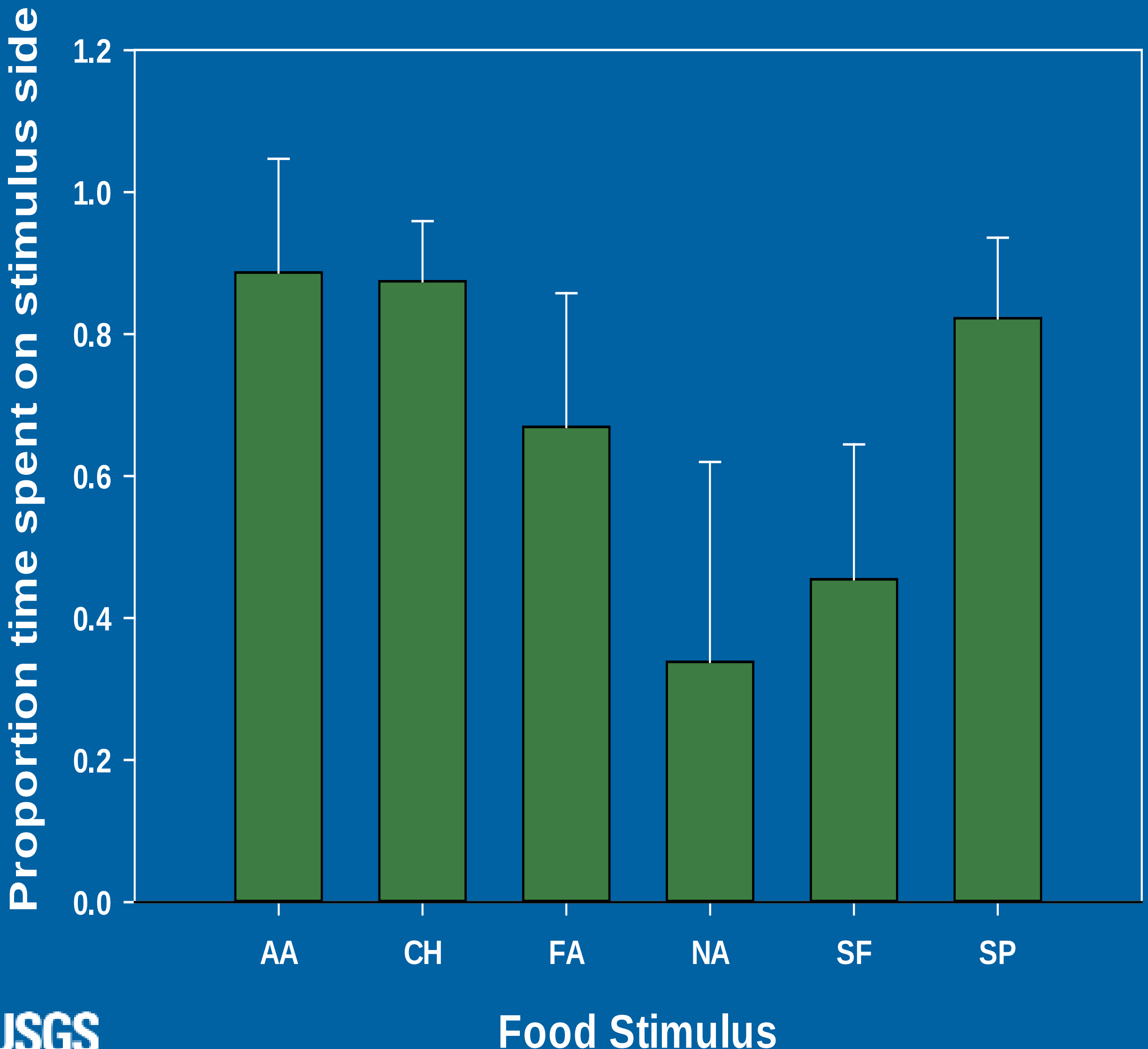
Research on the Identification of Asian Carp Attraction/Repulsion: Chemical Lures & Pheromones *Use in control strategies*

- **Natural chemical stimuli are being evaluated as attractant lures for Asian carp**
- **Laboratory and field studies show fish are very responsive to algal mixtures containing powdered Spirulina and Chlorella**
- **These stimuli are presently being used to condition Asian carp to feeding stations along tributaries of the Missouri River**
- **This approach maybe useful in aggregating and removing Asian carp from aquatic systems**

USGS

Chemical Lures for Asian Carp

Preferred Mixtures



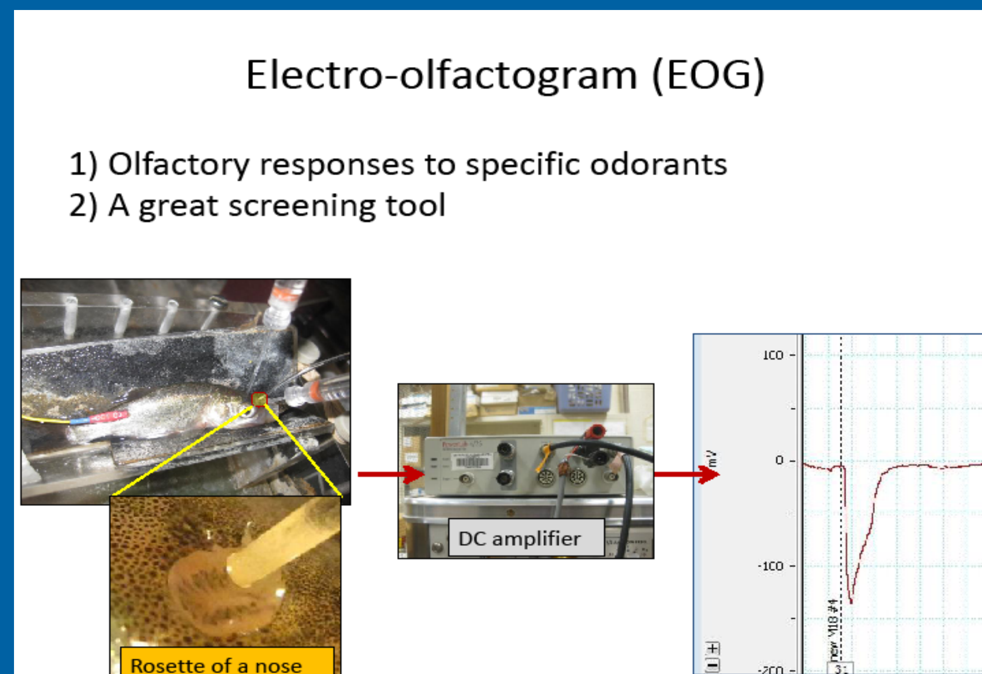
AA- Algae A
CH- Chlorella
FA- Filtered Algae A
NA- Nano
SF- Shellfish
SP- Spirulina

USGS

Research on the Identification of Asian Carp Attraction/Repulsion: Chemical Lures & Pheromones *Use in control strategies*

- **Asian carp are highly sensitiveto sex hormone metabolites and are behaviorally attracted to them.**

- **Female carp can be hormonally induced to release sex pheromones that are effective as olfactory stimulants and induce attraction.**



Environmental DNA Calibration Study – USGS research

- Compared DNA extraction kits
  - Found cheaper, faster kit that extracts more DNA than
  - USGS will coordinate interlab study to validate results
- Determined that piscivorous birds can be vector of eDNA
- Determined potential for Asian carp carcasses to be sources of eDNA
- Determined persistence of silver carp DNA in eagle feces and in fish slime under ambient “summer” conditions
- Designed >20 markers for silver carp and bighead carp for cPCR and qPCR
- Developed “stairstep” markers to determine mtDNA degradation

USGS

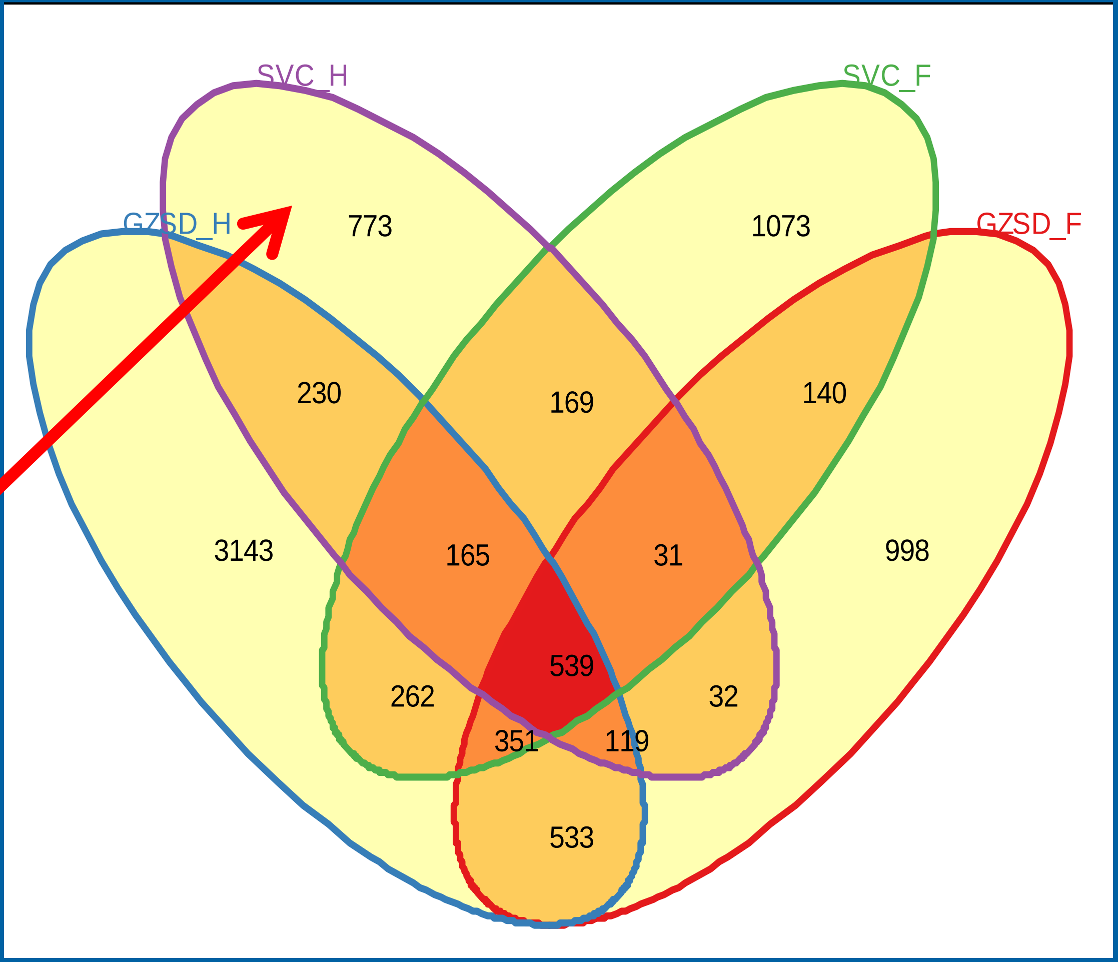
Development of a Rapid and Quantitative Genetic-Based Asian Carp Detection Method

- **We currently lack capacity to distinguish between DNA from live vs dead Asian carp**
- **The focus of this project is on tracking microbes associated with live Asian carp**
- **Samples processed from silver carp, bighead carp, grass carp, gizzard shad, bigmouth buffalo, smallmouth buffalo from Illinois, James, Mississippi, Missouri and Wabash Rivers**
- **Identified bacterial species unique to silver, bighead and grass carp and developed initial markers to identify some of the unique bacteria**

USGS

Microbial Source Tracking Marker for Rapid Detection

- Complements eDNA work
- Collaboration with Univ IL
- 700 unique species of bacteria in silver carp hindgut



USGS

Development of a Rapid and Quantitative Genetic-Based Asian Carp Detection Method – *Next Steps*

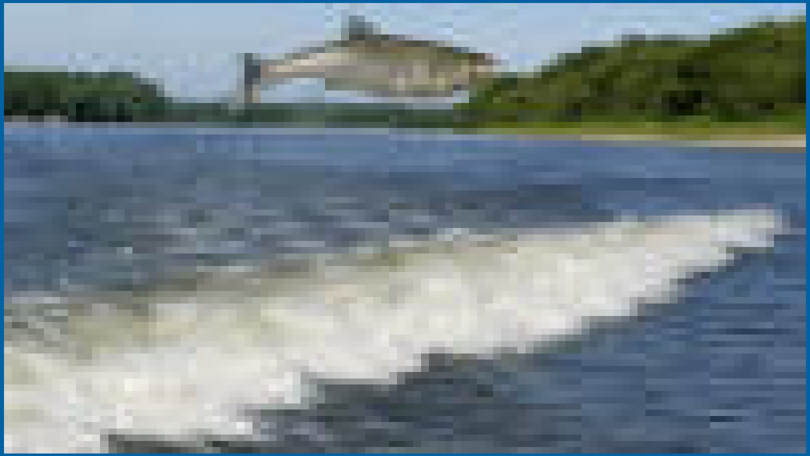
- **Sampling in 2013 to determine the presence of these bacteria in background environmental samples**
- **Target bacterial sequences and information to interpret the results could be available in 2014**
- **Workshop planned for 2014 to facilitate transfer of sample processing techniques**
- **eDNA symposium – MWFW conference 1/2014**
- **Incorporate with eDNA sampling (FWS)**

USGS

New Projects in FY2013

Focus on:

- Detection
- Control
  - Habitat (YOY)
  - Life-cycle
  - Hydrology
  - Aggregation



- Demos & Field Tests

USGS

Detection

- eDNA Monitoring Project to Correlate Asian Carp eDNA and/or Microbial Tracking Technologies with Telemetry Data in the Wabash River
- Real-Time Asian Carp Detection at USGS Stream gages–Supports Wabash eDNA Project
- Removing uncertainty of eDNA monitoring for invasive species in the upper Miss. Basin
- Use of acoustic video and side-scan technology to determine behavior of Asian carps, especially net avoidance behavior
- Hydraulic and Water Quality Evaluation of the Upper Illinois River

USGS

Habitat and Life-Cycle

- **Validation of drift-model-based river risk assessments using reservoirs currently containing Asian carps**
- **Movement, habitat selection and behaviors of Asian carp and native planktivores in a newly invaded river segment: Implications for control**
- **Understanding Feeding Habits of Asian Carp and Native Planktivores in a Newly Invaded River Segments to Inform Chemical Control Strategies**
- **Assessing natural recruitment constraints on Asian carp in river reaches with established and potentially emerging populations: Implications for control**

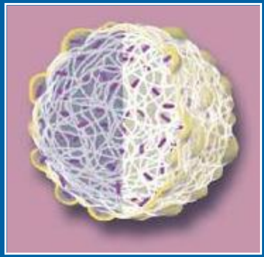
Deterrence and Control

- **Establishment of Demonstration/Evaluation Sites for New Technologies for the Control and Monitoring of Asian Carp**
- **Field deployment of carbon dioxide barrier to deter Asian carp – Led by IL DNR and UIUC**
- **Field evaluation of chemical attractants to control Asian carp and development of protocols for field verification of response**
- **Field Use of Seismic Technology to Divert Asian Carp**
- **Field testing of Oral Delivery Platforms**

USGS

USGS and Partner Objectives

- Developing new management tools to control or remove Asian carp
- Building a framework for tool development
  - Standardize creation of methods and tools which can then be tailored and applied to control other invasive species
- Actively and efficiently manage the science




USGS

Questions?

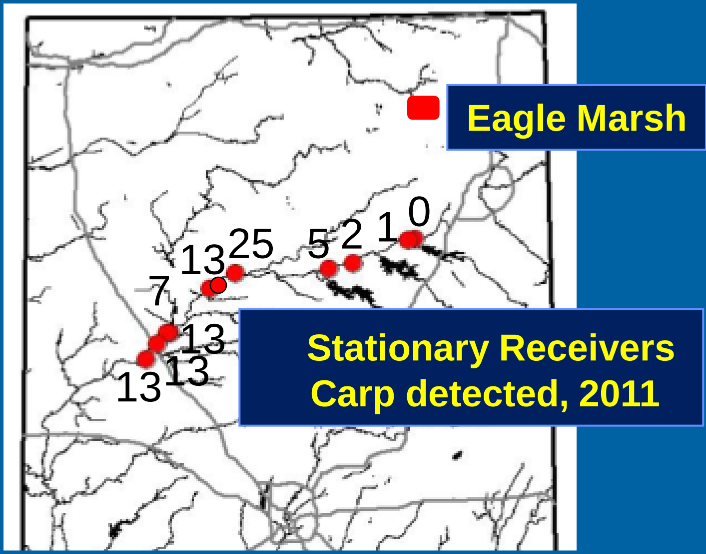
eDNA Monitoring Project to Correlate Asian Carp eDNA and/or Microbial Tracking Technologies with Telemetry Data in the Wabash River

- This is a new project for 2013 that leverages Purdue's telemetry of Asian carp in the Wabash River
- This project represents our first attempt to correlate eDNA (AC microbes ID'd in Microbial Source Tracking project) with detections of a telemetered fish population
- Data generated in this study should inform other eDNA sampling efforts for Asian carp
- Field work will consist of collecting water samples in the Wabash River. Purdue University will coordinate fish movement monitoring and sample collections.

USGS

Real-Time Asian Carp Detection at USGS Stream gages–Supports Wabash eDNA Project

- **USGS to install REAL-TIME detectors at 3-4 existing USGS streamgages to detect tagged AC in Wabash R.**
- **Use as trigger for eDNA and microbial tracking samples; validate results**
- **Provide data to evaluate relation of AC migration to flow and temperature changes**
- **Project completed in 2014; Online data release to USGS NWIS-Web, 2013**
- **Prepare short publication to help managers use the data, as resources available**



Graphics from Coulter and Goforth, (2011)

http://www.in.gov/dnr/fishwild/files/fw-PurdueAsianCarpMovements2011Report.pdf

Removing uncertainty of eDNA monitoring for invasive species in the upper Miss. Basin

New Project in 2013

- eDNA surveillance for Asian carp is being applied to areas where diverse fish populations are not well represented in genetic databases
- Assistance in 2013 - Agencies along the UMR can provide fish samples to USGS for mtDNA sequencing
- Sequence information will be posted to GenBank
- Sequences will be compared to existing and future Asian carp markers
- How might project evolve once completed? Native species eDNA monitoring could support native species monitoring before/after Asian carp control

USGS

Field deployment of carbon dioxide barrier to deter Asian carp – Led by IL DNR and UIUC

New Project in 2013

- Alternative barrier systems can provide redundant systems to existing barriers and could be deployed where electrical barriers or water guns cannot
- USGS will work with UIUC and IL DNR to test CO2 barriers in our outdoor experimental pond complex.
- Potential demo at UMESC in summer 2013
- UIUC, IL DNR and USGS will collaborate to provide methods/procedures to natural resource agencies to deploy barriers
- If classified as a pesticide, could require EPA FIFRA registration

USGS

Assessing natural recruitment constraints on Asian carp in river reaches with established and potentially emerging populations: Implications for control. New Project in 2013

- Determine habitat selection of post-drift larval and juvenile Asian carp to inform development of control measures (e.g., microparticles) and inform habitat management actions.
- Determine the effectiveness of predators under variable conditions (e.g., turbidity, depth, vegetation abundance) to prey on larval and juvenile Asian carp to inform possible control measures (e.g., predator stocking and habitat management).

Assessing natural recruitment constraints on Asian carp in river reaches with established and potentially emerging populations: Implications for control.

- This information will be applicable to all areas that Asian carp have invaded or might invade in the future.
- Project completion and final results expected in 2015
- May need sampling assistance in 2013
- Future: Similar studies could be deployed at other invasion fronts or in areas with established populations as necessary to provide more specific information.

USGS

Movement, habitat selection and behaviors of Asian carp and native planktivores in a newly invaded river segment: Implications for control.

- Determine where, when and why Asian carp and native planktivores are moving and congregating in newly invaded areas to inform the application of control measures (e.g., microparticles).
- Determine if telemetered Asian carp can be used to locate congregations of free-ranging Asian carp for control purposes in newly invaded areas.

USGS

Movement, habitat selection and behaviors of Asian carp and native planktivores in a newly invaded river segment: Implications for control.

- This information and tool will be applicable to other areas that are newly invaded and with established populations of AC, both in and out of the GL basin.
- Project completion and final results expected in 2015
- Publications will disseminate results, while results will be directly used by the control team formulating microparticle application strategies and managers implementing any control measures
- USGS would continue to support partners in the application of this info to control strategies

USGS

Understanding Feeding Habits of Asian Carp and Native Planktivores in a Newly Invaded River Segments to Inform Chemical Control Strategies – New Project in 2013

- Will provide information important to the application of control agents through a microparticle delivery system in & out of the GL basin.
- Designed to determine where/when Asian carp are feeding – inform where/when to apply microparticles
- Also identify foods Asian carp are exploiting and thus where microparticles could be applied
- Identify major energy pathways, areas and taxa most vulnerable to Asian carp.

USGS

Understanding Feeding Habits of Asian Carp and Native Planktivores in a Newly Invaded River Segments to Inform Chemical Control Strategies

- Final results are expected to be delivered in 2015 but preliminary findings will be applied to microparticle development efforts as available.
- How could project evolve? Continued research could focus on:
  - How carp-induced nutrient recycling is important for enhancing and sustaining carp populations
  - The importance of system productivity (e.g., nutrient enrichment) on invasibility of aquatic systems by carp
  - Better understanding the mechanisms of detrimental competitive interactions among carp and native fishes

USGS

Validation of drift-model-based river risk assessments using reservoirs currently containing Asian carps- New in 2013

- Linked to Trib assessment project
- Assess reproduction and establishment of bigheaded carps and grass carp in reservoirs where they have been introduced, and apply models developed for use in Great Lakes tributaries to test predictive ability of models.
- Requires an understanding of developmental rate of grass carp similar to that developed for the bigheaded carps. This part of the project to be completed in early 2013
- This information will be applicable to all areas that Asian carp have invaded or might invade in the future.
- Project will be completed in 2015

USGS

Use of acoustic video and side-scan technology to determine behavior of Asian carps, especially net avoidance behavior - New Project in 2013

- **Use DIDSON and sidescan technology with different capture gear to determine effectiveness rate of different gears and assess behaviors used to avoid gears, in order to improve harvest tactics and strategies.**
- **This information will be applicable to all areas that Asian carp have invaded or might invade in the future.**
- **This project will be completed in 2015**

USGS

Hydraulic and Water Quality Evaluation of the Upper Illinois River – New in FY2013

- Migration of Asian carp up the Illinois River moved rapidly from 1986 until about 2002 when the population front "stalled" in the Marseilles/Dresden Pools of the Illinois River (Kolar, et al, 2007).
- This project will collect detailed hydraulic and water quality data and collaborate with partner agencies collecting fish telemetry data to determine what factors that may be stalling the migration.
- Project will be completed Sept. 2014.

Confluence of the Des Plaines and Kankakee Rivers—approximate location of “stalled “Asian carp migration front

USGS

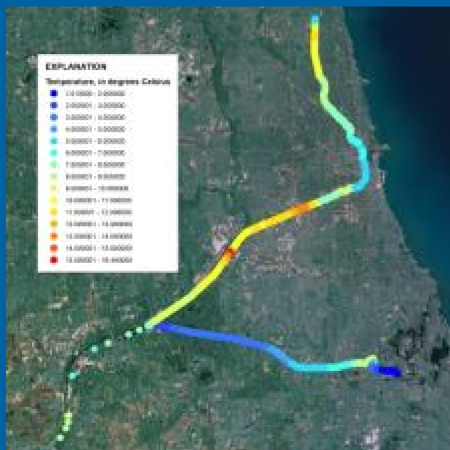
USGS Science Focus: Management Tools

EXCLUSION – DETECTION – ATTRACTION – REMOVAL

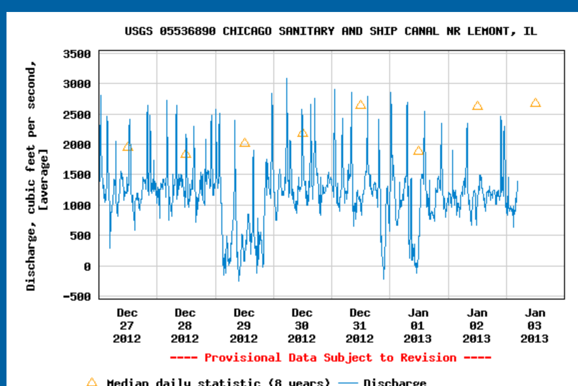
- **Asian carp biology**
  - **Life history knowledge is essential for risk assessment, surveillance, and control development and application**
- **Asian carp control technologies**
  - **Tools to keep them from moving into the Great Lakes and to reduce or push back current populations**
- **Asian carp monitoring**
  - **eDNA and rapid microbial methods to detect carp and trigger management response/action**
- **Critical hydrologic expertise**

USGS

Chicago Area Waterway System data



- Synoptic water temperature survey

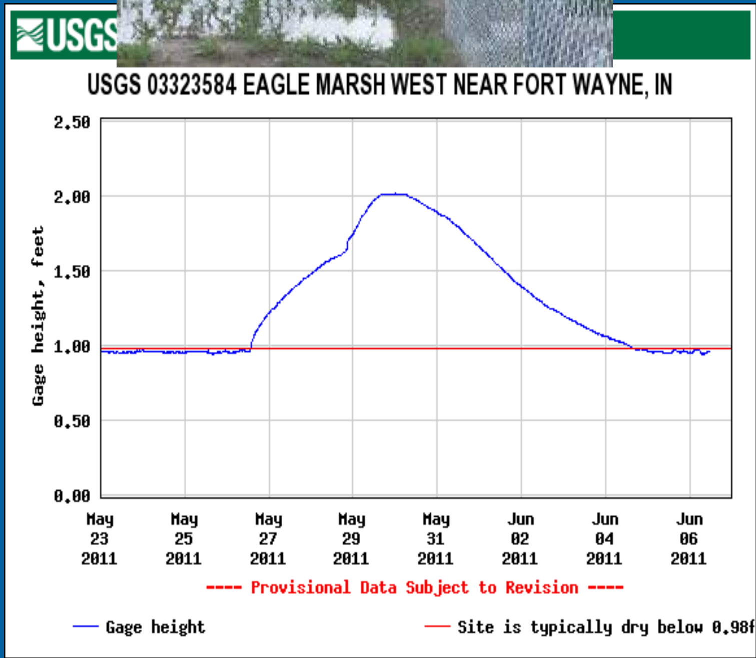


- Reverse flows in CSSC near electric fish barrier

USGS

Wabash-Maumee Interbasin Connection 1.
Next Steps

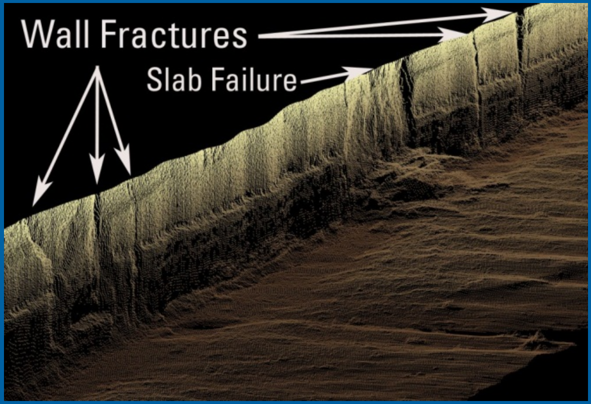
- Post May 2014 AC fence/USGS alert support possible
- Streamflow data critical for modeling efforts



USGS

“Groundwater” conduit between the Chicago Sanitary and Ship Canal (CSSC) and Des Plaines River

- Although there are fractures, appears to be little hydraulic connection
- Particles as large as eggs and larvae are very unlikely to move between the river and the CSSC through fractures
- Study Completed
- Report in Preparation



USGS

Asian carp- bluegreen algae relationships

- Applied improved bioenergetic model to remote-sensed bluegreen and green algae concentrations to map timing and availability of planktonic food resources for bigheaded carps in Lake Erie
- Measured uptake by Asian carp and measured their growth

USGS

Trib Assessment Tool: Asian carp early life history: developmental rate and behavior- *Next Steps*

- Second manuscript on complete developmental and behavioral data in journal review
- Model used to estimate spawning locations of Asian carps in published manuscript
- Manuscript describing sinking rate and implications for required river velocity in prep
- Further studies on survival of settled eggs planned for 2013
- Once project is completed, USGS will continue to support partners in the application of the models

USGS

Environmental DNA Calibration Study – USGS research – *Next Steps*

- USGS coordination of interlab comparison of selected eDNA extraction methods (with USACE, FWS)
- Pending the completion of the interlab trial, new extraction procedures would be available for 2013
- No field work planned for 2013
- Information on vector and carcass work was provided to the Council of Lakes Committees and the USFWS genetics workgroup. Work will be summarized in the ECALS interim report and journal articles
- Plan for eDNA session – MWFW conference 1/2014

USGS

Environmental DNA Calibration Study – USGS research – *Next Steps*

How will we help managers utilize the tools/information in their state?

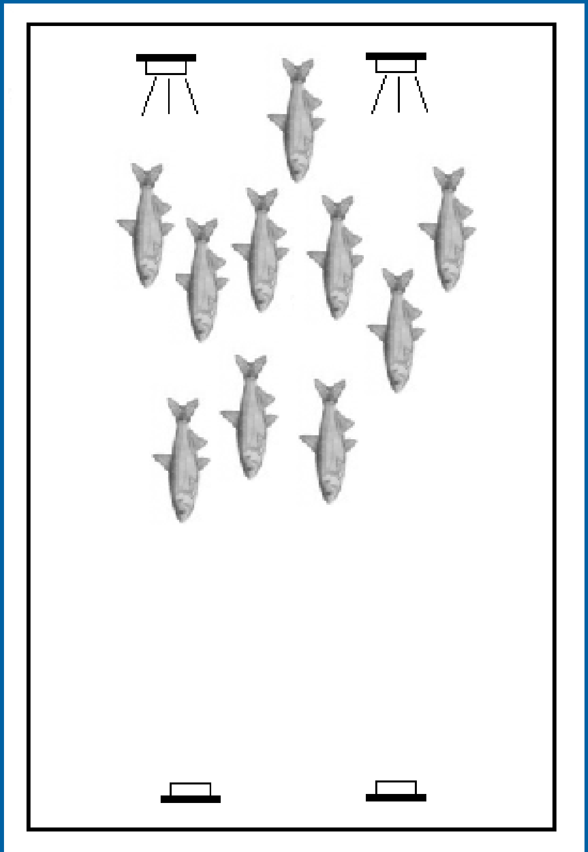
- **Results will inform ECALS probabilistic models**
- **ECALS eDNA workshop last month**

How will this project evolve once complete?

- **We need to better understand how DNA degrades as it is shed from live fish.**
- **Develop linkages to other detection systems, e.g. MST**

USGS

Evaluating sound as a tool to herd or serve as a barrier to Asian carp



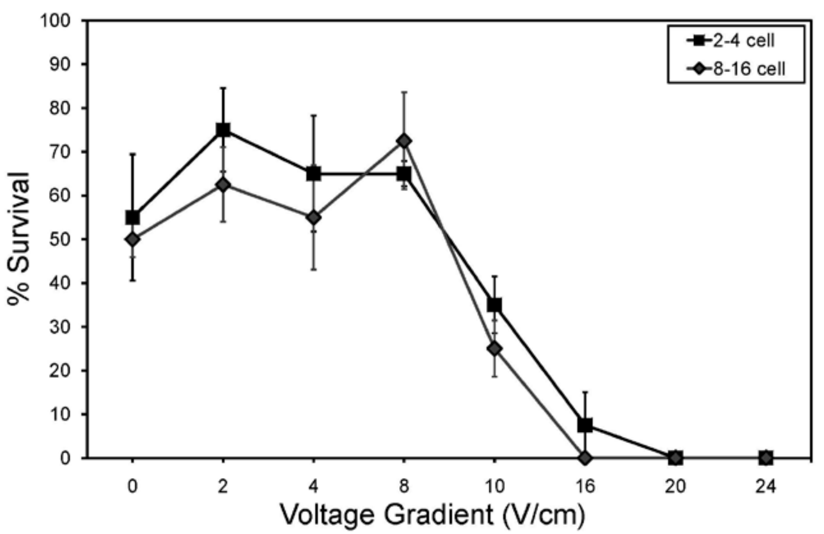
speaker location

USGS

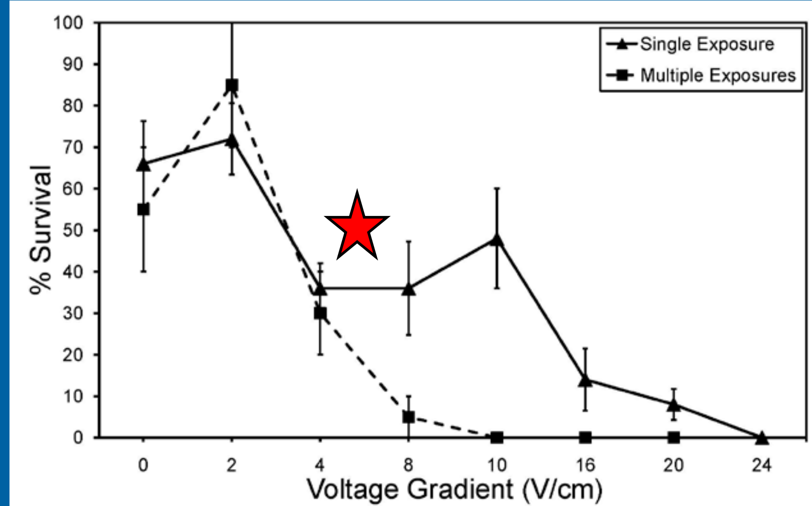
Use of Seismic Technology to Divert and Eradicate Asian Carp – *Next Steps*

- Watergun Demo at LaCrosse ponds – Spring 2013
- Field trials, Summer 2013 – Assistance from other agencies needed to help select test locations, select surrogate seismic monitoring locations, field support
- Watergun results and the pressure map will be summarized into USGS fact sheets & on USGS web site
- USGS can help managers use waterguns by assisting with planning barrier installations; Designing deployment arrays & provide pressure monitoring support to evaluate arrays

USGS

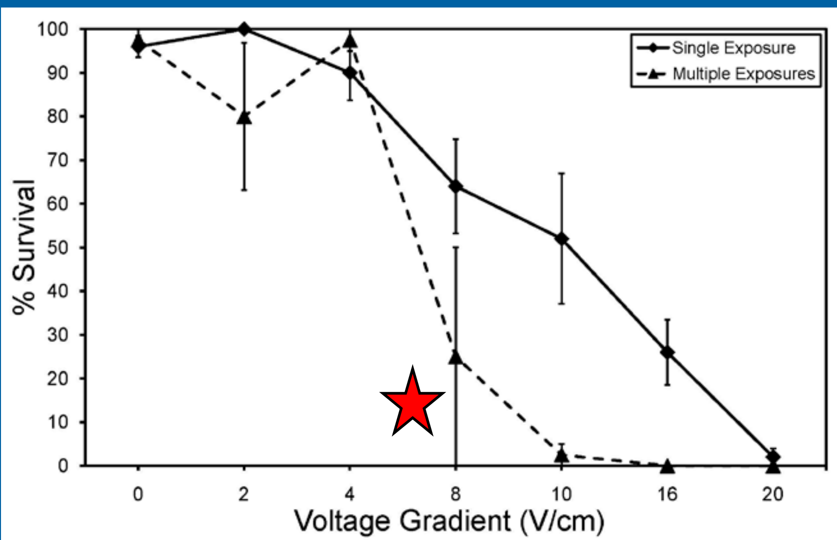


<16-cell embryo

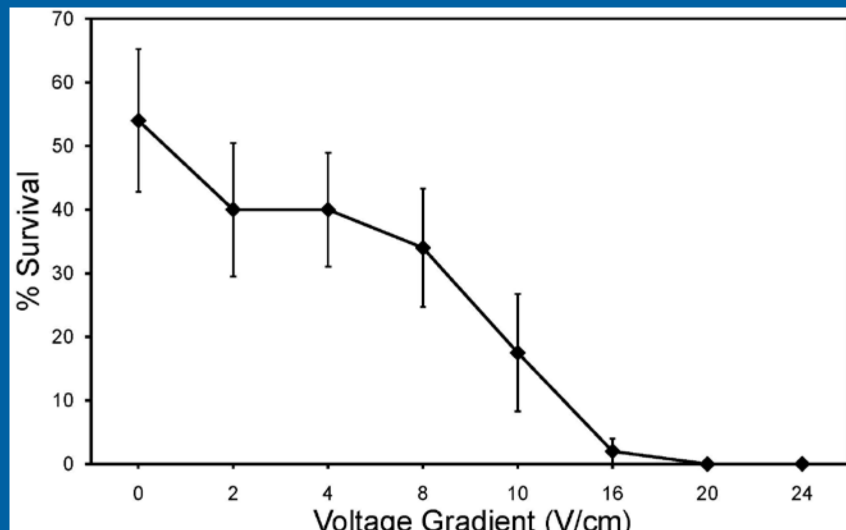


Embryo at blastulation

Survival of bighead carp exposed to pulsed DC voltage gradients during embryo/larvae development



2-week old larvae



Embryo at gastrulation

Controlling Asian carp through physical disruption of early life history stages

Next Steps

- Determine the minimum exposure duration/frequency to reduce blastula-stage embryo and <2-wk old larvae survival
- Develop engineering solutions to integrate the tributary assessment tool developed by USGS (e.g. egg transport/location in water column) with methods (electrical/physical) to decrease embryo survival

USGS

Chemical Lures for Asian Carp – *Next Steps*

- **Various methods of delivery are being evaluated for the release of the algal stimulus**
- **This feeding station approach will be evaluated under different field conditions in 2013 to demonstrate utility in attracting carp**
- **Management application will be facilitated through meeting or workshop presentations and interagency collaboration studies**

USGS

eDNA monitoring – Minnesota DNR

Re-evaluate sites that where sampled in 2011

- St. Croix River (50 samples / site)
  - Above Dam at Taylor Falls
  - Below Dam at Taylor Falls
- Mississippi River (50 samples / site)
  - Above Coon Rapids Dam
  - Below Coon Rapids Dam
  - Below Lock and Dam 1

Collected at 2 isolated sites as negative controls

- Riley Lake near Minneapolis (50 samples / site)
- Square Lake near St. Paul (50 samples / site)

Collected at 1 site infested with Asian carp

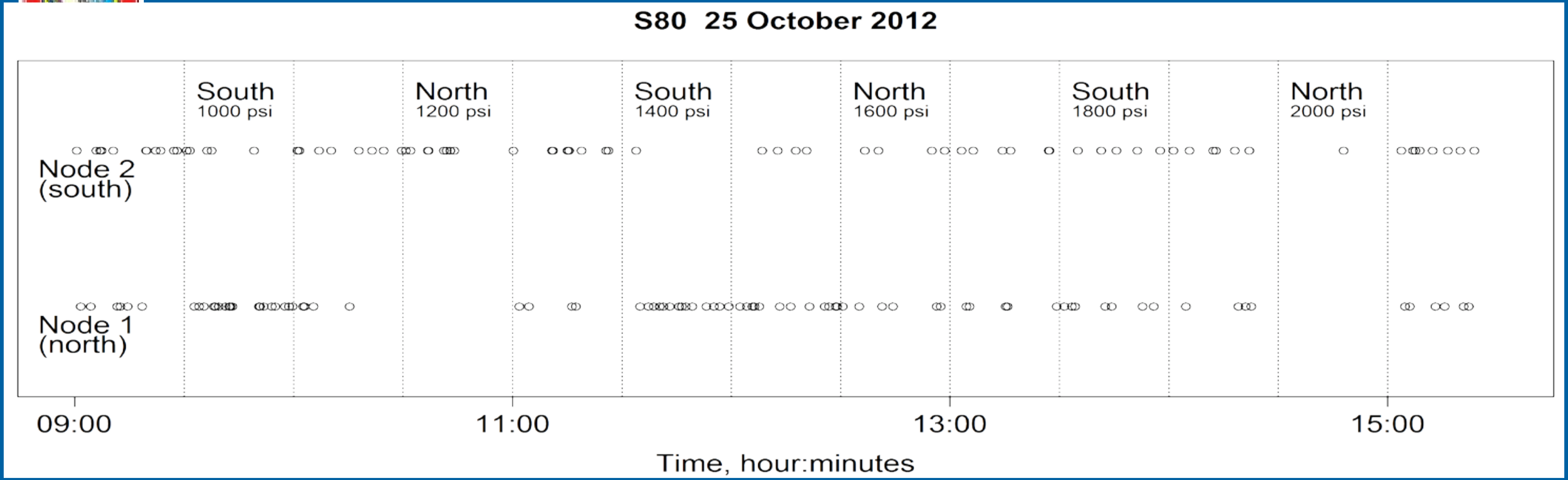
- Below Lock and Dam 19 near Keokuk, IA (50 samples / site)

Use of Seismic Technology to Divert and Eradicate Asian Carp

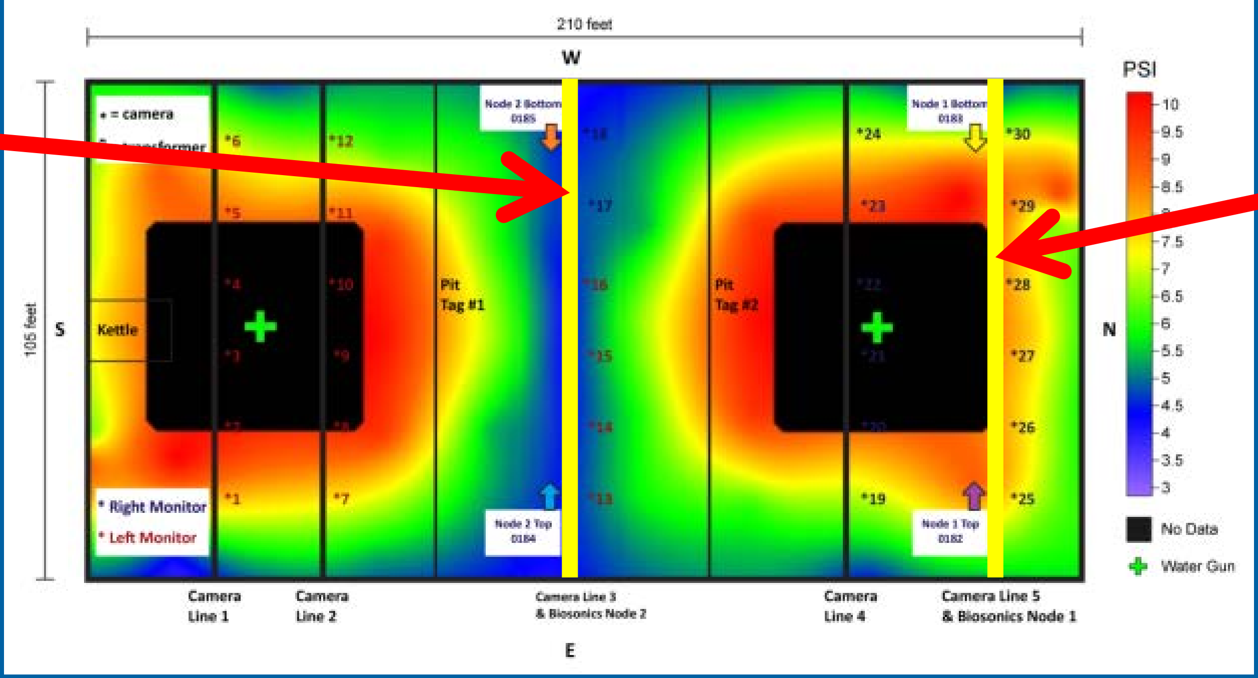
- Observed fish behavioral and movement response
  - under field conditions – backwater area of IL River
  - under controlled mesocosm conditions – USGS UMESC
- Supported electric fish barrier clearing operations
- Mapped pressure gradient of the 120-in^3 water guns operated at maximum pressure (2,000 psi)
- Recorded seismic data during operation in/near glacial deposits, bedrock and constructed pond
- Initiated work to evaluate the response of Asian carp to pure and complex sounds in mesocosm and laboratory conditions

USGS

Sound Pressure



Node 2



Node 1

Evaluating sound as a tool to herd or serve as a barrier to Asian Carp

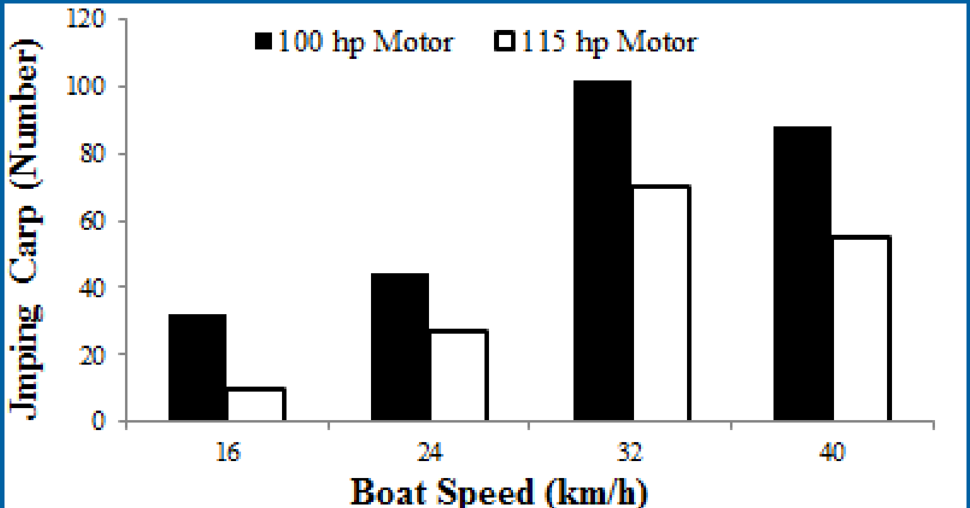


Fig. 2: Number of silver carp jumping in response to 100 hp and 115 hp boat motors driving across 100m at four speeds.

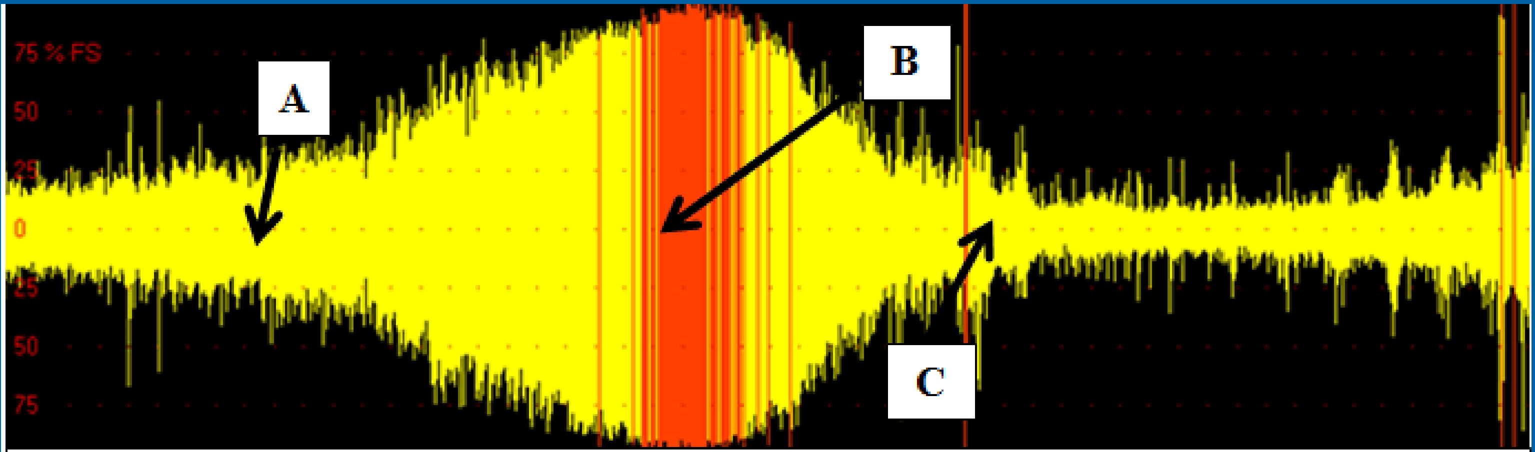


Fig 1: Hydrophone recording of 100 hp motor at 32 km/h. A: Fish begin jumping, **B:** Peak of fish jumping frequency, **C:** Termination of fish jumping

USGS

Controlling Asian carp through physical disruption of early life history stages

Collaboration with Purdue University (Reuben Goforth):

- Evaluated survival of zebrafish, goldfish, fathead minnow and bighead carp embryos at multiple developmental stages to pulsed DC and virtual DC voltage gradients
- Field strengths >16 V/cm were required to significantly reduce survival of embryos of zebrafish, goldfish and fathead minnow embryo
- Field strengths of 4-8 V/cm caused substantial mortality in certain stages of bighead carp embryo development

USGS