



# **Innovative Approaches to Restoration: Mussel Propagation in Kentucky:**

Natural History, Conservation, Protection, and Enhancement of  
the Most Endangered Group of Animals in North America  
*Funded in part by KDFWR, USFWS, and SWG*





# Global Distribution of Freshwater Mussel Diversity

## Mussel Species Diversity

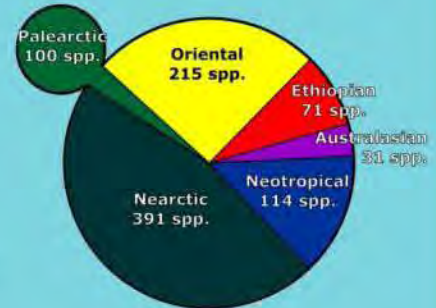


**We currently estimate the consensus tally of global freshwater mussel diversity to be 918 species.**

his estimate is based upon various published and published sources (see numerous references, listed on our web site). Freshwater mussels are distributed globally but not uniformly. The two greatest areas of diversity are the Nearctic and the Oriental biogeographic provinces, with other continental areas of lower diversity, including the tropics of South America and Africa (see pie chart, right). The map (below), divided into subcontinental political entities, is color-coded for the number of freshwater mussel species known to inhabit

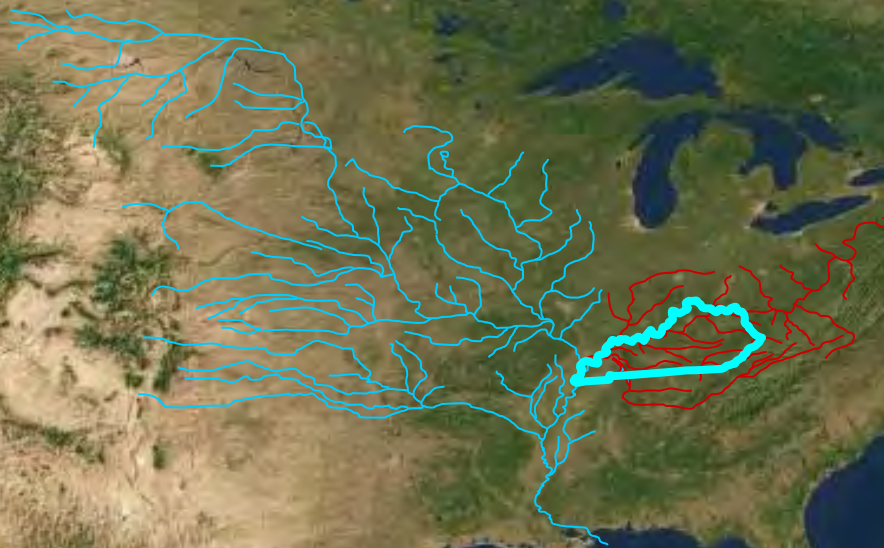
each unit. This map depicts the "hot spots" of unionoid diversity: Brazil, with both the Amazon and La Plata basins; the Democratic Republic of the Congo at the intersection of the Congo and the upper Nile watersheds; southeastern Asia from China south into Indochina; and, especially, southeastern North America where the magnitude of freshwater mussel diversity is unparalleled anywhere else in the world.

The tally of 918 freshwater mussel species is far from final. In every area of the world, including the well-studied USA, there are taxonomic issues in need of resolution. For example, in the eastern portion of the Palearctic, Russian malacologists have introduced as many as 77 superfluous nominal species that perhaps describe only phenotypic variations rather than real biological entities. Those spurious taxa are represented by the bulbous protrusion from the Palearctic wedge in the pie chart to the right. As the MUSSEL Project moves forward, our estimate of global freshwater mussel diversity will be continually refined.



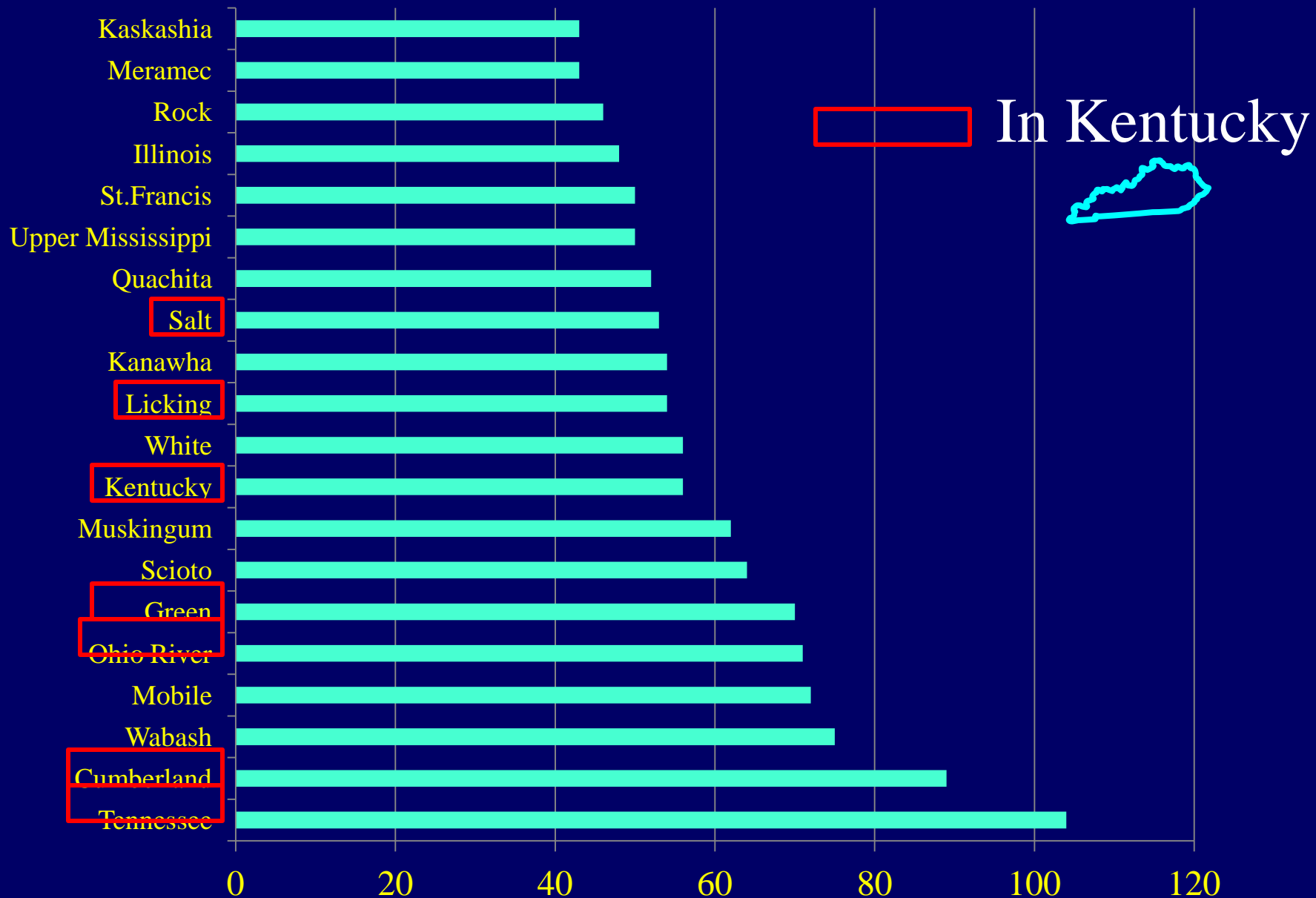


# North America's diverse aquatic fauna



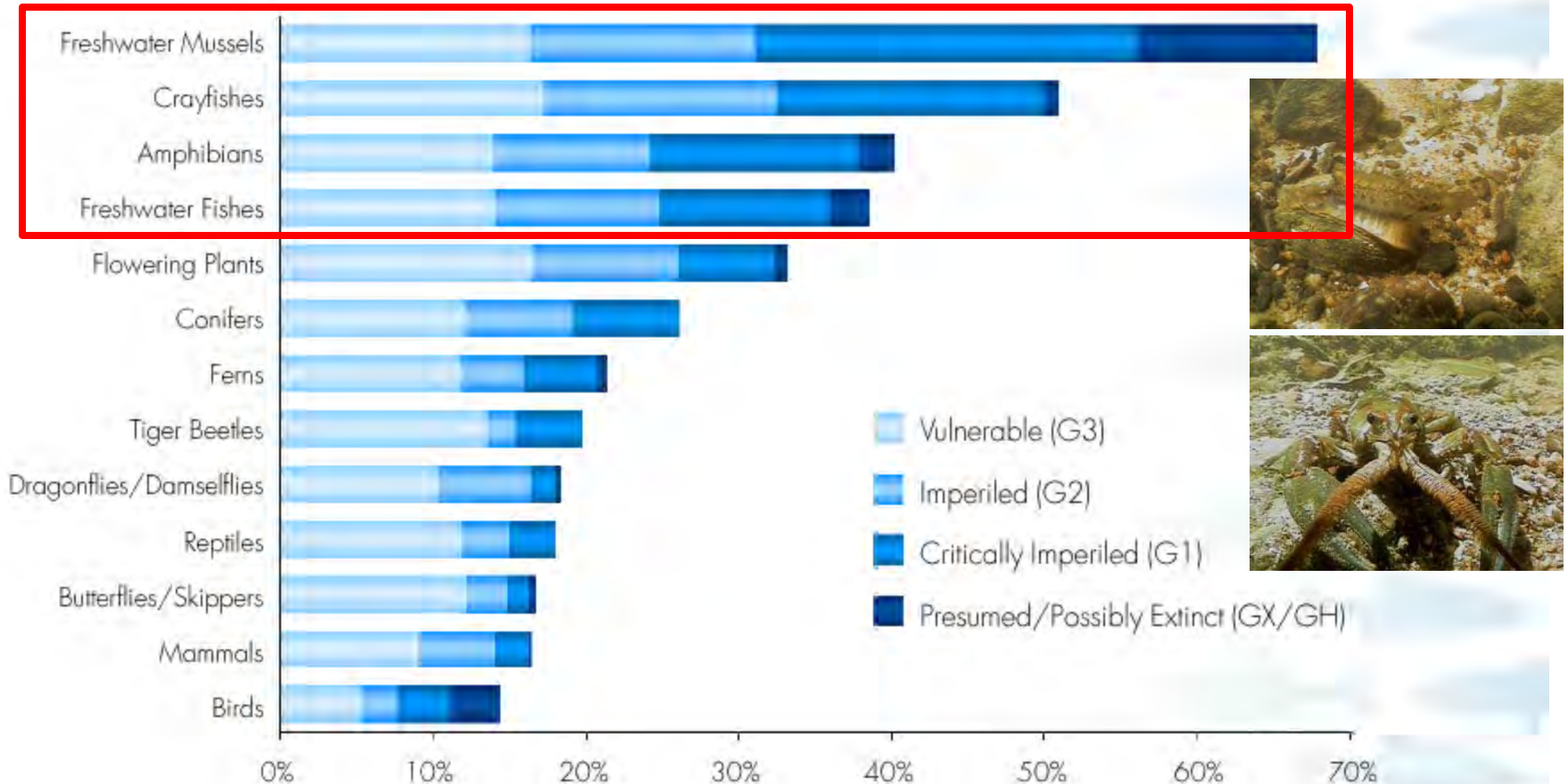
Mississippi &  
Ohio River  
systems

# Number of Mussel Species in US by Major Drainage



## Figure 1. Proportion of U.S. Species at Risk

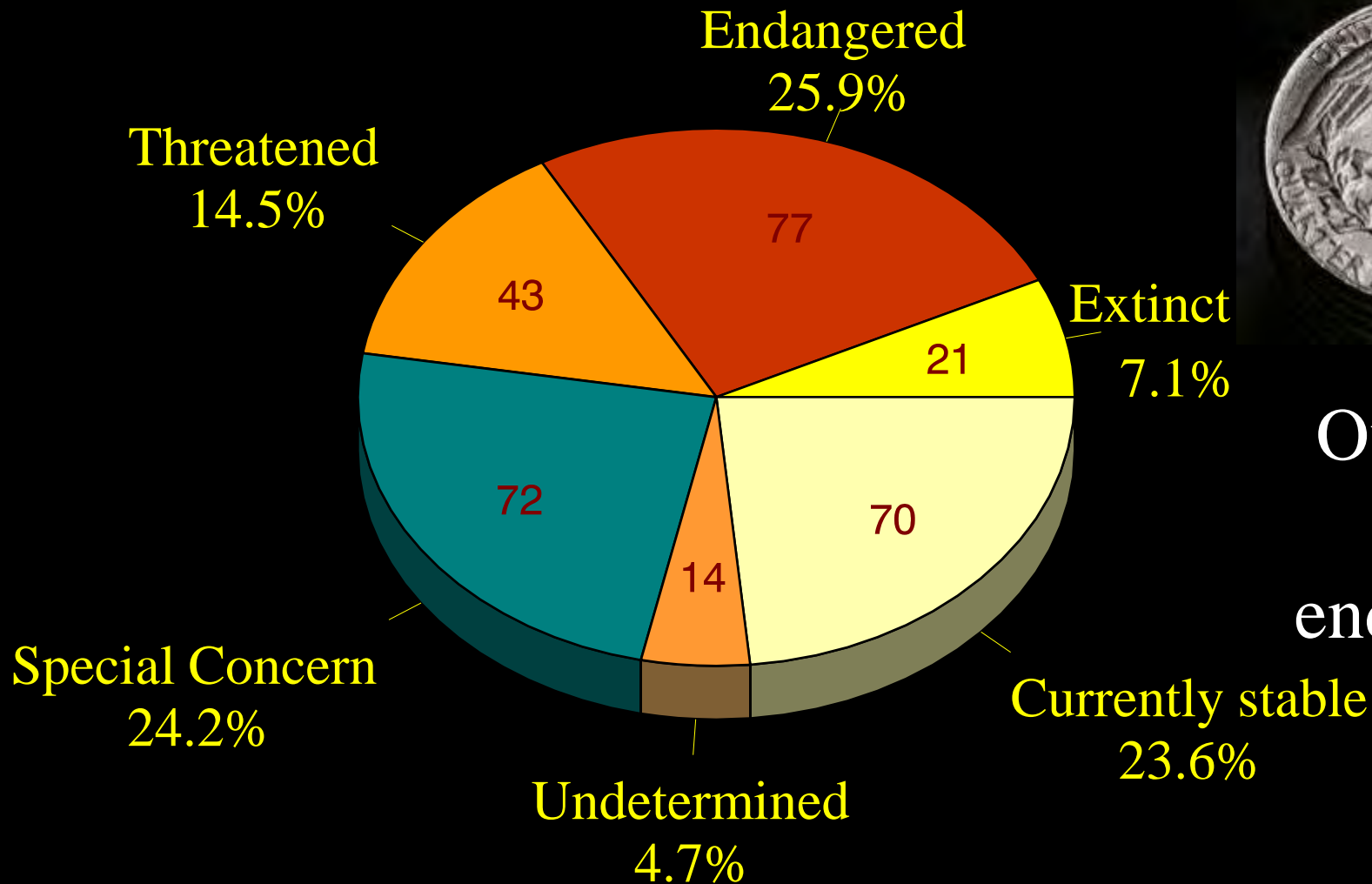
The species groups that are proportionately the most imperiled—mussels, crayfishes, and amphibians—consist entirely or primarily of freshwater species. (Source: 1997 Species Report Card<sup>21</sup>)



# Aquatic Fauna in the US in trouble

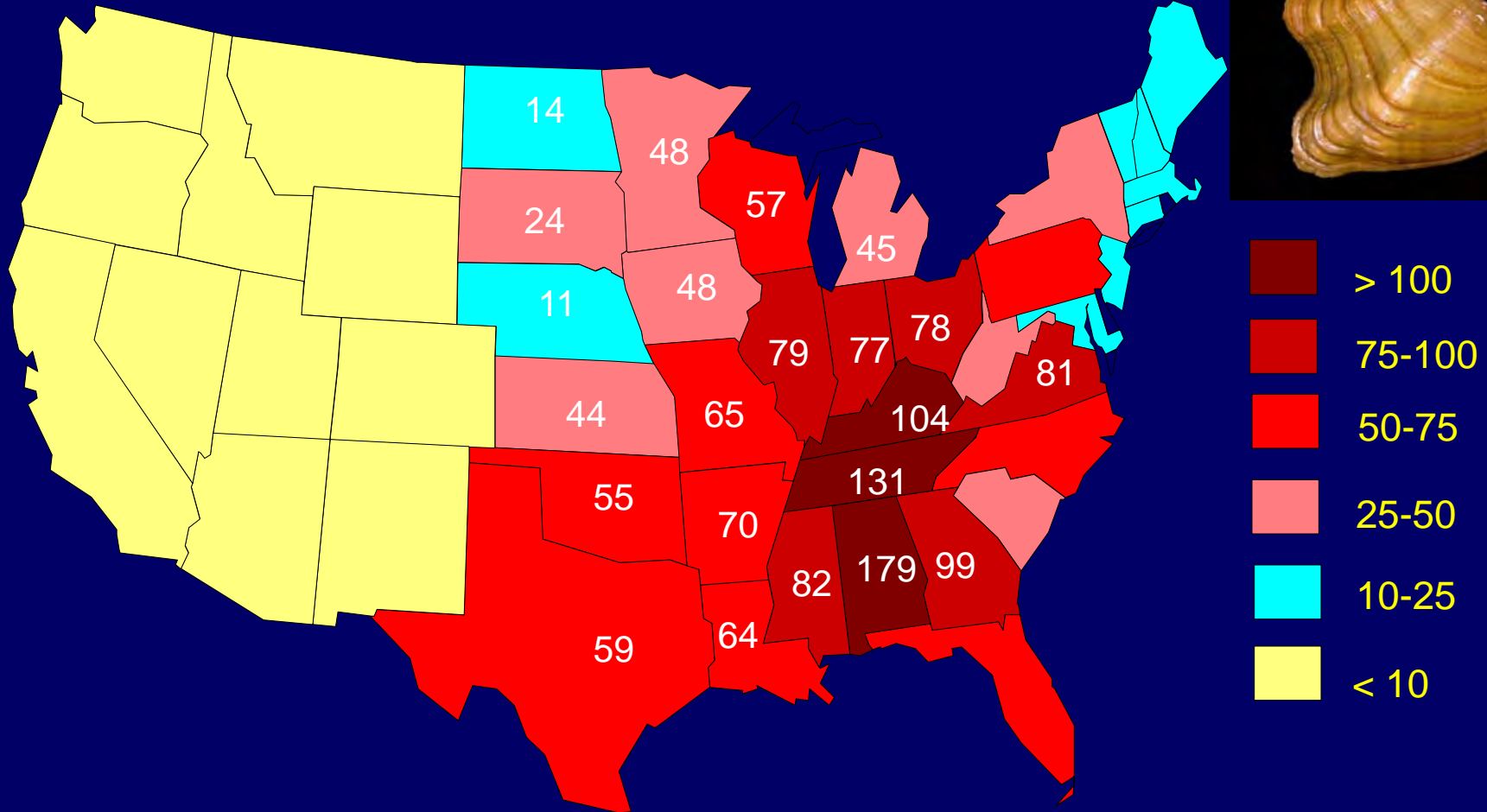


# Conservation status of freshwater mussels in the United States



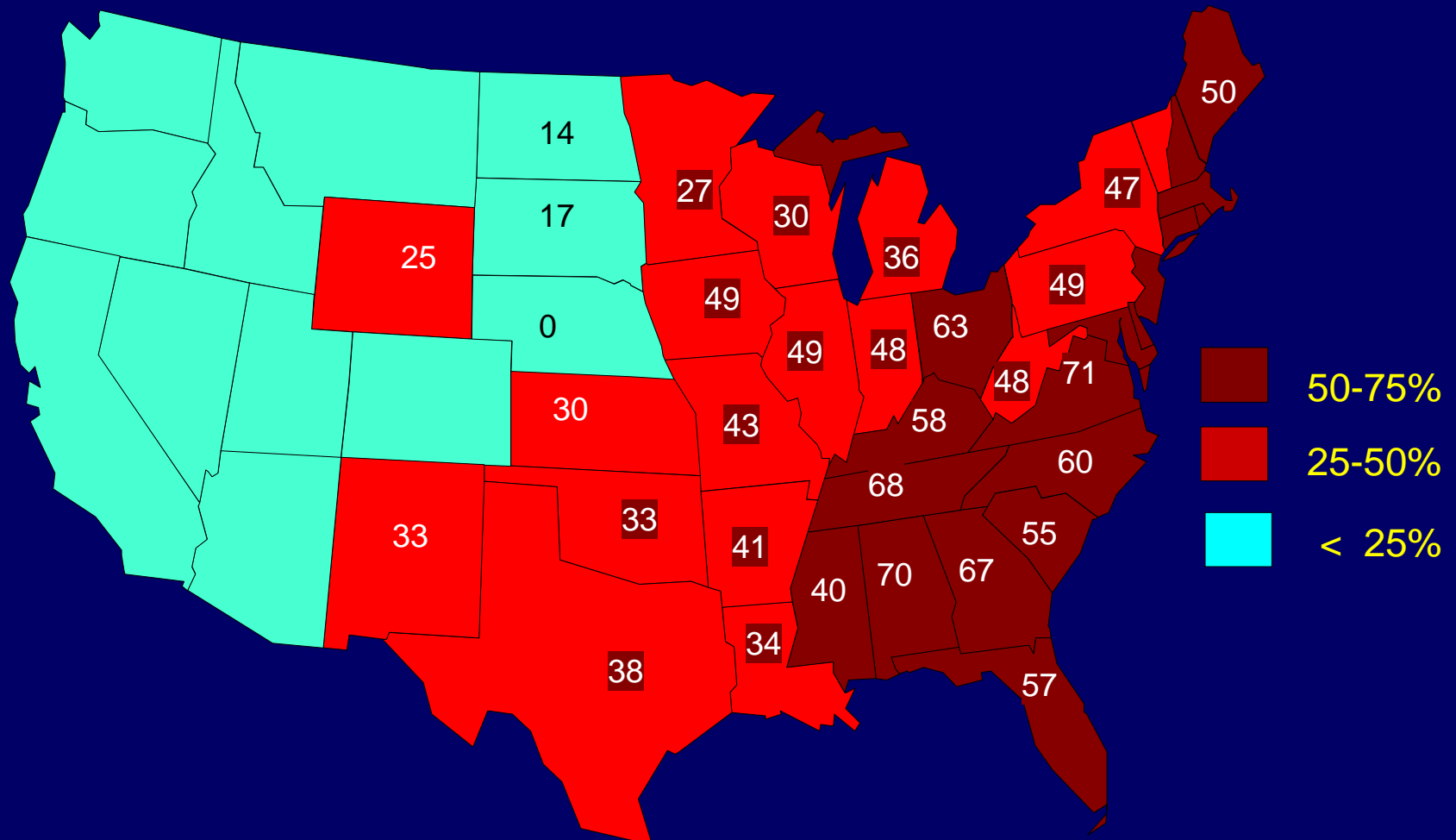
Over 1/4<sup>th</sup>  
are  
endangered

# Number of mussel species by state



Diversity similar to that of fishes and crayfishes

# Percentage of Mussel Species Imperiled





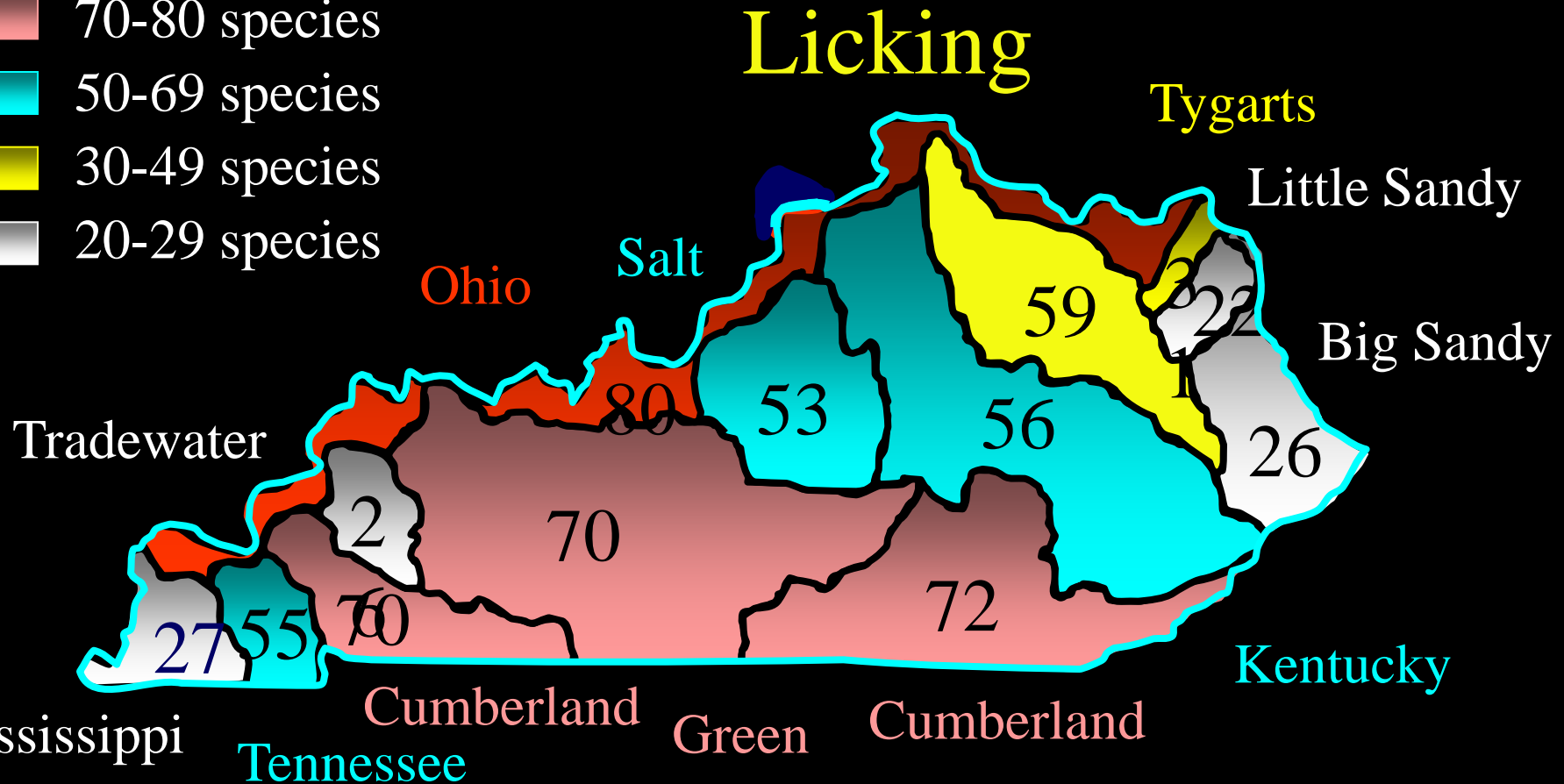
# Kentucky's Mussel Fauna

- 104 species
- 25 T&E
- 13 extinct
- 46 SGCN
- 8 extirpated
- 84 extant

## Kentucky's Endangered Mussels

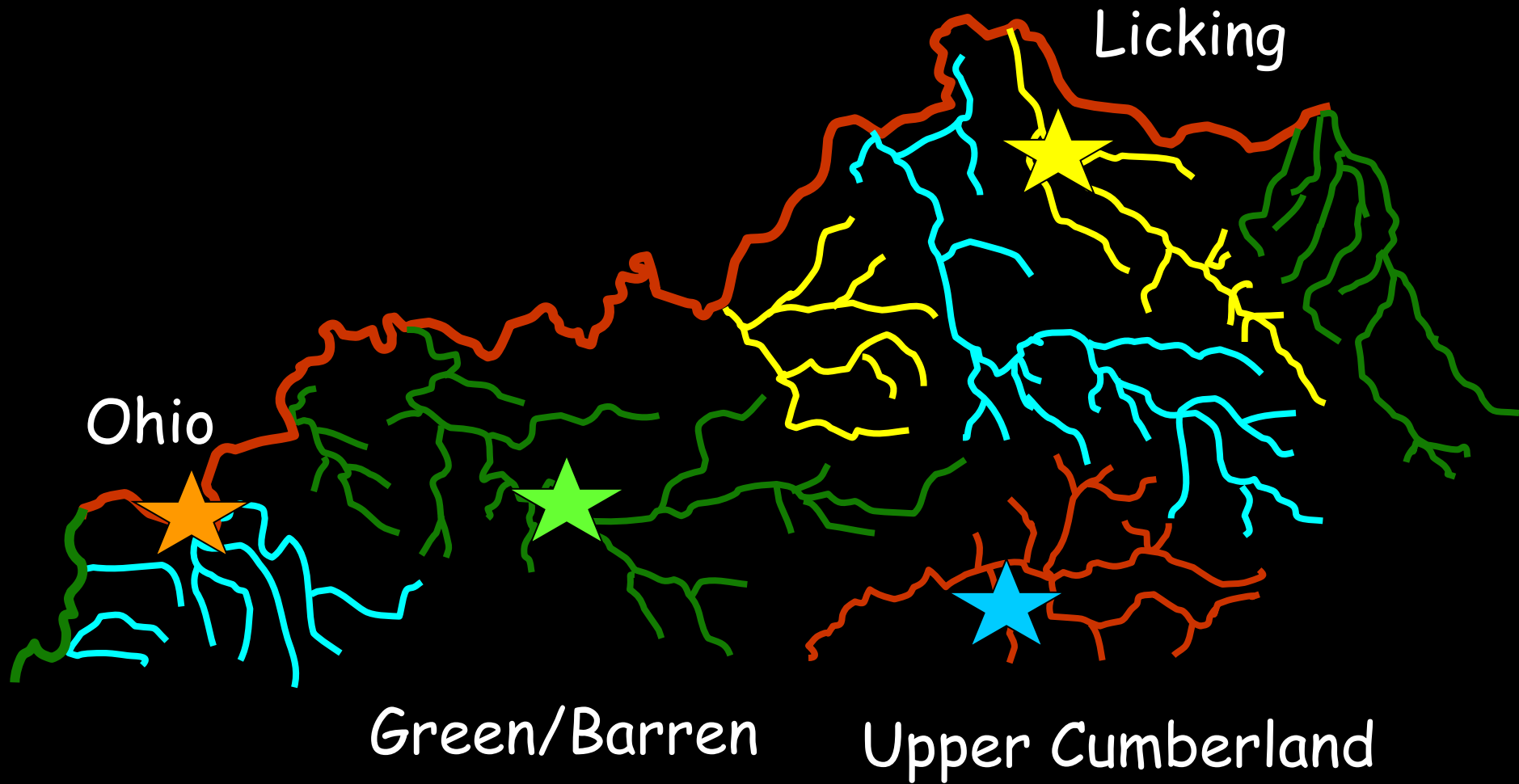


# Kentucky Mussel Species by Watershed



Number of species within major watersheds. KY ranks nationally in the top three for its diversity of fishes and mussels

# Kentucky watersheds with high numbers of at risk fish & mussels





Glochidia



Internal egg  
development



♀



♂

*Obovaria subrotunda*

Round hickorynut mussel

Mussel Life  
Cycle



Fish host



Glochidia attachment  
on gills



Juvenile mussel

Mussels are dependent on finding a host fish

## Mussel Life Cycle



♀

♂

Research conducted by KDFWR

Hundreds of thousands of larvae  
(glochidia) produced by the female



Glochidia



*Obovaria subrotunda*

Internal egg  
development



Mussel Life  
Cycle



♀



♂

Research conducted by KDFWR

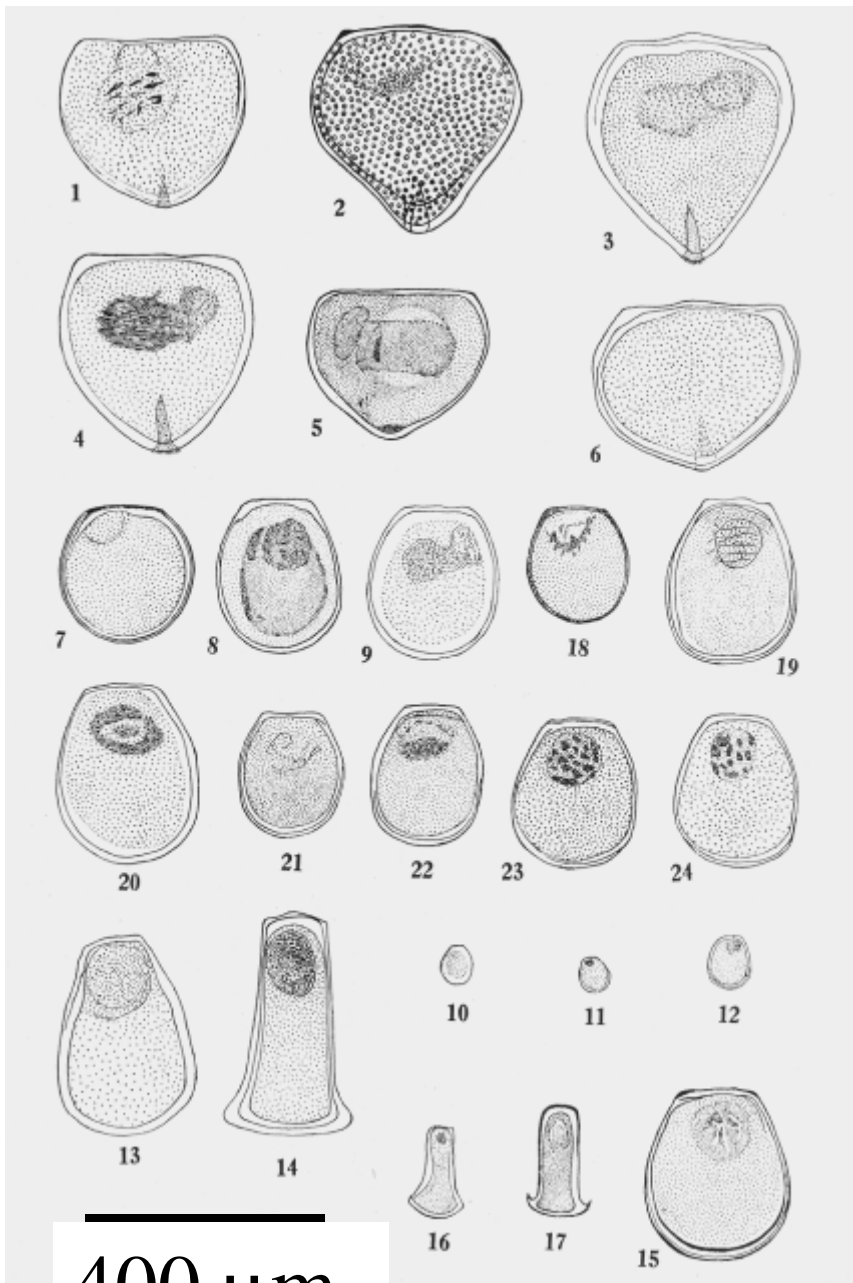


# Plight of Mussels



wavyrayed lampmussel lure

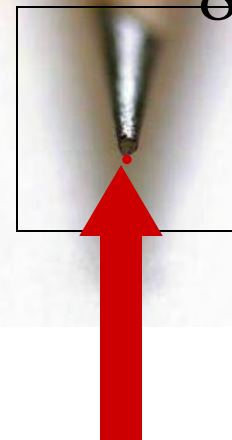
Mussels larvae (size: 50 to 400  $\mu\text{m}$ ) come in all shapes and sizes

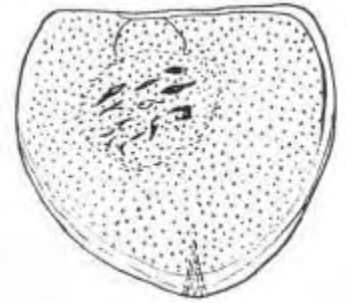


400  $\mu\text{m}$

8,000  $\mu\text{m}$  pencil

800  $\mu\text{m}$  tip





1 glochidium  
250 microns

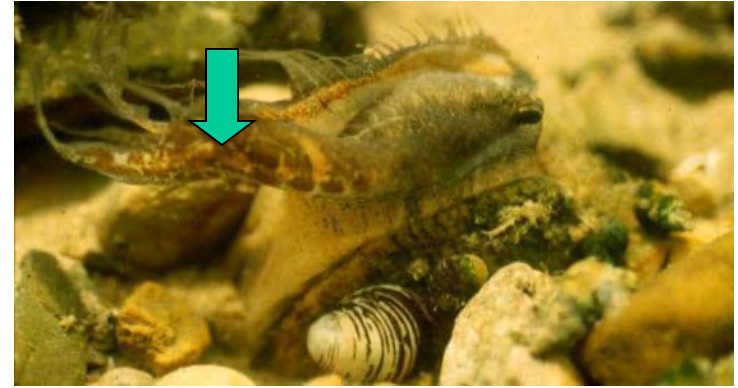
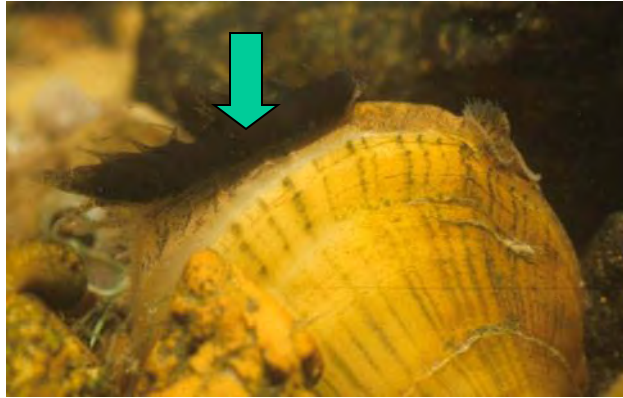


11 average sized  
glochidia can fit on  
the tip of a pencil



# Extraordinary methods of reproduction

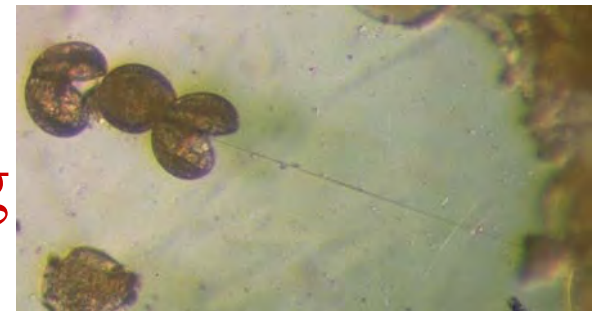
Mantle  
flaps

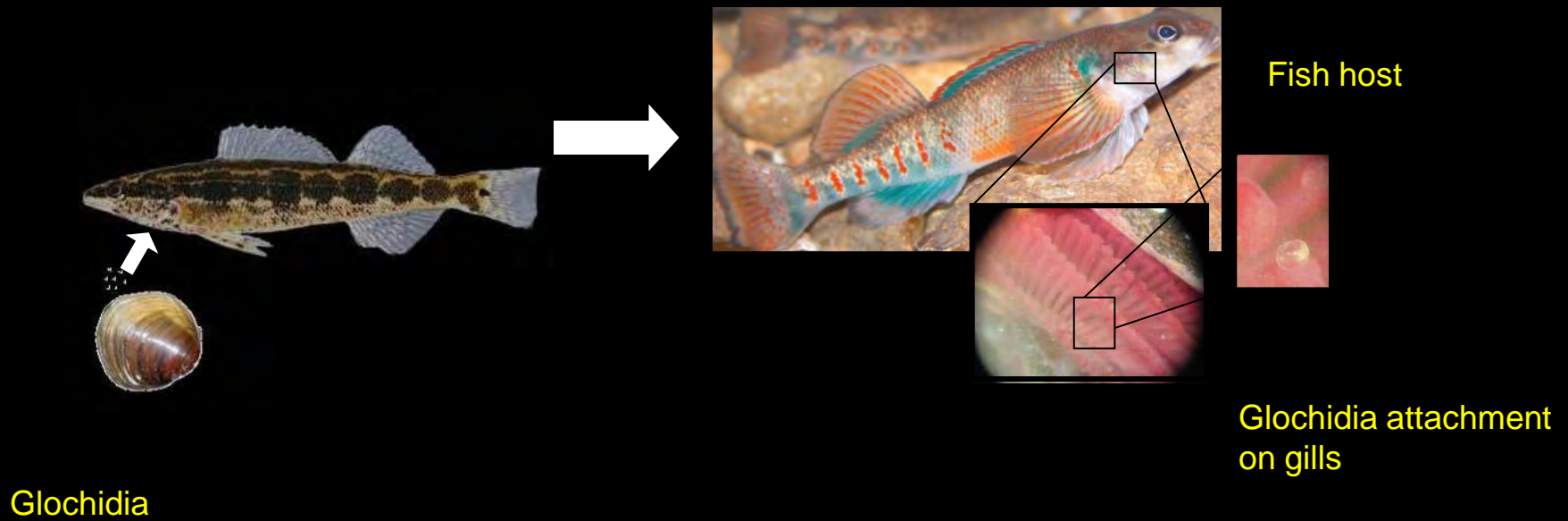


Package of  
larvae



Fishing string  
“thread”





*Obovaria subrotunda*

## Mussel Life Cycle

Not any fish host will work

Research conducted by KDFWR

Host fish relationship often very specific or unknown



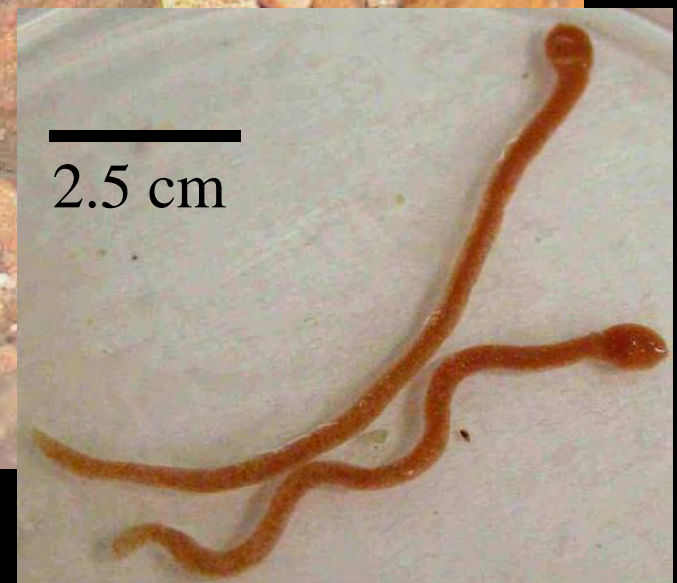
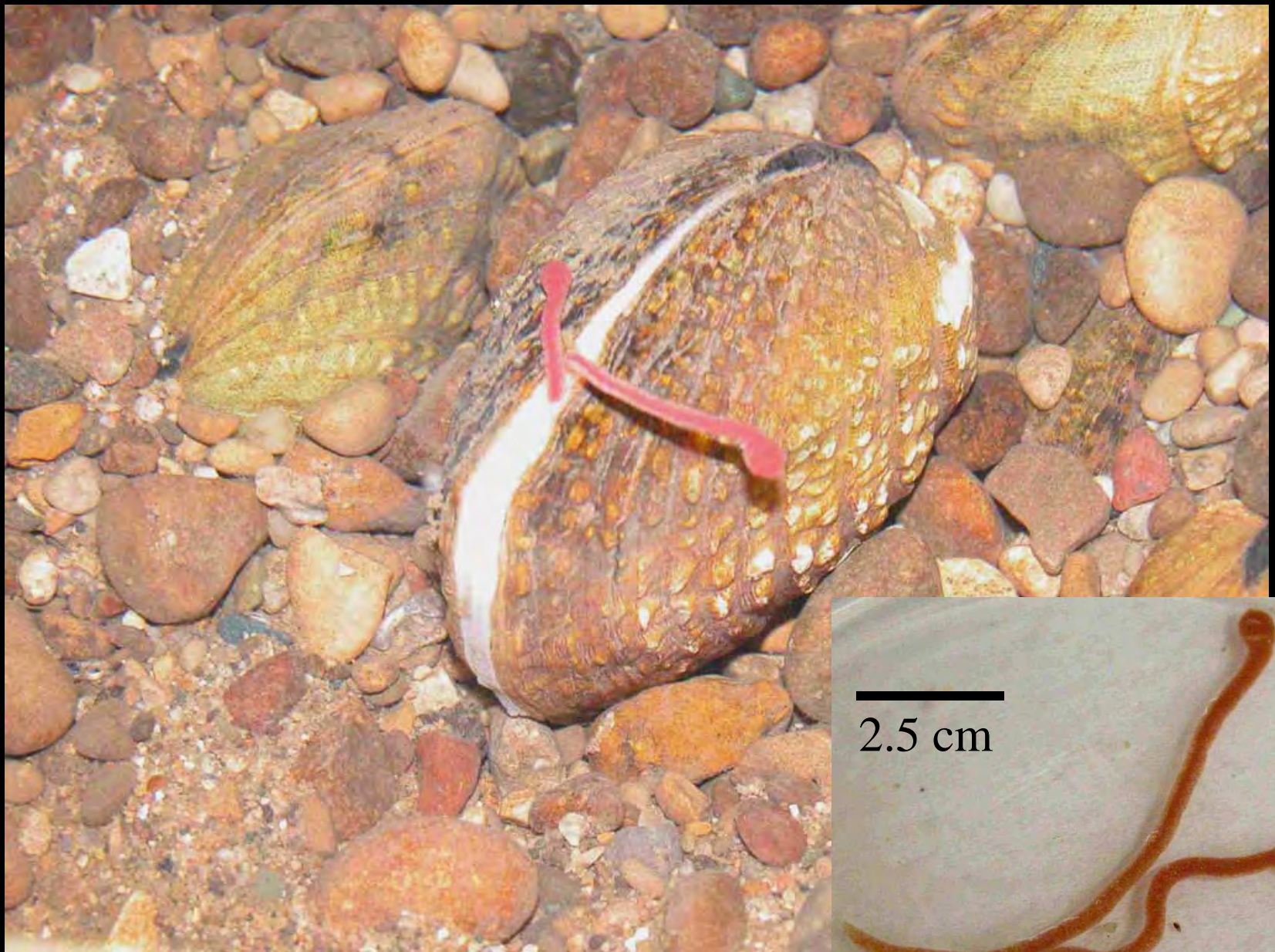
Frecklebelly darter, *Percina stictogaster*,  
1 of 5 darters as host for the round  
hickorynut, *Obovaria subrotunda*.



# Fish hosts







fanshell *Cyprogenia stegaria*

# Endangered fanshell lure





# Plain Pocketbook lure



A photograph of a snuffbox mussel, *Epioblasma triquetra*, resting on a bed of small, light-colored pebbles. The mussel is positioned in the center of the frame, slightly below the title. It has a yellowish-brown, textured shell with a distinct hinge. The pebbles are small, rounded, and vary in shades of light brown, tan, and off-white.

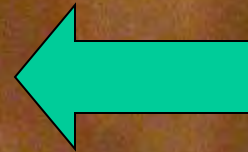
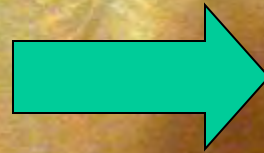
# Host capture by snuffbox mussel

*Epioblasma triquetra*

M. C. Barnhart 2005



Encystment in the gills

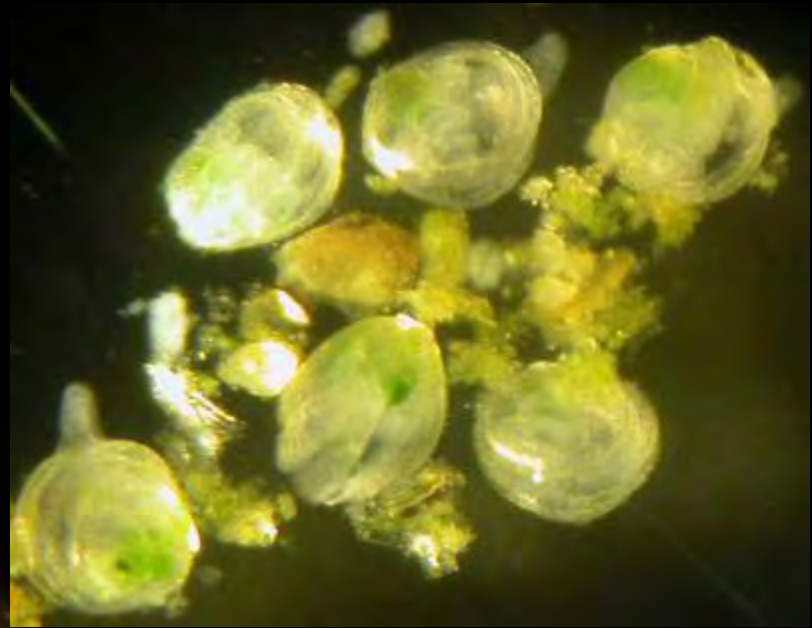


Fin attachment

# The juvenile mussel



Juvenile *Lampsilis* mussel  
(6 months) ( $\sim 1/4$ " inch)



Juvenile pink mussels,  
< 2 weeks old

# Major factors contributing to mussel declines

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- Habitat loss
- Restricted host fish migration
- Pollution
- Over-exploitation
- Disease
- Exotic Species
- Sedimentation





# Overharvest or overexploitation of native mussels in last 140 years



Fig. 2. Mountains of shells rose up alongside the Mississippi as clammers made a living harvesting mussels to supply the button industry. From the Oscar Grossheim Collection, courtesy Musser Public Library, Muscatine, Iowa.



# Mussel Fishing through the Ice near Princeton, Iowa, on the Mississippi River, Winter of 1898-99



# Mussel resources unveiled

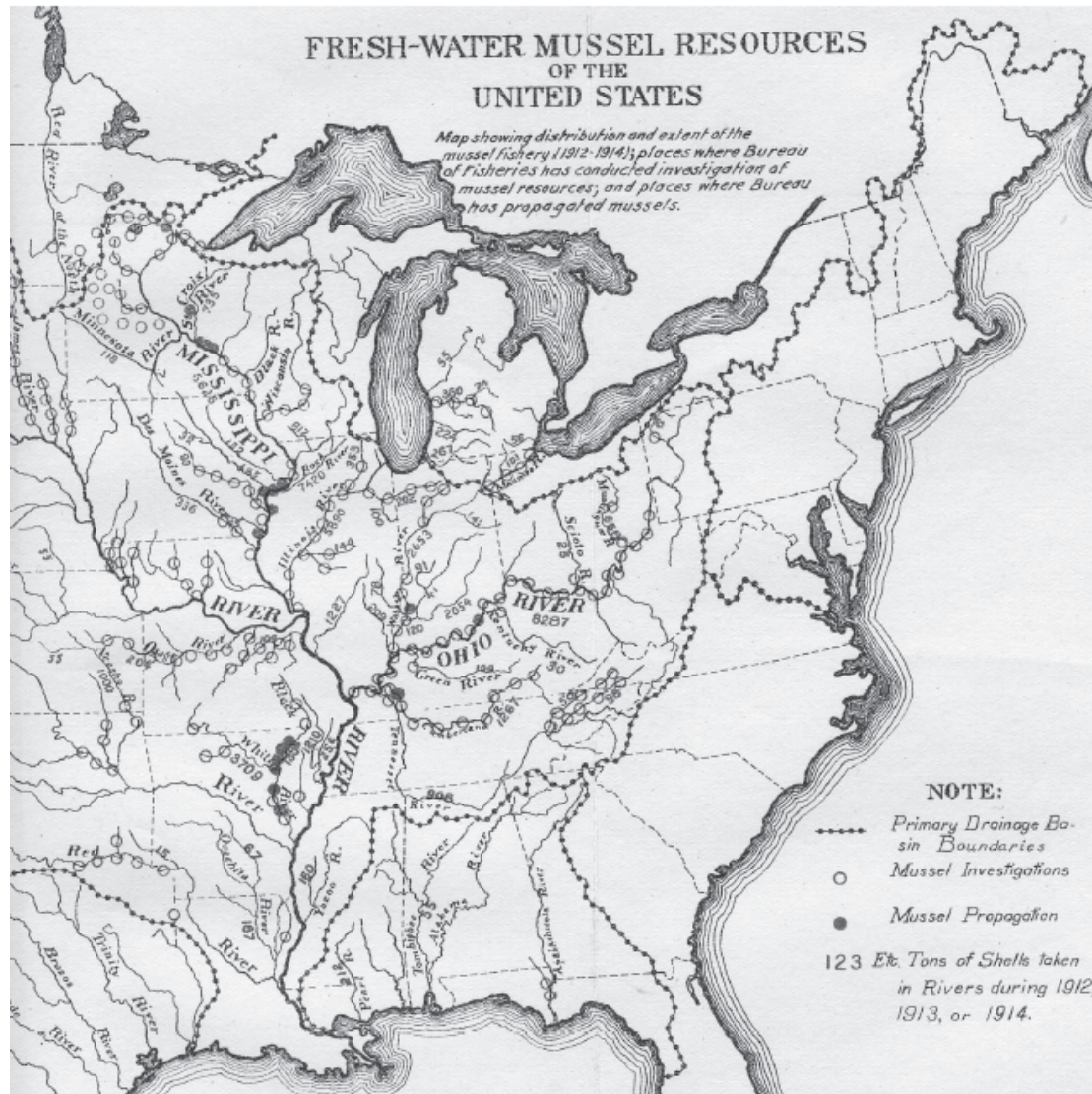


Fig. 14. By 1914, surveys had revealed a great deal about mussel resources.  
From R.E. Coker, "Fresh-Water Mussels and Mussel Industries" (1921).



J. Boepple late 1800's



In 1884, a German by the name of J.F. Boepple founded the Mississippi River pearl button industry by applying his native trade to the abundant Mississippi River mussels. By 1890, Muscatine was known as the *Pearl Button Capital of the World*. 2,500 workers were employed in 43 different button-related businesses.

<http://www.greatriver.com/pearls.htm>

# The Button Industry early 1900's



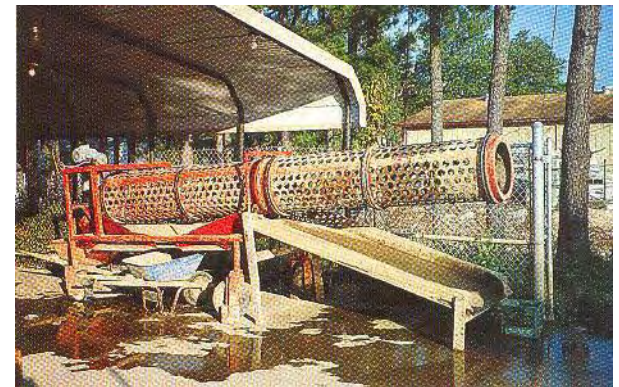
Over 40 button manufacturing companies in the Muscatine, Iowa area in the late 1800's



# Use of freshwater mussels in Kentucky in the early 1900's

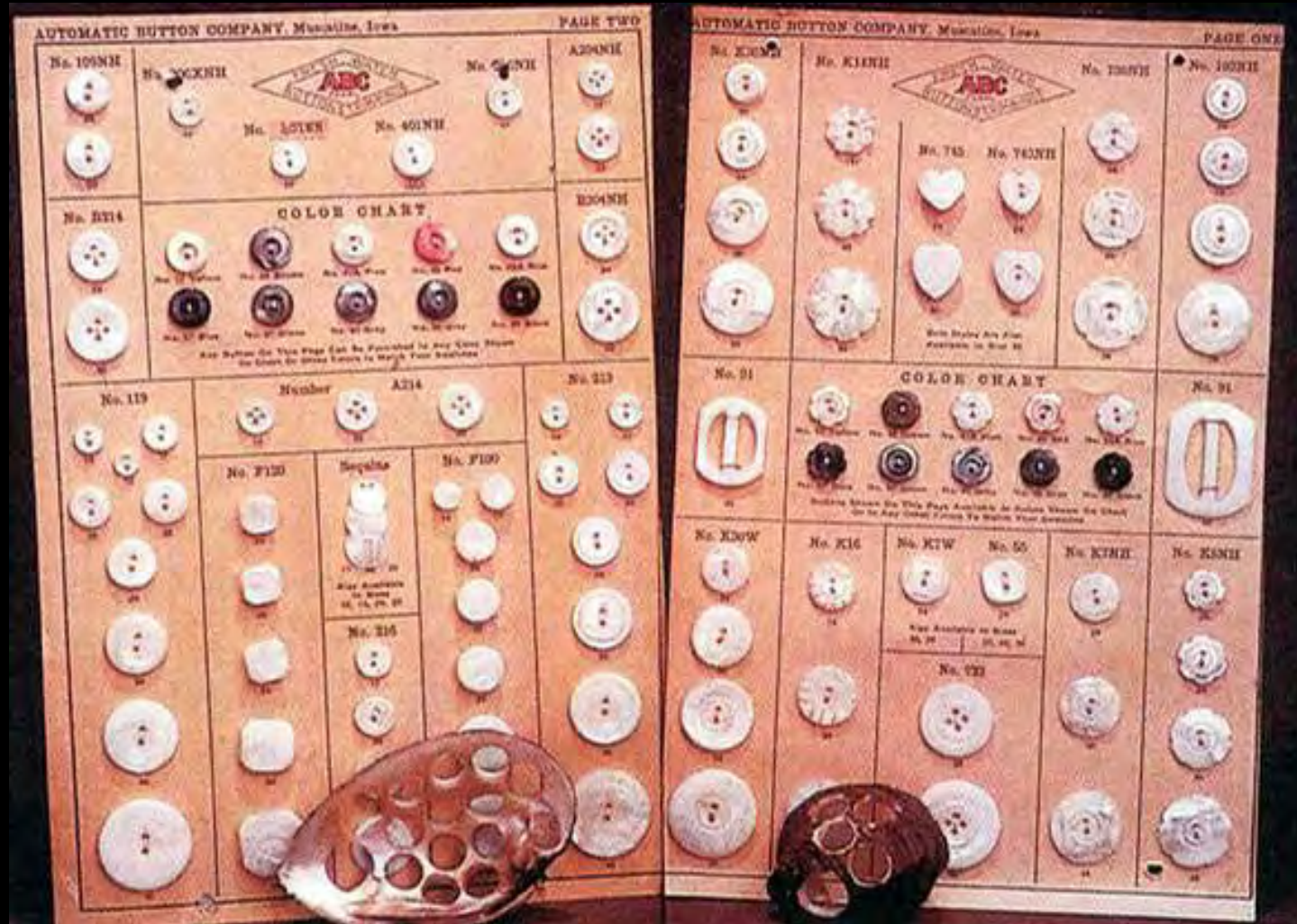


Button industry, Cultured pearls





# Buttons from mussel shells





Photograph by Ralph Stock, National Geographic Stock

## Tennessee Dive Rig



# The brailboat used to harvest mussels in large rivers

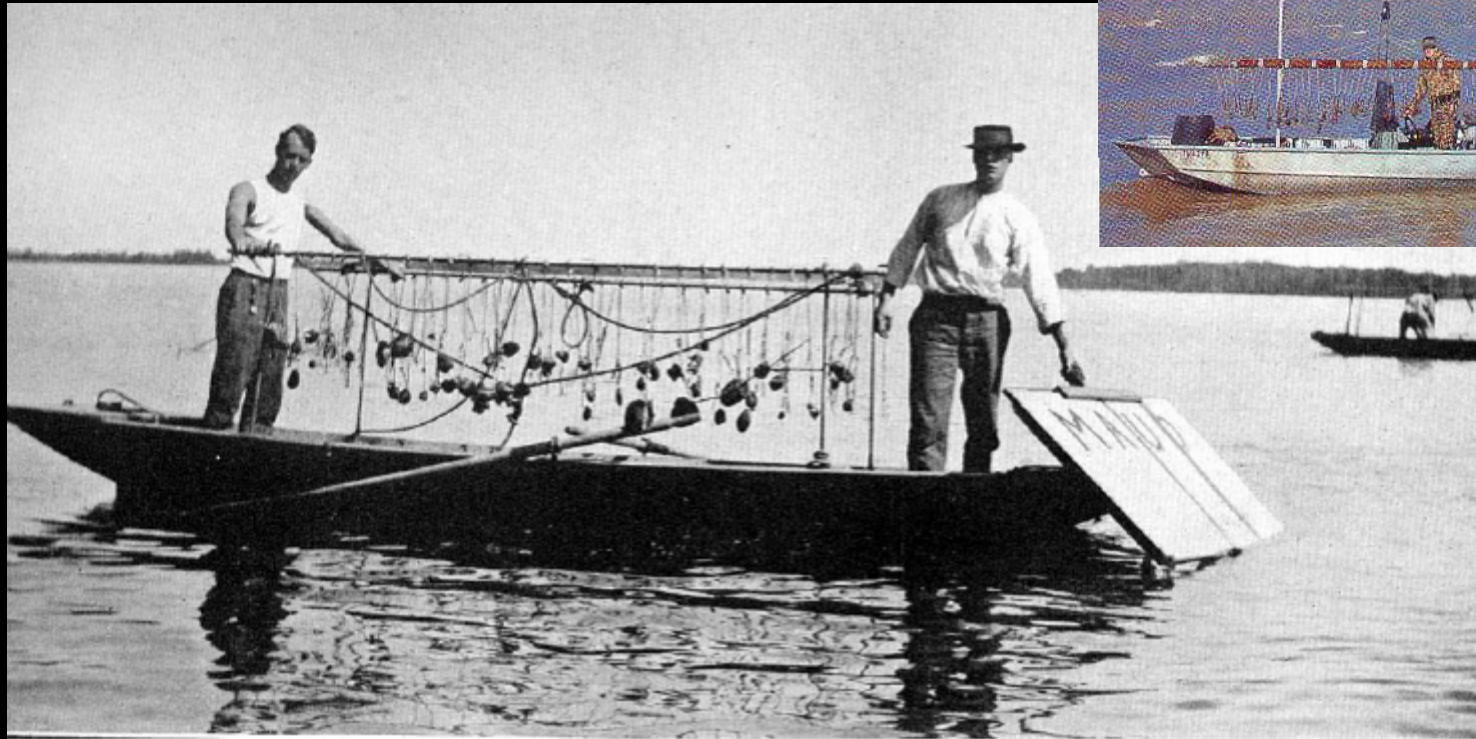


Fig. 7. The crowfoot bar was employed by scientists as well as clambers. Scientists gathered specimens themselves, but also procured mussels from fishermen. From Lefevre and Curtis, "Studies" (1910), fig. 69.



A large mussel processing operation near Muscatine, Iowa (1986). The mussels were being processed (cooked), bagged for shipment to Japan for use in the cultured pearl industry. Source: Jerry Rasmussen, U.S. Fish & Wildlife Service. 1986





Kokichi Mikimoto (photo: Mikimoto Company, Japan)

### Cultured pearls



Photo: Mikimoto Company, Japan

# Cultured Pearl Industry

### The cultured pearl process



The cultured pearl process.

Photo: Tom Watters, Ohio State University





## South Sea Cultured Pearl Farm in The Philippines











Many North American pearl mussels produce high-quality pearls. Use of these pearls for jewelry and decorative objects dates back at least 2,000 years, to the ancient Hopewell culture in Ohio. But subsequently, American freshwater pearls went almost unnoticed until the mid-1800s, when several people reported finding spectacular pearls in rivers and streams around the United States. Those discoveries triggered the beginning of large-scale harvesting--first for pearls, later for mother-of-pearl to be used in buttons, and today for shells to produce nuclei for cultured pearls.

Source American Museum of Natural History

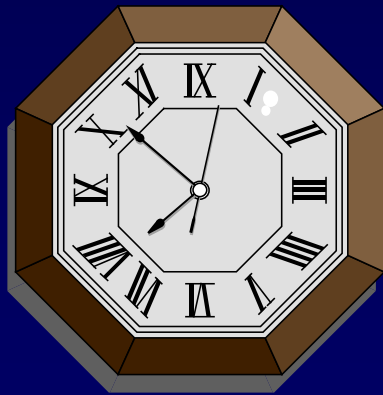


American Freshwater Pearl Mussels. Clockwise from top left: Yellow Sandshell (*Lampsilis teres*); Wartyback (*Quadrula nodulata*); Washboard (*Megaloniaias nervosa*); Ebonyshell (*Fusconaia ebena*); Pigtoe (*Pleurobema cordatum*); center: Monkeyface (*Quadrula metanevra*).



# What's Next?

Conservation, Management,  
and Recovery of Rare and  
Imperiled Mussels



Timing is Critical



# Early 1900 Recovery Research with the US Bureau of Fisheries



Fig. 13. "Interior of station at North La Cross, equipped as a laboratory."  
Fig. 66 in Lefevre and Curtis, "Studies" (1910).

**Management**



# Early 1900 management practices



Fig. 18. Seining black bass in overflowed lands near La Crosse, to be infected with glochidia. Fig. 67 in Lefevre and Curtis "Studies" (1910).

# Center for Mollusk Conservation



Kentucky Department of Fish  
& Wildlife Resources

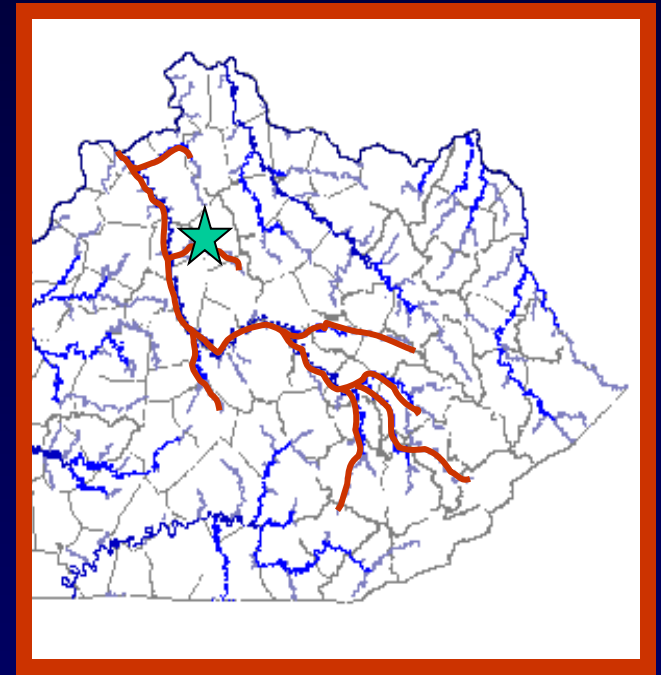


Established 2002

# Center for Mollusk Conservation

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- Located in Frankfort, KY
- Flow through facilities
- Elkhorn Creek★watershed (~20 species)
- Culture tanks for fish and mussels
- Adult/juvenile holding facilities
- Juvenile growout facilities-



Kentucky River  
system (56 species)





Center for Mollusk Conservation





**Holding tanks**  
**Quarantine tanks**  
**Algae culture**  
**Aquaponics**







Outside raceways





## New Fish-tank Rooms 2011



# New Greenhouse II, opened 2011





# Juvenile Mussel Research Building with *in vitro* Culture Lab 2012







# Culture Technique

# Living Stream Tank



Educating about rivers and endangered species





**Minor Clark Hatchery in Eastern KY**



**13) Isom, Billy G., and Robert G. Hudson, "In Vitro Culture of Parasitic Freshwater Mussel Glochidia," *The Nautilus* 96 (No. 4, 1982): 147-51.**

Vol. 96(4)

October 29, 1982

THE NAUTILUS 147

IN VITRO CULTURE OF PARASITIC  
FRESHWATER MUSSEL GLOCHIDIA

**Billy G. Isom**

Division of Water Resources  
Office of Natural Resources  
Tennessee Valley Authority  
Muscle Shoals, AL 35660

and

**Robert G. Hudson**

Associate Professor of Biology  
Department of Biology  
Presbyterian College  
Clinton, SC 29325

**The Future**

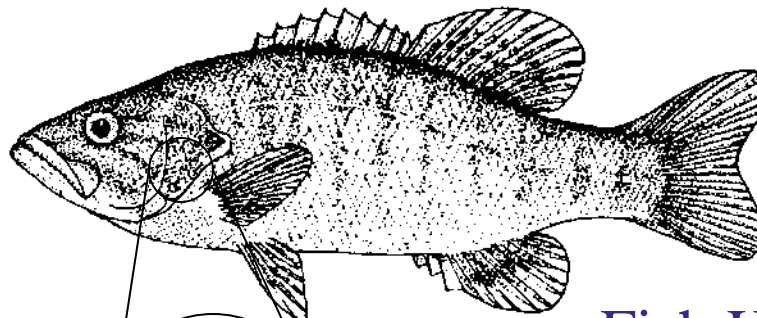
*Ellis and Ellis (1926 and 1927) reported transformation of freshwater mussel glochidia in vitro culture. However, their methodology was never published. This report gives a new method for the in vitro culture of mussel glochidia to juveniles rather than by their natural fish host encystment. The medium consists of physiological salts, amino acids, glucose, vitamins, antibiotics, and a nonspecific component of fish blood plasma. The relative concentration of fish plasma required for optimum results was 33 percent. In vitro culture may prove very beneficial in reestablishing the populations of endangered mussels, management of species used by the pearl culture industry, and culturing of stocks for bioassays, genetic studies, or other uses.*

Application of in vitro culture for the purpose of sustaining endangered mussels of commercial importance is obvious, since usually an unknown fish host is required for larval development in nature. The female mussel has her eggs ferti-

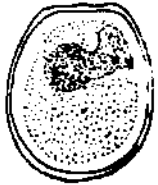
also currently being used for the culturing of freshwater pearls in the United States. The harvesting as a result of demand for such pearls has contributed significantly to the depletion of freshwater mussel resources in the



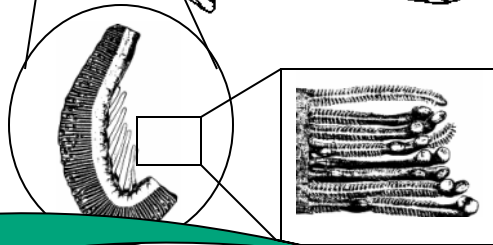
**The Future**



Fish Host



Glochidia

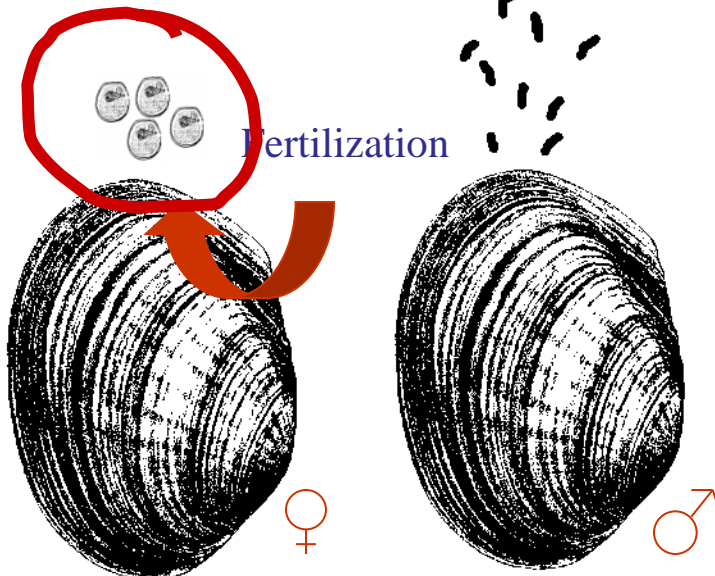


Glochidia on Gills



Juvenile

# Mussel Life Cycle



Adult mucket



# The father of the *in vitro* culture method, Max Mapes Ellis



Fig. 38. Max Mapes Ellis, inspecting bottom samples on the Mississippi River near Oquawka, Illinois, 1931. Courtesy of Cornelia Motley and Philip V. Scarpino.

Out of the box thinking, but in the realm of good science



\$5-6,000

CO<sup>2</sup> incubator





**Thousands of stockable  
sized mussels from  
*in vitro* culture**





# Restoration







tag reader



Pit tagged mussel



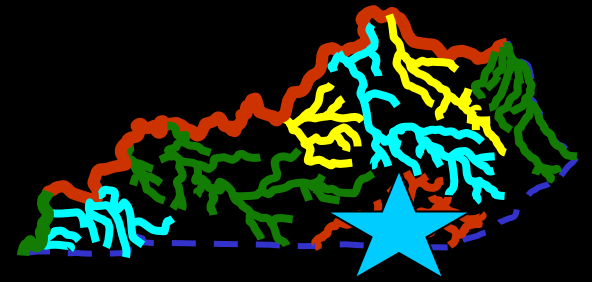


**snuffbox restoration**  
**2009**



Photo: Matt Thomas

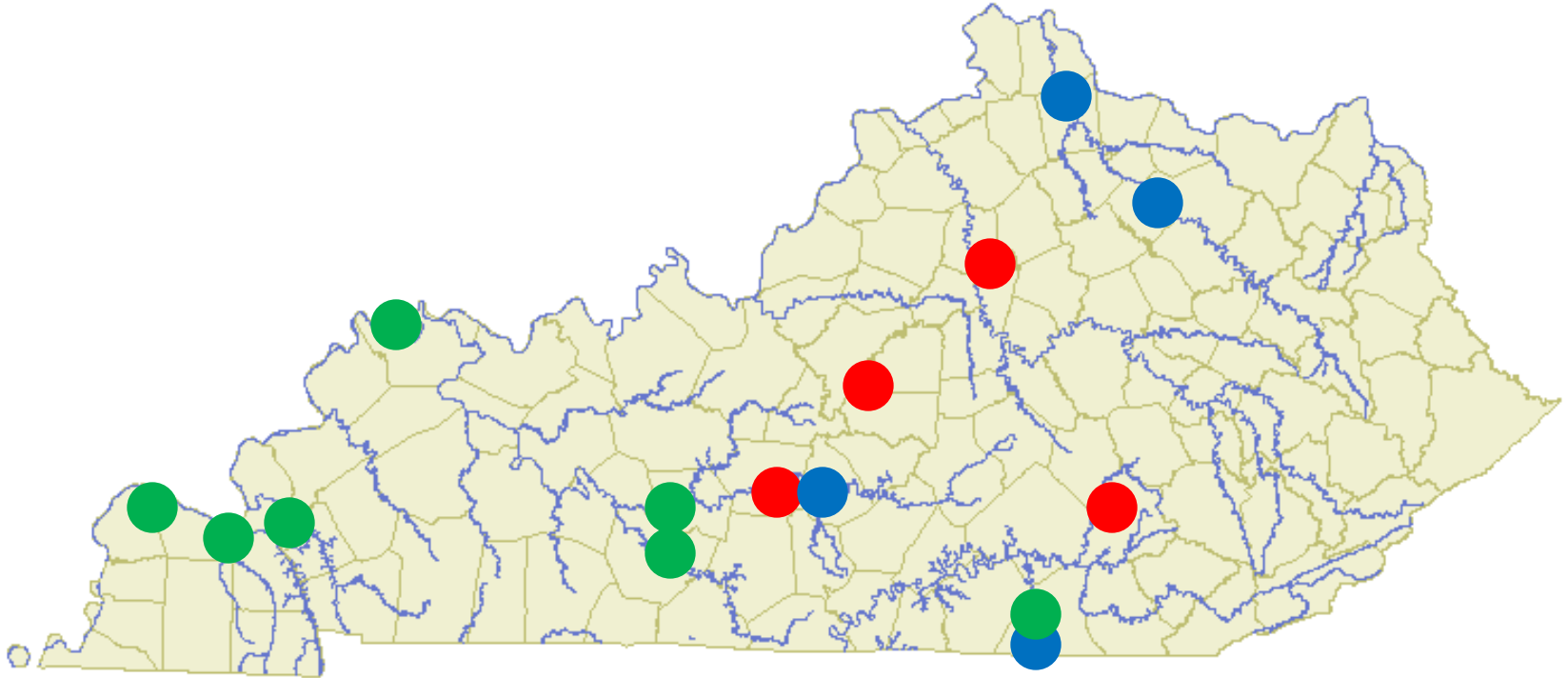
host (striped darter) reared and released at same site



hatchery propagated juveniles of the  
endangered Cumberland bean released in 2009



- Monitoring & Future Augmentation Site
- Augmentation/Monitoring Site
- Reintroduction/Augmentation/Monitoring Site



Augmentation, Monitoring, and Reintroduction  
Sites for Mussel Releases in Kentucky

Lots of people (50-75% volunteers)

# Population Monitoring

1 day per site

Lots of quadrats ( $m^2$ )







# Restoration via Translocation





# Licking River Upstream View at Site

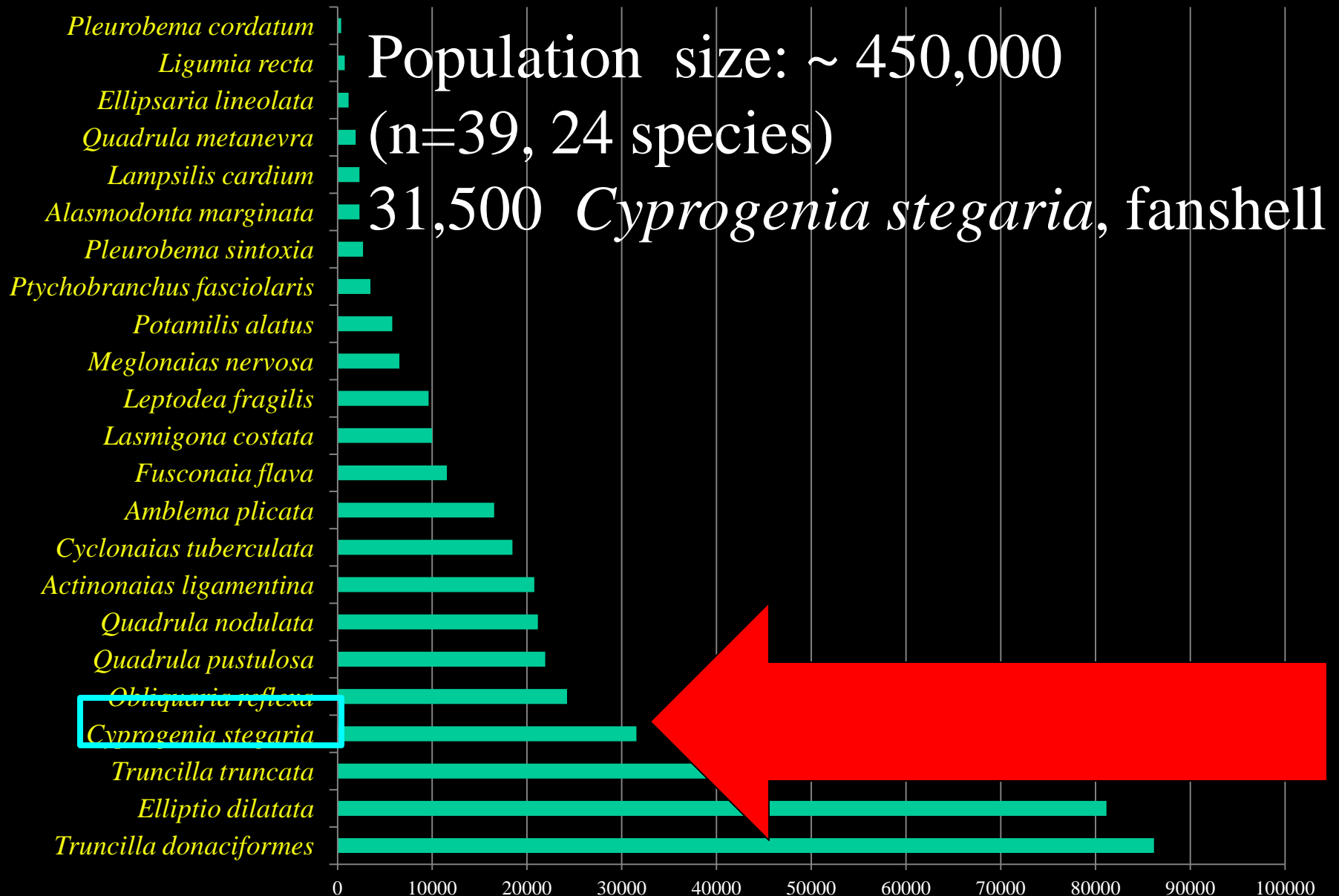


# Mussel Monitoring and Recovery

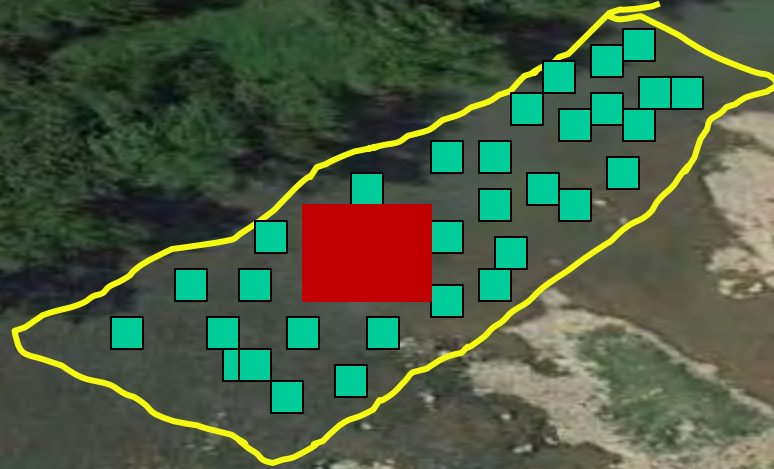
- 2007 initiated a study to monitor several sites
- Site chosen for sampling in Lower Licking
- Site examined in 2007 (quantitative random sampling)
- 2010: site selected for the endangered fanshell, *Cyprogenia stegaria*, recovery
- 2010: 5x5m non-random grid chosen for fanshell removal in 2010
- Joint effort with OH, WV, KY to establish 4 new populations of the fanshell
- 2012: exact 5x5 grid reexamined for recovery of fanshells







# Lower Licking River Site



1 m<sup>2</sup> random sampling location



5x5 grid (2010 & 2012)



20x40 grid (2007) 39 samples



# Grid Comparison

	2010	2012
# species	23	22
% overlap	87%	91%
# mussels	912	784
Avg Density	36.5	31.4
Avg SR per Grid	11.2	9.4
# Fanshells	104	30
Avg Fanshell Density	4.16	1.2
# Grids w/ Fanshells	25	17
% Grids w/Fanshells	100%	68%



Mussel silo

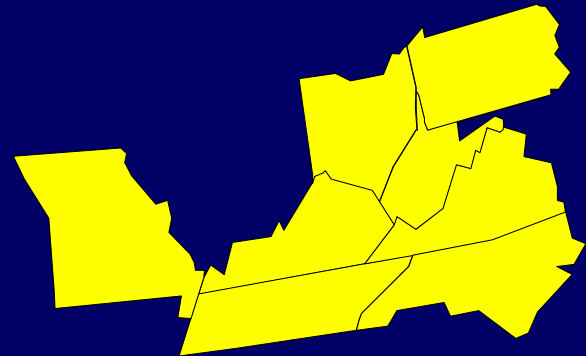


## In stream monitoring

Hatchery reared *L. abrupta* released into the Green River in 2011 & 2012 (cooperative effort with TWRA, MCNP and TN Tech)

# KDFWR Cooperative Restoration Efforts with State, USFWS and Federal Partners

- Translocation of the northern riffleshell from PA to KY (2012)
- Fanshell restoration to WV and OH (2010)
- Translocation of 4 endangered mussels from TN to KY
- Pink mucket propagation with TN
- Purple catspaw propagation with OH and WV
- Cooperative propagation efforts with VA, TN, WV, OH, MO, NC, and PA
- Cooperative propagation using *in vitro* culture techniques from mussels shipped in culture media





# Summary of Mussel Conservation at CMC

- 104 mussel species in KY
- 84 mussel species still found in the state
- 27 federal endangered species (projected in 2013)
- 54 mussel species cultured at CMC (working with more than 12 species now)
- 20 mussel species cultured to a tagable size  
(6 species (3 FE) released in the wild, up to 8-10 species may be released in 2012)
- >30 mussel species cultured using *invitro*-culture methods
- Translocated 5 species (3 FE) from other states to KY and 1 FE species to two other states

# Am I going to survive?

With a little help,  
I think you will  
make it.

