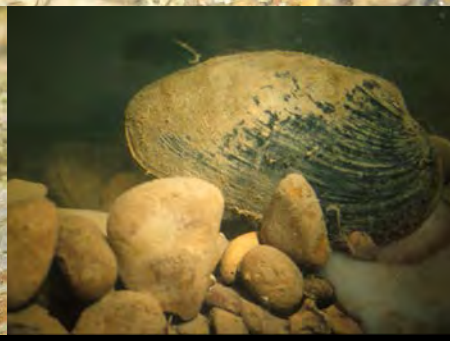
Conservation of freshwater mussels: taxonomy, captive breeding and wild restoration











mncn museonacionaldecienciasnaturales

Rafael Araujo. Museo Nacional de Ciencias Naturales-CSIC. Madrid



1. INTRODUCTION
2. TAXONOMY
3. DISTRIBUTION
4. THREATS
5. CAPTIVE BREEDING
6. RELEASE AND MONITORING

Ecological role of freshwater bivalves

1- **Nutrient dynamics of the aquatic systems removing phytoplancton, bacteria and organic material from water and sediments**

- **Food (filtration and suspension)**
- **Excretion and biodeposición**

2- **Biomodification of sediments increasing their oxygen levels**



50 liters water/day

- Strayer DL *et al* 1999. Transformation of freshwater ecosystems by bivalves. *BioScience* 49.

- Vaughn CC & Hakenkamp CC. 2001. The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology* 46.

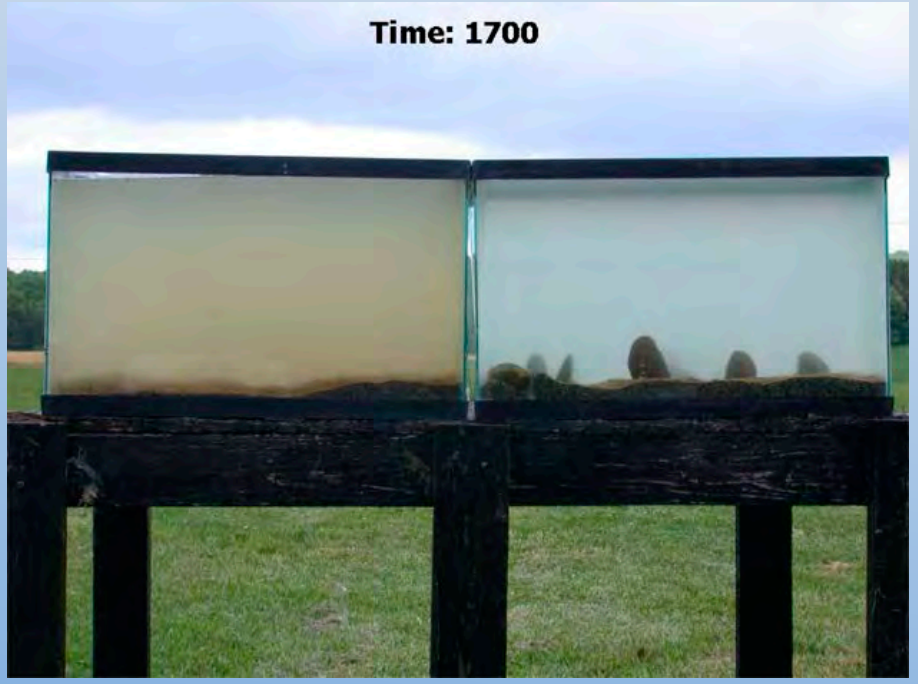
Time: 0900

8 Eastern Elliptios, *Elliptio complanata*

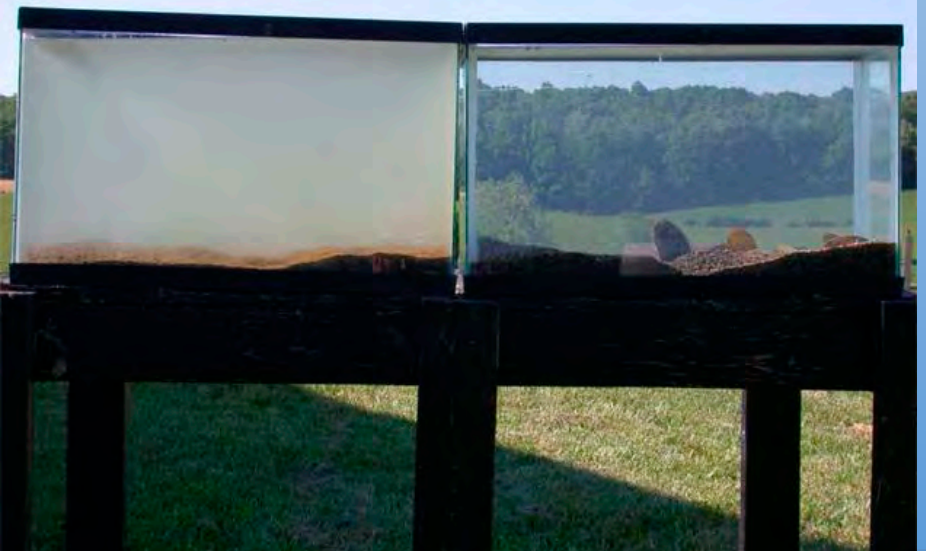
In 10 gallons of water at ≈ 22°C

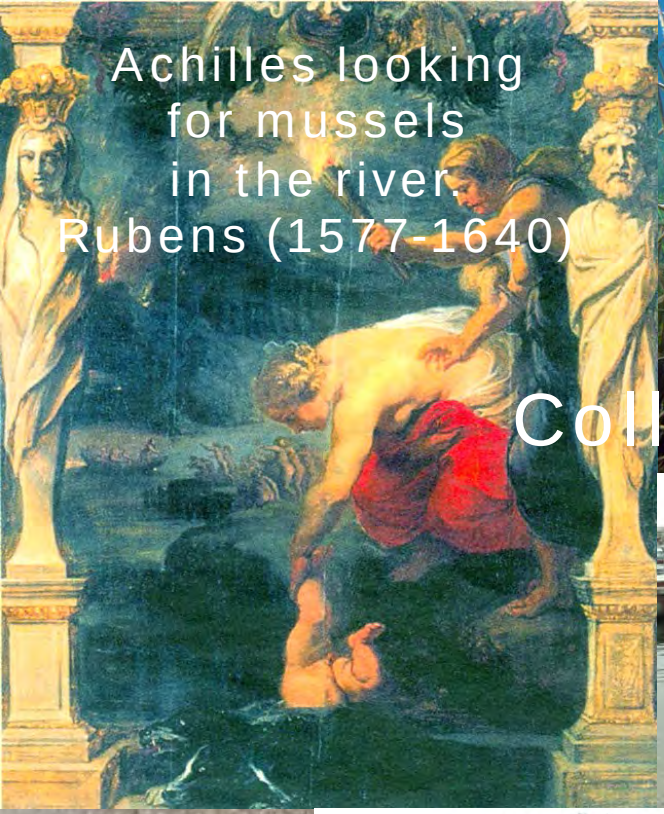


Time: 1700



Time: 0900





Collecting mussels





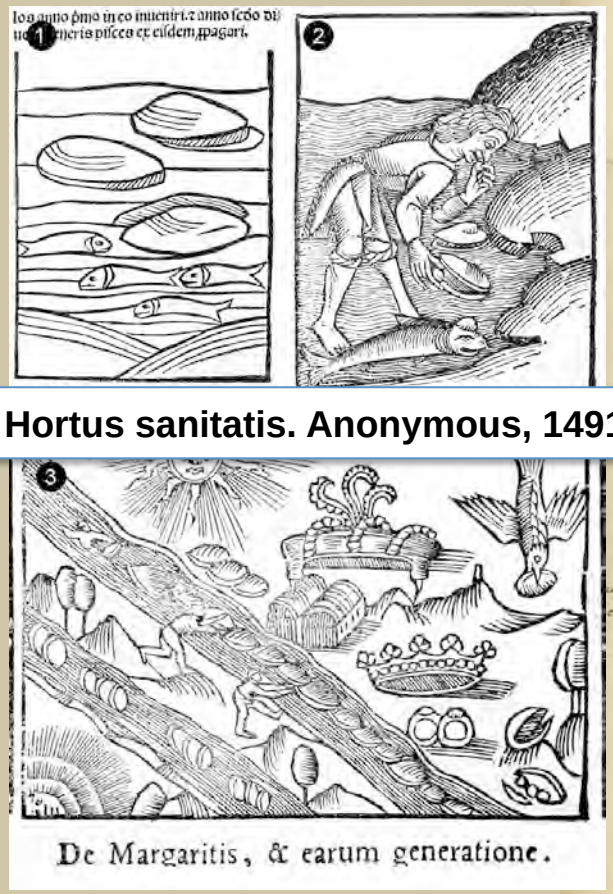






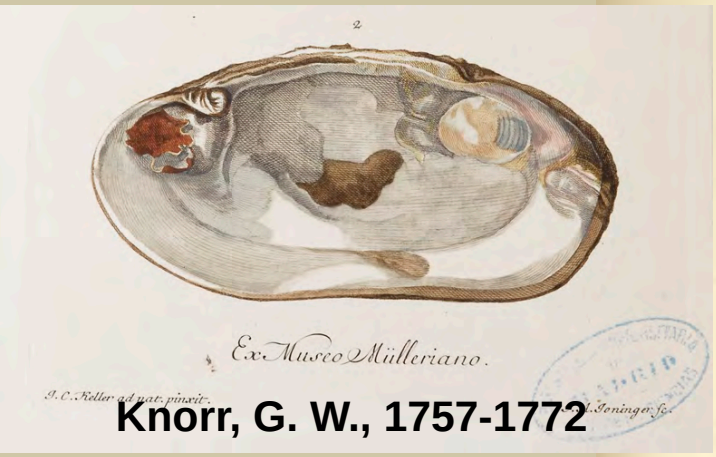






Hortus sanitatis. Anonymous, 1491

Magnus, 1555



Knorr, G. W., 1757-1772



Historiae Conchyliorum. Lister, 1685-1692



1736. Warning at the Schwienau River (Saxony, Germany) “Preserve your hands”



Elizabeth I of England 1533-1603

The Story of My Life by "Billie Button"



MY name is Billie Button, and my home is now at La Crosse, Wisconsin, but I expect to make a trip pretty soon and where I'll bring up Heaven only knows. On the waist of Sidney Christopher Sparks, at Punxsutawney, Pennsylvania, maybe, or perhaps bedecking the dress that little Lucille Dunwoody, of Tulare, California, wears to her birthday party.

But while I am waiting for my call to start on the succession of journeys that will bring me to my final destination and career of usefulness, I've been asked by Mr. MacWillie, of the Wisconsin Pearl Button Company, to tell you the story of my life.

Now, I'm not a Kipling or an Ernest Seton-Thompson, but if you'll be patient and let me spin my yarn in my own way, I think you'll find it interesting, for I don't believe you have ever seen anything just like it in print before.

Now for a fresh start. My name, as I told you, is Billie Button—of the Pearl-Button family, if you please, distantly connected with the Ivory-Buttons, the Bone-Buttons, and the Brass-Buttons, but with quite a different history, I assure you.

In telling you about us Pearl-Buttons I am going to go clear back to the beginning of things, in the days of Old Mother Clam, on the mud flats of the Mississippi. I'll have something to say about how the festive clam lives—and dies—how its shell gets into the button factory and what happens to it there. There will be interesting chapters on my experience in the big, modern plant of the Wisconsin Pearl Button Company, at La Crosse.

And while my adventures may not bring you all the chills and thrills that follow Deadwood Dick or Sherlock Holmes, I believe that you will find them sufficiently varied and interesting to keep you from napping and to make you look forward to each successive chapter with a real hankering.

See here, though, THIS isn't my STORY—it's only the ADVANCE NOTICE of it, written (in true fifteen cent magazine fashion) by the author. The real story will start with next month's calendar.

The Story of My Life by "Billie Button"

CHAPTER I. *The author tells of his ancestry and pedigree, with side-lights on the habits of the care-free clam.*

IN telling my story, I, Billie Button, must go back to the time long before I was I at all. Come with me, kind reader, to the Mississippi River, somewhere between Iowa and Wisconsin. Take a peep beneath its waters a little way off shore. Clams—Clams—Clams—millions of them, waiting in a "bed" a mile or more long, with their faces turned upstream and their mouths open, for the clam "toils not, neither does he spin," but waits for the big river to bring his food right down into his jaws. Not the clams that make your mouth water at the famous Clam Bakes of Long Island Sound, but their cousins, the Fresh-Water-Clams.

High-sounding names they have in the mouths of the scientific gentlemen who write Fisheries Bulletins and Zoologies. Take a long breath before you tackle them: Lampsilis Adontonoides, Anodonta Corpulenta, Plagiola Donaciformis, Quadrula Pustulata, and a lot of others that sound like the combatants in a Sicilian vendetta. Your clammer and longshore urchin, though, don't bother with scientific terminology, but dub them Slop Bucket, Nigger Head, Mucket, Pig Toe and other expressive if inelegant nicknames.



Ask that clammer over there to show you what his "crow foot" has brought up. Look carefully at the dozen or so specimens he dumps into your boat. Maybe they'll be all of one family, or just as likely every one will be different in appearance and name.

Pick up one and rip off its shell ruthlessly. (What's a clam's feelings to the pursuit of knowledge?) If it's breeding time and you have chanced on a mother clam, you will find the gills doing double duty, for besides performing their natural breathing functions they will be distended (wholly or in part) with a jelly-like mass containing thousands of tiny eggs, becoming what the scientists call the "marsupium" or brood pouch. If your specimen answers to the name of Paper Shell or Lampsilis Laevissima, these eggs will be pale or colorless. If it chances to be a Quadrula Ebena, they will be pink or bright red.

The Story of My Life by "Billie Button"

CHAPTER II. *Here you meet the Glochidium. Don't be afraid of his name. He is harmless*

MAYBE the next clam you investigate will have no eggs at all—then you can gamble that it is a father clam, useful, but with a history that, as Kipling says, "is another story."

Number three opens up differently. The brood pouch is full, but not of eggs. Instead you'll see, if you look closely, myriads of little embryo clams or glochidia (how these professors do love to parade their Latin). These glochidia are hatched from the eggs in the brood pouch, but remain there, packed snug and tight, for periods of from a few weeks to several months, according to the particular kind of clam from which they spring. The glochidium is tiny, of course, and its shell is soft, but it has all the essential features of the adult clam, and in the brood pouch of the old mother clam its organs develop and it grows up toward perfect clamhood.



A GLOCHIDIUM

Now drop the rest of your clams into the bottom of your boat, row off down stream and try your luck as an angler. If fate is kind and bait attractive, you'll land a brace of rock bass or a sheepshead—at worst you can count on pulling in a few sunfish.

What has that to do with clams? Let's see.

Take a close look at that fish you caught last. Along its fins and gills you will find little rough humps and bunches—all closed over with "skin." Don't be suspicious. Mr. Fish isn't diseased—these are not boils or bunions, but another step in the life history of the clam, and a shining example of Dame Nature's way of caring for her helpless children.

When you open these bunches you will find that each contains a glochidium just like one of those you saw in the clam's brood pouch a half hour back, only a little better developed. You have stumbled on the clam in its "parasitic" stage.

The story of Billie Button

Wisconsin Pearl Button Co.

G. Lefevre

W. C. Curtis

H. M. Smith

R. E. Coker

A. F. Shira

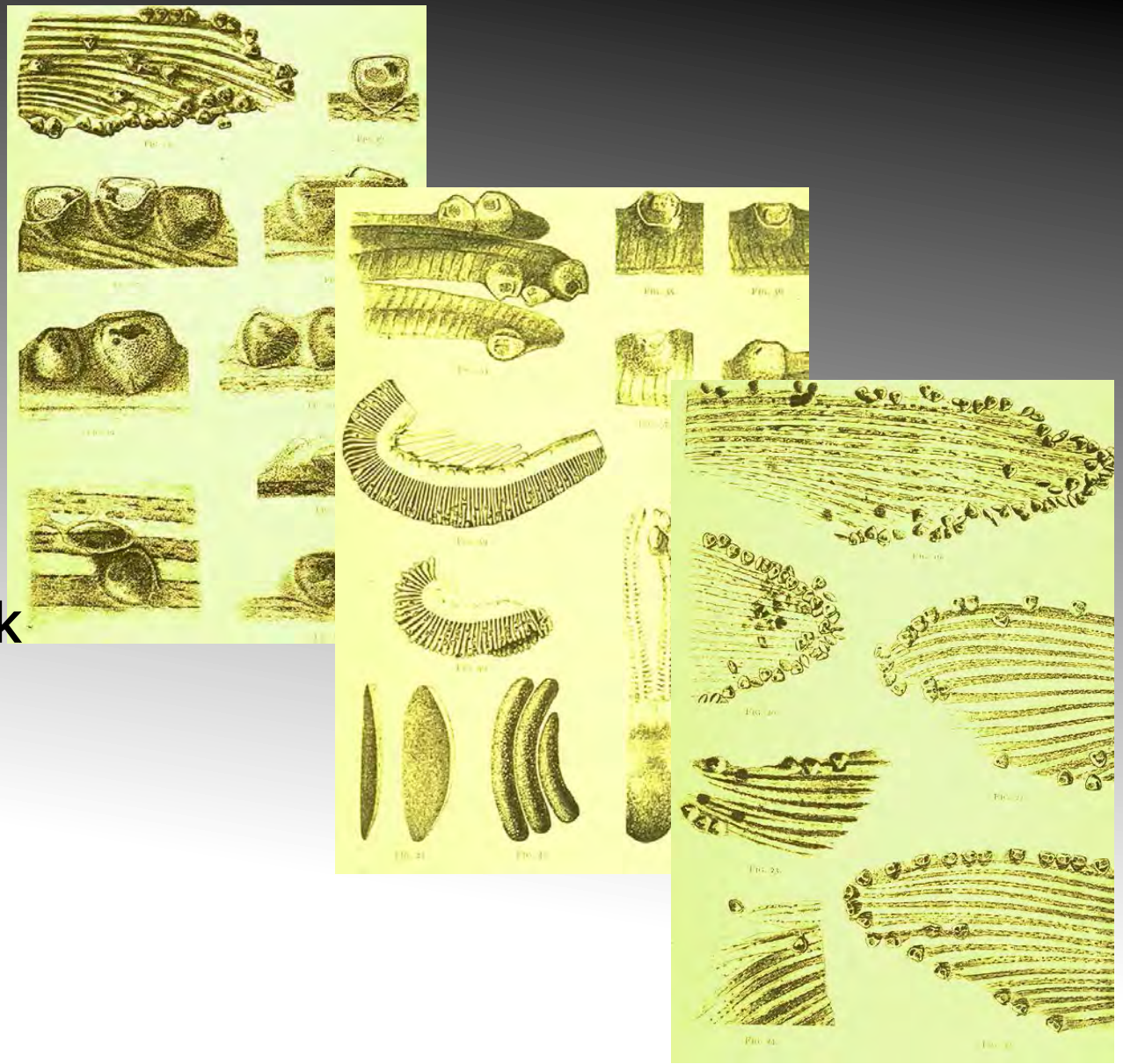
H. Walton Clark

A. D. Howard

W. R. Allen

F. C. Baker

F. B. Isely



Lefevre G & Curtis WC. 1912. Studies on the reproduction and artificial propagation of freshwater mussels. *Bull. Bur. Fish*. 30: 105-201

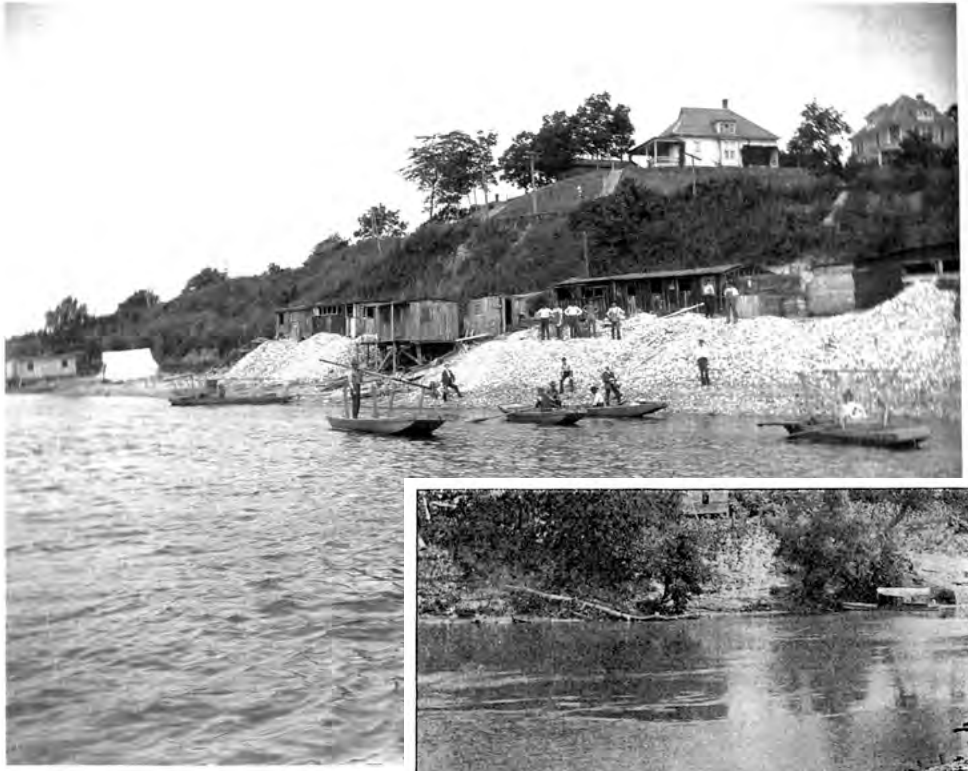


FIGURE 4. Musselers, clamming boats, and saw
Library [Muscatine, Iowa]; Oscar Grossheim Coll



irtesy of Musser Public Library [Muscatine, Iowa]; Oscar

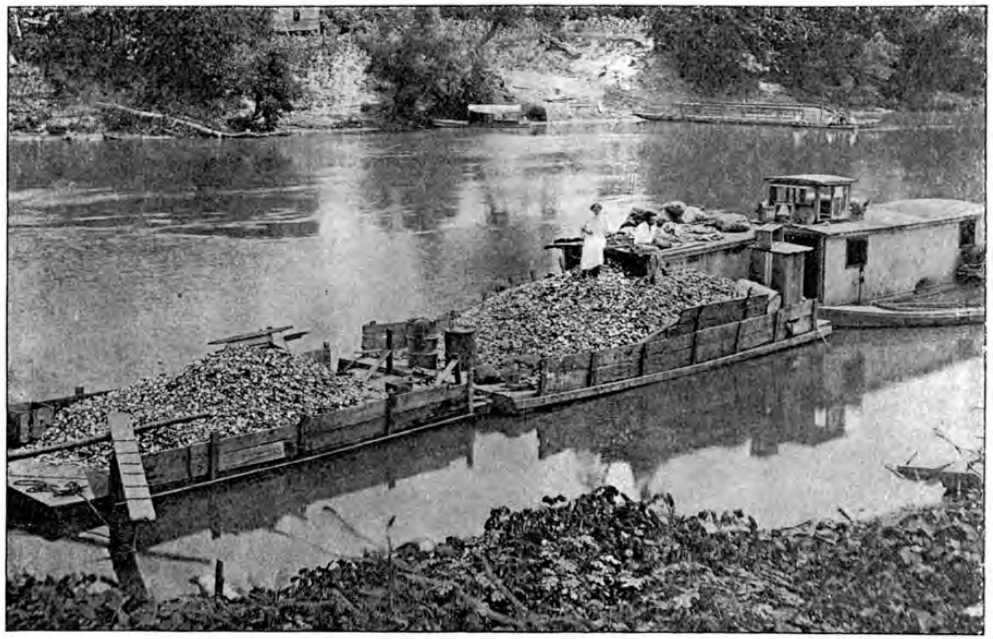
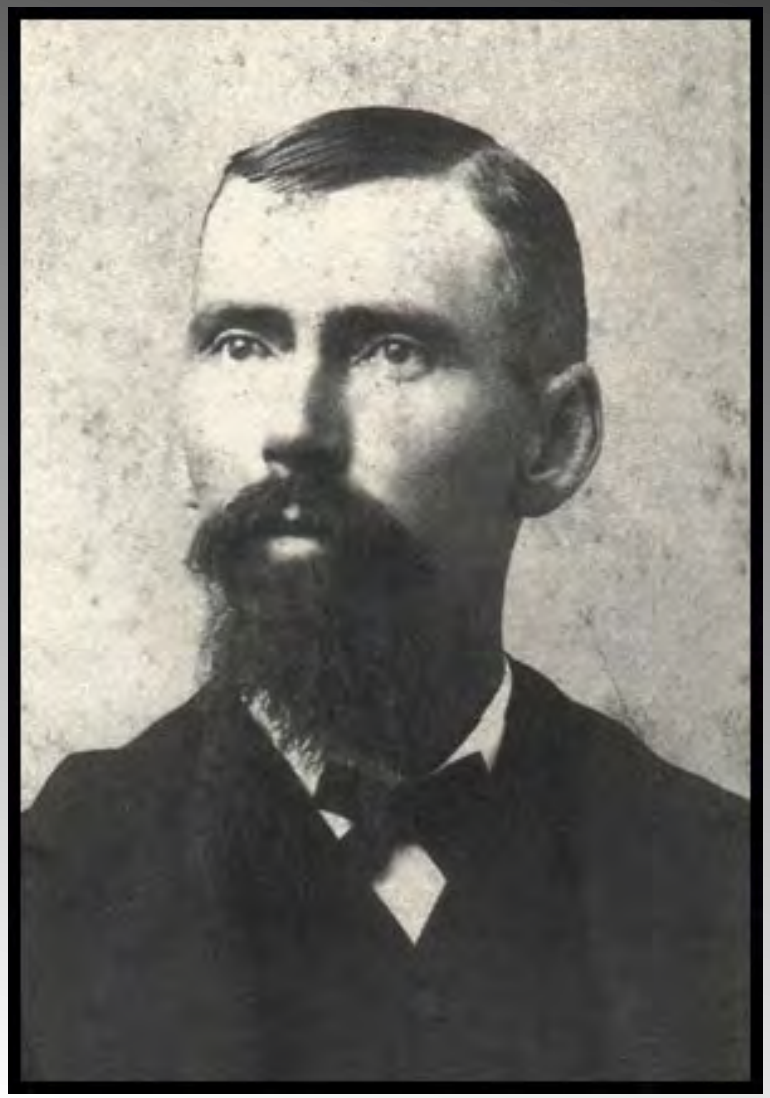


FIG. 1.—Black River, Ark., a very productive mussel stream.

Coker RE, Shira AF, Walton H & Howard AD. 1921. Natural history and propagation of fresh-water mussel. *Bulletin of the United States Bureau of Fisheries* 37:79-180

Claassen C. 1994. Washboards, pigtoes and muckets: historic musseling in the Mississippi watershed. *Historical Archaeology* 28(2): 1-145



C. T. Simpson
(1846-1932)



A. E. Ortmann
(1863-1927)

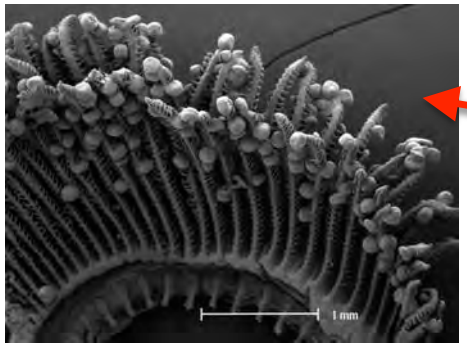




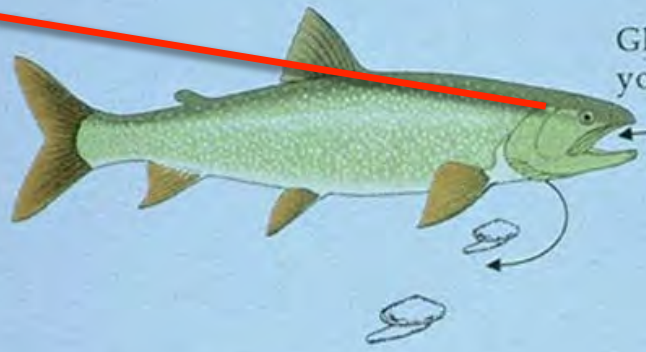


Locard **Bourguignat** **Fritz Haas**

- **Nouvelle École: Bourguignat, Locard, Servain (XIX Century)**
- **Superfamilia Unionacea. Haas (1969)**



4

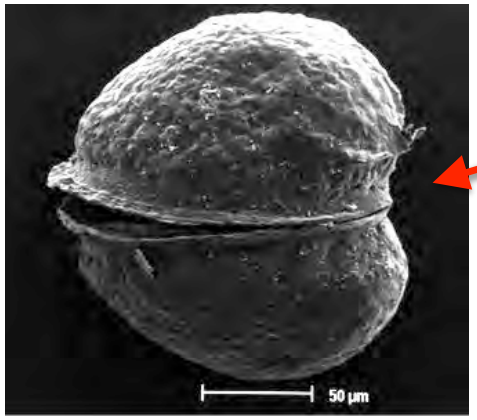


Glochidia attach to young trout or salmon



3

5



Juvenile mussels drop off and bury into the gravel

Glochidia released by female mussel



Sperm taken in by female mussel

Young mussels visible after approx. 5 years

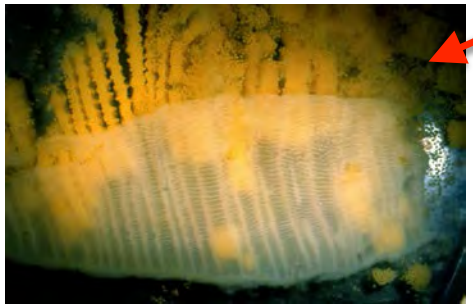


FEMALE

MALE

Adult Pearl Mussel in gravel

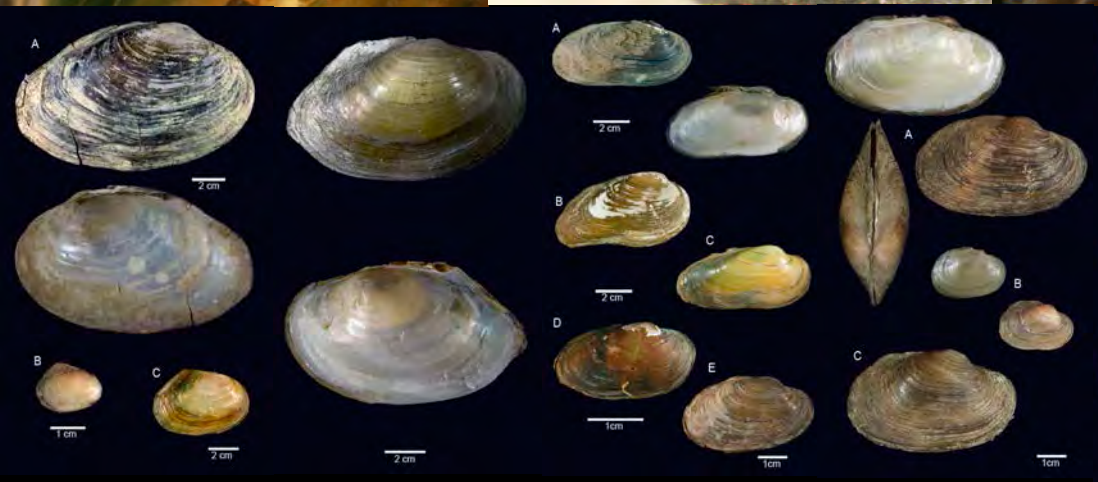
2



1





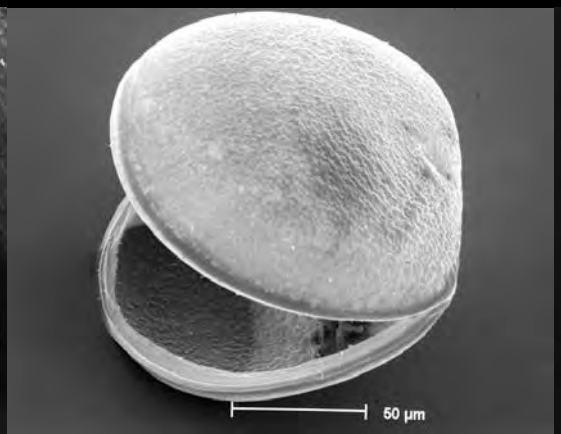



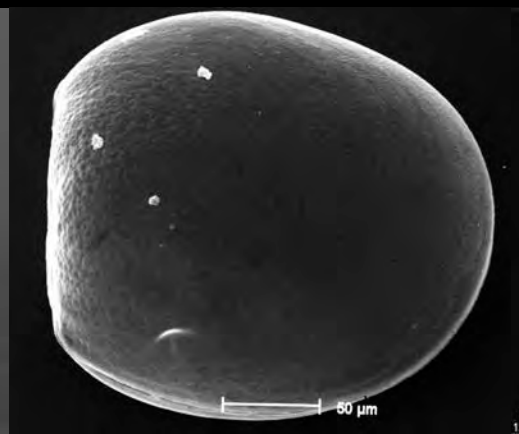


Anatomy

Biology

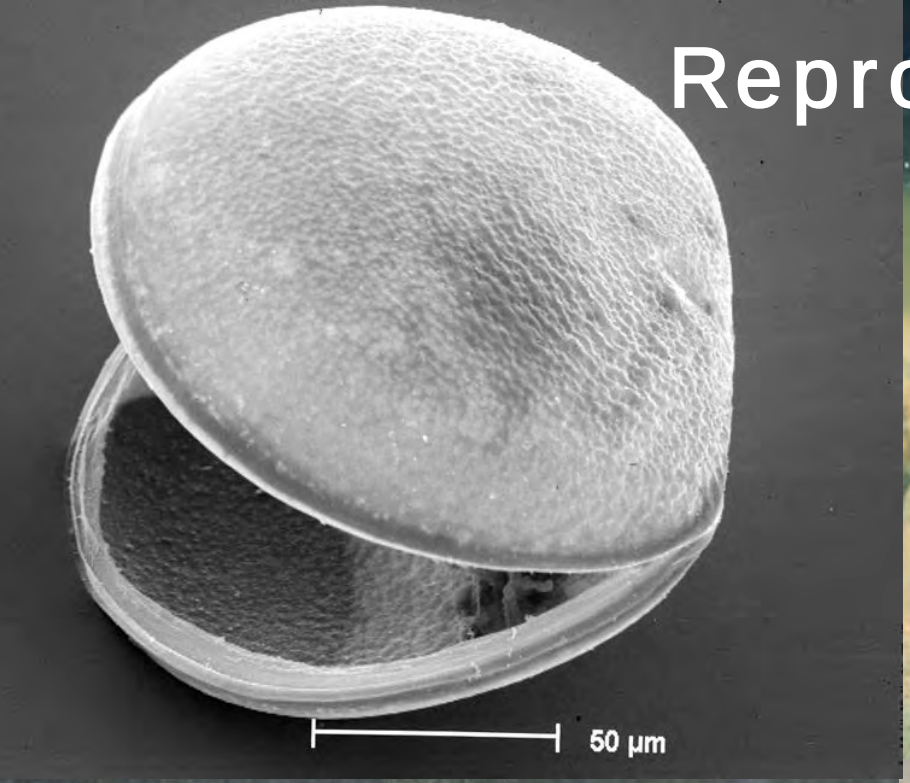








Reproduction







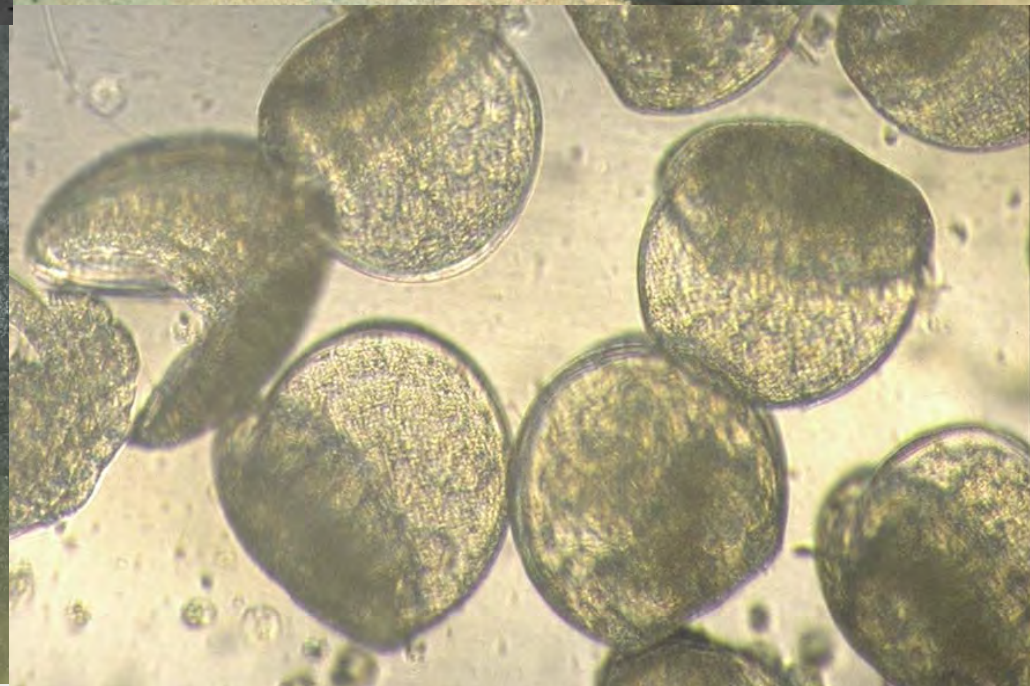




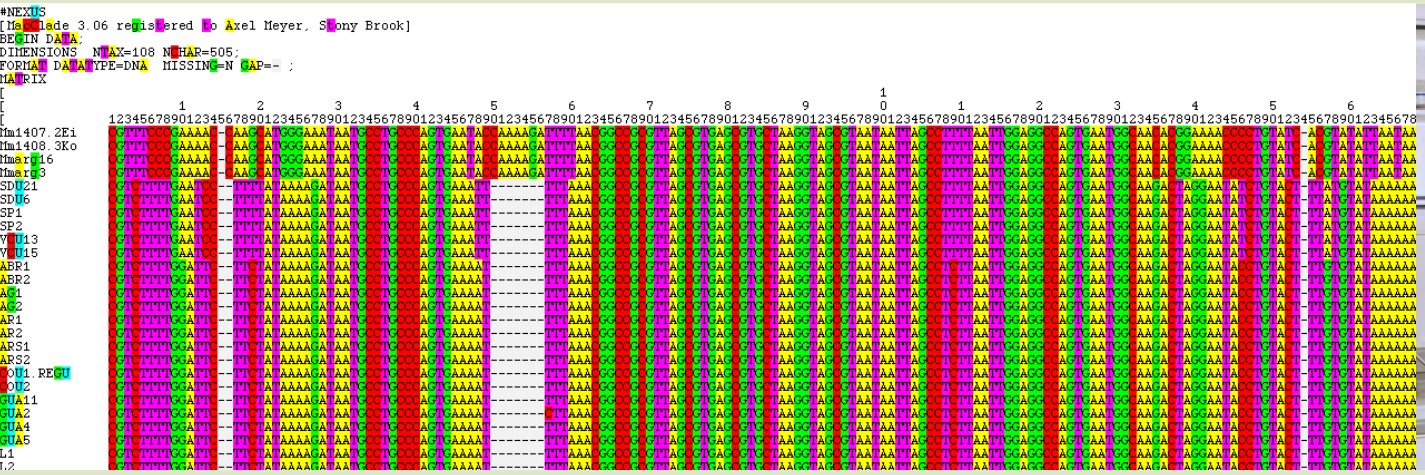
Plate 14. Demersal conglutinates. (a) Ouachita kidneyshell, *Ptychobranchus occidentalis*, larval fish or insect mimic. (b) Fluted kidneyshell, *P. subtentum*, blackfly pupa mimic. (c) Triangular kidneyshell, *P. greenii*, fish egg mimic (conglutinates within gill). (d) Western fanshell, *Cyprogenia aberti*, worm mimic. (c, Paul Hartfield, photo; all others, Chris Barnhart).



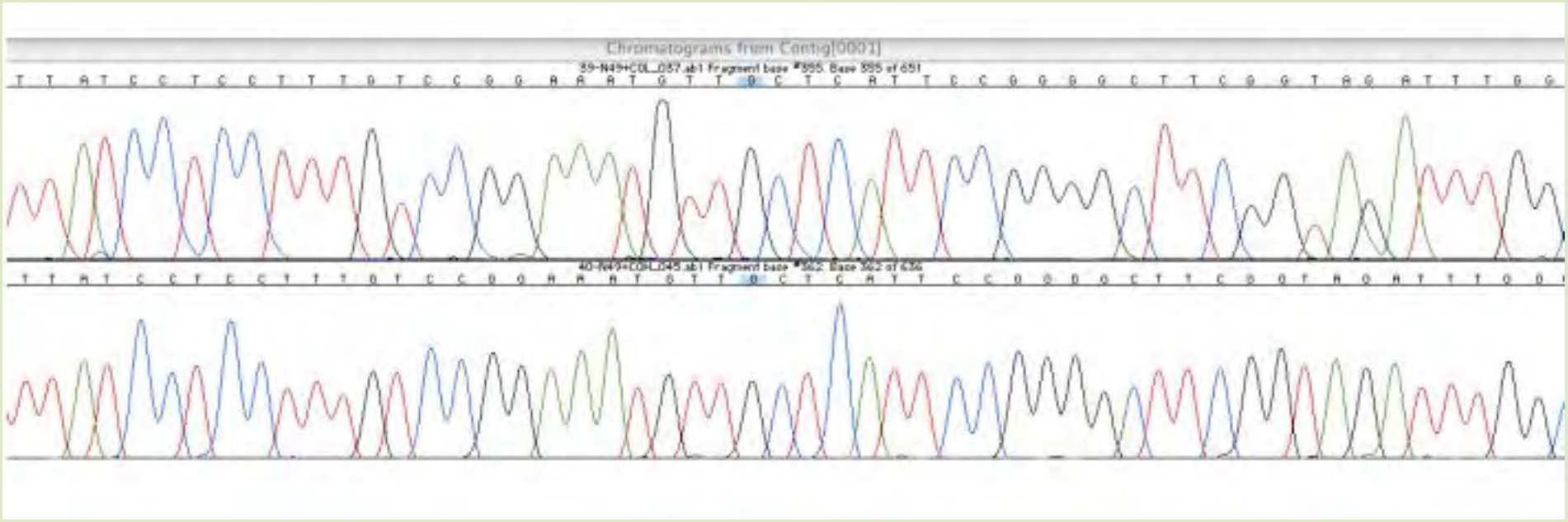
Plate 15. Superconglutinates of the orange-nacre mucket, *Hamiota perovalis*. (a–c) Early stages of release showing paired superconglutinates; mucus tube tethering superconglutinates to female is not visible. (d) Later stage of release showing single superconglutinate after disassociation; mucus tube is indicated by arrow. (a, b, Chris Barnhart, photos; c, d, William Roston, photos).

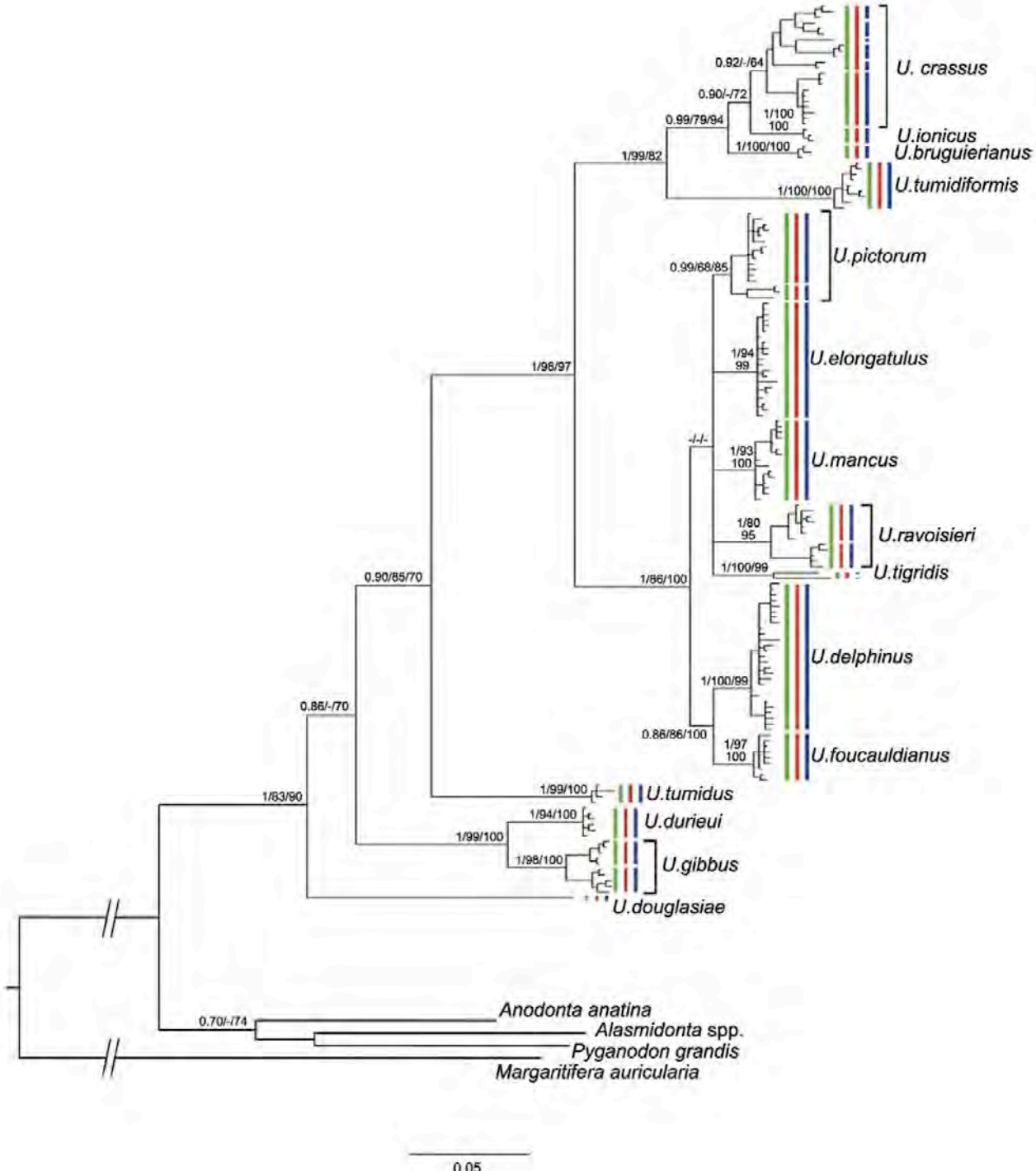
Molecular tools

Mitochondrial and nuclear DNA








0.92/-/64
0.90/-/72
0.99/79/94
1/100
100
1/100/100
U. crassus
U.ionicus
U.bruguierianus
1/99/82
1/100/100
U.tumidiformis
U.pictorum
0.99/68/85
1/98/97
1/94
99
U.elongatulus
-/-/-
1/93
100
U.mancus
1/80
95
U.ravoisieri
1/100/99
U.tigridis
0.90/85/70
1/86/100
U.delphinus
1/100/99
0.86/-/70
0.86/86/100
1/97
100
U.foucauldianus
1/99/100
U.tumidus
1/63/90
1/94/100
U.durieui
1/99/100
1/98/100
U.gibbus
U.douglasiae
Anodonta anatina
0.70/-/74
Alasmidonta spp.
Pyganodon grandis
Margaritifera auricularia
0.05

Margaritifera auricularia



Margaritifera margaritifera

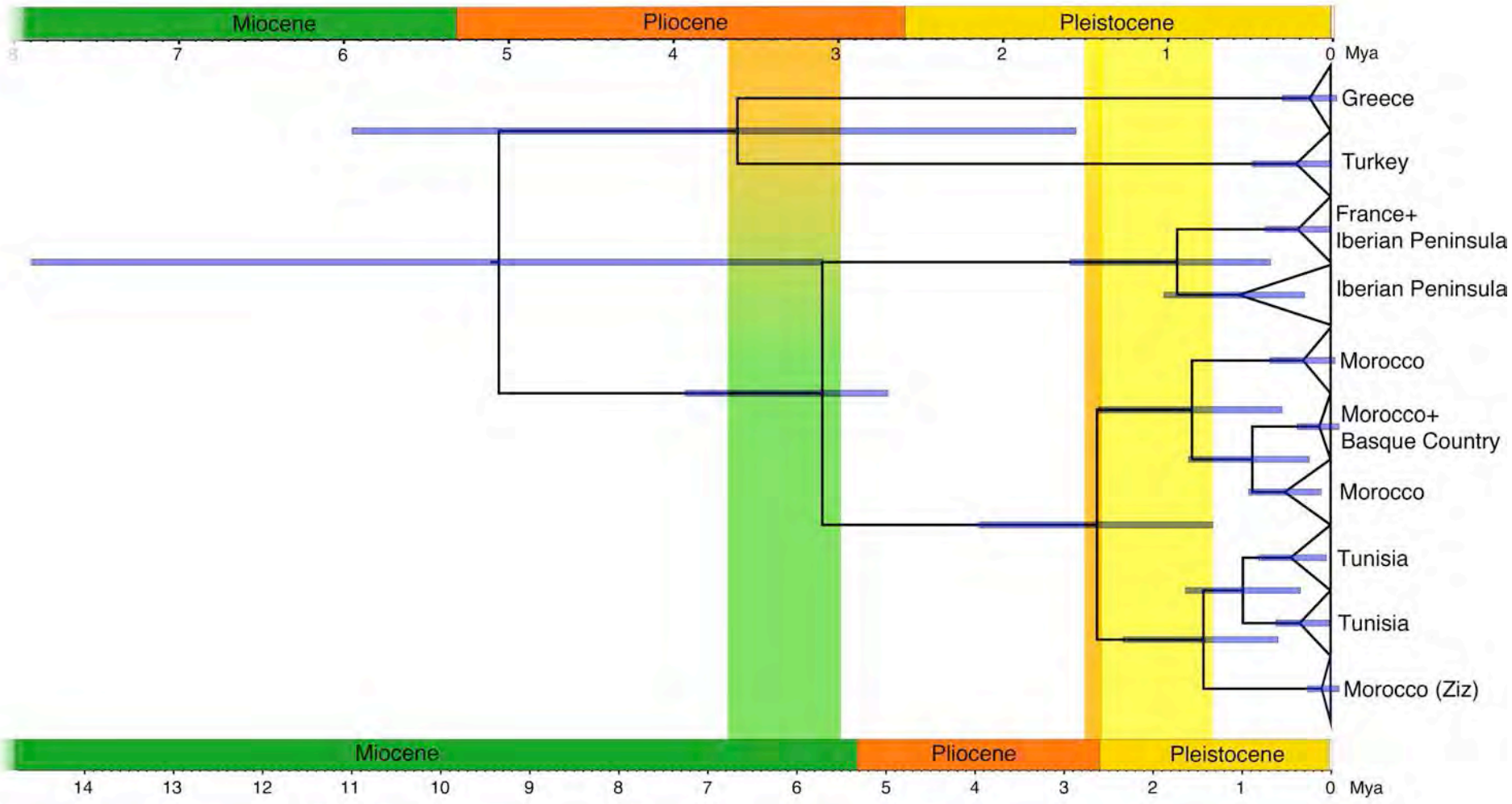




Margaritifera marocana

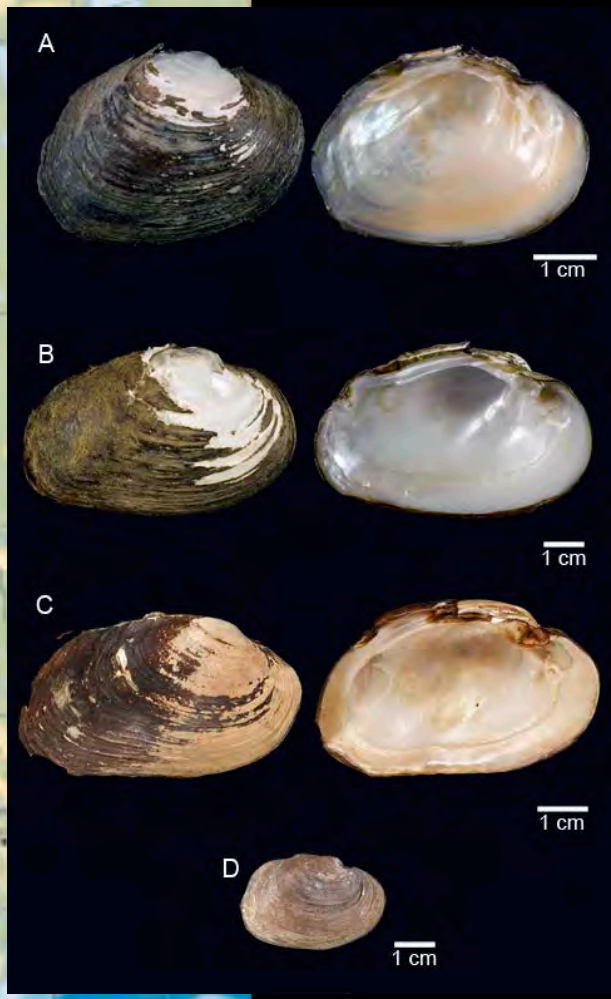


Time estimations of *Potomida* lineages



Araujo R, Buckley D, Nagel KO & Machordom A. 2017. *Potomida littoralis* (Bivalvia, Unionidae) evolutionary history. Slow evolution or recent speciation? *Zoological Journal of the Linnean Society* 179(2): 277-290. doi: 10.1111/zoj.12470

Potomida littoralis



Potomida acarnanica



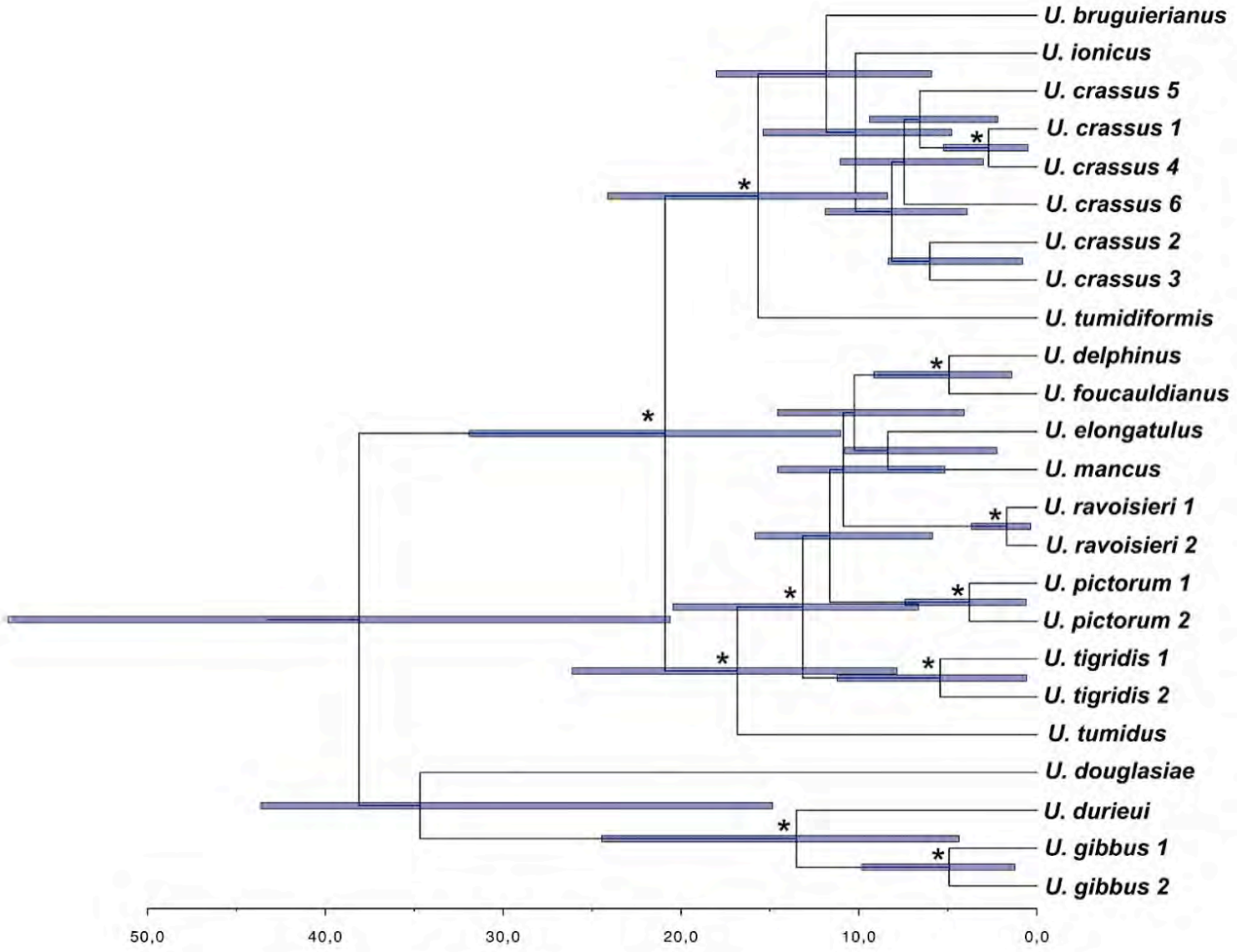
Potomida semirugata



Microcondylaea bonellii



Time estimations of *Unio* lineages

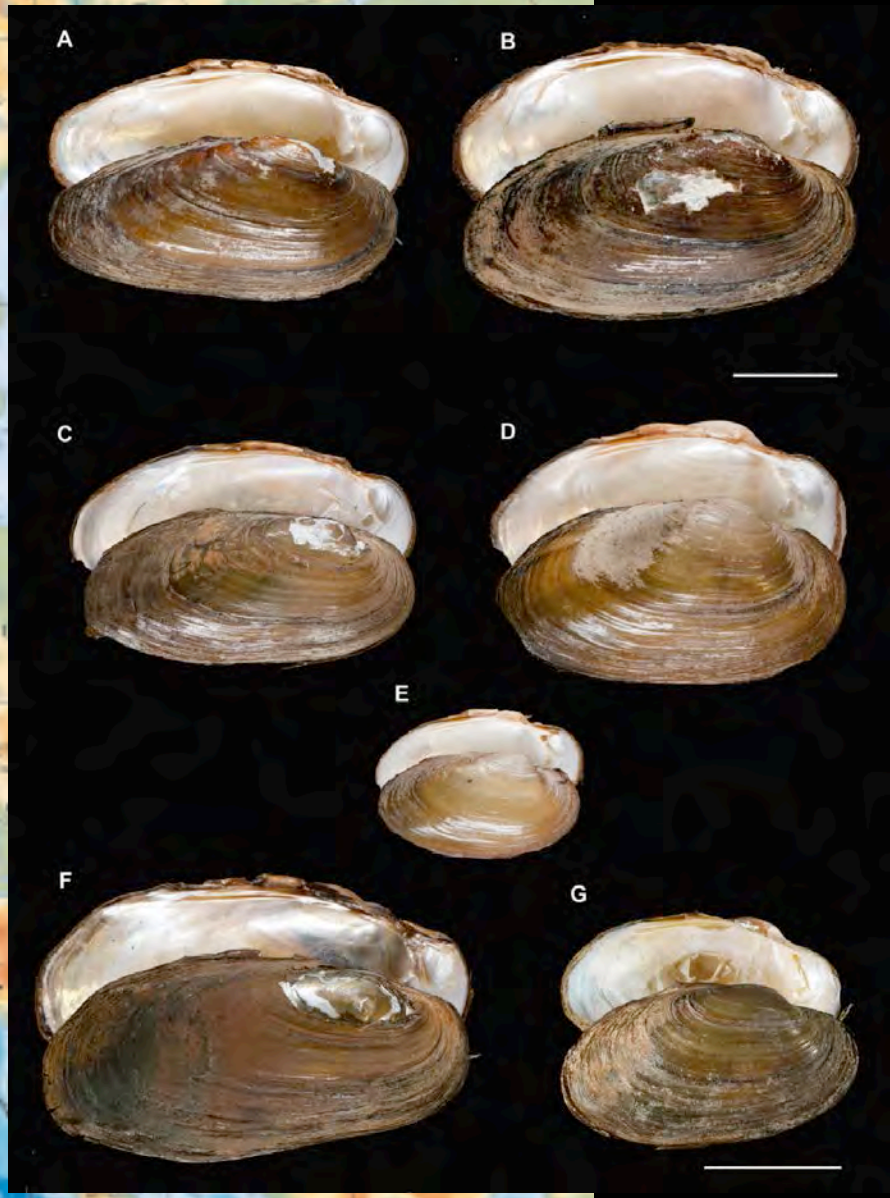


Araujo R, Buckley D, Nagel KO, García-Jiménez R & Machordom A. 2018. Species boundaries, geographic distribution and evolutionary history of the Western Palaeartic freshwater mussels *Unio* (Bivalvia, Unionidae). *Zoological Journal of the Linnean Society* 182: 275-299. doi.org/10.1093/zoolinnean/zlx039

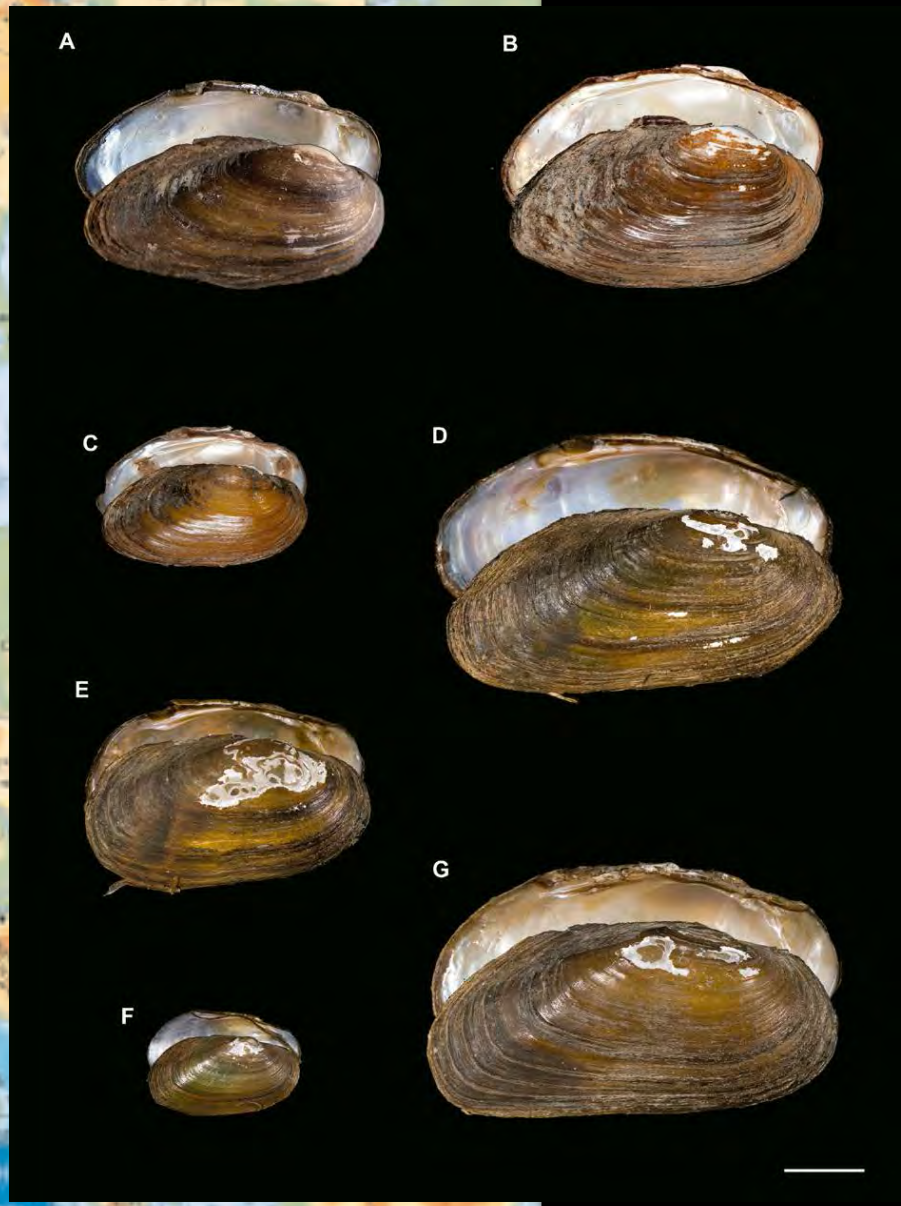
Unio delphinus



Unio foucauldianus



Unio mancus



Unio ravoisieri



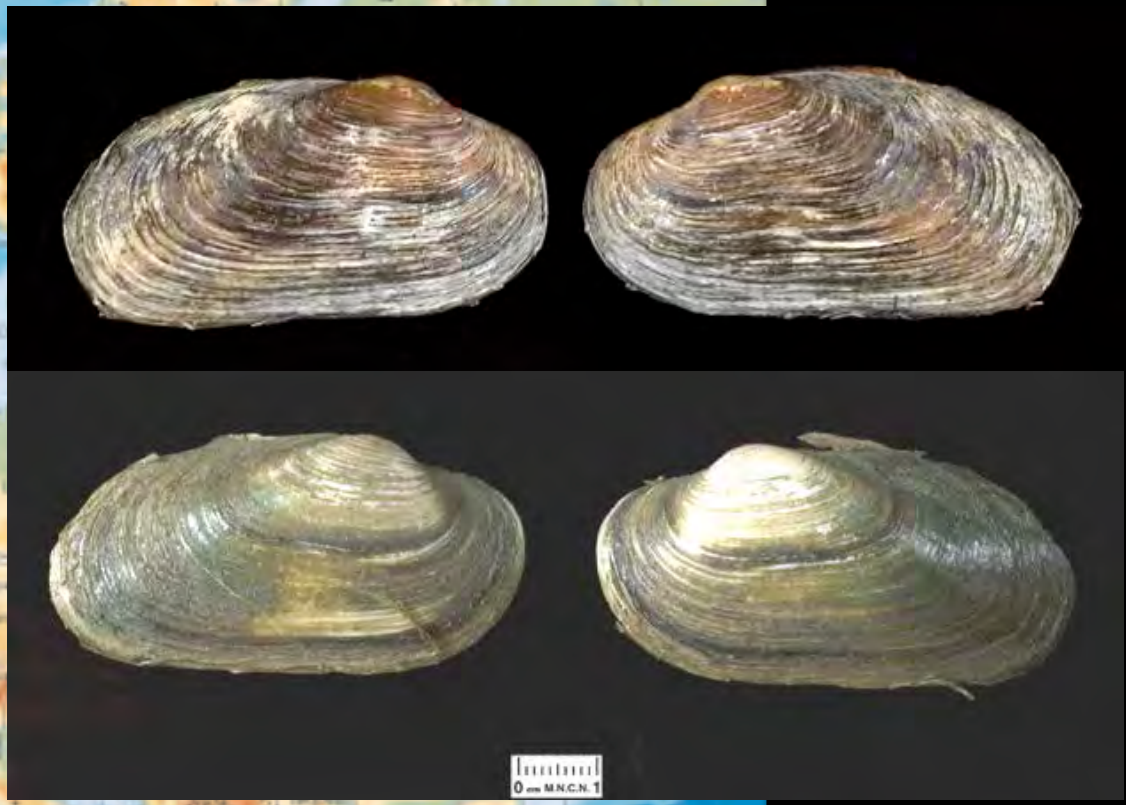
Unio gibbus



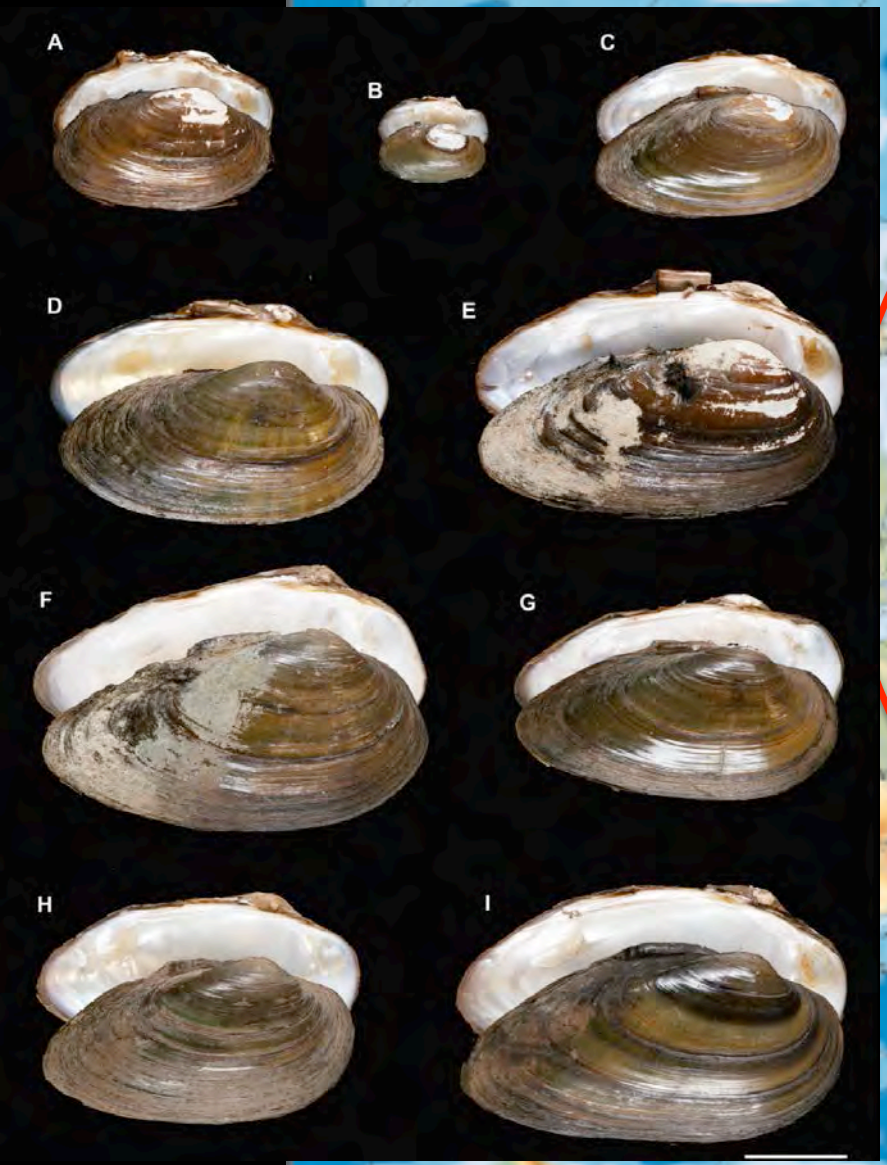
Unio tumidiformis



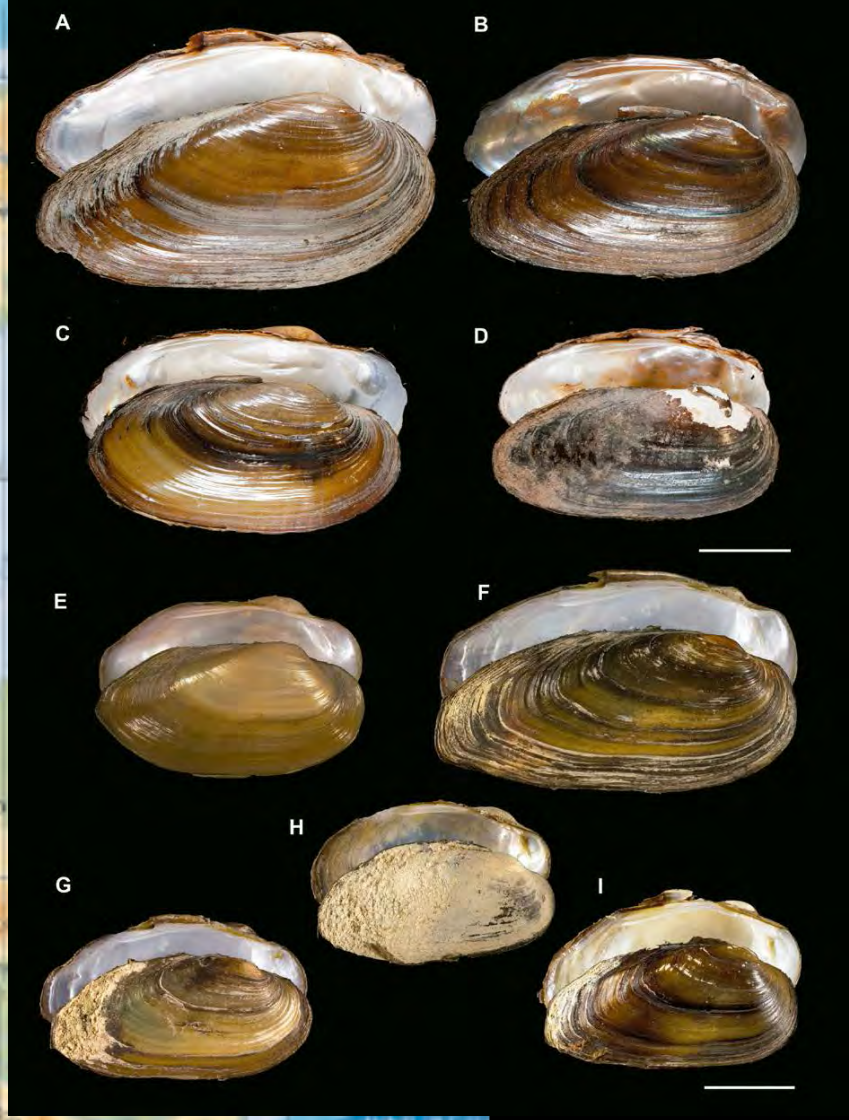
Unio durieui



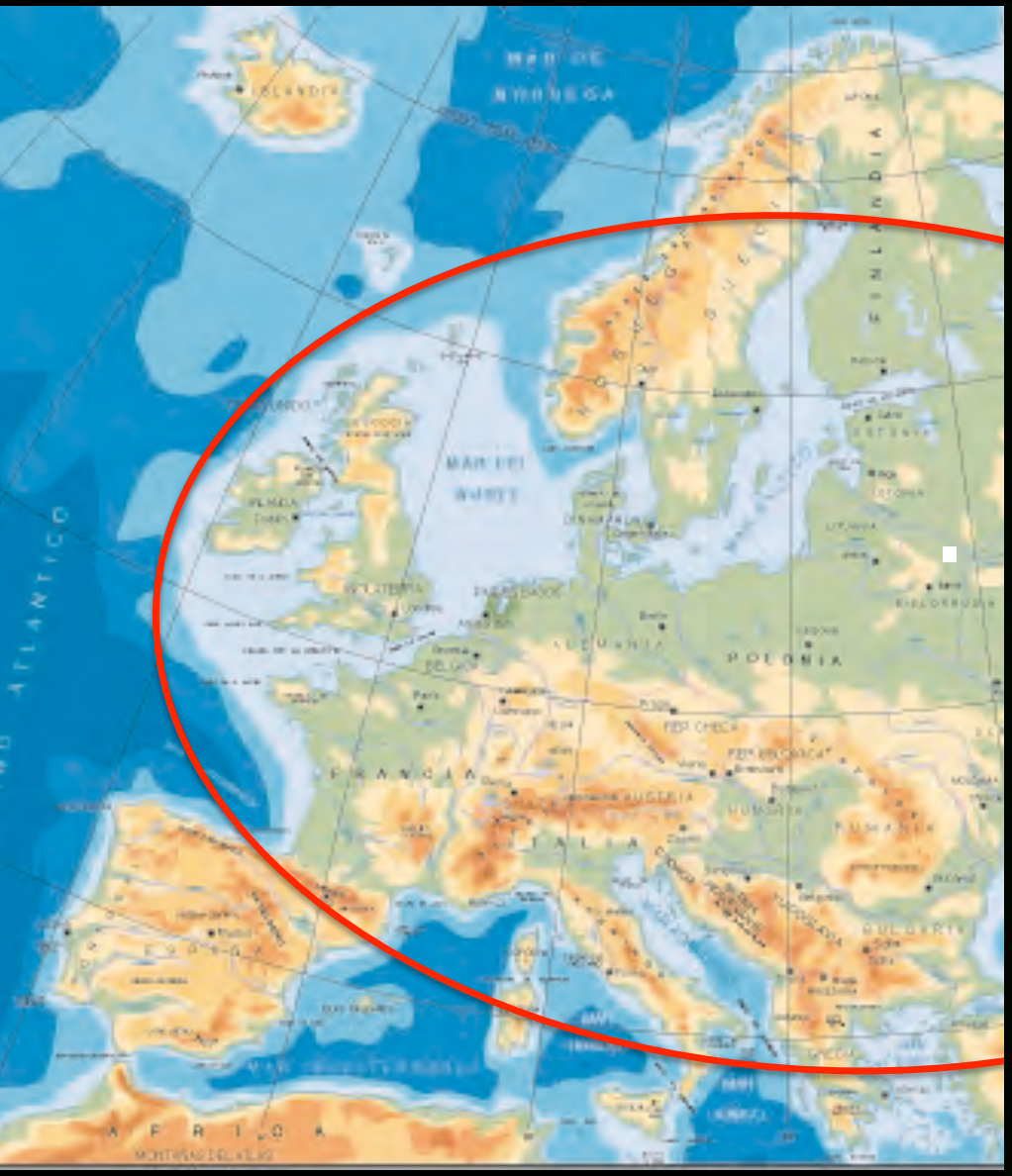
Unio tumidus



Unio elongatulus

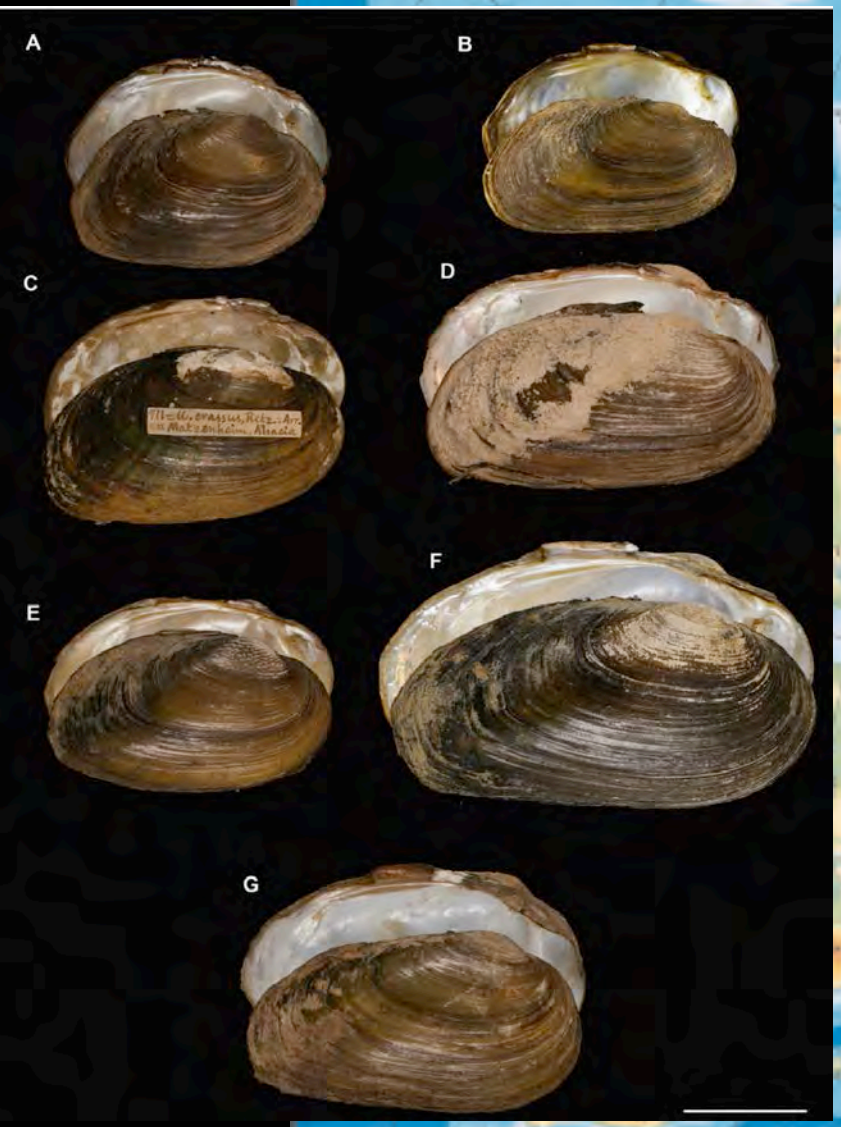


Unio pictorum

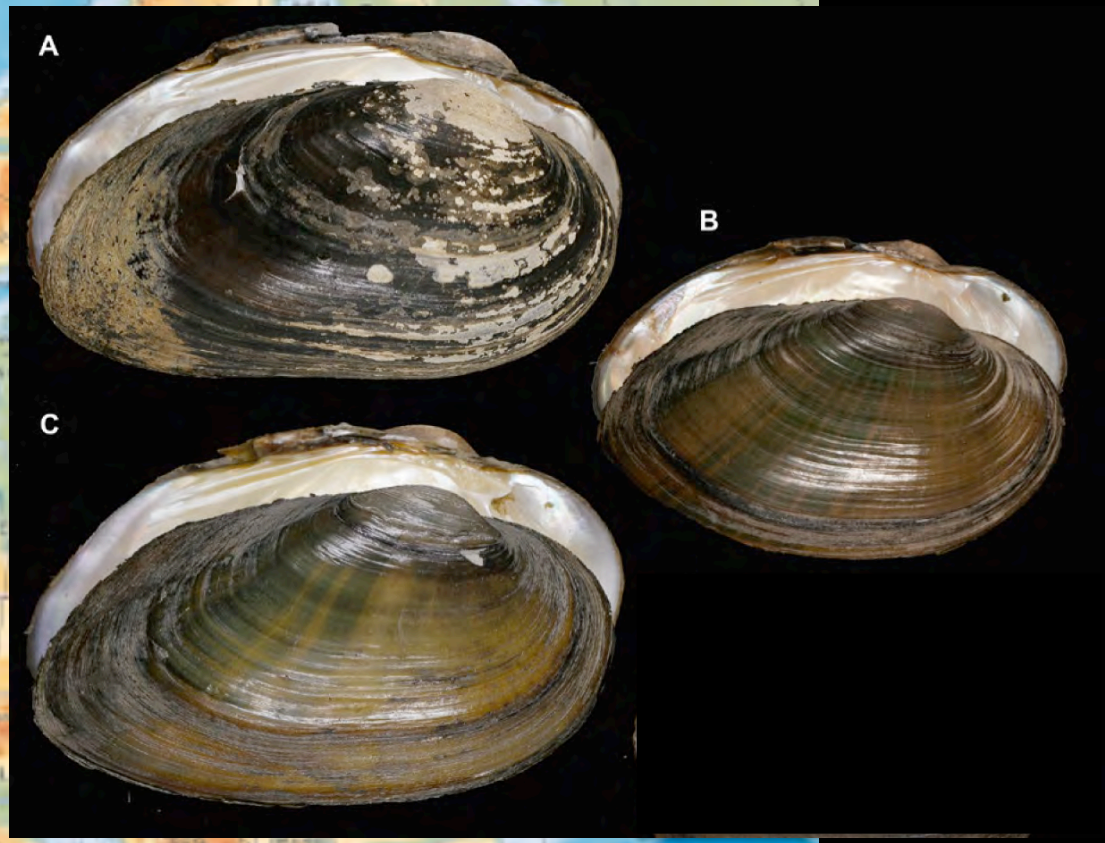




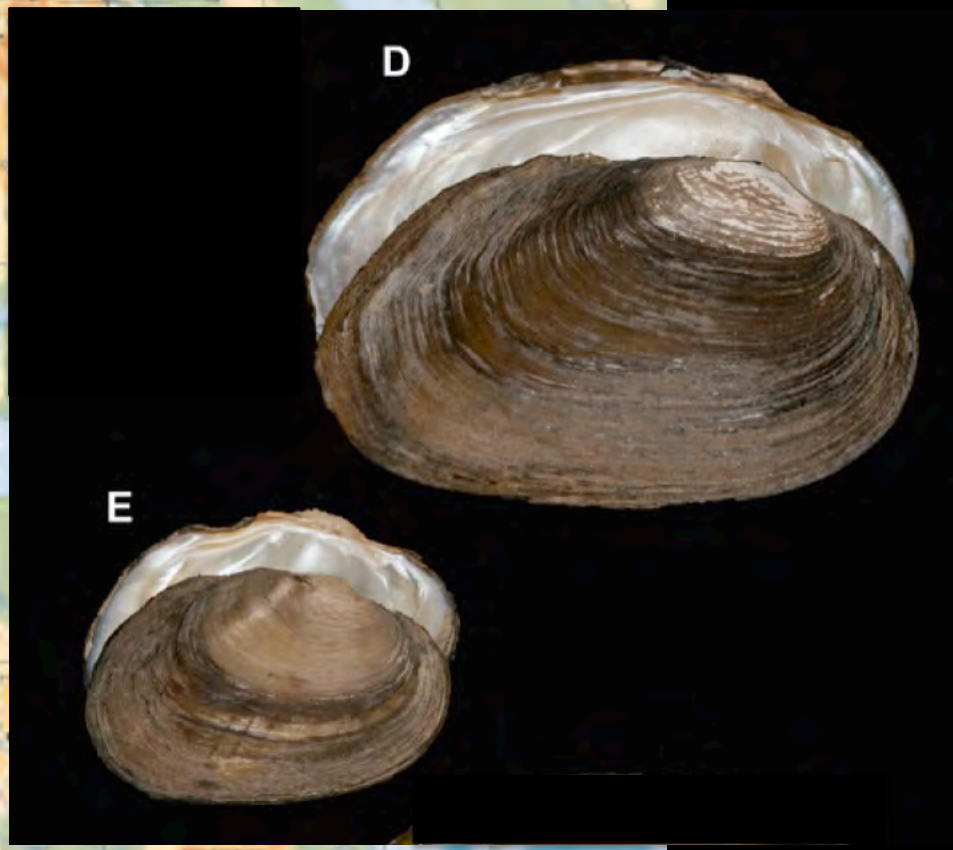
Unio crassus



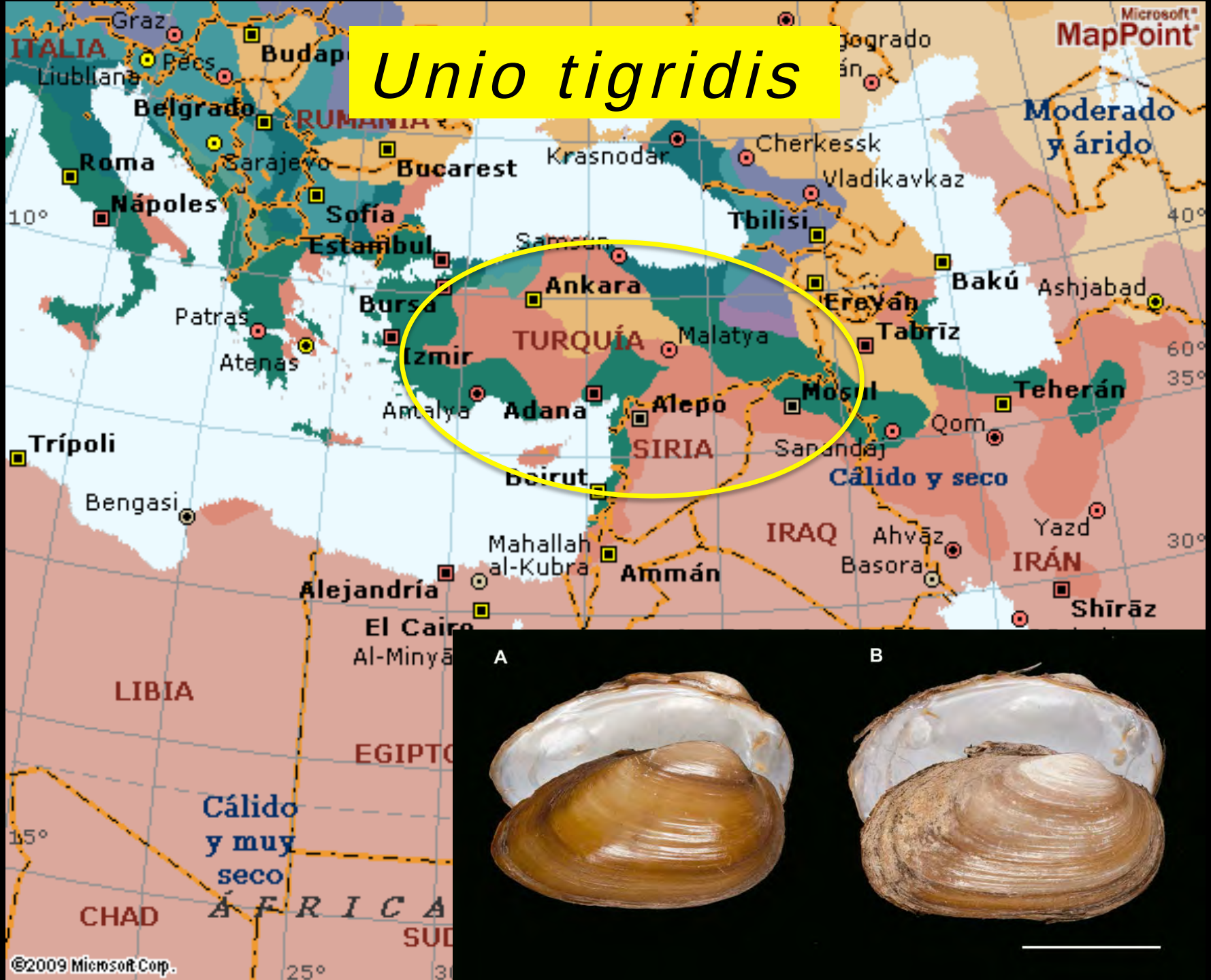
Unio bruguierianus



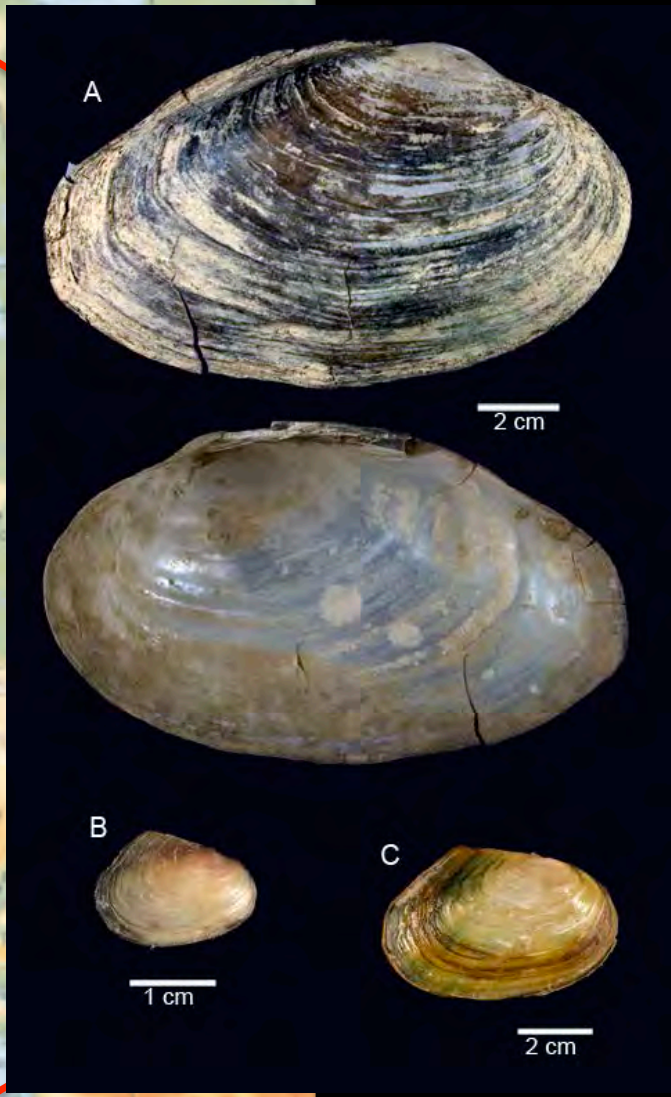
Unio ionicus



Unio tigridis



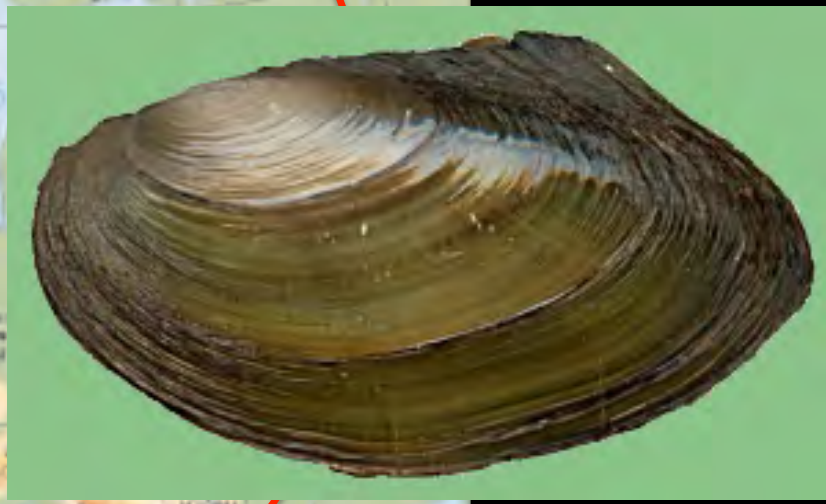
Anodonta anatina



Anodonta cygnea



Pseudanodonta complanata



Sinanodonta woodiana



Table 3.1. *Worldwide diversity of freshwater mussels (Order Unionoida)*

Region	Number of taxa
Nearctic	**302**
Mississippi River basin	133
Gulf Coast drainages	147
Atlantic Coast drainages	52
Peninsular Florida	14
Pacific Coast drainages	7
Neotropica	**172**
Mesoamerica	102
Amazon–Orinoco basins	42
Paranà–Paraguay basins	41
Afrotropica	**85**
Congo River basin	34
Nile River basin	26
Western Africa	22
Palearctica	**45**
Japan–Sakhalin	17
Amur–Beringia	14
Europe	11
Indotropica	**219**
Indochina	91
Yangtze–Huang basins	63
India–Burma	54
Australasia	**33**

Note: Major geographic regions (in bold) are followed by example subregions. Totals for subregions do not equal region totals due to overlap in species distributions among subregions and omission of some subregions (adapted from Graf and Cummings 2007).

24 ssp in Europe
and
North Africa

One of the most threatened animals groups



Main threats

Water detraction

Unsustainable urbanistic development

Habitat fragmentation

Fire and mining

Channeling of natural rivers

Polution and overexploitation of aquifers and rivers

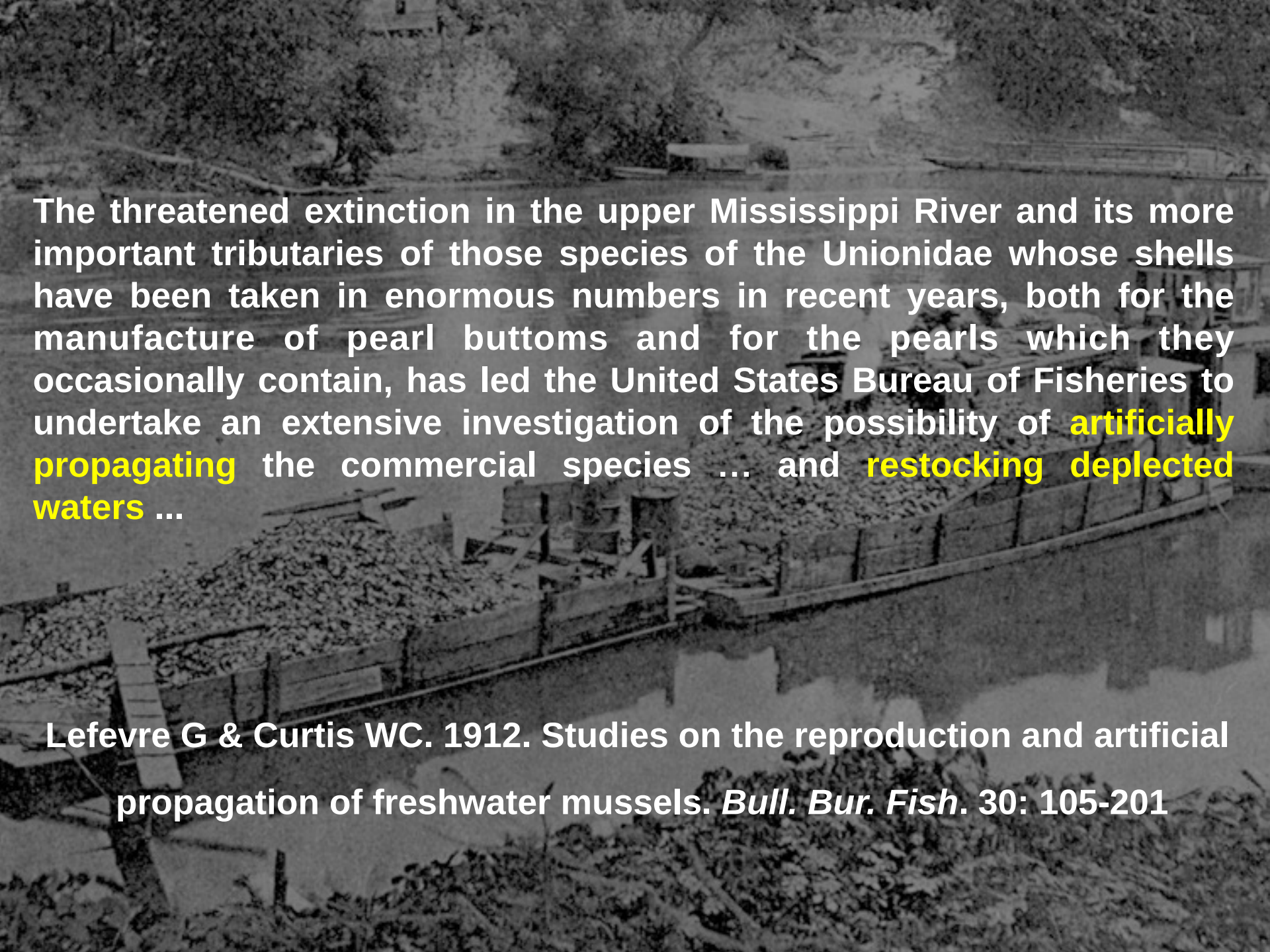
Extinction of native fish

Exotic and invasive species









The threatened extinction in the upper Mississippi River and its more important tributaries of those species of the Unionidae whose shells have been taken in enormous numbers in recent years, both for the manufacture of pearl buttoms and for the pearls which they occasionally contain, has led the United States Bureau of Fisheries to undertake an extensive investigation of the possibility of **artificially propagating** the commercial species … and **restocking deplected waters** ...

Lefevre G & Curtis WC. 1912. Studies on the reproduction and artificial propagation of freshwater mussels. *Bull. Bur. Fish*. 30: 105-201

LIFE Projects involving freshwater mussels 2015

Margaritifera margaritifera

LIFE13 NAT/FR/000506	FR
LIFE13 NAT/SE/000116	SE
LIFE11 NAT/UK/000383	UK
LIFE10 NAT/SE/000045	SE
LIFE09 NAT/ES/000514	ES
LIFE09 NAT/IE/000220	IE
LIFE10 NAT/PT/000073	PT

Margaritifera auricularia

LIFE13 BIO/FR/001162	FR

Unio ravoisieri and *U. mancus* (*elongatulus*)

LIFE12 NAT/ES/001091	ES

Unio crassus

LIFE13 NAT/PL/000009	PL
LIFE11 NAT/PL/000431	PL
LIFE10 NAT/AT/000015	AT
LIFE10 NAT/SE/000046	SE
LIFE11 NAT/LU/000857	LU
LIFE10 NAT/PT/000073	PT



CAPTIVE BREEDING

1. Fish maintenance (in vitro culture)

2. Source of glochidia (gravid naiads)

3. Fish infectation

4. Juvenile collecting

5. Juvenile rearing

6. Restocking in the wild

1. Fish maintenance

- Stocked previously to infectation (one month)

- Free of glochidia

- Free of diseases and parasites



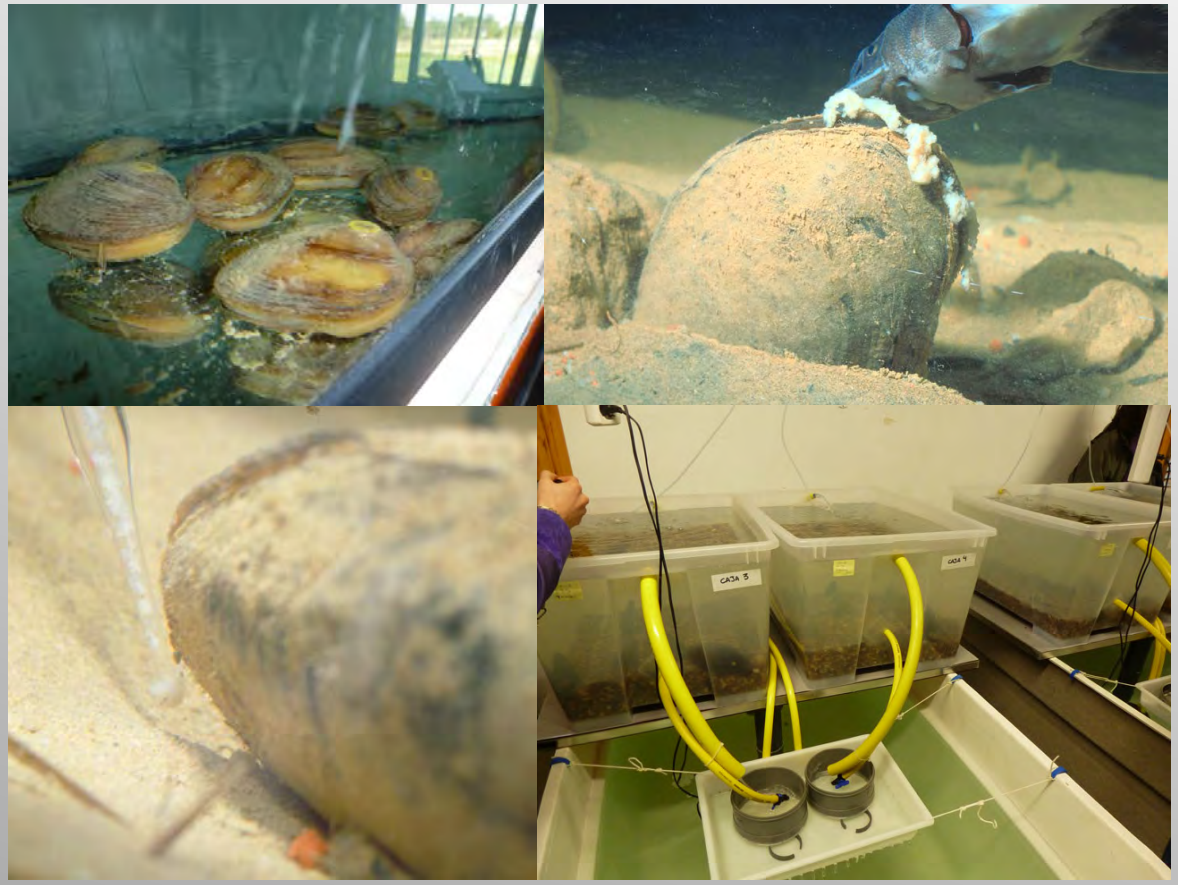








2. Source of the glochidia. Gravid naiads



- At the lab during reproductive season
- River water

3. Fish infectation

At the river banks

- releasing to the (restored) wild

At the lab

- raceways, tanks or aquaria

- controlled cages

- releasing to the (restored) wild



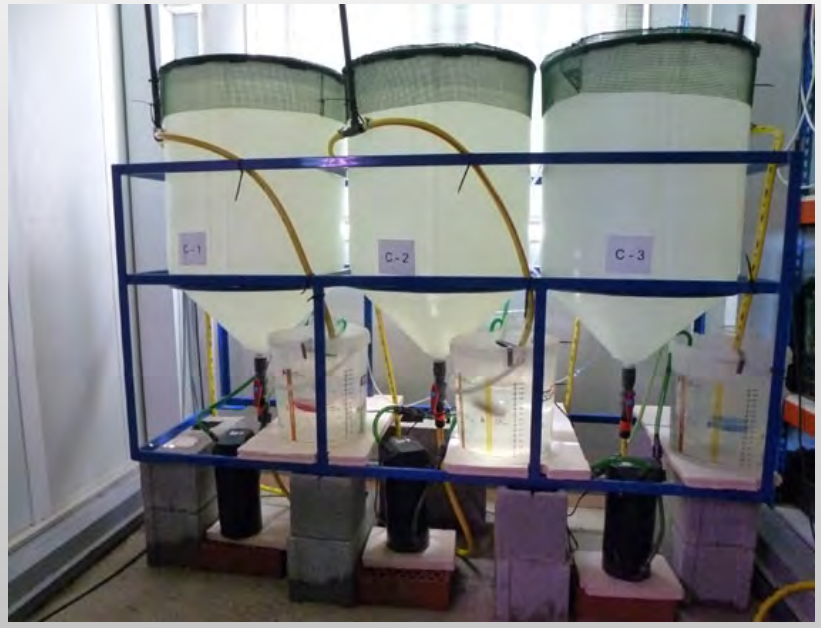




3. Fish infectation and metamorphosis

Facilities

- Natural channels
- Artificial channels
- Raceways
- Laboratory with aquaria and tanks









Degree/days is the relation between the temperature (number of degrees) and the number of days for the metamorphosis

***Unio ravoisieri*: 8-26 days at 17-23º**

***Unio mancus*: 7-26 days at 15-22º**

***Unio tumidiformis*: 6-14 days at 22-26º**

***Unio crassus*: 26-53 days at 12º**

***Margaritifera auricularia*: 30- 60 days at 16-24º**

***Margaritifera margaritifera*: 6-11 months**






4. Juvenile collecting




Degree/days

5. Juvenile rearing

Artificial

Seminatural

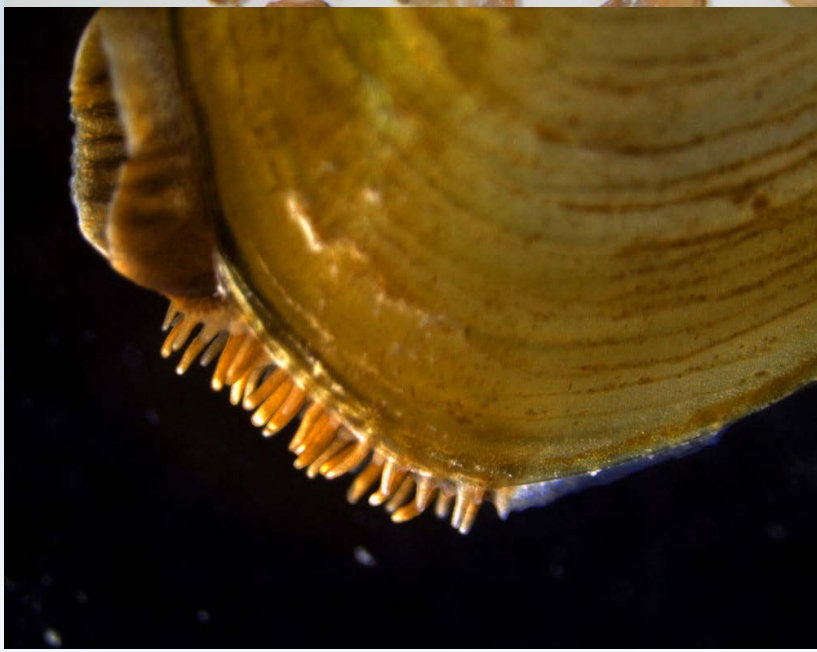
Natural

Diet !!!!

Water !!!

Sediment !





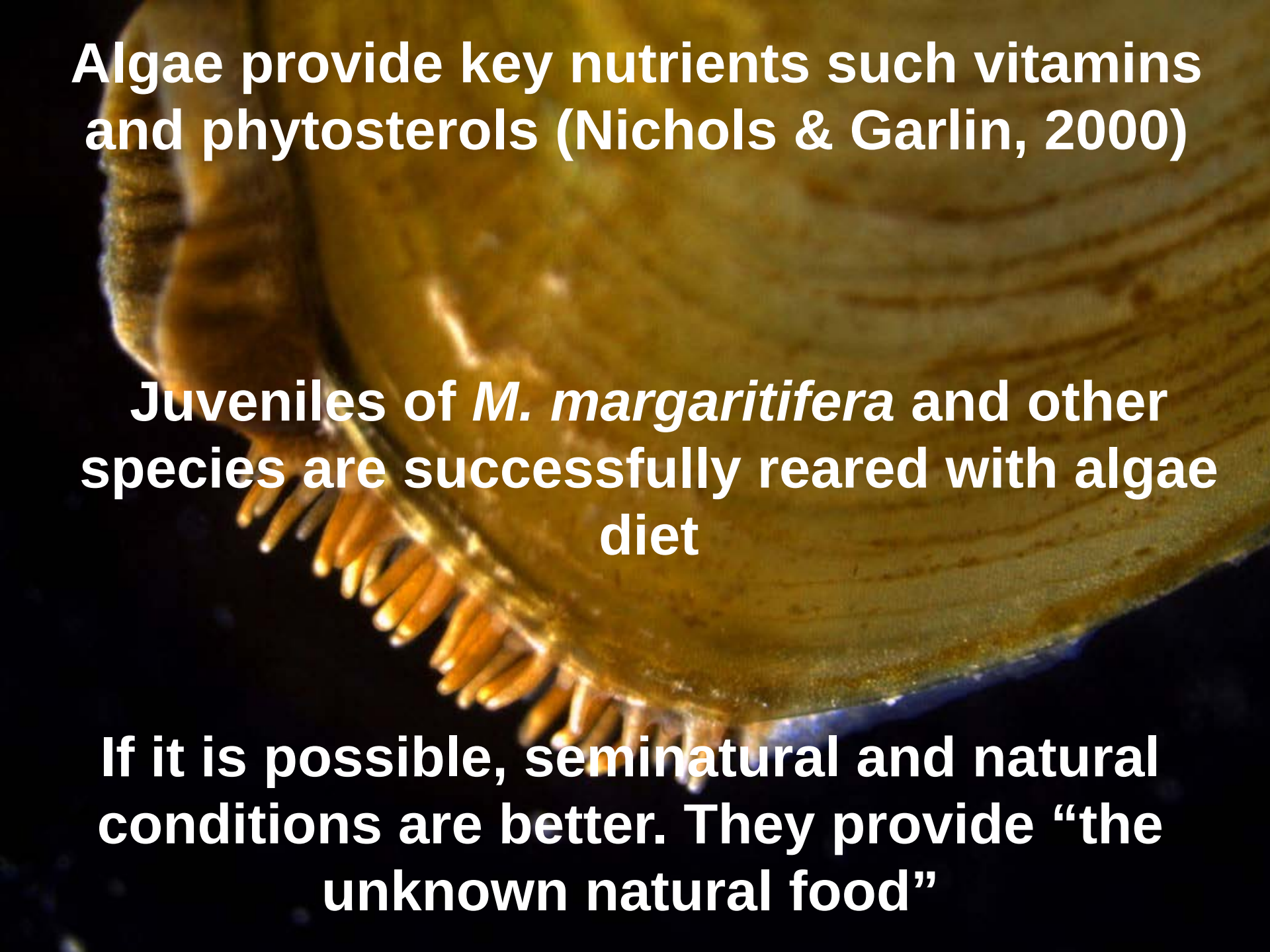
5. Juvenile rearing

Algae diet

All unionids from both the river and lake used bacterial carbons, not algal carbons, as their main dietary source.

Bacteria, not algae, were the main dietary item for unionids.

Nichols SJ & Garling D. 2000. Food-web dynamics and trophic-level interactions in a multispecies comunity of freshwater unionids. *Canadian Journal of Zoology* 78: 871-882



Algae provide key nutrients such vitamins and phytosterols (Nichols & Garlin, 2000)

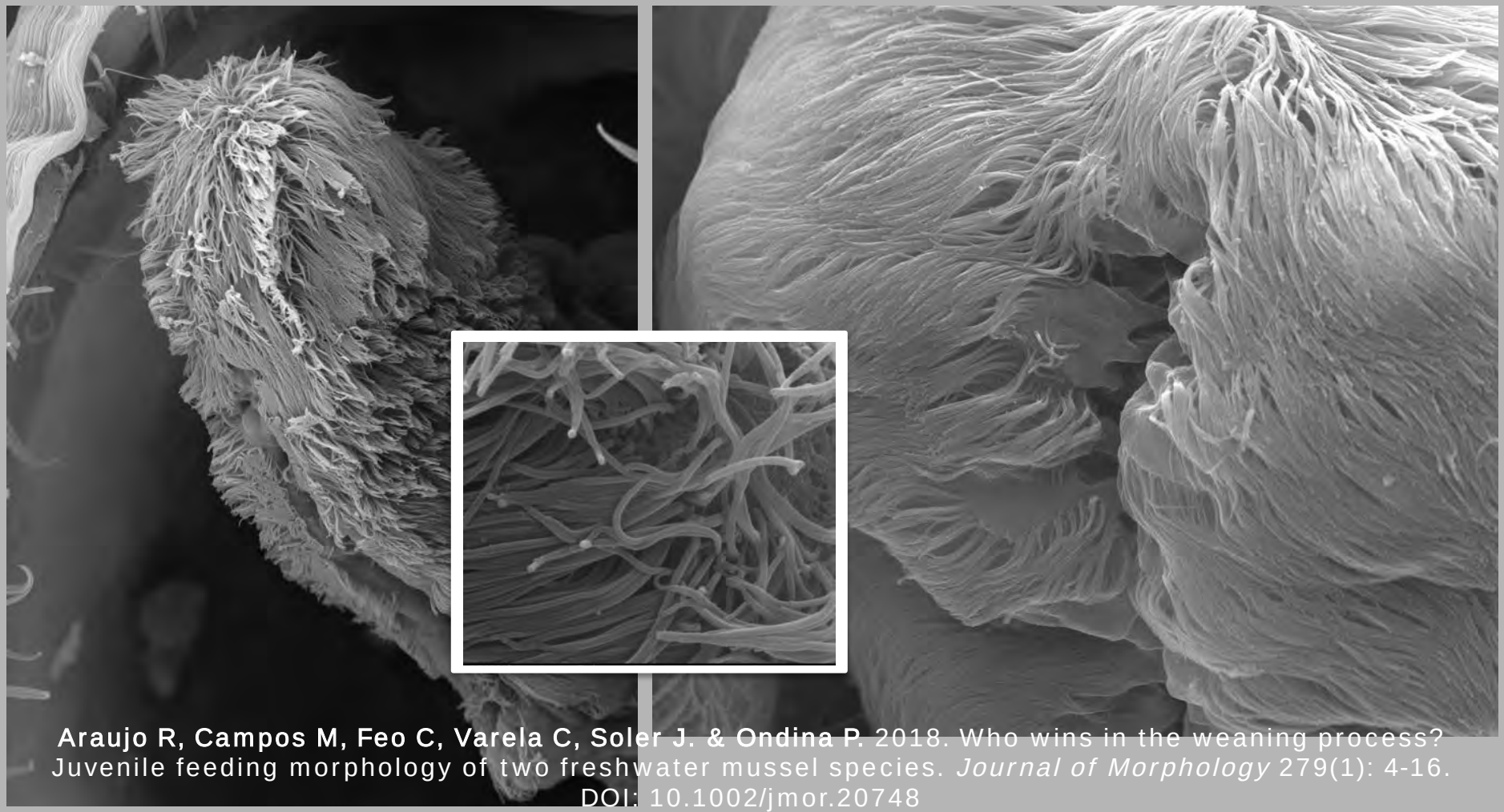
Juveniles of *M. margaritifera* and other species are successfully reared with algae diet

If it is possible, seminatural and natural conditions are better. They provide “the unknown natural food”

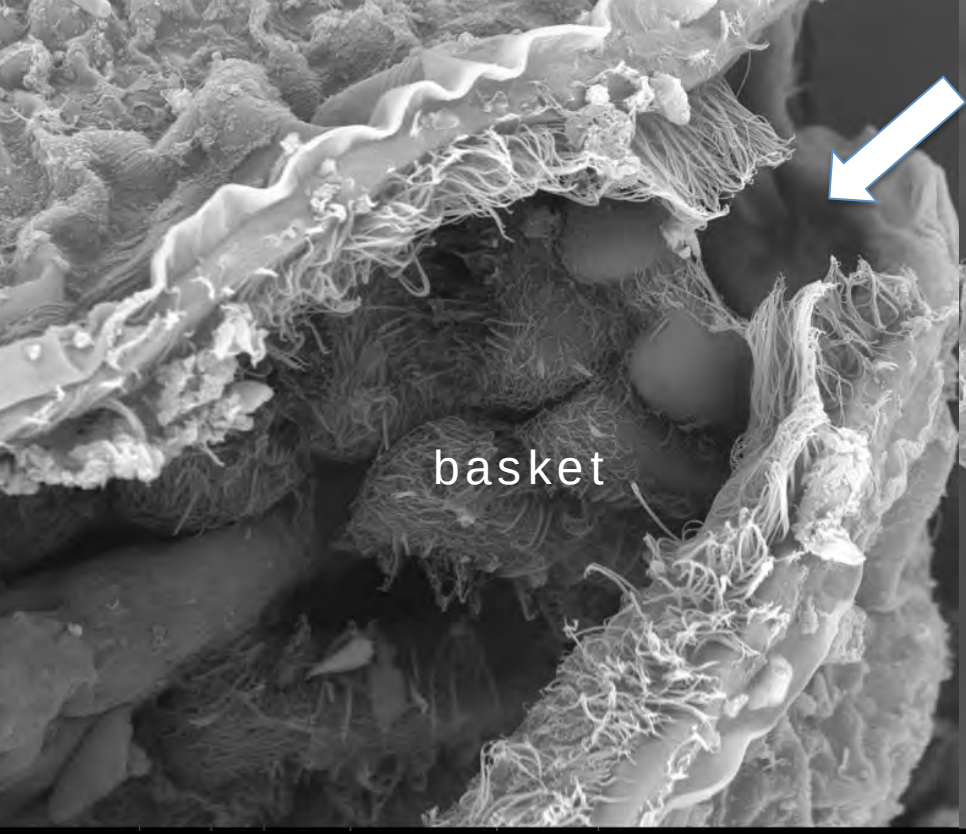
Newly emerged juveniles of both *M. margaritifera* and *Unio mancus* first feed by pedal feeding using the cilia of the foot and of the mantle margin

M. margaritifera 0 days

U. mancus 0 days



Araujo R, Campos M, Feo C, Varela C, Soler J. & Ondina P. 2018. Who wins in the weaning process? Juvenile feeding morphology of two freshwater mussel species. *Journal of Morphology* 279(1): 4-16. DOI: 10.1002/jmor.20748





Initiation of suspension feeding begins when the cilia of the posterior parts of the inner gill filaments join to form a virtual hole for water current.

The gill filaments build a basket with a dorsal and a ventral chamber with a ciliated posterior opening. This opening allows water to enter, facilitated by the mantle and gill cilia, similar to the ulterior inhalant aperture in the adult.

Days of appearance of feeding structures in juvenile freshwater mussels

	Inner gill cirri	Basket	Inner gill bent	Ventral groove	Labial palps	Outer gill	Inhalant papillae
M. margaritifera[1]	0	10	130	180	180	240	300
M. margaritifera[2]	-	-	1.1-2.2 mm*	2.2-4.5 mm*	-	4.5 mm*	-
U. mancus[1]	0	10	40-60	60	70	180	300
V. iris[6]	-	14	-	-	-	-	after 272
L. ventricosa[7]	0	-	after 56	-	2	-	-
U. imbecillis[8]	0	7-28	113	-	3	130	175
H. myersiana[9]	0	10	30	after 50	-	90	50

Starting of the filter feeding

Definitive filter feeding

Hyriopsis myersiana is the fastest growing species, reaching 1.8 mm in 60 days, 24 mm in 150 days and 85 mm in one year. The feeding organs of this species also develop more rapidly, having well-developed gills and siphons by 50 days post-emergence

For the other Unionidae species mentioned, these organs develop at similar times

In the case of *M. margaritifera*, these organs develop much more slowly

Early juvenile mortality can be due to the inability to successfully transition feeding modes

The transition from pedal feeding to filter feeding occurs around 150-200 days post-emergence in *M. margaritifera* and around 70 days in *U. mancus*, after juveniles are greater than 1 mm in length, which coincides with the timing of reported high mortality

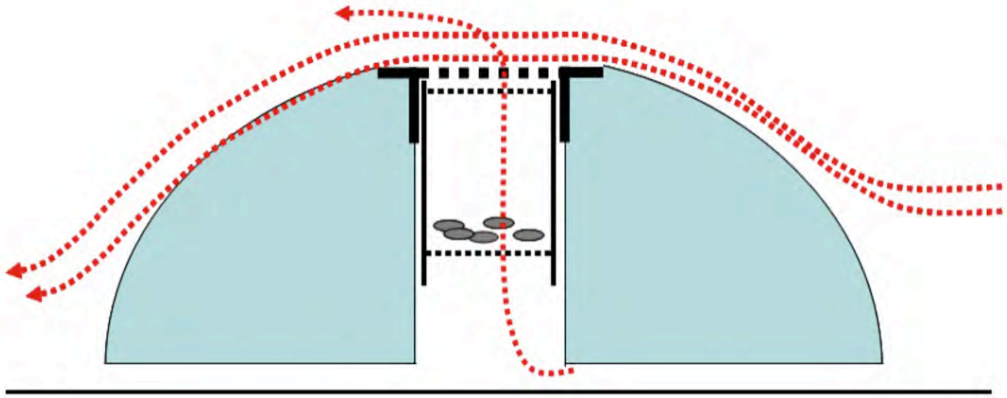
Once this feeding metamorphosis is complete, juvenile mortality decreases

5. Seminatural and natural rearing









5. Seminatural and natural juvenile rearing. Facilities









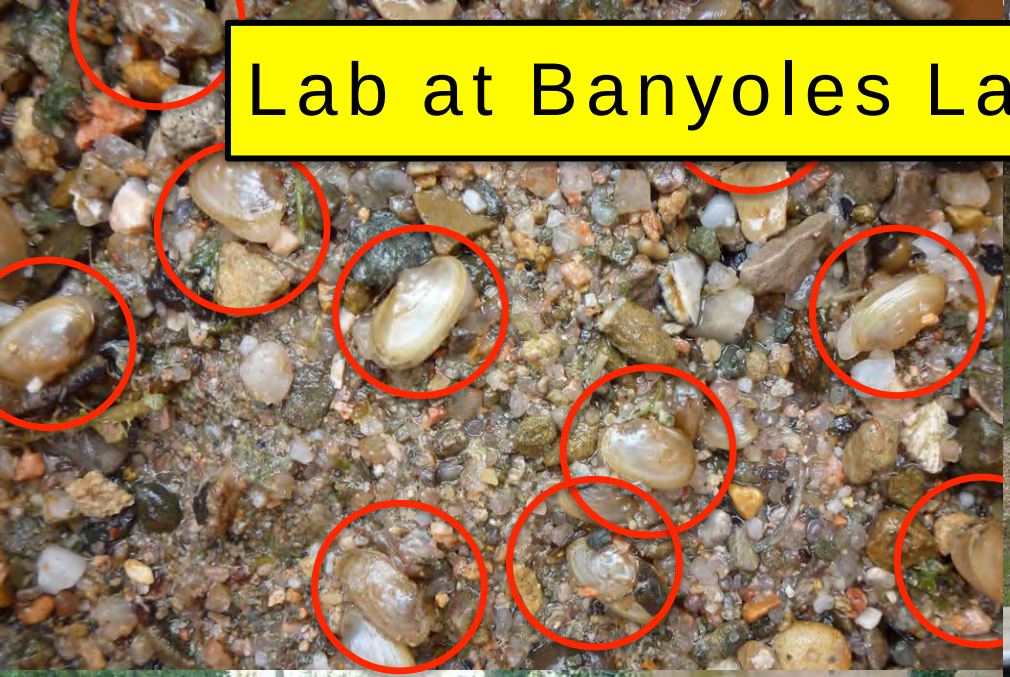








Lab at Banyoles Lake (Girona, Spain)









Juveniles of *Unio mancus* and *Unio ravoisieri* in pools and trays

Natural water and sediment

Table 2. Number of juveniles obtained from host fish, and numbers seeded in the different systems. Uma = *Unio mancus*. Ura = *U. ravoisieri*.

	2011		2012		2013		Total	
	Uma	Ura	Uma	Ura	Uma	Ura	Uma	Ura
Aquaria	329		489				**818**	**0**
Indoor plastic tray	7464	1592	15 447	875			**22 911**	**2467**
Outdoor pools	~3000	~1800	28 831	9719	36 140	4318	**67 971**	**15 837**
Outdoor channels					7766	9119	**7766**	**9119**
Tuppers			2653		601		**3254**	**0**
Lake			517				**517**	**0**
Lake streams and channels			5638				5638	**0**
TOTAL	**10 793**	**3392**	**53 575**	**10 594**	**44 507**	**13 437**	**108 875**	**27 423**



Araujo R, Feo C, Pou Q & Campos M. 2015. Conservation of two endangered European freshwater mussels. A three year semi-natural breeding experiment. *The Nautilus* 129(3): 126-135.

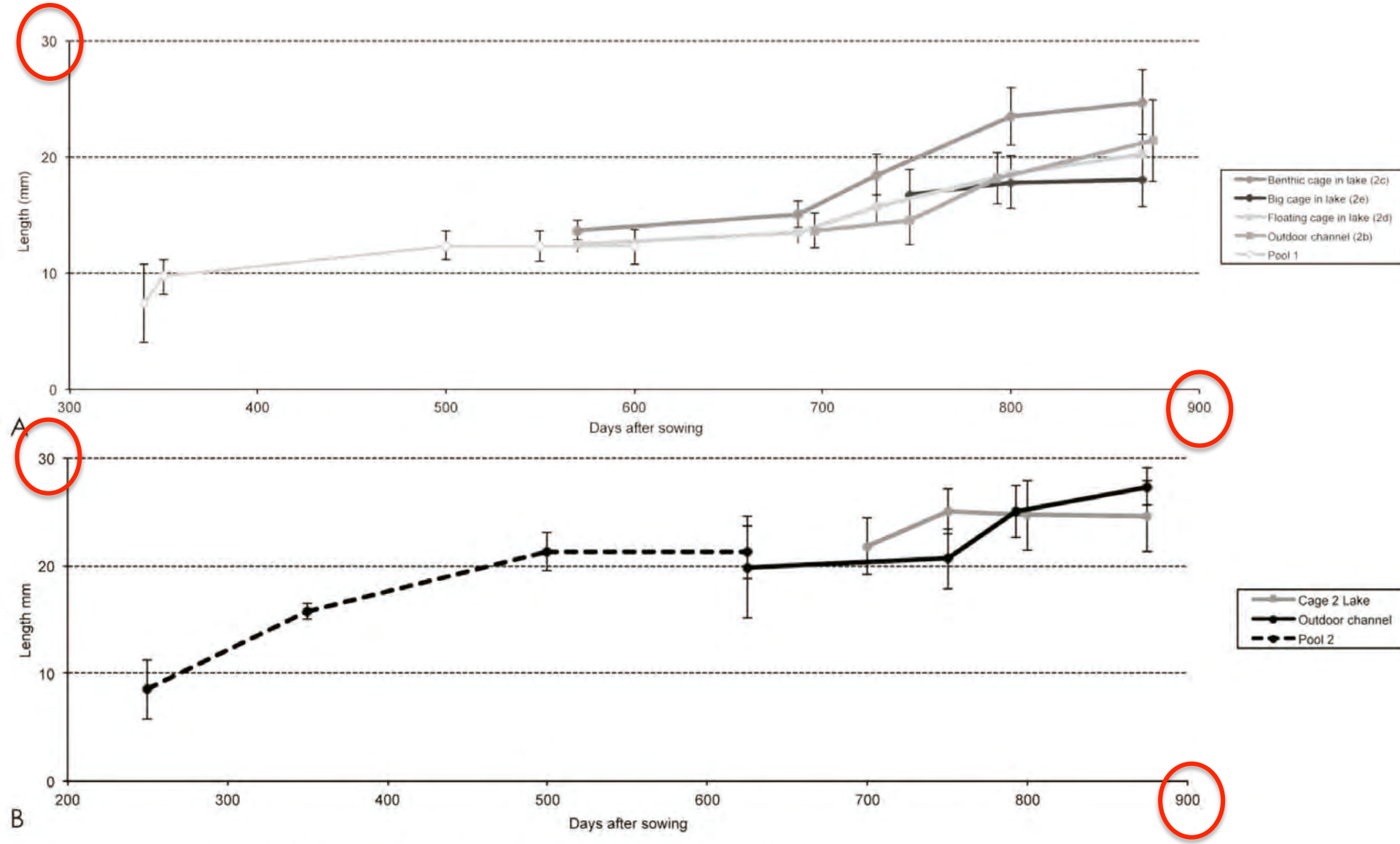


Figure 4. Juvenile growth in the different systems. A. *Unio mancus*. B. *U. ravoisieri*.

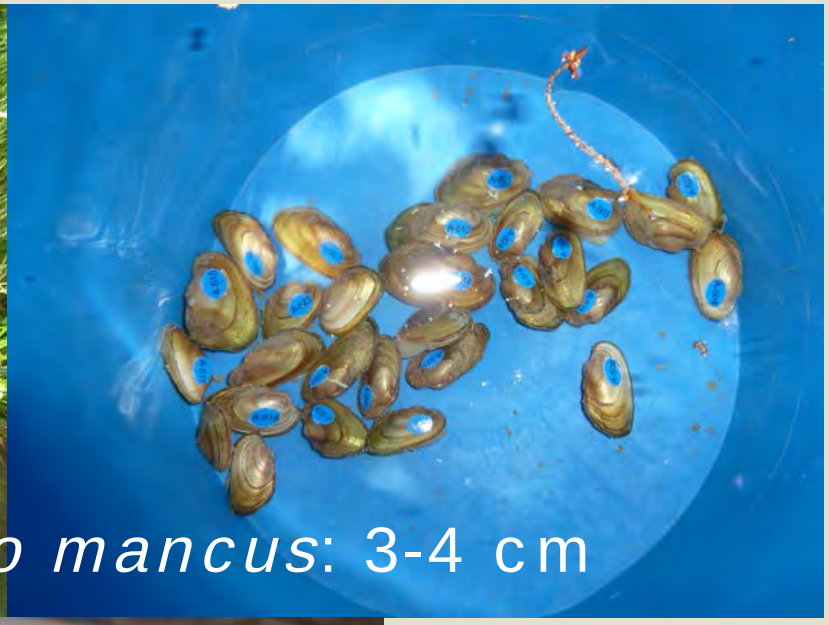




Survival 50-60 %

6. Minimum size for juvenile mussel release





In the case of *Unio mancus*: 3-4 cm



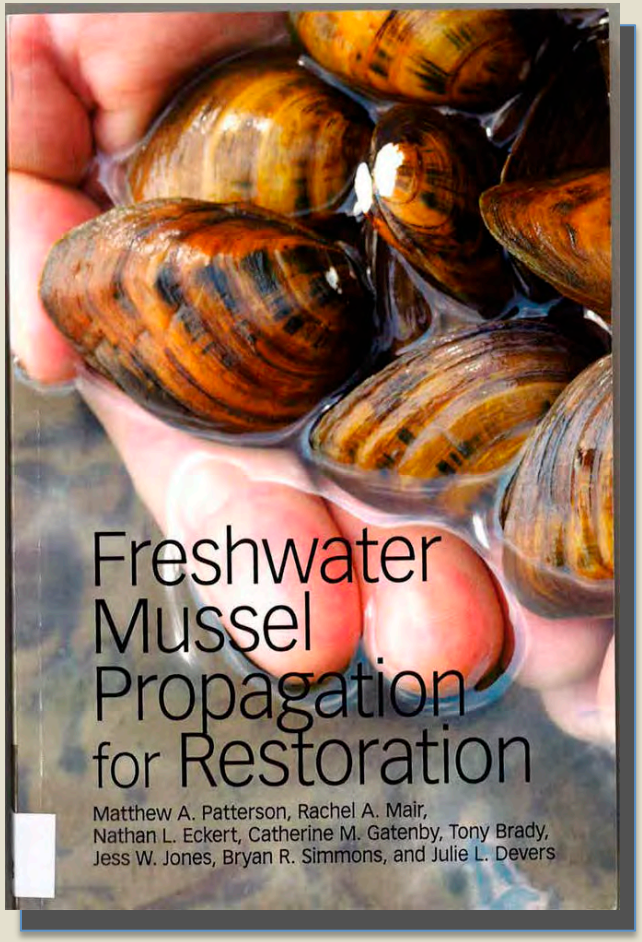
6. Juvenile mussel release and monitoring

1. Patterson MA, Mair RA, Eckert NL, Gatenby CM, Brady T, Jones JW, Simmons BR & Devers J. 2018. Eds. *Freshwater mussel propagation for restoration*. Cambridge University Press, Cambridge. 320 pp.

2. Hanshue S, Rathbun J, Badra P, Bettaso J, Hosler B, Pruden J & Grabarkiewicz J. 2018. Michigan Freshwater Mussel Survey Protocols and Relocation Procedures.

Essential premise

Any plan to propagate and release juvenile freshwater mussels
must include a plan for long-term monitoring



6. Juvenile mussel release and monitoring

Release juvenile freshwater mussels to the wild is the most important step in the entire propagation mussel

(Simmons, Patterson & Jones, 2018)

The general goals of mussel relocation efforts

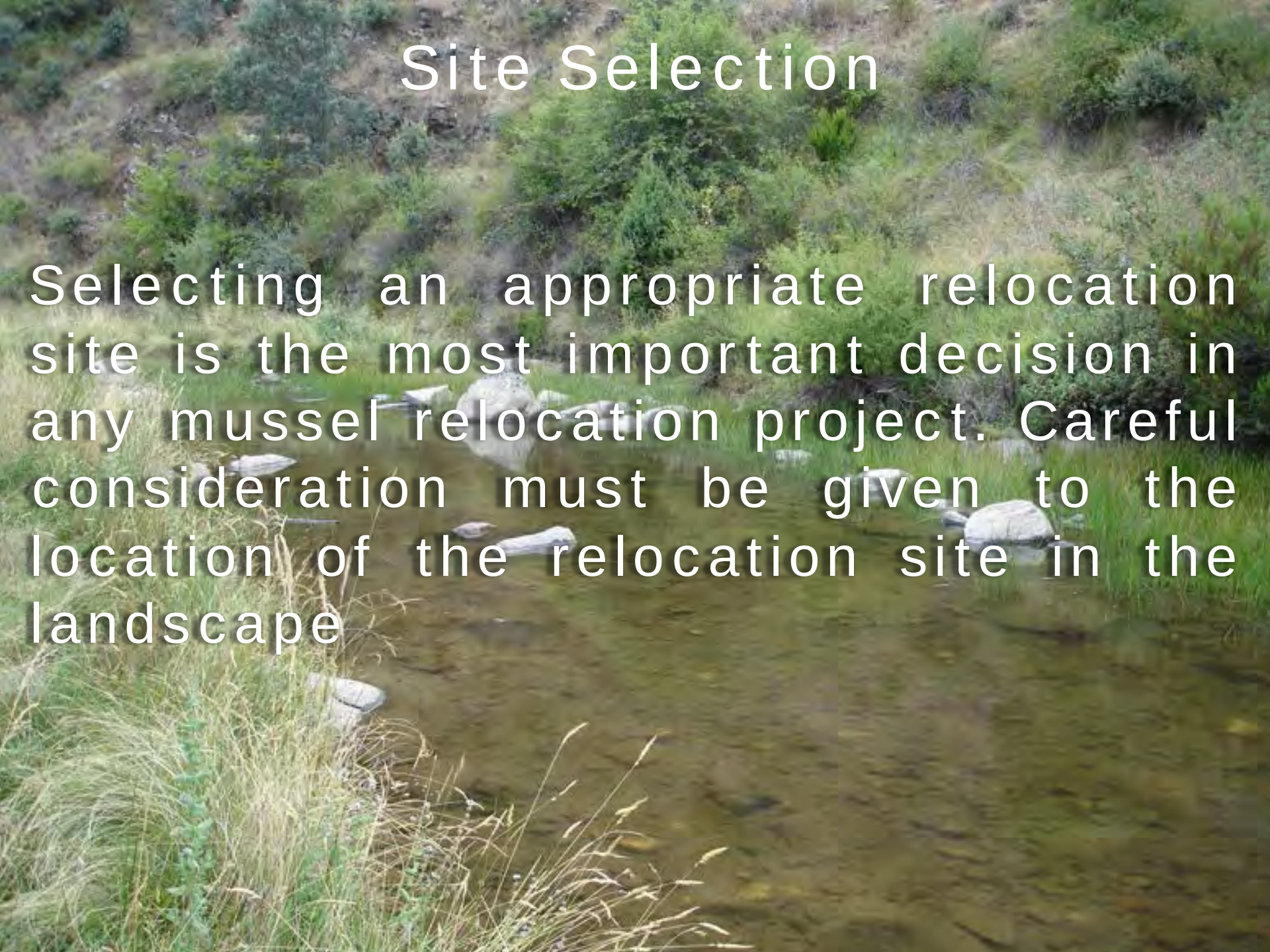
- Maximize survival and fitness of the relocated individuals, including genetic diversity

- Minimize risk to the resident mussel fauna at the relocation site. Invasive species!!!

- Document relocation outcomes to inform future relocation efforts

Site Selection

Selecting an appropriate relocation site is the most important decision in any mussel relocation project. Careful consideration must be given to the location of the relocation site in the landscape



A hierarchy of preferred destinations (modified from the U. S. Fish and Wildlife Service, 2008)

1. Within the same watershed (same of different reach). Select more than one relocation site

2. Habitat conditions should be as similar as possible to the project site (sediment composition and stability, water quality, water depth, flow regime, distribution of habitat)

3. Presence of a diverse mussel community reflective of the community from which the individual was moved and that has evidence of recruitment (survey performed at least 5 years before)

4. The relocation site survey may need to be repeated if an event or impact (e.g. a chemical spill) has occurred during the time between the original survey and the proposed relocation

5. Appropriate fish host species must be present

6. Juvenile release

1. Tagging (glue, pit tags, dremel,....)

- To differentiate between propagated mussels and natural recruitment

- Tagged mussels are models for ecological, evolutionary and toxicological studies





2. Transport

3. Release techniques. Record information and inform the public

4. Include long term monitoring. Select key areas. Schedule: prior to release juveniles, 3 months, 1-2 years and 4-6 years post release



Good luck with your research and thank you very much for your attention