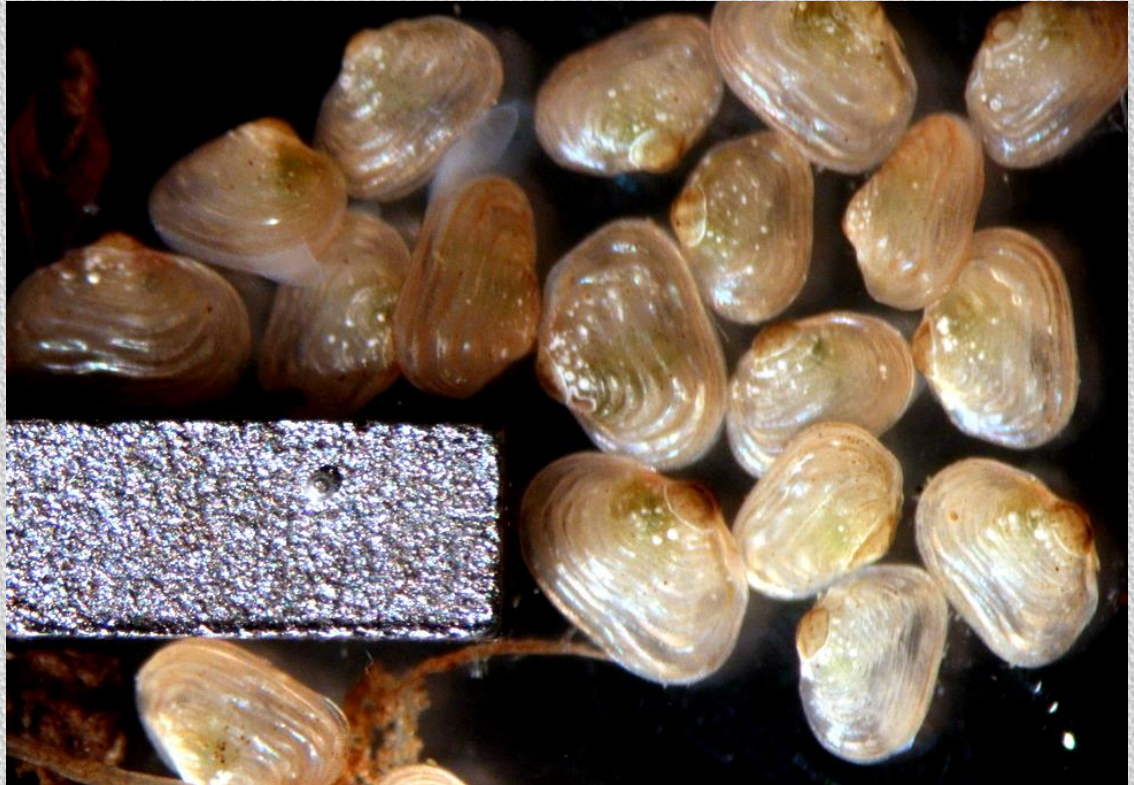


Margaritiferid Culture

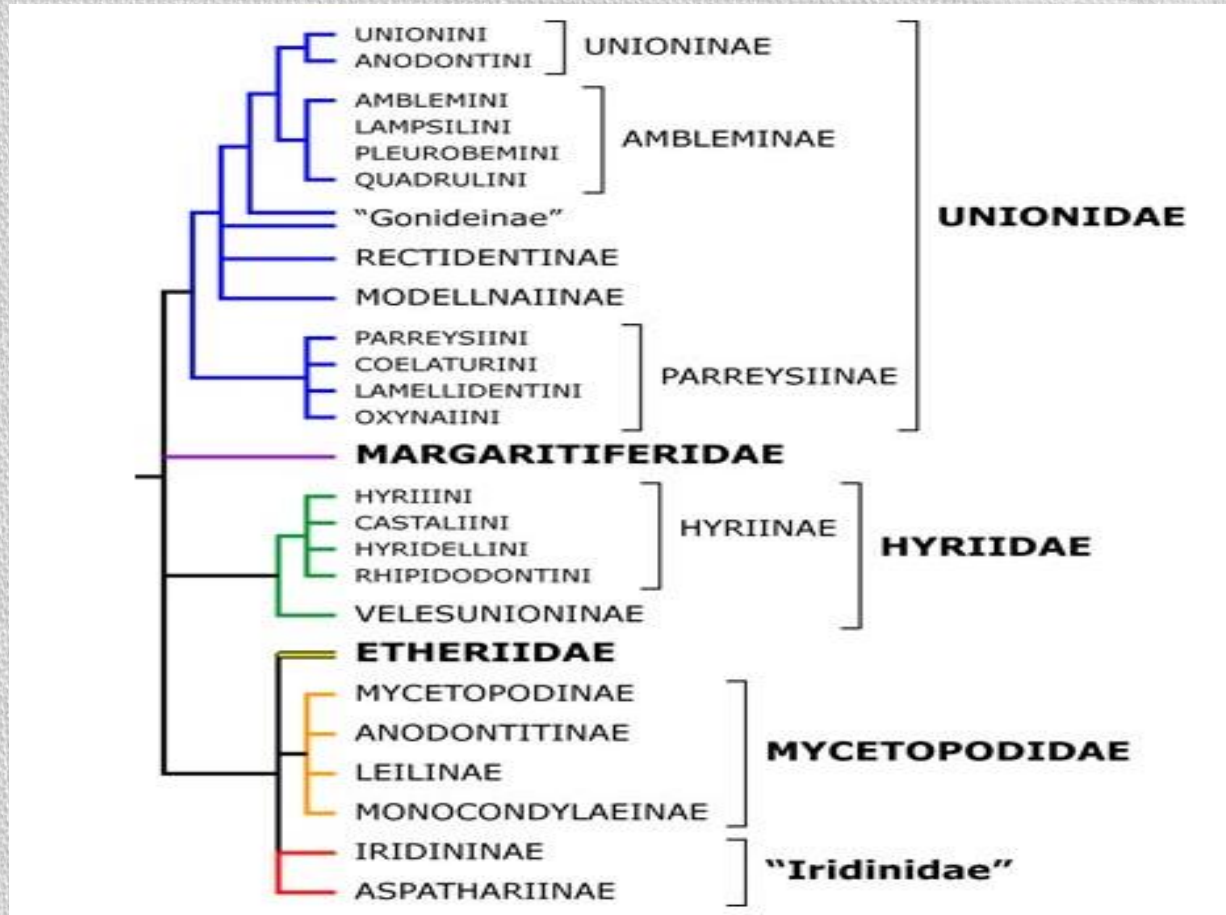
Beth Glidewell

Chris Barnhart

Missouri State University

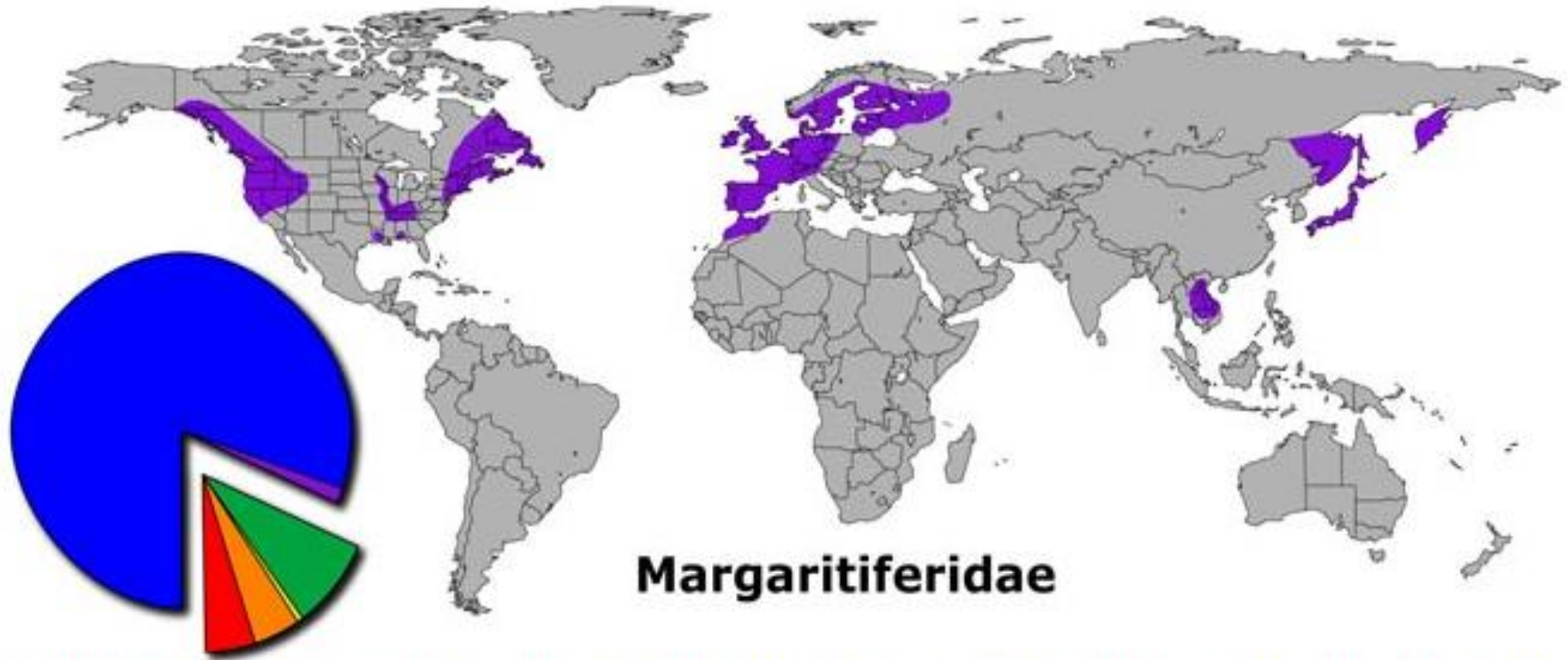


Family Margaritiferidae



Distribution of Margaritiferidae

Family MARGARITIFERIDAE



Margaritiferidae

Global Distribution of the Margaritiferidae. The thin purple wedge of the pie depicts the relative species diversity of the family (13 of 887).

Working with *M. falcata*: where to start...

European research, primarily with *Margaritifera margaritifera*

1989 Buddensiek 'sheet cages'

1990- Hruska, static boxes

Labor intensive

Utilize in situ conditions

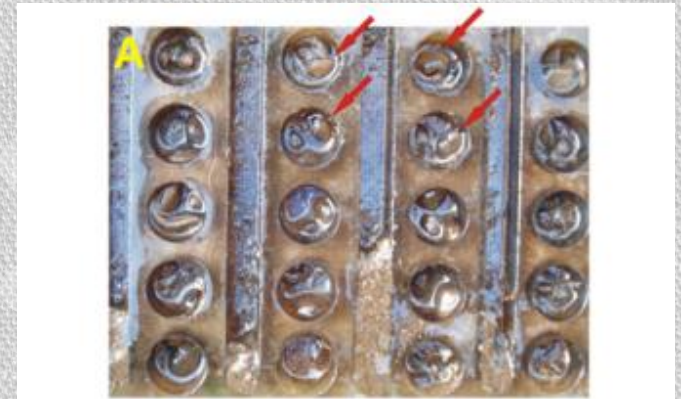
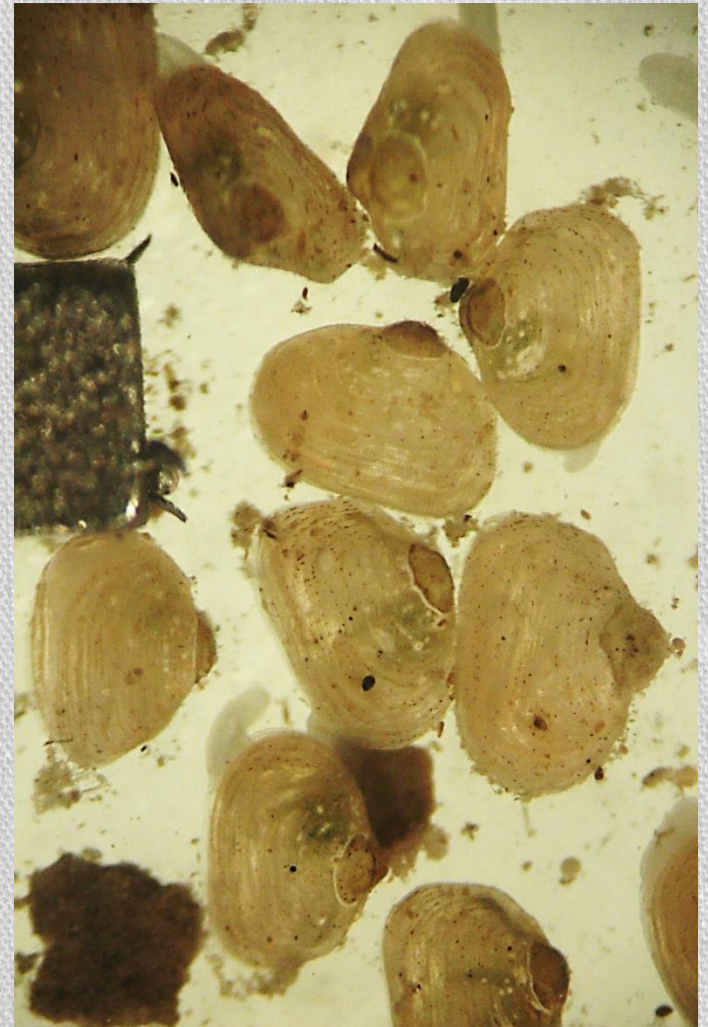


Figure 1. Successfully reared several-year-old juvenile freshwater pearl mussels (*Margaritifera margaritifera*) in sheet cages (A) and gravel boxes (B) reared in a stream of the Haarbach (Weiße Elster-Labe system, Saxony, Germany). The arrows denote juvenile mussels. The number of mussels visible in the sheet cage (A) and the box (B) is 21 and 32, respectively.

Gum, B., Lange, M., & Geist, J. (2011). A critical reflection on the success of rearing and culturing juvenile freshwater mussels with a focus on the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 21(7), 743-751.

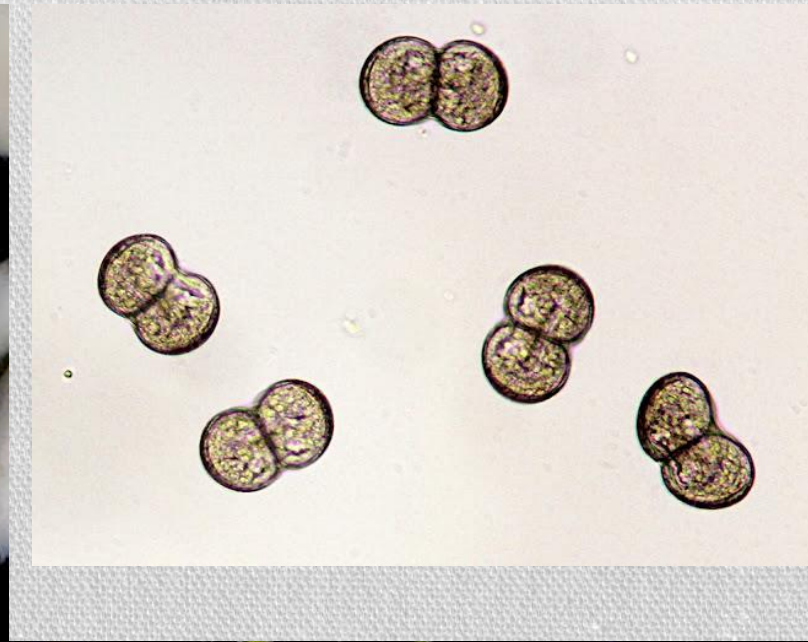
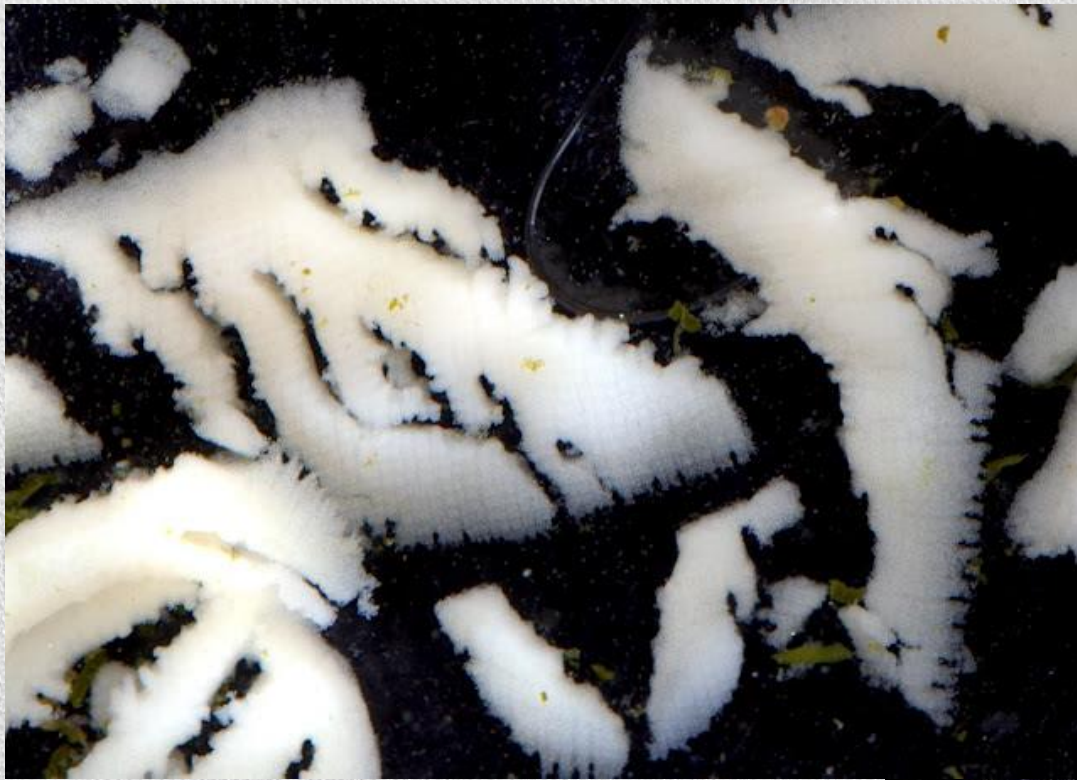
2012 and 2013 cohorts of *M. falcata*

- To produce juveniles
 - 1) for toxicology work
 - 2) for culture method development
- South Fork, Eel River, California
water temp 9-13°C early May
- Inoculation on Rainbow trout
(*Oncorhynchus mykiss*)
- Fish kept at 11° to 15°C



Margaritifera falcata conglutinates, Jeanette Howard





Conglutinates of eggs
containing mature
larvae, released at 13C



1 day

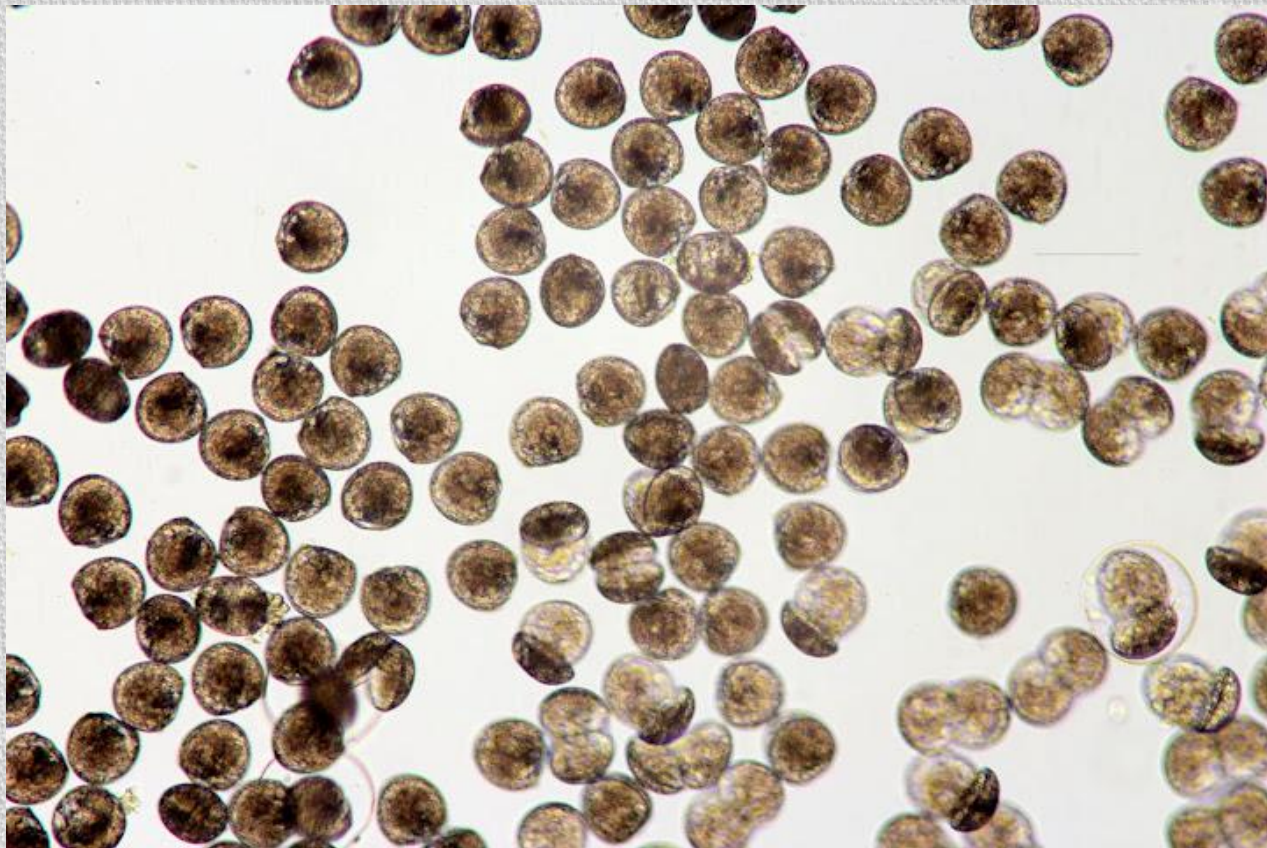
M. falcata and *M. margaritifera* larvae grow from ~65 μm to ~300 μm during encapsulation

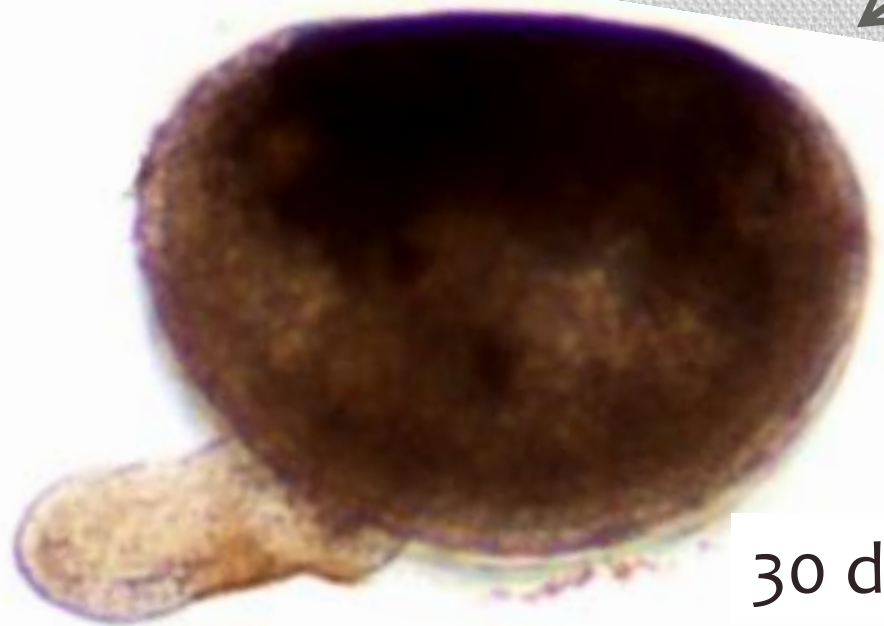
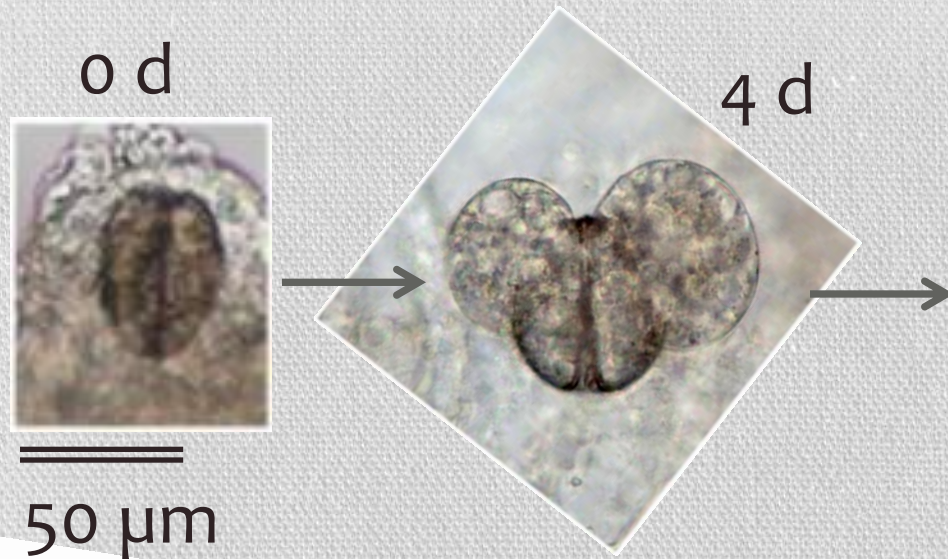
250 μm

4 weeks

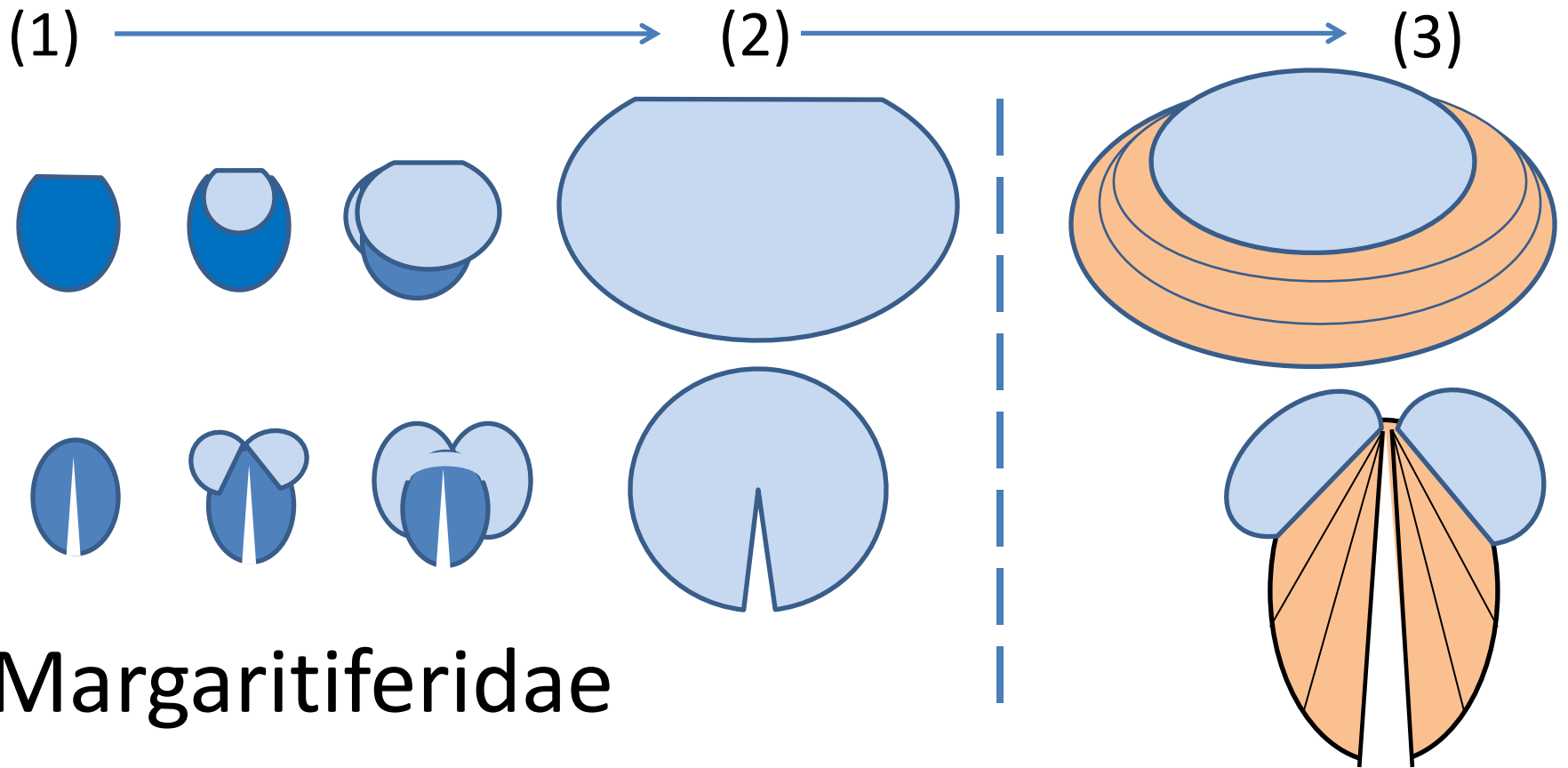
4.5 weeks

The larva looks like a glochidium, but it is not...





Growth of
encapsulated
Margaritifera falcata

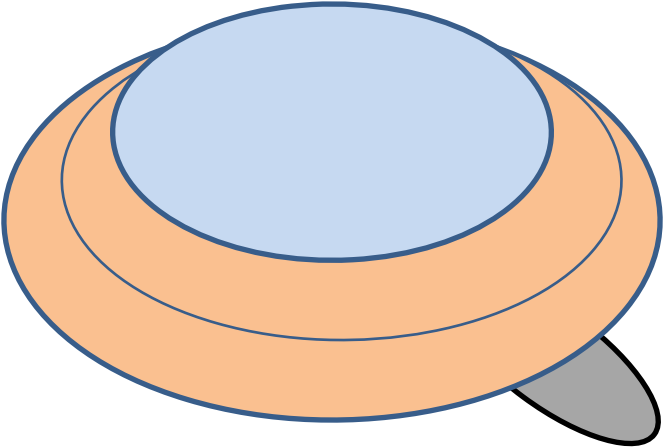
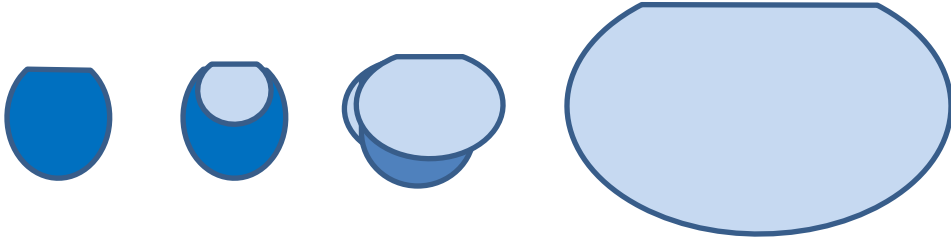


Margaritiferidae

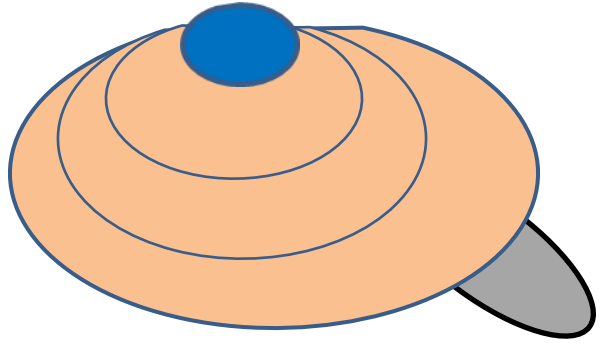
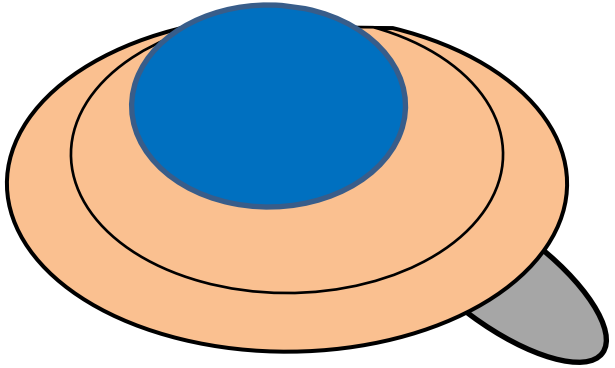
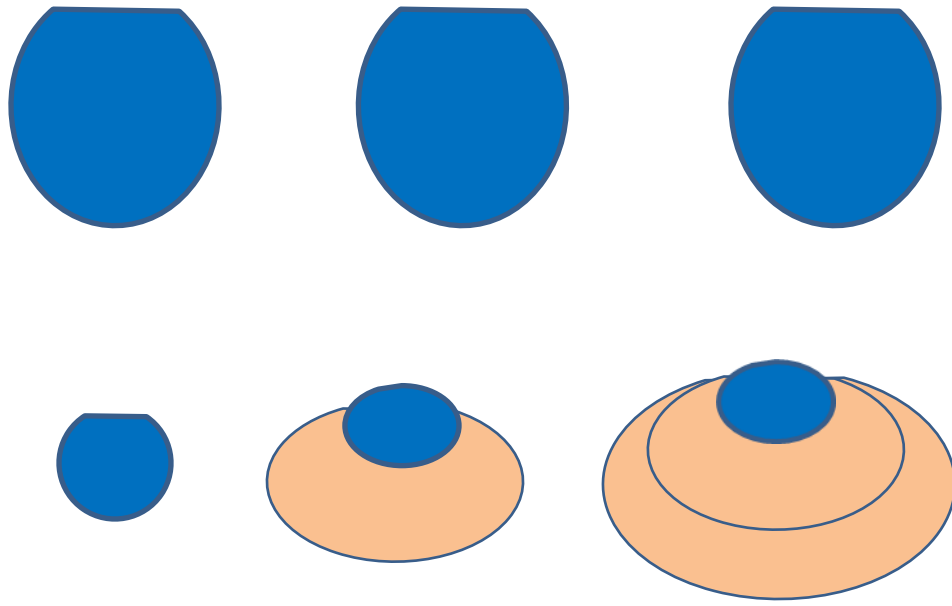
Primary larva (1) grows by inflation of dorso-lateral lobes to become secondary larva (2).

Secondary larva metamorphoses to juvenile (3), which grows by accretion at shell margin.

Margaritiferidae



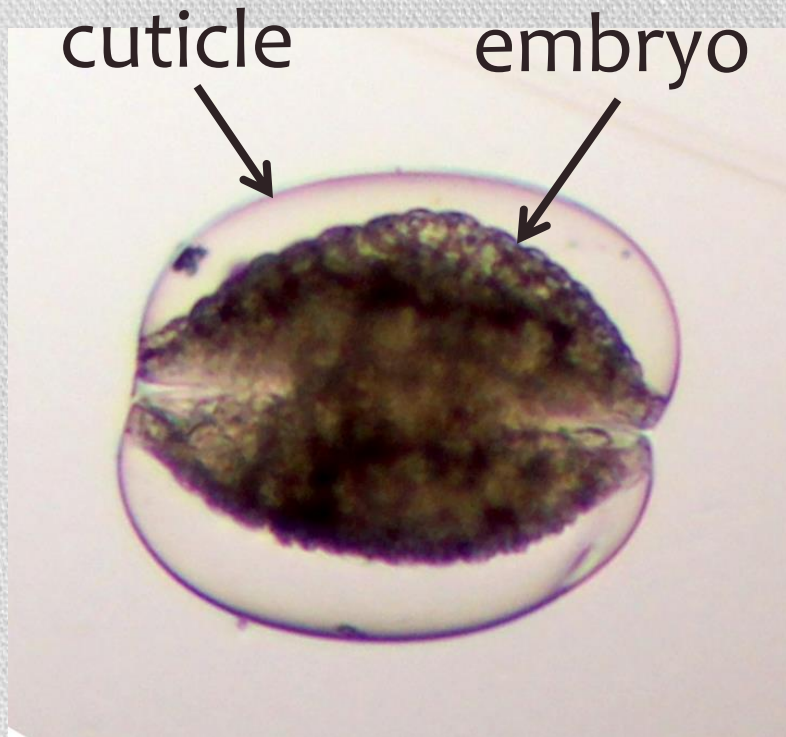
Unionidae



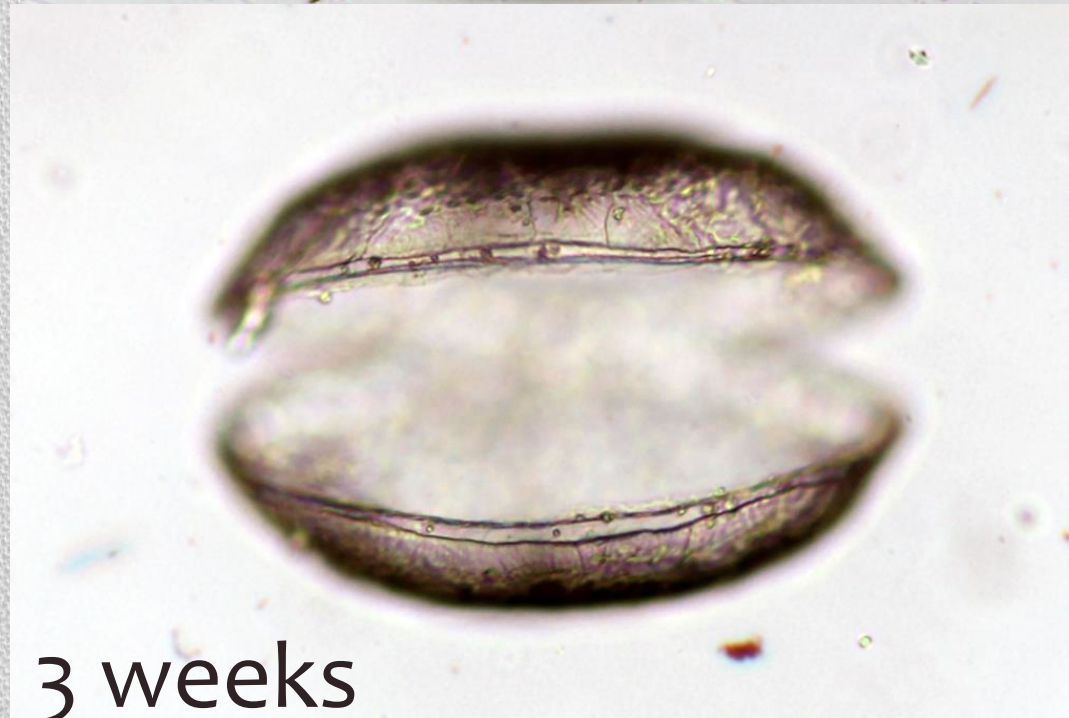
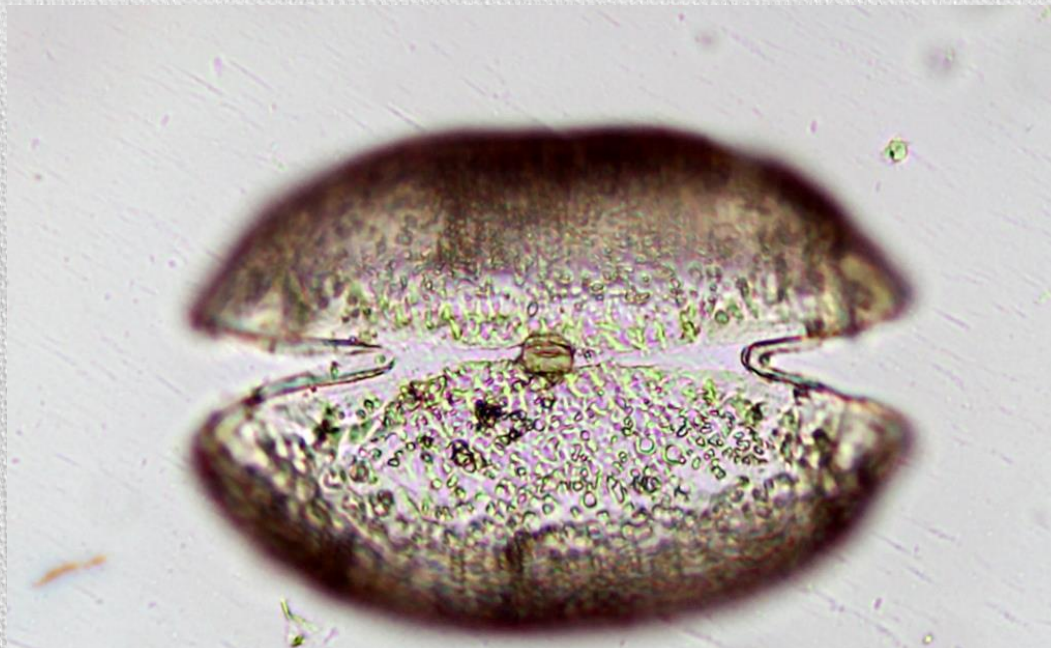
Larvae recovered from host gills at 4-5 days post-inoculation



Cuticle of secondary larva is initially uncalcified

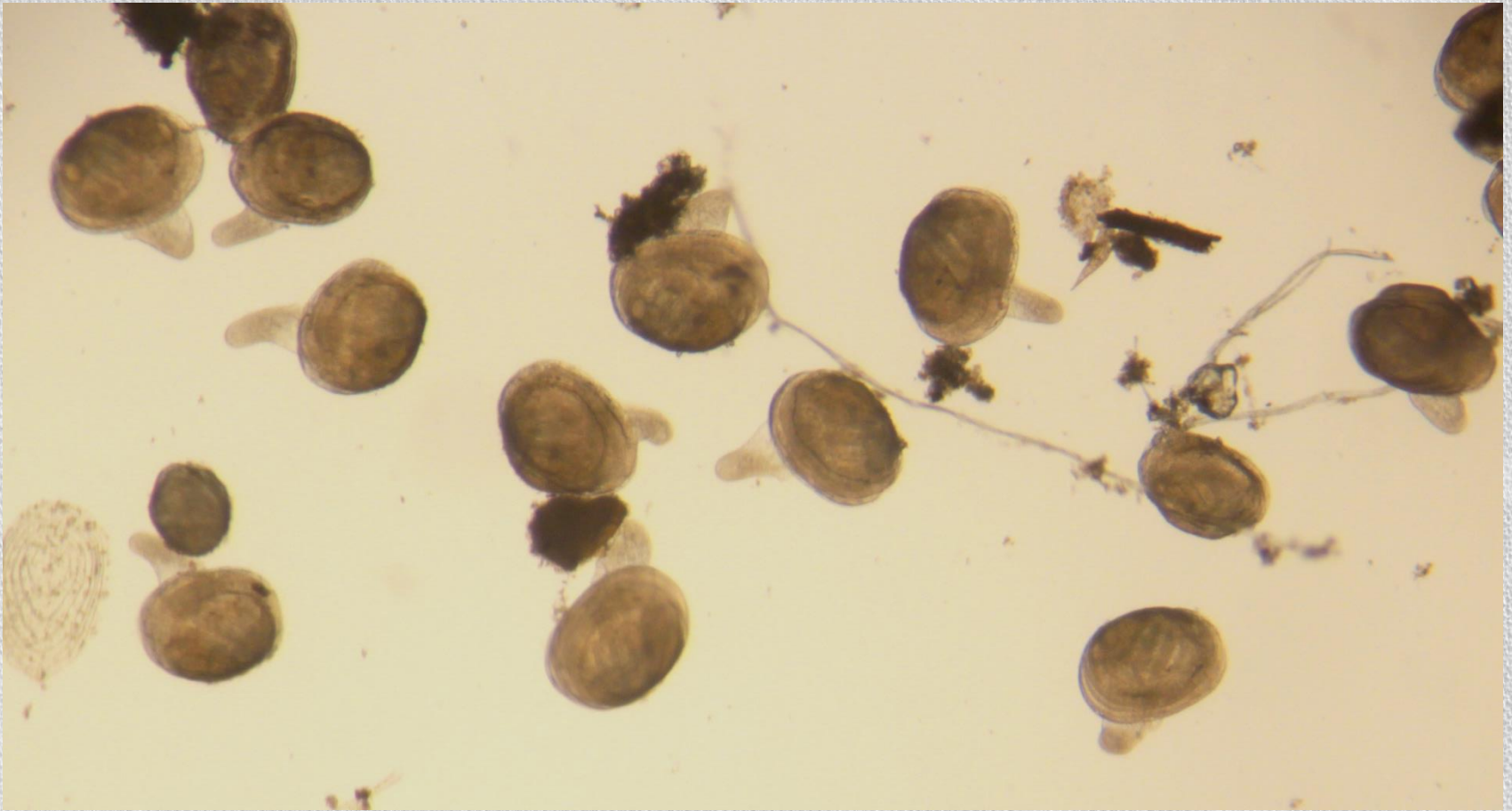


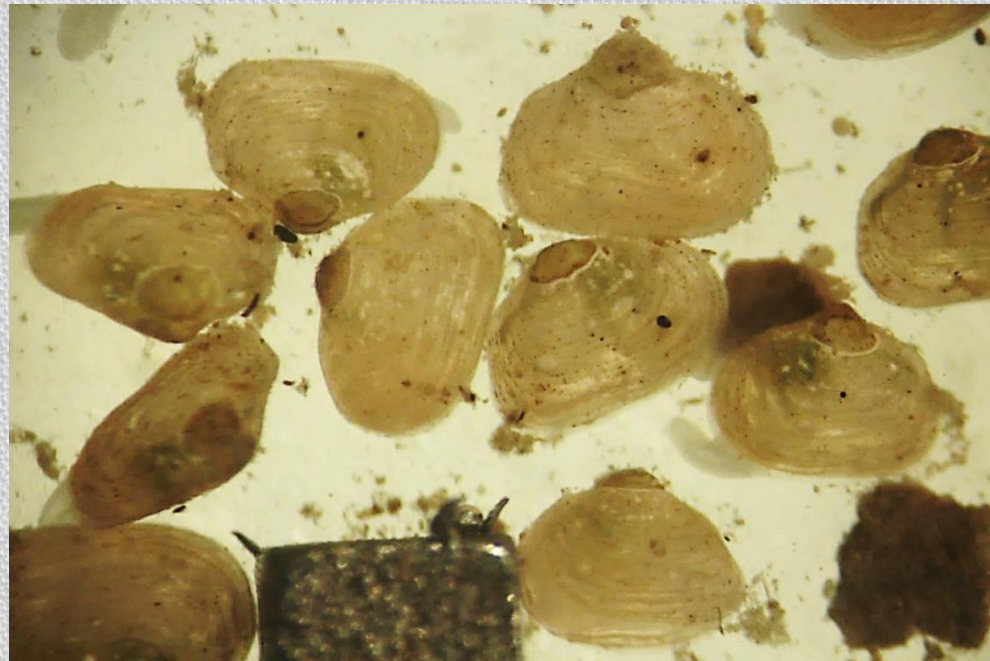
2 weeks



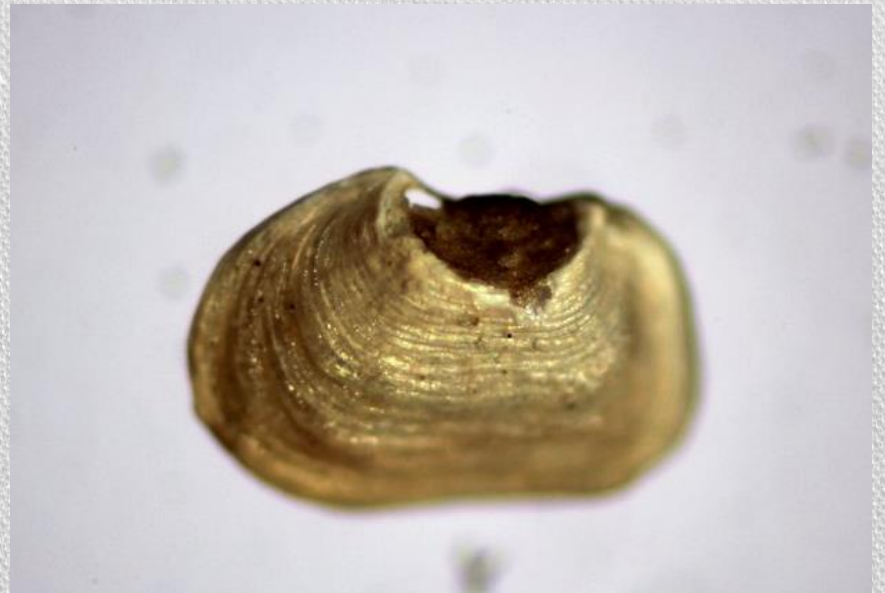
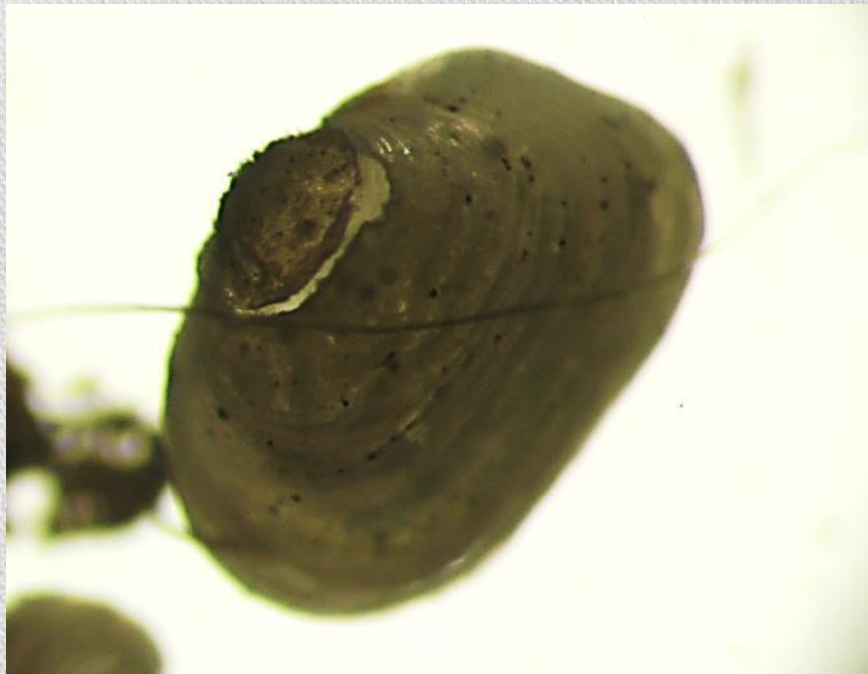
3 weeks

The cuticle calcifies, but remains very fragile...

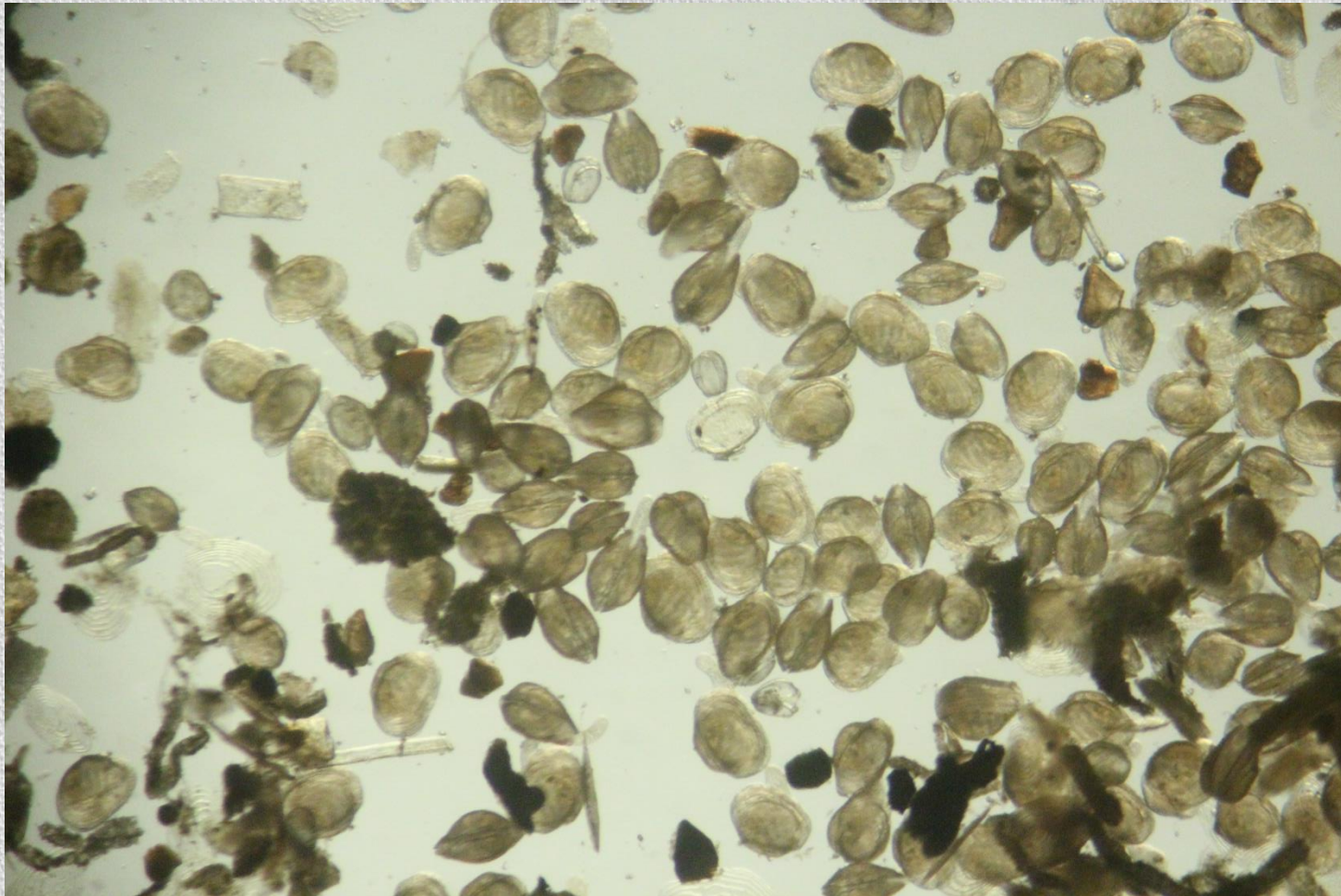




Secondary larval shell
with weak attachment
to accreted juvenile
shell



Culture techniques



'Mucket Buckets'

Sediment-free mucket buckets:
too dynamic

500 juveniles / cup

150 μ m nitex screen

Terminated after 3 weeks

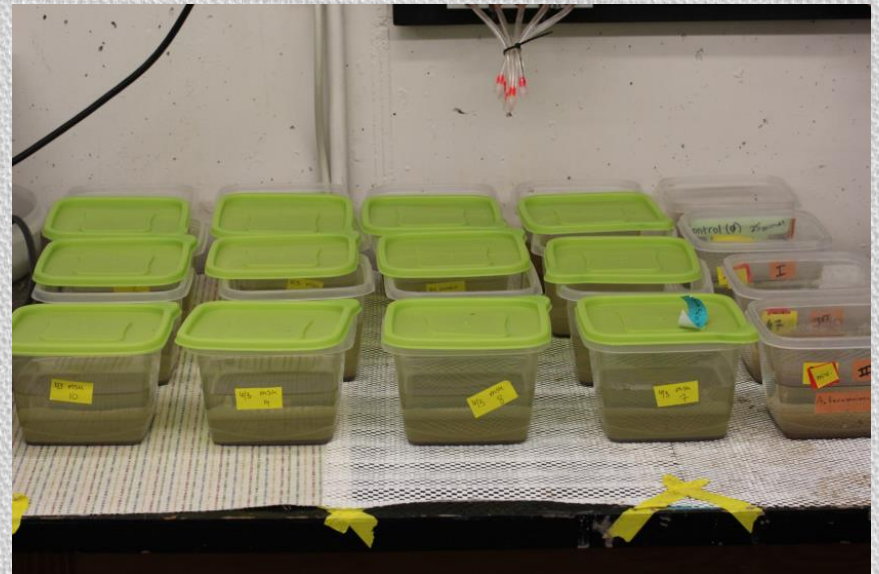


Hruška sediment culture



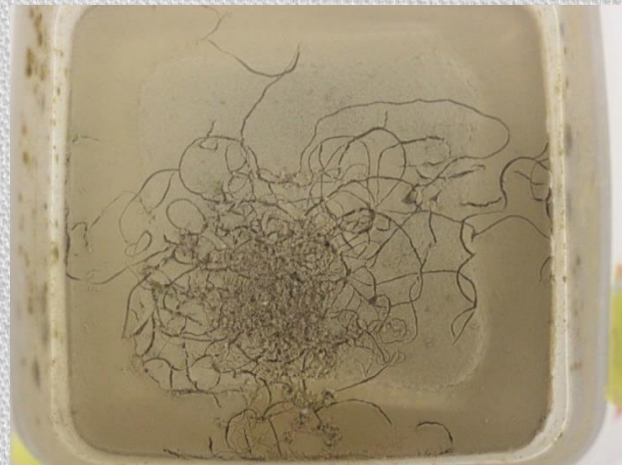
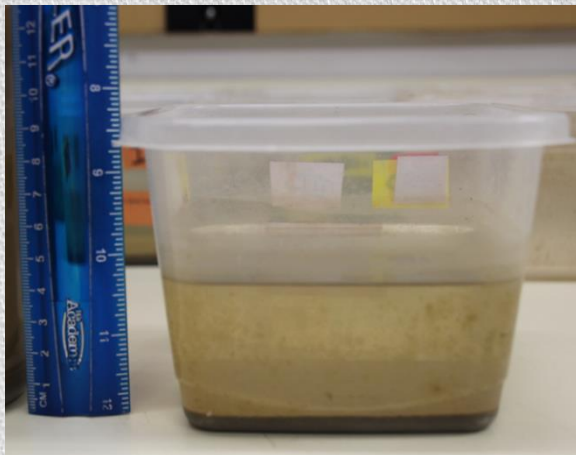
- 1-L plastic boxes
- 20mL sieved fresh sediment (<math><150\ \mu\text{m}</math>)
- 500mL food water ~18-20C
- 4 drops Nanno + 120 μl SFD per 10 L H₂O
- 8 drops Nanno + 240 μl SFD per 10 L H₂O

Eybe, T., Thielen, F., Bohn, T., & Sures, B. (2013). The first millimetre-rearing juvenile freshwater pearl mussels (*Margaritifera margaritifera* L.) in plastic boxes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 23(6), 964-975.



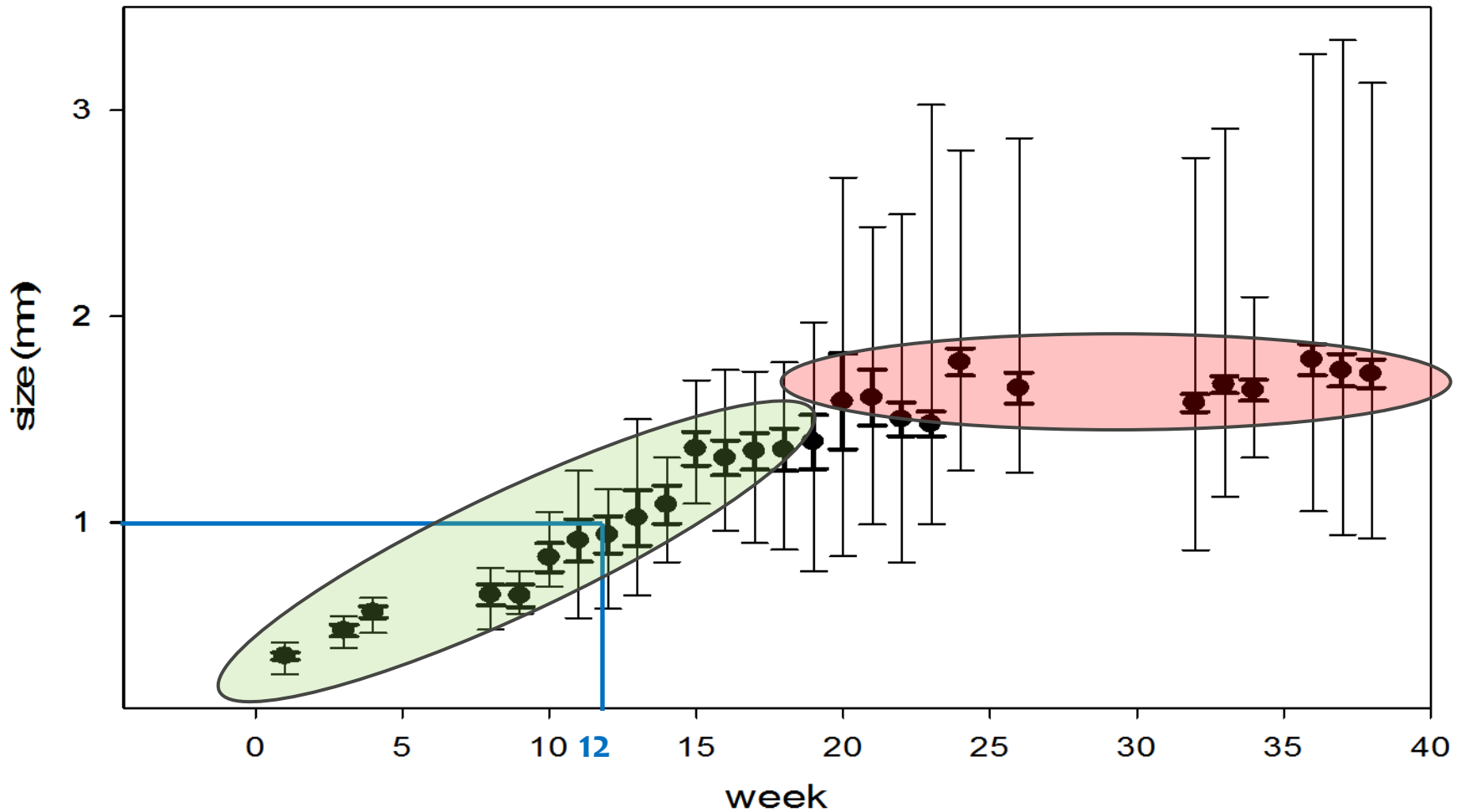
Weekly media change

- Sediment loosened with pipette
- All media sieved through 150 μ m screen
- Juveniles counted/measured
- New food water, new sediment
- Juveniles replaced

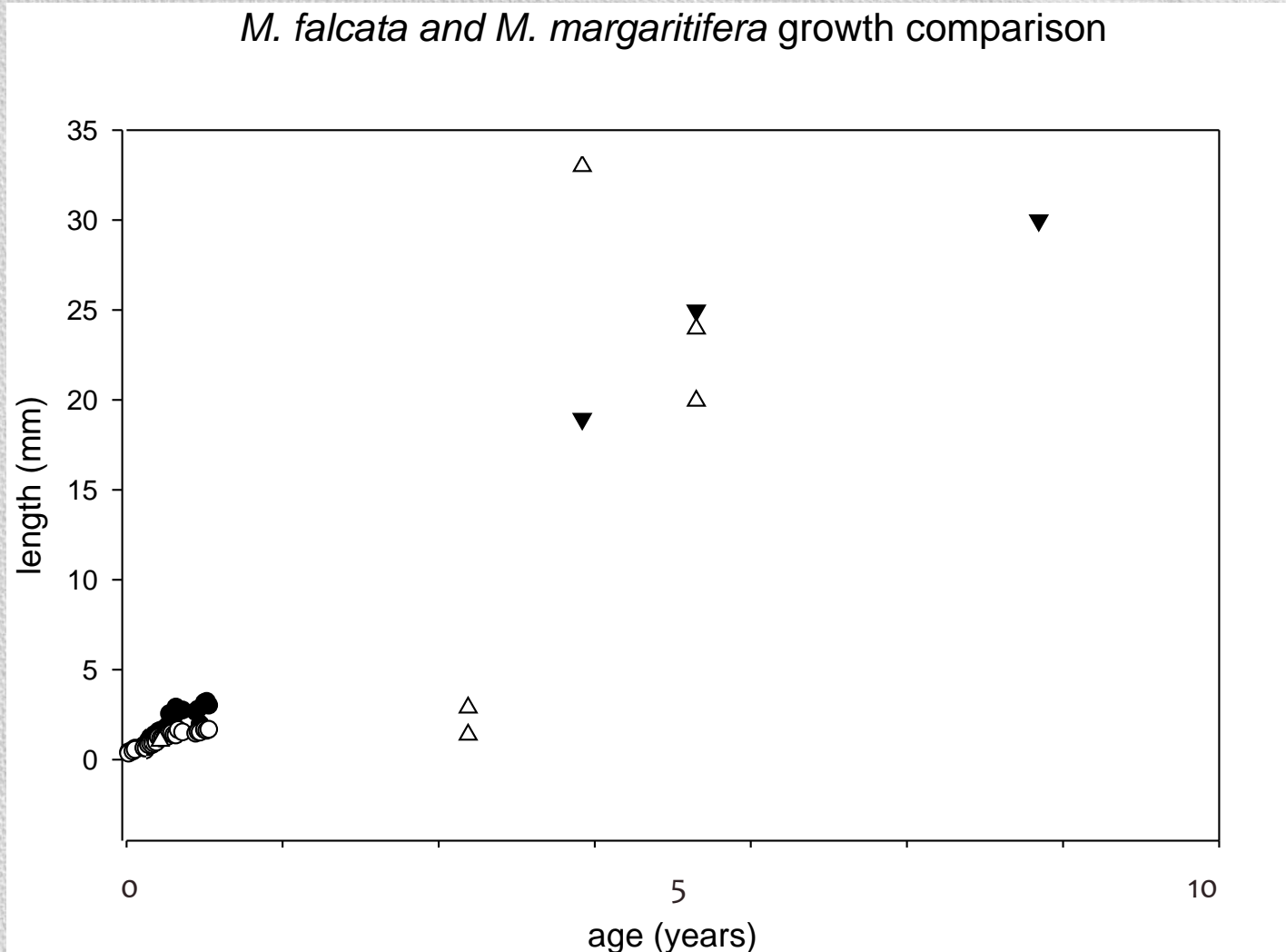


Growth in sediment

M. falcata size (mm)
mean \pm 95% CI, maximum - minimum

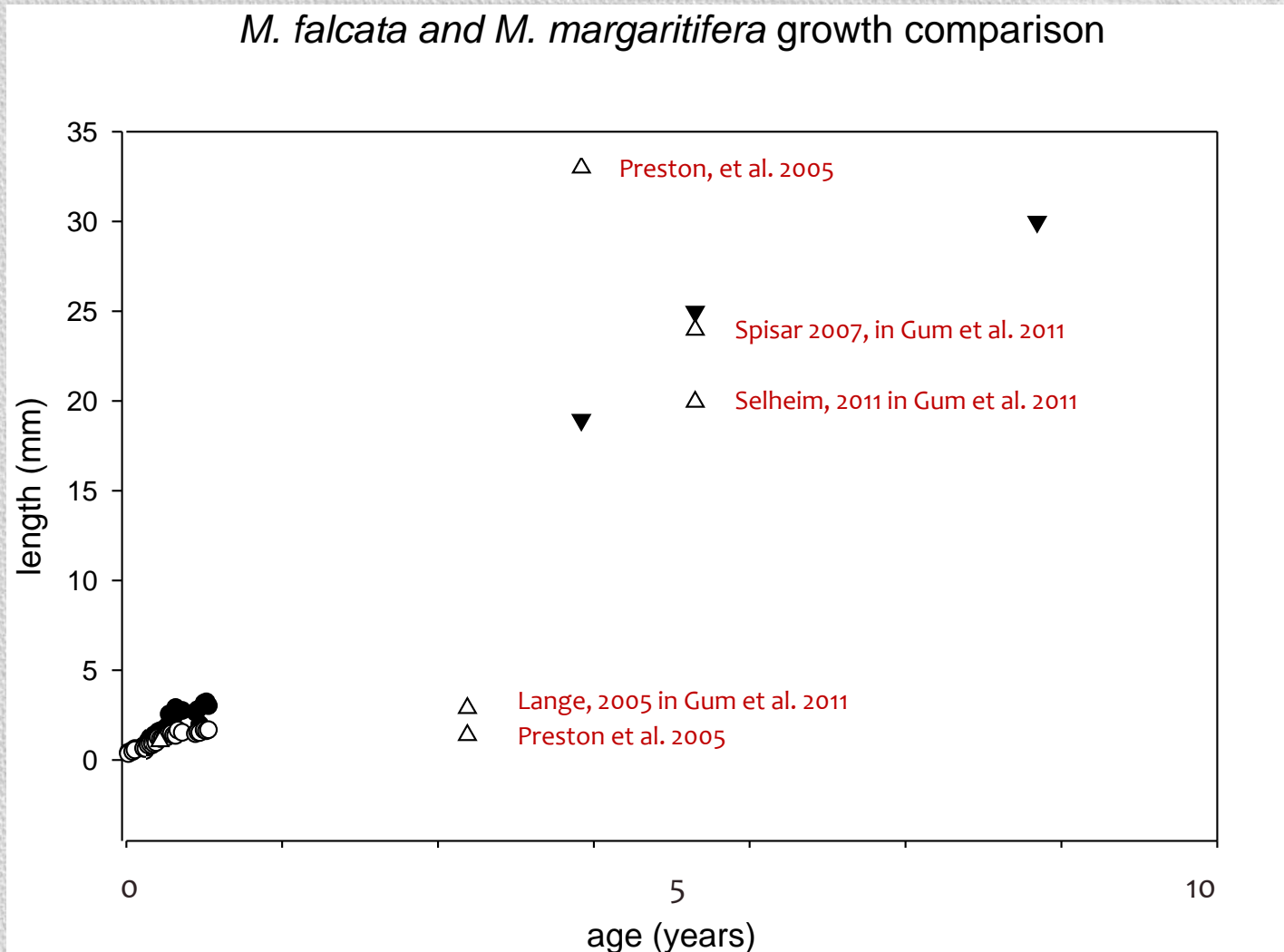


Growth comparison



Culture 0-9 months, Eel River, 4,5 and 8 years (from Howard and Cuffey, 2006)

Growth comparison

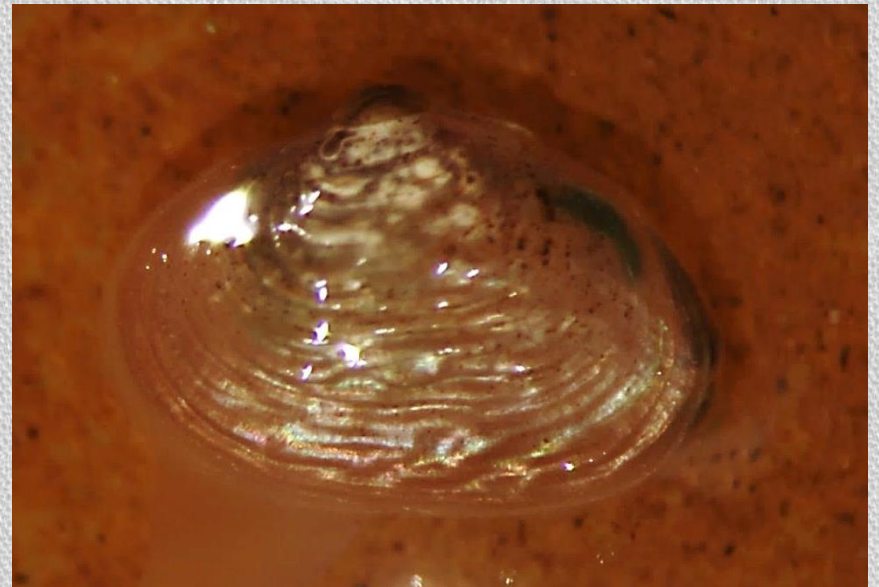


Culture 0-9 months, Eel River, 4,5 and 8 years (from Howard and Cuffey2006)

Results

- 2012
 - 4000 juveniles produced
 - For toxicology
 - Stage 1 culture successful

- 2013
 - 30,000 juveniles produced
 - For toxicology
 - Many lost in culture
 - ~1000/25,000 remained at 3.5mo



Alabama Aquatic Biodiversity Center

- *Margaritifera marrianae* (Alabama pearlshell)
- Static sediment boxes
 - 600mL pond water
 - 10-25 g of fine sediment
 - 53,000 cells/mL Shellfish Diet 1800 and 906,000 cells/mL Nanno
- Switched to twice weekly water and sediment changes, and survivorship improved, though growth slow
- Slow growth and total mortality in mucket buckets

Poster 7 CAPTIVE PROPAGATION OF ALABAMA PEARLSHELL (MARGARITIFERA MARRIANAE, R.I. JOHNSON, 1983). Michael L. Buntin, Todd B. Fobian, and Paul D. Johnson. Alabama Department on Conservation and Natural Resources, Alabama Aquatic Biodiversity Center, Marion, AL

Eybe, Thielen et al.

<http://www.unio.lu/>



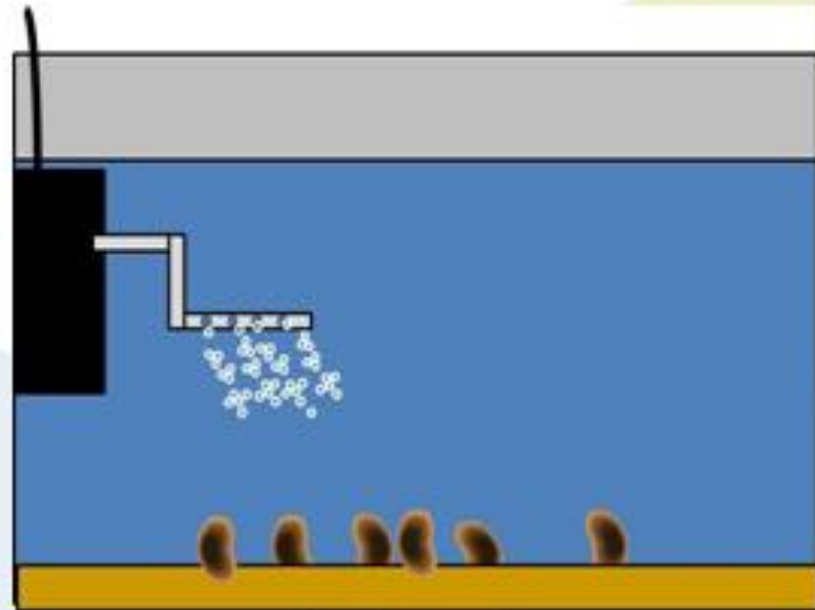
Eybe, Thielen et al.

> 1mm juveniles

zusammen für d'natur

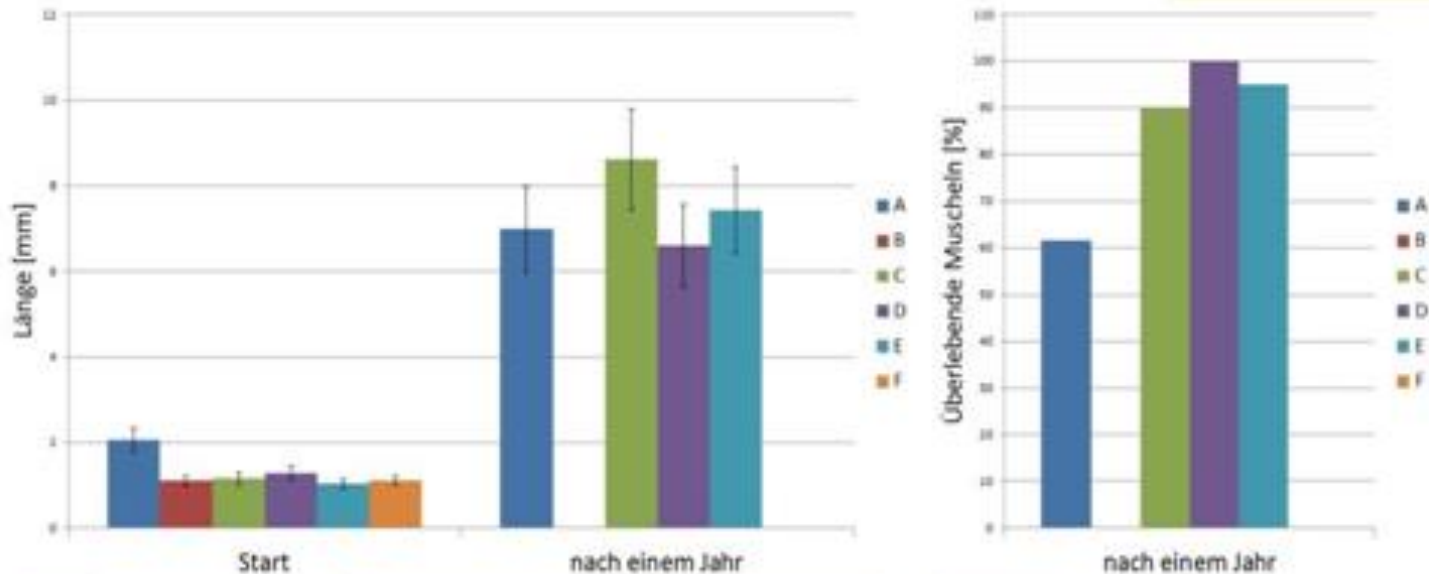
Experiment 4 / Sand Aquaria

Rearing of juvenile Mussels >1mm

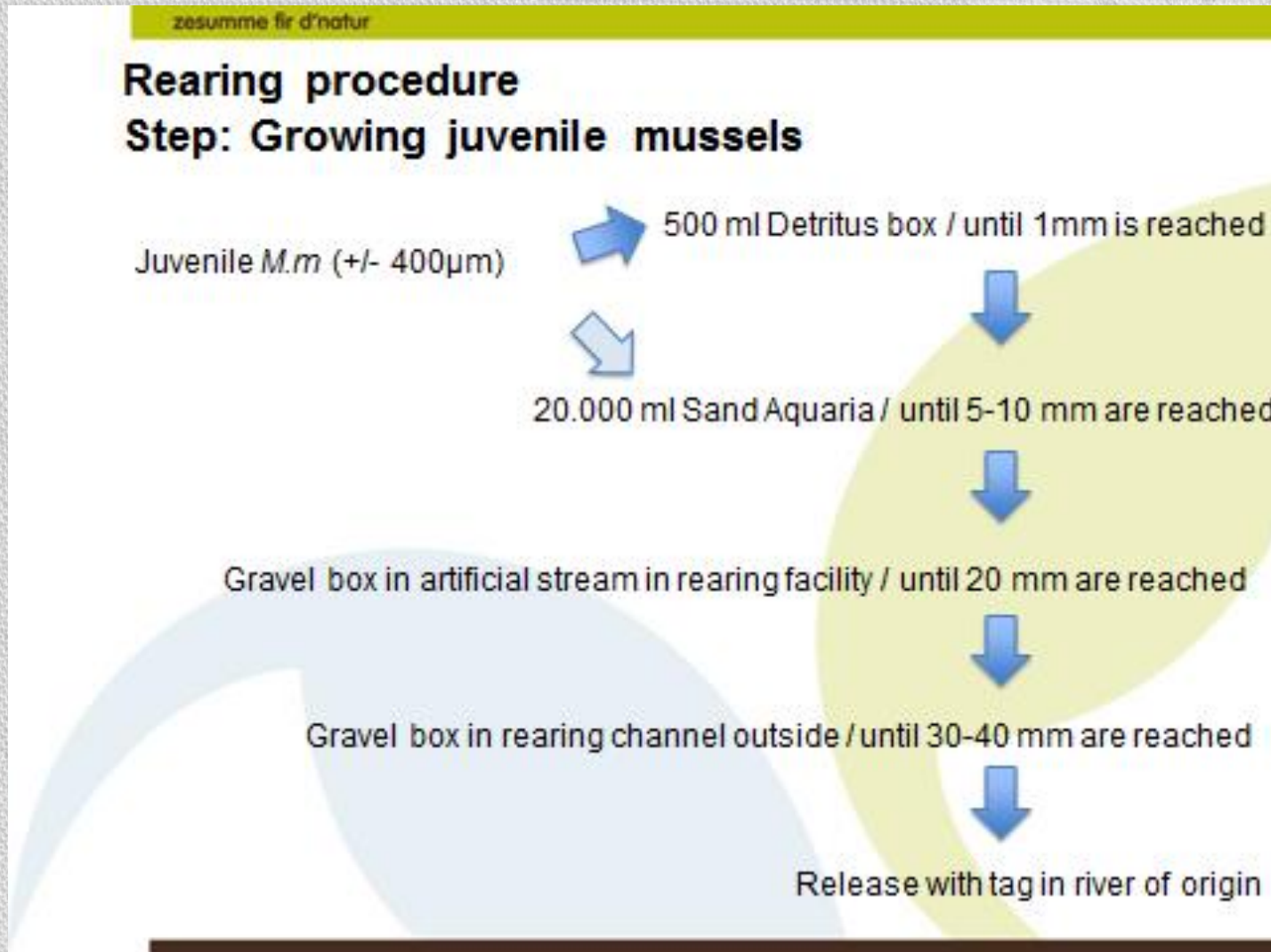


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Results after one year

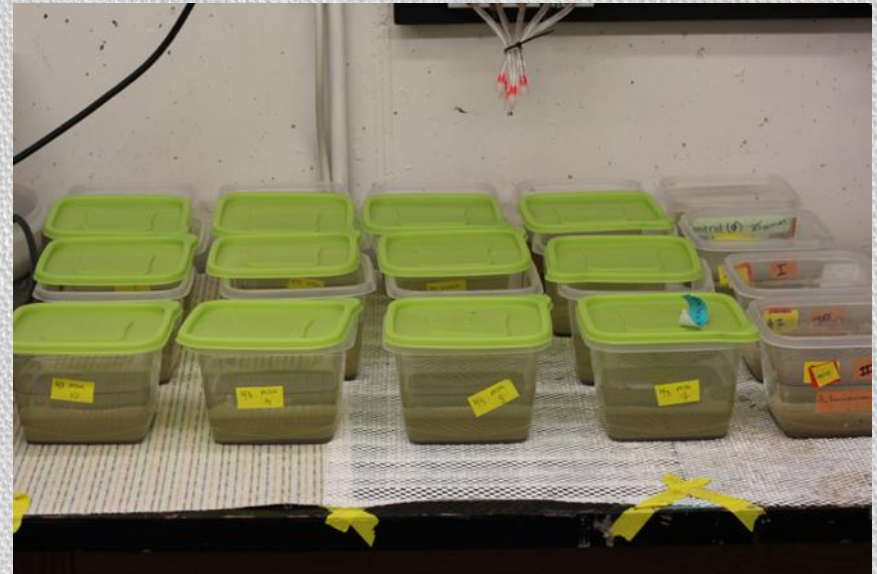


After 1 year

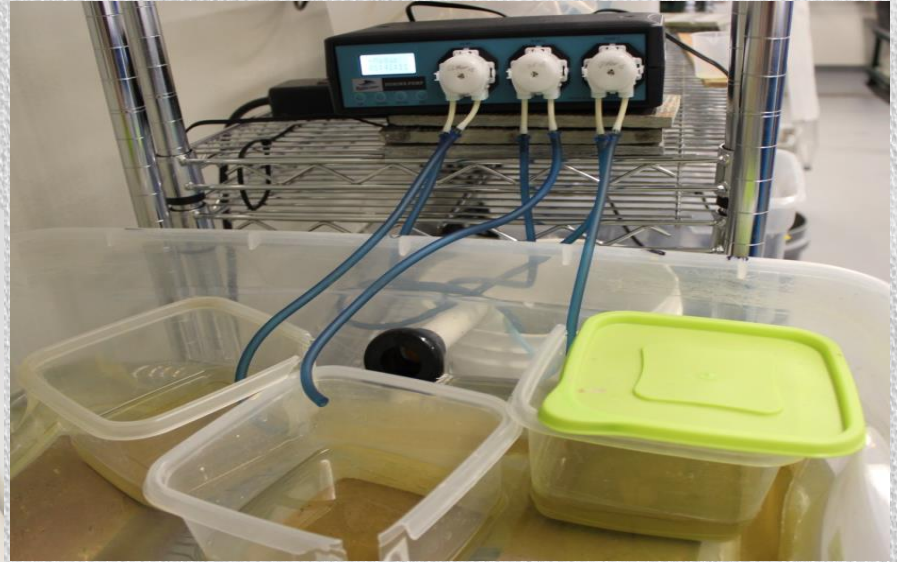
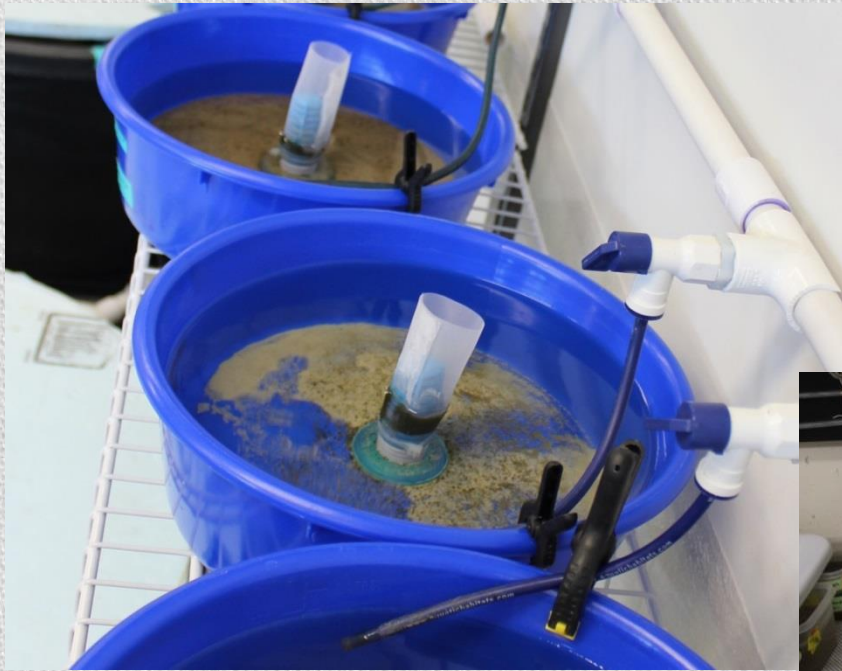


Hruška sediment culture- summary

- Moderately successful for early stages (stage 1) (~3 months/1-2mm)
- Possible low DO at sediment interface?
- Inadequate food levels?
- Labor intensive for large numbers
- Temperature considerations and
- Laboratory space



Next stage culture methods





Buddensiek, V. (1995). The culture of juvenile freshwater pearl mussels *Margaritifera margaritifera* L. in cages: a contribution to conservation programmes and the knowledge of habitat requirements. *Biological Conservation*, 74(1), 33-40.

Eybe, T., Thielen, F., Bohn, T., & Sures, B. (2013). The first millimetre-rearing juvenile freshwater pearl mussels (*Margaritifera margaritifera* L.) in plastic boxes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 23(6), 964-975

Gum, B., Lange, M., & Geist, J. (2011). A critical reflection on the success of rearing and culturing juvenile freshwater mussels with a focus on the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 21(7), 743-751.

Howard, J. K., & Cuffey, K. M. (2006). Factors controlling the age structure of *Margaritifera falcata* in 2 northern California streams. *Journal of the North American Benthological Society*, 25(3), 677-690.

Hruska, J. (1992). The freshwater pearl mussel in South Bohemia: evaluation of the effect of temperature on reproduction, growth and age structure of the population. *Archiv für Hydrobiologie*, 126(2), 181-191.

The MUSSEL project. <http://mussel-project.uwsp.edu/>

Questions?

