Mussel Propagation: from Capture to Culture

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FMCS Propagation Workshop
St. Charles 2015

Deciding on host(s)

- Published reports, Ellipsaria, OSU database are a good place to start, but don’t assume too much.
- If possible, test and quantify your attachment and metamorphosis success.
- Metamorphosis can be highly variable among species pairs, and also among population pairs.

Testing host compatibility (% metamorphosis success)

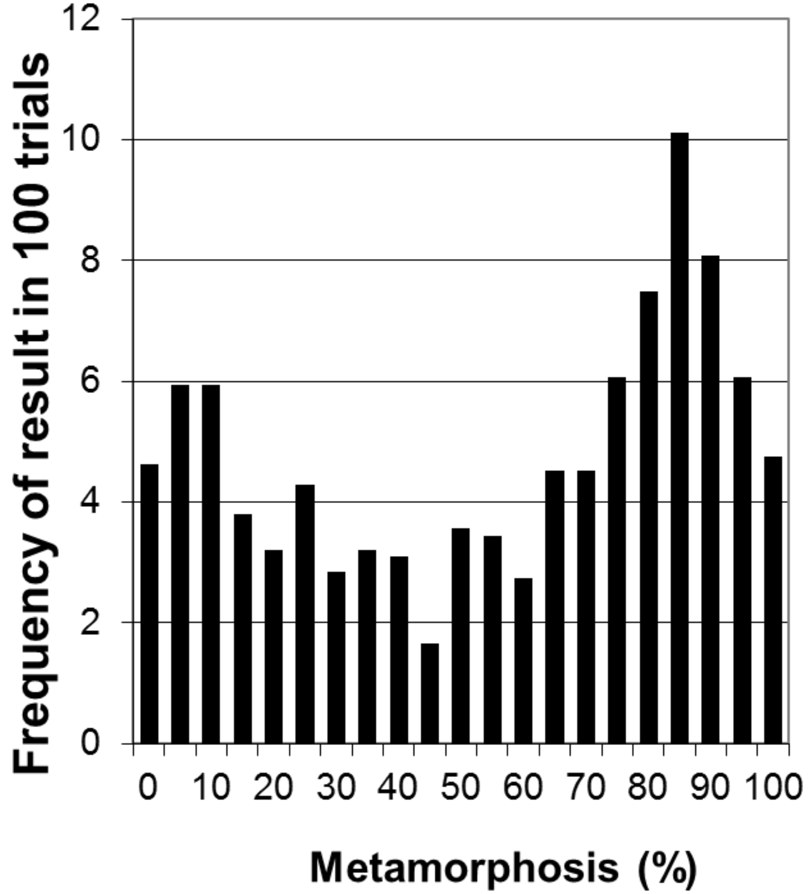
Metamorphosis success

- 25 mussel species
- 41 mussel-host pairs
- 100 trials
- 969 individual fish

Metamorphosis success varied from zero to nearly 100%

Sources of variation

- Among mussel-host species pairs (host specificity)
- Among trials within species-pairs
- Among individual fish within trials

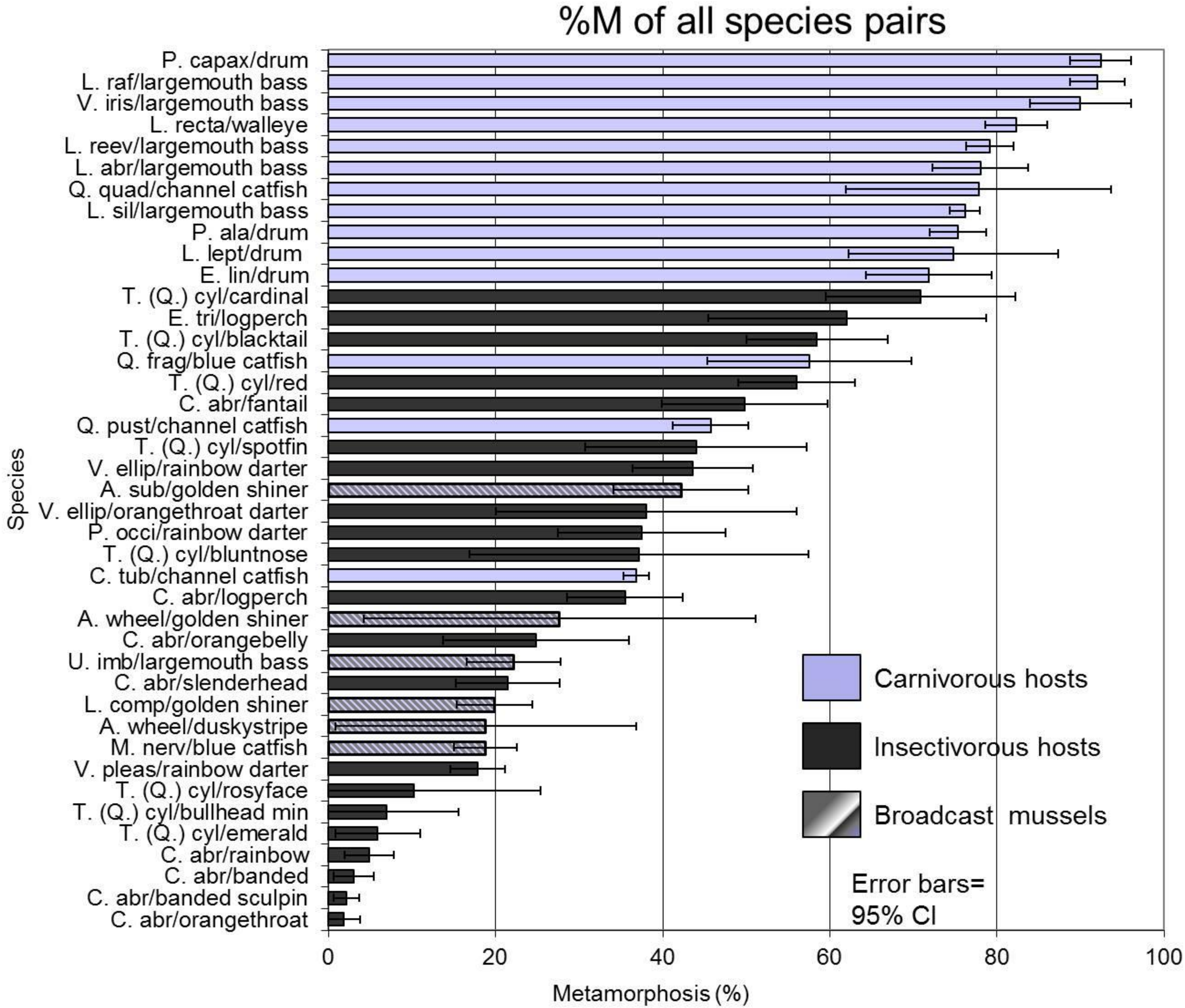


A. Crownhart (Fritts) thesis

Partitioning variation in M%

- General linear model ANOVA
  - **65% among mussel-host species pairs** (=host specificity) $p<0.001$
  - **16% among trials within species-pairs** $p<0.001$
  - **19% among individual fish within trials**

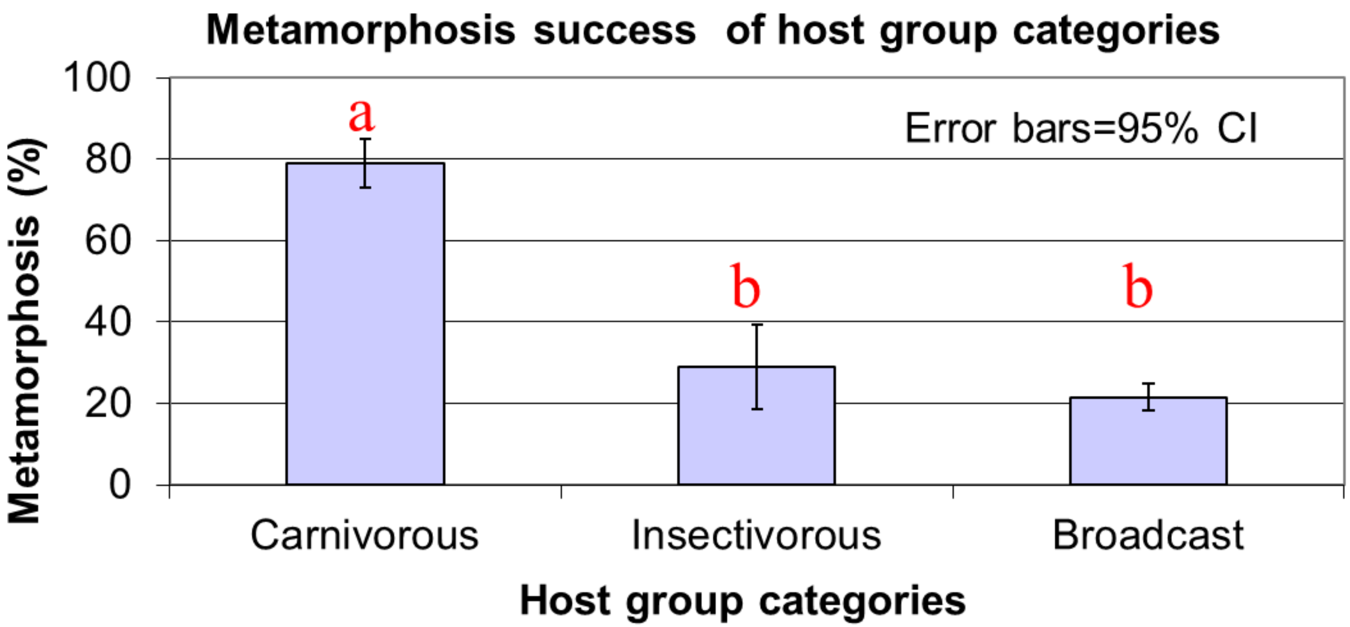
A. Crownhart (Fritts) thesis



%M of all species pairs
P. capax/drum
L. raf/largemouth bass
V. iris/largemouth bass
L. recta/walleye
L. reev/largemouth bass
L. abr/largemouth bass
Q. quad/channel catfish
L. sil/largemouth bass
P. ala/drum
L. lept/drum
E. lin/drum
T. (Q.) cyl/cardinal
E. tri/logperch
T. (Q.) cyl/blacktail
Q. frag/blue catfish
T. (Q.) cyl/red
C. abr/fantail
Q. pust/channel catfish
T. (Q.) cyl/spotfin
V. ellip/rainbow darter
A. sub/golden shiner
V. ellip/orangethroat darter
P. occi/rainbow darter
T. (Q.) cyl/bluntnose
C. tub/channel catfish
C. abr/logperch
A. wheel/golden shiner
C. abr/orangebelly
U. imb/largemouth bass
C. abr/slenderhead
L. comp/golden shiner
A. wheel/duskystripe
M. nerv/blue catfish
V. pleas/rainbow darter
T. (Q.) cyl/rosyface
T. (Q.) cyl/bullhead min
T. (Q.) cyl/emerald
C. abr/rainbow
C. abr/banded
C. abr/banded sculpin
C. abr/orangethroat
Species
Carnivorous hosts
Insectivorous hosts
Broadcast mussels
Error bars= 95% CI
0
20
40
60
80
100
Metamorphosis (%)

Metamorphosis success by host group categories

	Mean M% ± SD	
Carnivorous	79.1 ± 10.1	(range up to 97%)
Insectivorous	29.0 ± 23.1	(range up to 70%)
Broadcast	21.5 ± 3.7	(range up to 42%)



Andrea Crownhart (Fritts) thesis

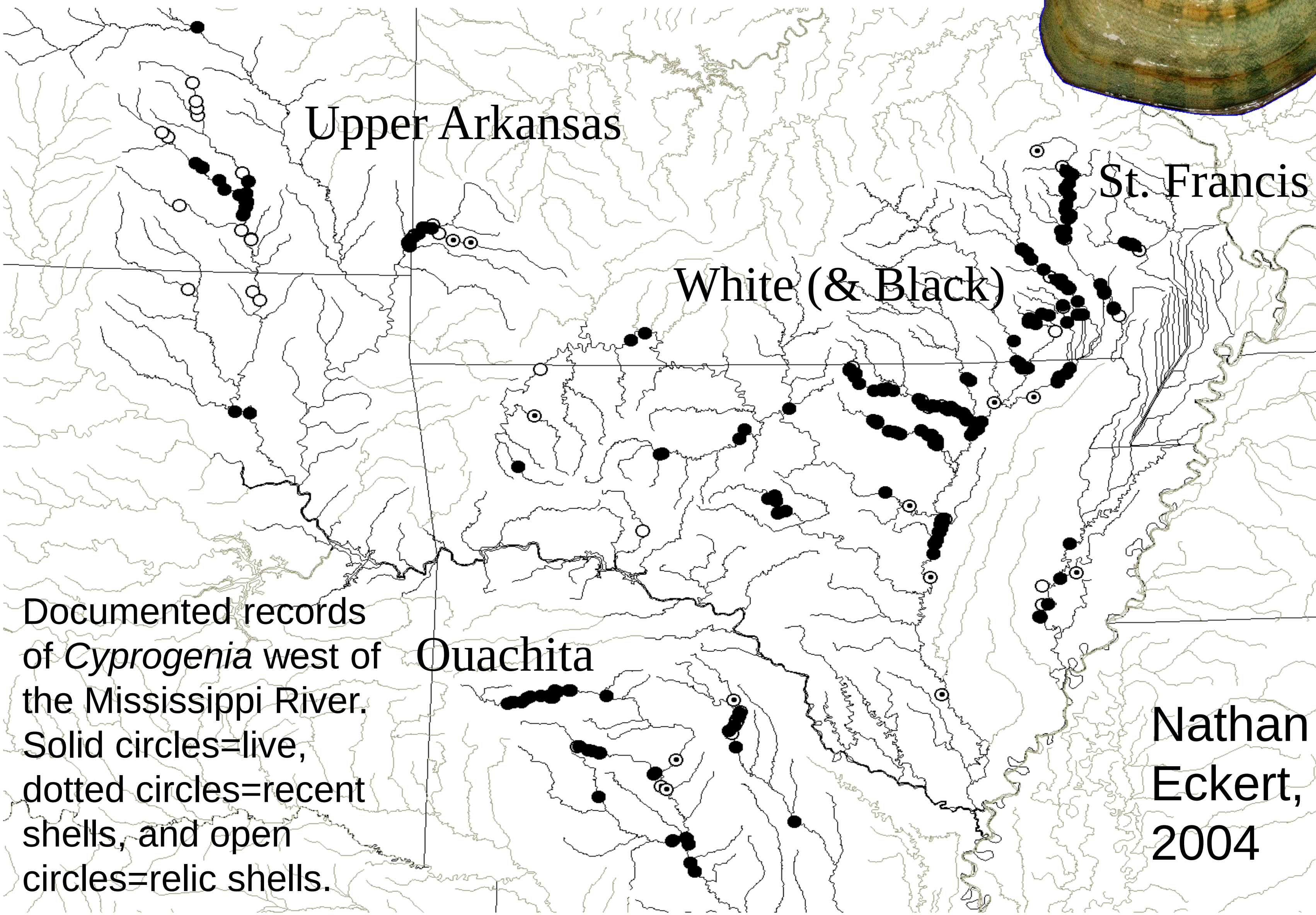
Generalizations

- Mussels that attract large carnivorous hosts (bass, walleye, drum, ictalurids) tend to be host specialists and have higher %M.
- Mussels that broadcast glochidia or conglutinates generally have a broader range of hosts and lower M%

Local host adaptation

- Widespread mussel taxa may adapt locally to different hosts, particularly if the hosts are darters.
- Examples: *Venustaconcha pleasii* and rainbow darter, *Cyprogenia aberti* and darters (Riusech 2000, Eckert 2003)
- *See Platform #32 Schneider (3pm Monday)*

Local hosts: *Cyprogenia aberti*



Documented records of *Cyprogenia* west of the Mississippi River. Solid circles=live, dotted circles=recent shells, and open circles=relic shells.

Nathan Eckert, 2004

Example- Verdigis vs. Ouachita

%M

Verdigris logperch

	N attached	N transformed	% Transformed
Ouachita fanshells	**144 ± 35**	101.1 ±34.7	65.2 ± 9.0
Verdigris fanshells	**213 ± 49**	107.3 ±28.0	52.1 ± 9.3

Verdigris slenderhead

	N attached	N transformed	% Transformed
Ouachita fanshells	190 ± 28	**20.60 ± 27.0**	**7.8 ± 8.2**
Verdigris fanshells	172 ± 27	**94.20 ± 28.7**	**53.5 ± 11.9**

Ouachita orangebelly

	N attached	N transformed	% Transformed
Ouachita fanshells	110 ± 20	**58.0 ± 21.8**	**48.4 ± 14.4**
Verdigris fanshells	94 ± 24	**1.6 ± 1.2**	**1.3 ± 0.8**

Ouachita banded

	N attached	N transformed	% Transformed
Ouachita fanshells	32 ± 6	1.5 ± 1.4	5.1 ± 4.8
Verdigris fanshells	29 ± 8	0.5 ± 0.5	1.1 ± 1.3

Means ± StDev, n = 15

Host condition: health

- Host health is critical for survival of the host during the parasitic phase.
- Also for clean recovery of juveniles, reducing mucus, scales, ammonia
- Hosts can be treated for disease with usual therapeutics (salt, formalin, antibiotics) during the parasitic stage (Rach 2006 N. Am. J. Aquaculture 68:348)

Flexibacter (*columnaris*) is a common problem. We treat with salt and Kanamycin

Favorite fish therapeutants

- NaCl (Blue Diamond) up to 0.3%
  - discourages ich, bacterial infections, others. Remove or reduce before drop-off.
- Kanamycin sulphate
  - for ich and *Aeromonas*
  - Absorbed via gills, can use as prophylactic soak during transport, kind to biofilters
- Praziquantel
  - treat wild fish prophylactically for flukes and tapeworms. Does NOT work for *Macrostomum*

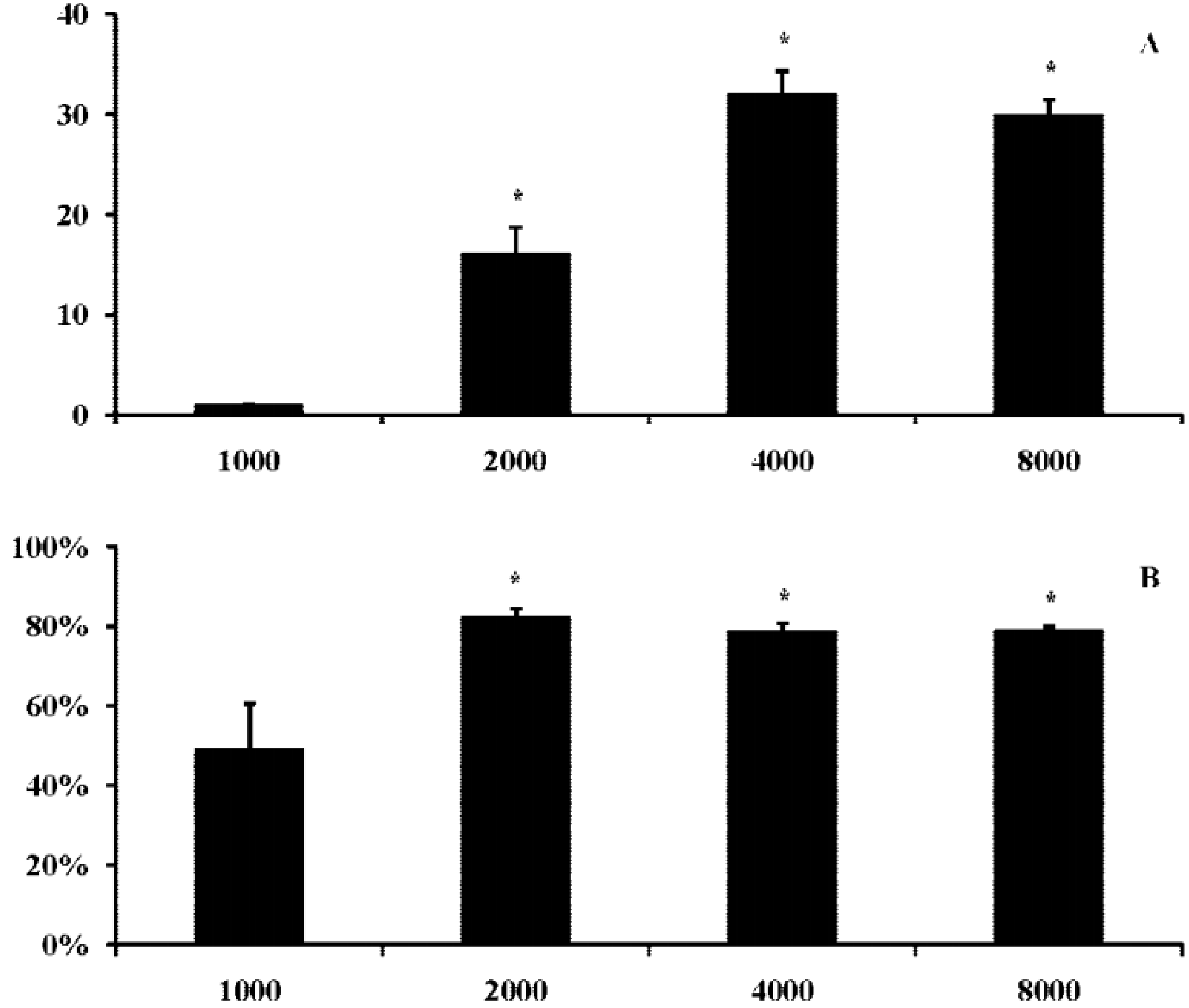
Host condition: stress

- Host stress may enhance M%
- *Utterbackia* M% on bluegill was 1/3 higher when plasma cortisol was elevated (Dubansky 2011 Biol. Bull. 220)
- Infection protocol affected cortisol and M%
- Recent test of fatmucket on bluegill showed smaller effect (see Platform #40 4:40 Monday Karel Douda).

Infection intensity and metamorphosis success

Attachment (glochidia/g)

Metamorphosis (percent of attached glochidia)



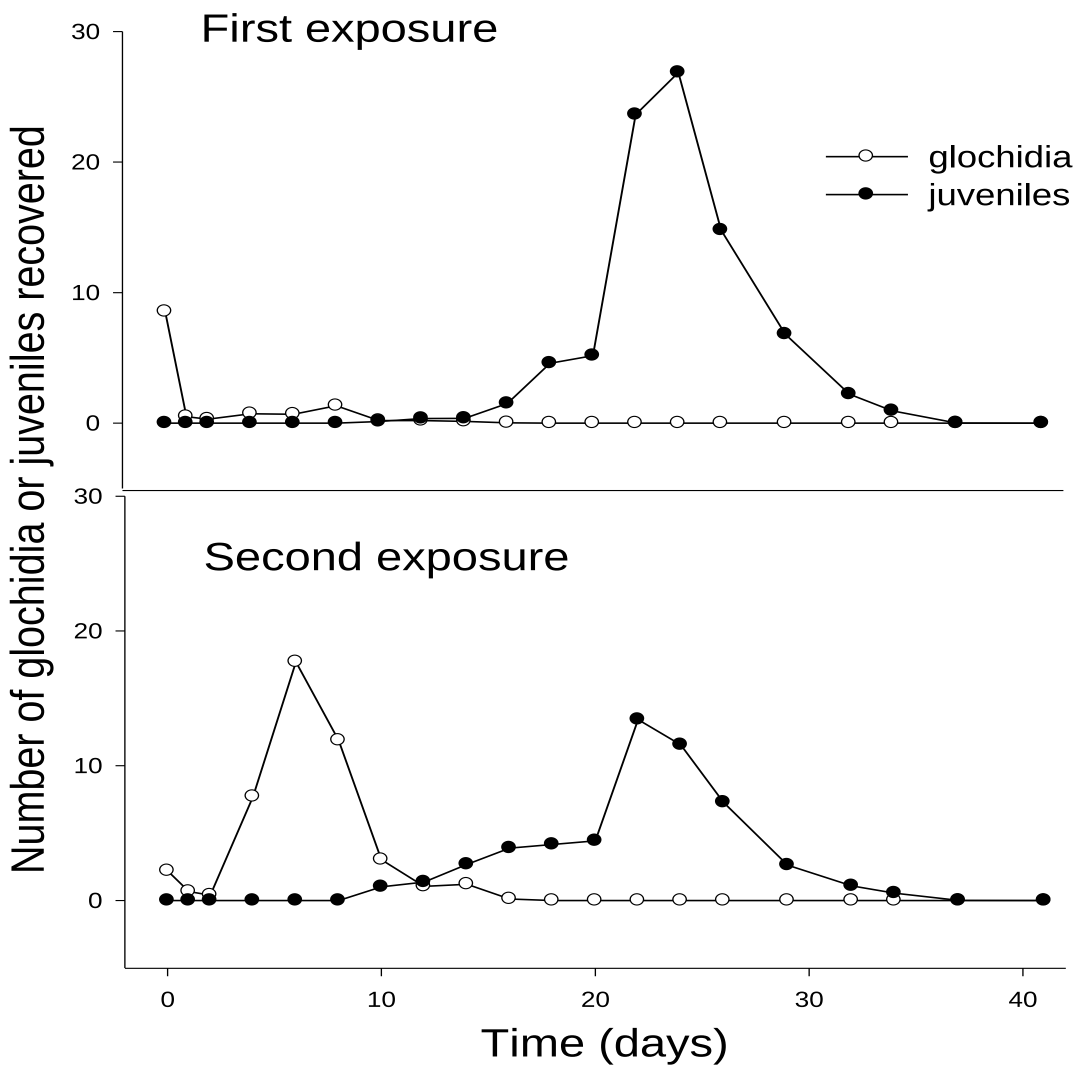
Inoculation (glochidia/L)

(Dubansky 2011 Biol. Bull. 220)

Immunity of fish to mussels

- Whether a fish species is a suitable host is determined mainly by the innate immune system (does not require previous exposure).
- Fish can also develop acquired immunity (antibody production) after repeated exposure. (e.g. Dodd et al. 2005)

Acquired immunity

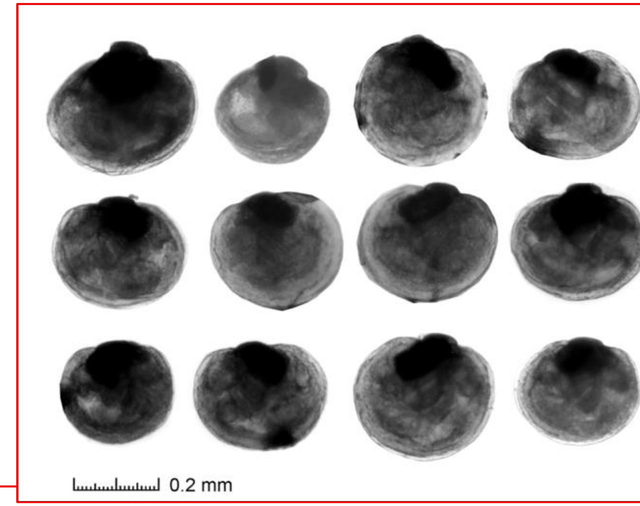


Temperature and metamorphosis

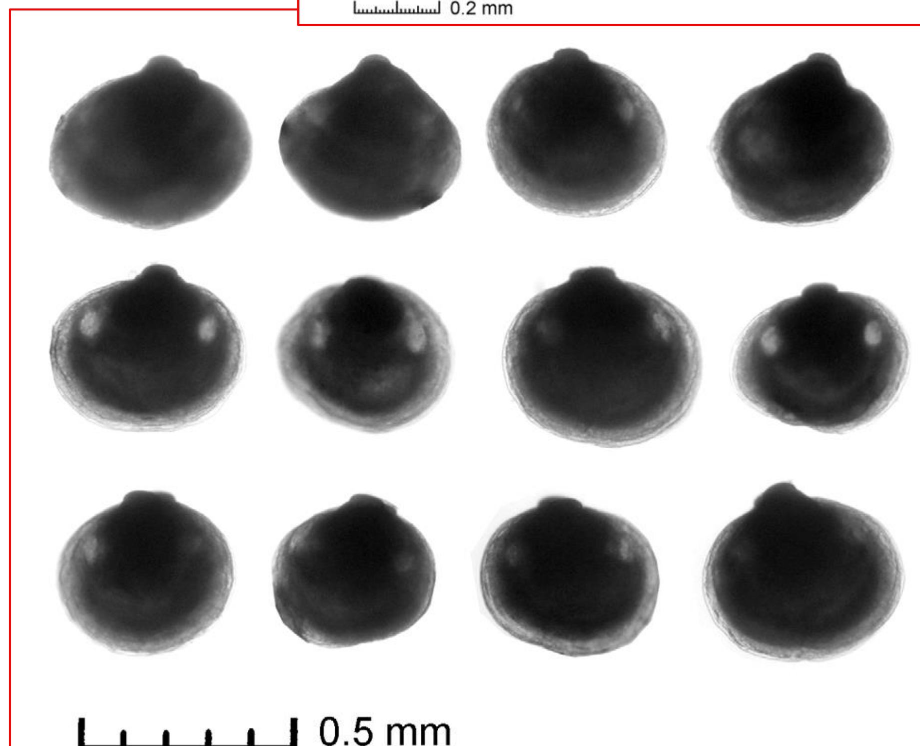
- Parasitic period can be prolonged by holding hosts at lower temperature.
- Very useful if you wish to recover juveniles at different times from the same batch of fish

Prolonging parasitic period with low temperature

A. Juvenile *Quadrula fragosa* (Saline River) from blue catfish kept at 23C. Metamorphosis was complete after about 12 days on the host. Mean length = 318 microns.



B. Same cohort but from host fish held at winter temperature. These juveniles spent about 4 months on the host. Mean length = 439 microns.



See also Steingraber et al. Am. Mid. Nat 157:297

Temperature and metamorphosis

- Lower temperature can also increase M% in some cases (e.g. Roberts JNABS 18:477)
- Recovery of juveniles is best achieved by raising temperature at the end for shorter drop-off period.

Getting glochidia

- Timing of mussel reproduction
- Transport and care of females
- Collecting glochidia
  - Premature vs mature
  - Glochidia maturity
  - Brood condition

- Spawning= release of gametes
- Brooding (=“gravid”)
  - Bradytictic= long-term brooder (~winter)
  - Tachytictic= short-term brooder (spring, summer, or fall)
- Release of glochidia
- Attachment
- Encapsulation (encystment)
- Drop-off of juveniles

Timing of reproduction

- Margaritiferidae
  - short-term spring brooders
  - *Cumberlandia monodonta* April-May
  - *Margaritifera falcata, M. hembeli, M. marrianae*, likewise April-May: recent observations by Jeanette Howard, Tony Brady, Paul Johnson

Jeanette Howard

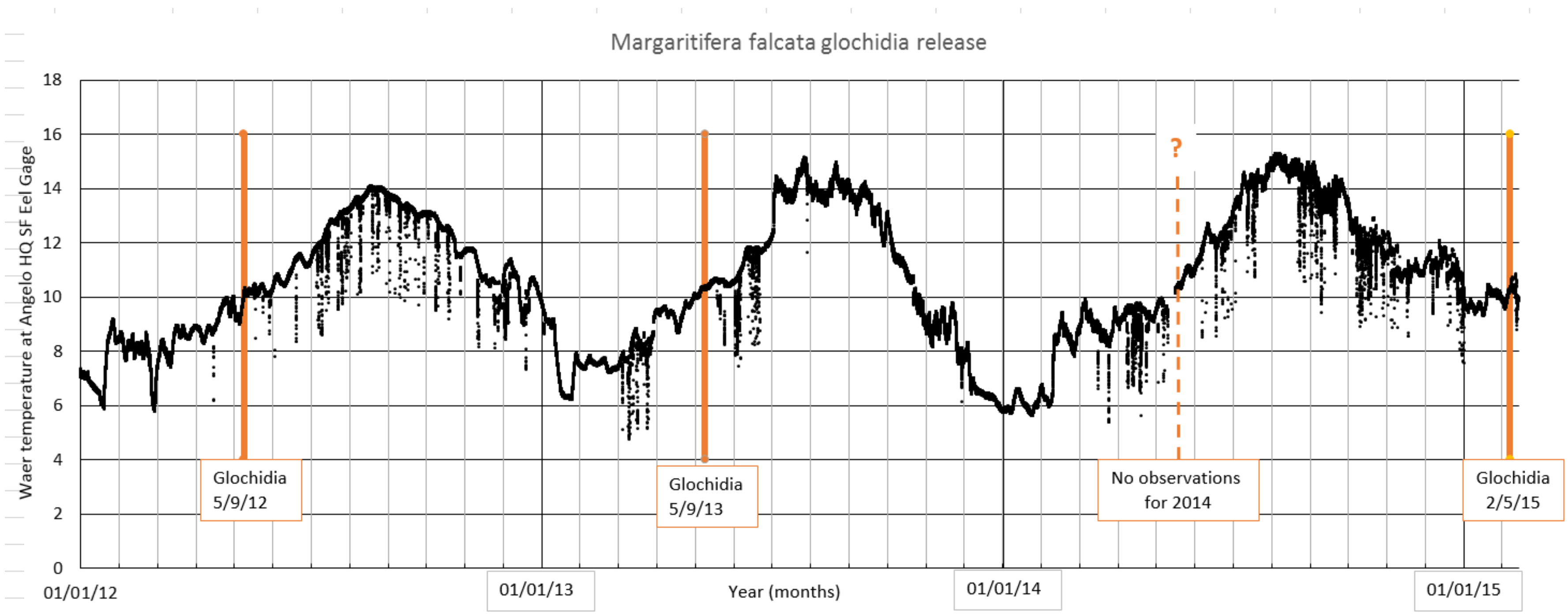
The Nature Conservancy

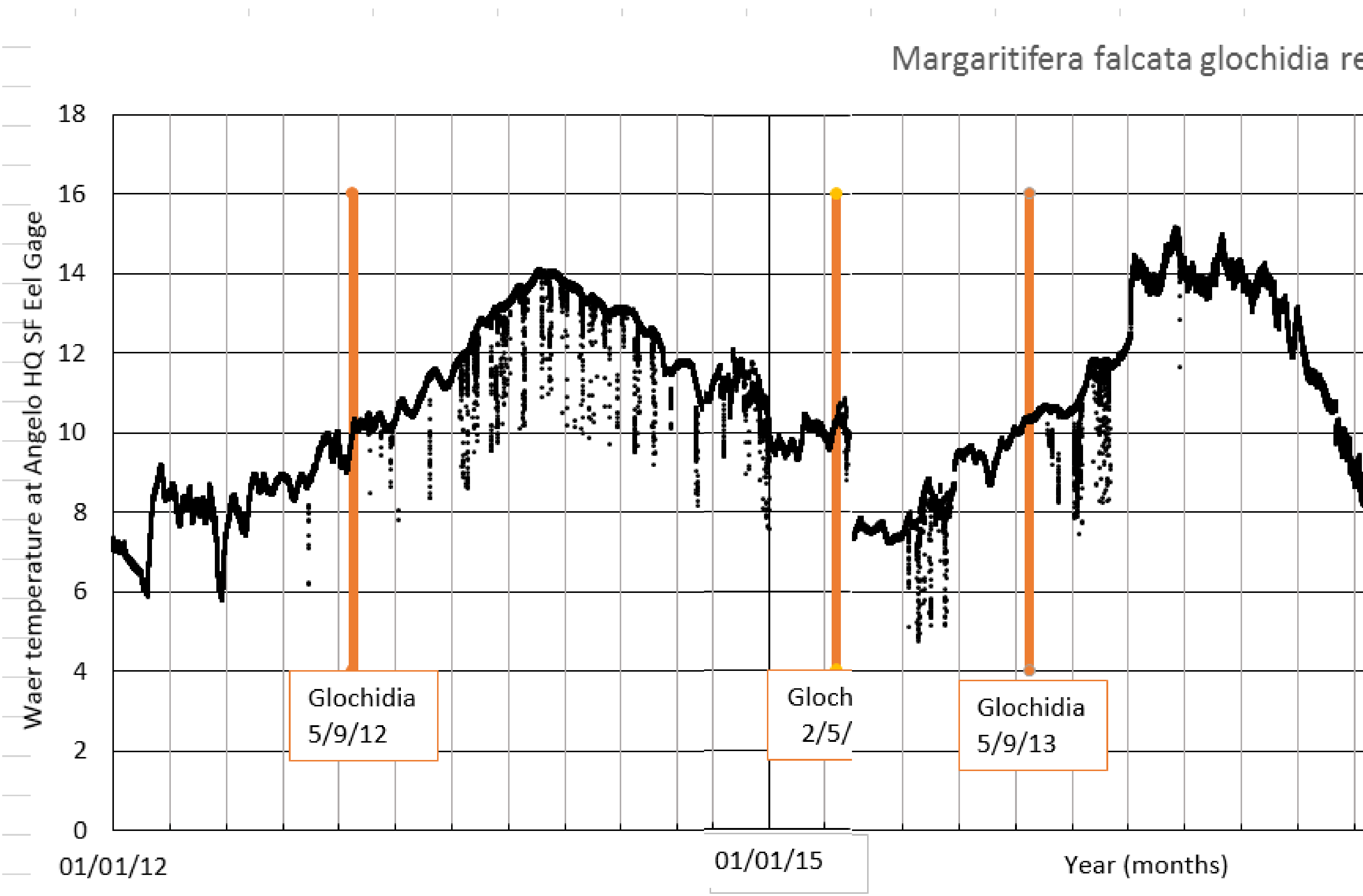
Margaritifera falcata

South Fork Eel River, Mendocino Co. CA

Margaritifera falcata

- Spawn in the spring release glochidia shortly after water temperature exceeds 10C.





Margaritifera falcata glochidia re
Waer temperature at Angelo HQ SF Eel Gage
18
16
14
12
10
8
6
4
2
0
Glochidia
5/9/12
Gloch
2/5/
Glochidia
5/9/13
01/01/12
01/01/15
Year (months)

Margaritifera falcata brooding females shipped to MSU,
Held in chilled recirc. system
11 C >>>>> 13 C

Conglutinates of eggs containing mature glochidia, released at 13C

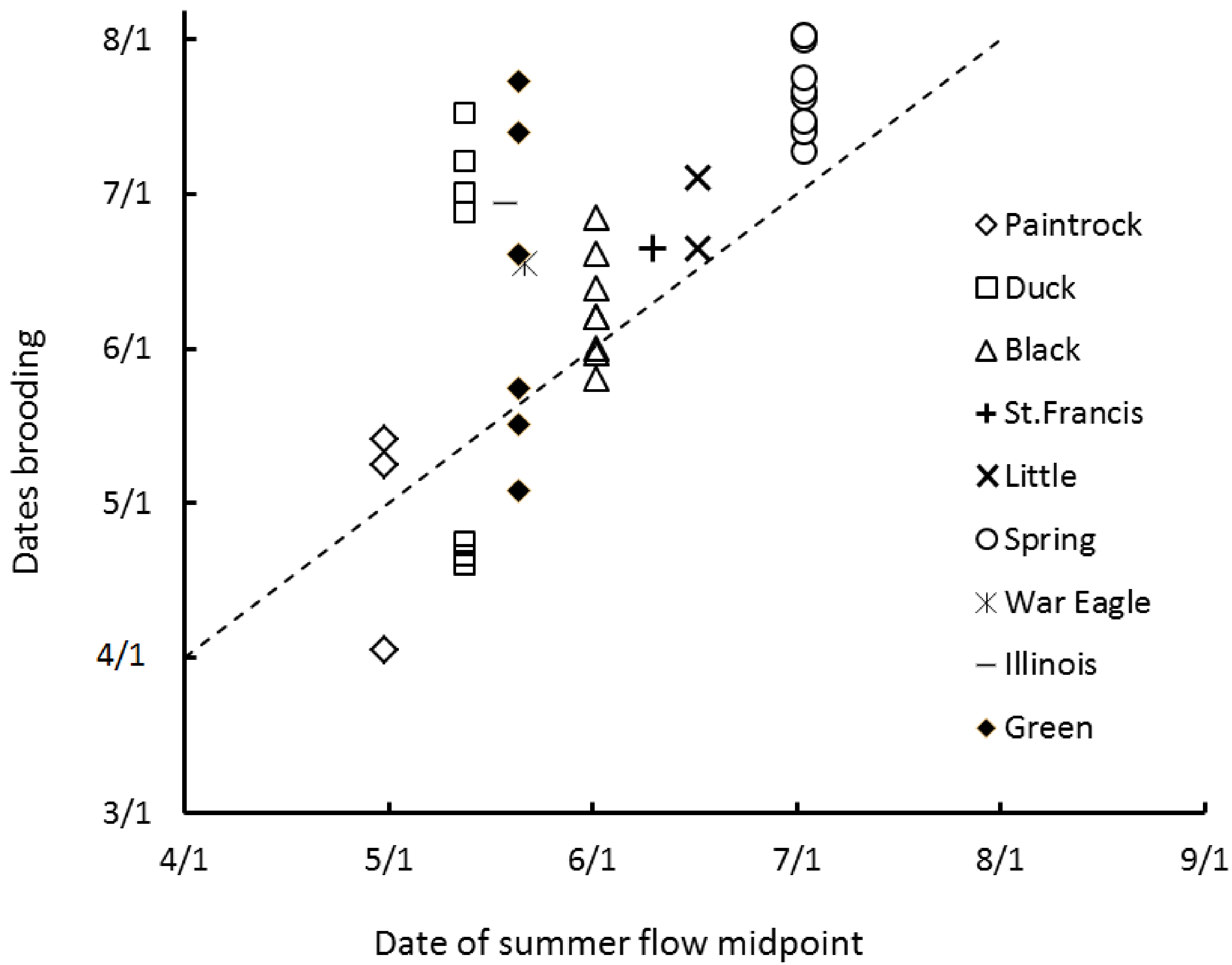
Timing of reproduction

- Lampsilini, Anodontini
  - Most are long term brooders: spawn in late summer, brood until following spring-midsummer
  - Examples of exceptions, e.g.
    Lampsilis rafinesqueana June-August
    Truncilla truncata April-June
    Obliquaria reflexa April-July

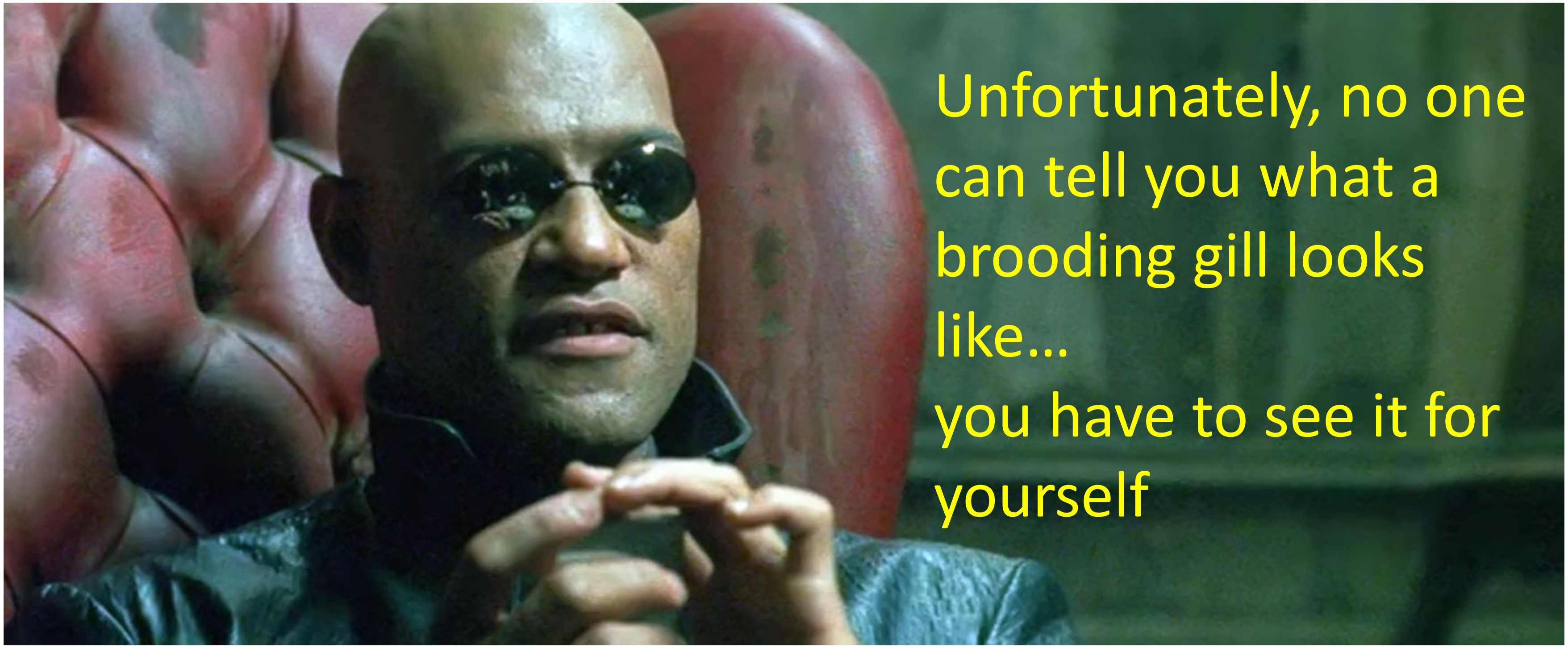
Timing of reproduction

- Quadrulini, Pleurobemini
  - Short-term brooders, timing is variable
  - *Q. pustulosa* in July
  - *Quadrula fragosa* in October
  - *Megalonaias nervosa* in November-December
  - *Quadrula cylindrica* very wide range April-July (see Poster #17)
  - Multiple broods in some *Elliptio* (Price and Eads)

Brooding in *Quadrula cylindrica*



Recognizing brooding females



TETRAGENOUS = brood in all four demibranchs
Margaritiferidae, Quadrulini, Pleurobemini, Gonideini

Washboard
Megalonaias nervosa (Quadrulini)

Anodontini, Lampsilini brood in outer demibranchs only (DIAGENOUS)

Flat floater

Anodonta suborbiculata

Gravid outer demibranch

Creeper

Strophitus undulatus

Gravid outer demibranch

Entire demibranch is marsupial in most Anodontini and Amblemines

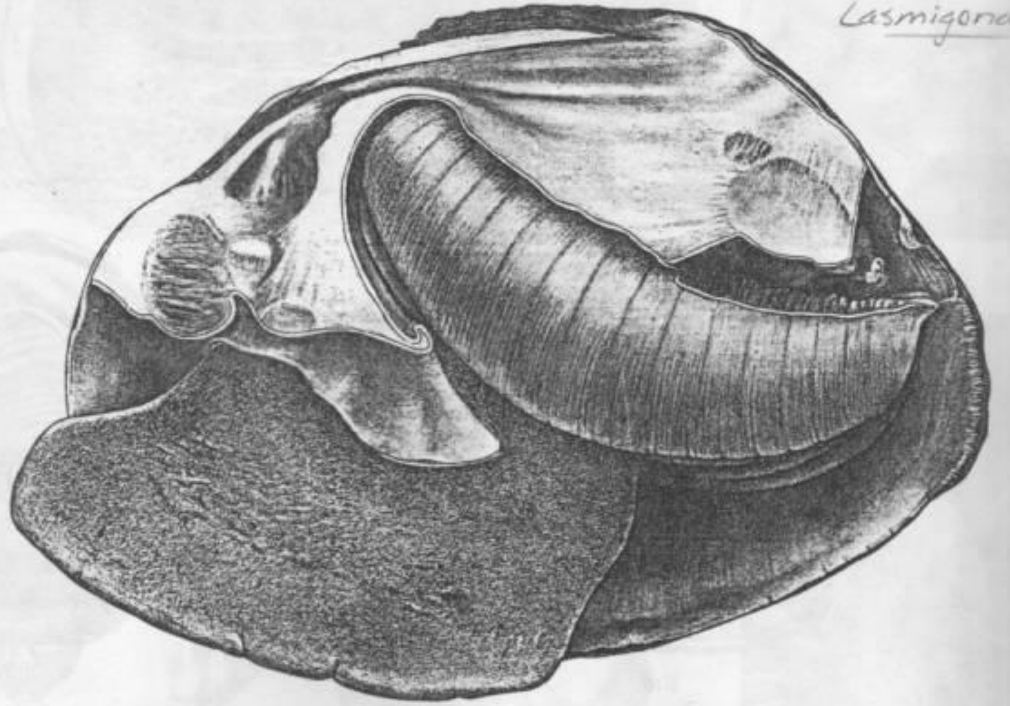


FIG. 3.

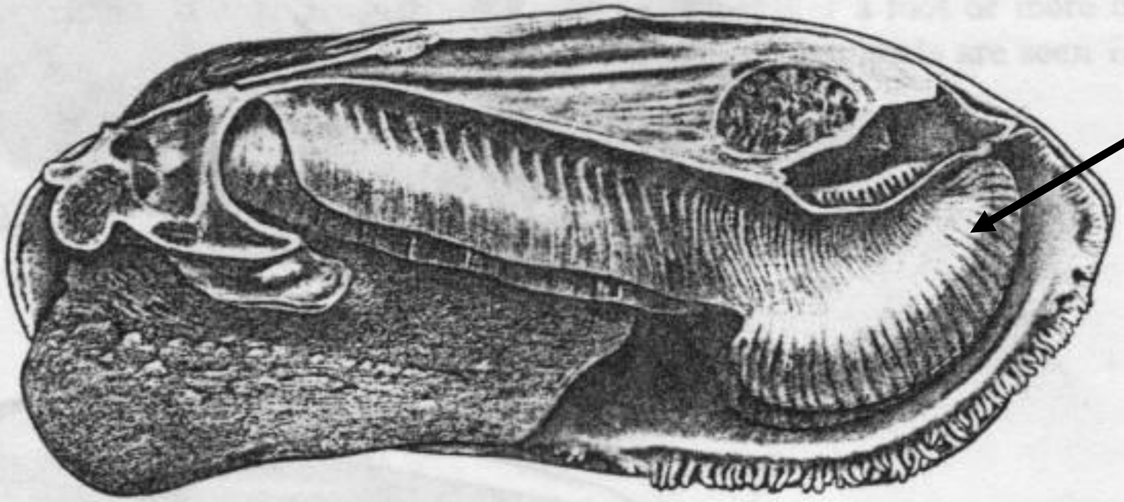


FIG. 6.

Only posterior portion is marsupial in most Lampsilini

Most Lampsilini use only the posterior portion of the outer demibranchs

Yellow sandshell
Lampsilis teres

Gravid gill of *Obliquaria*

Getting glochidia

- Timing of mussel reproduction
- Recognizing brooding females
- Transport and care of females
- Collecting glochidia
  - Premature vs mature
  - Brood maturity and condition
  - Removing larvae
  - Freeing larvae from conglutinates

Getting glochidia

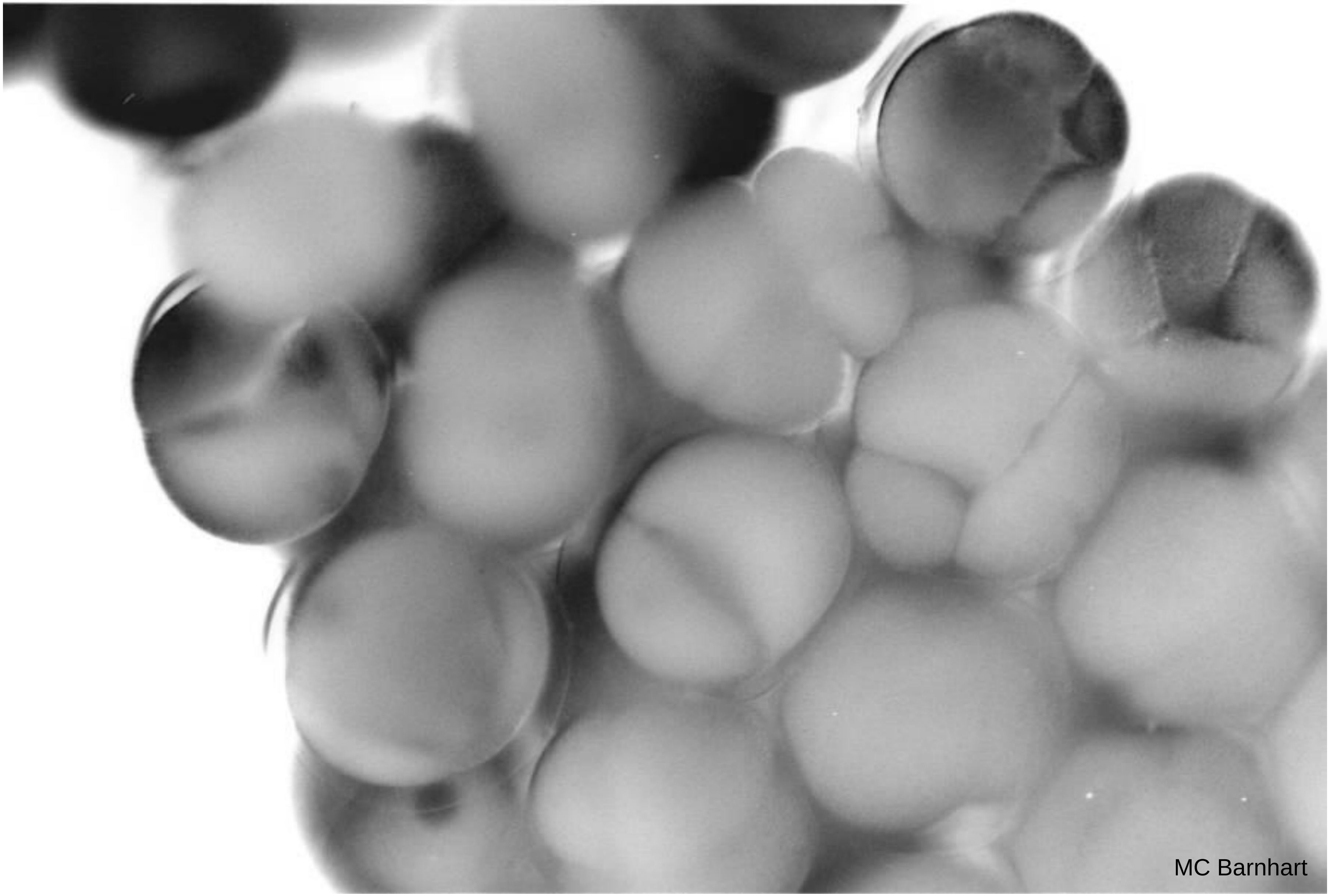
- Timing of mussel reproduction
- Recognizing brooding females
- Transport and care of females
- Collecting glochidia
  - Premature vs mature
  - Glochidia maturity
  - Brood condition

Development

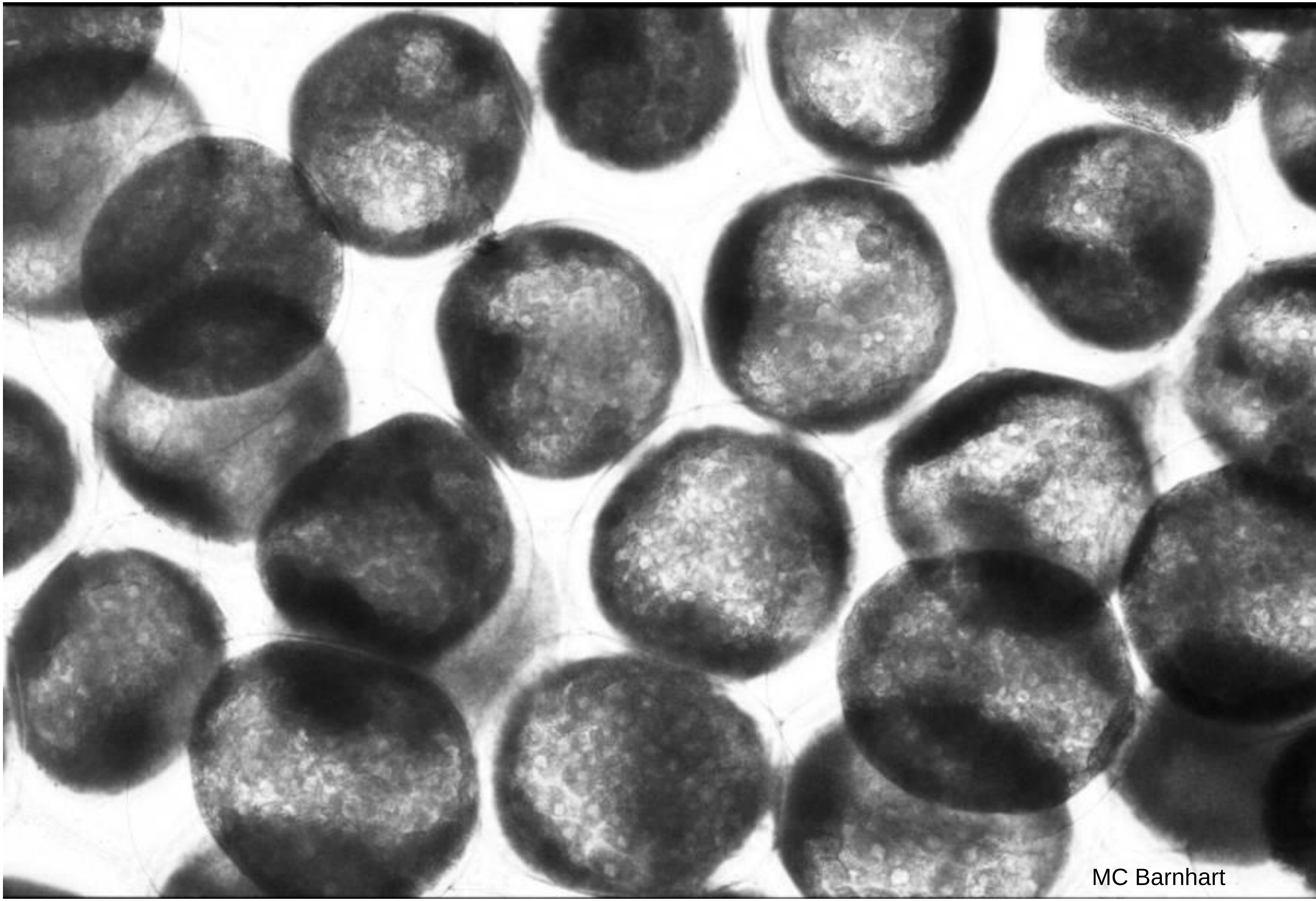
- Development of larvae in eggs requires ~2-4 weeks depending on species & temperature
- This is slow compared to marine bivalves (e.g. 24 h in oyster)
- Development is important issue especially when working with short-term brooders

1. Cleavage

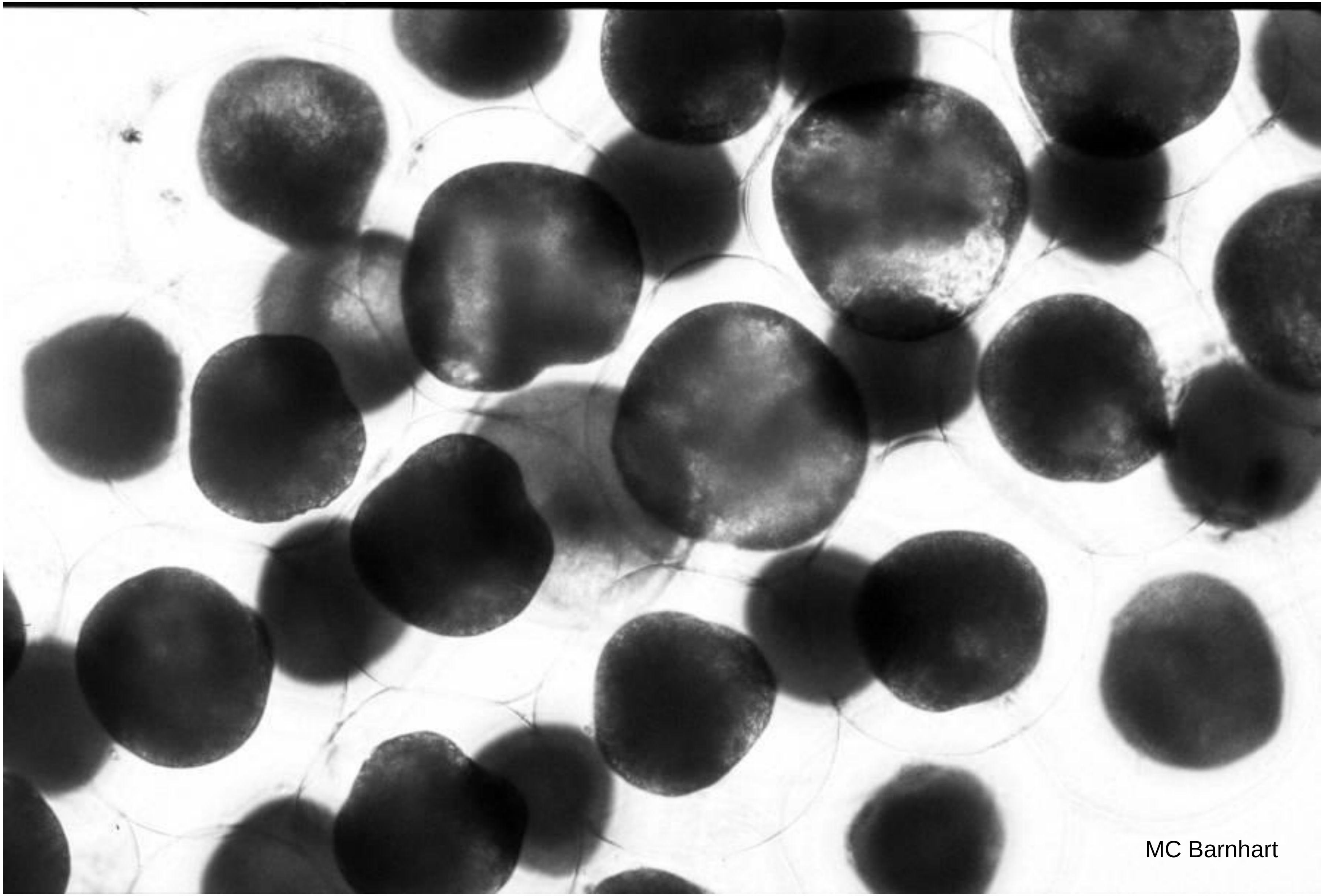
Elliptio dilatata



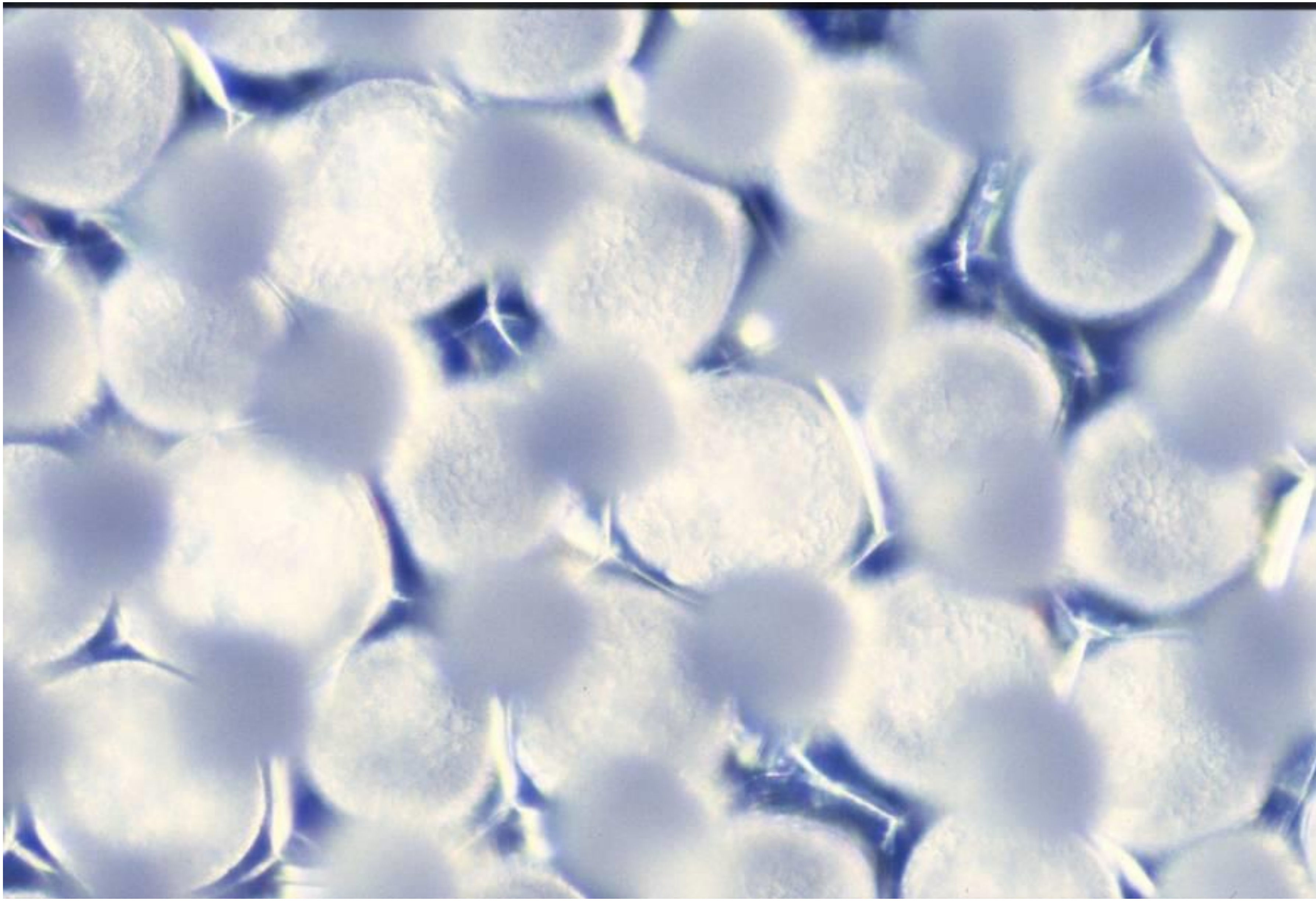
2. Blastulae (1-2 days) *Elliptio dilatata*



3. Gastrulation (gastrulae smaller and darker)

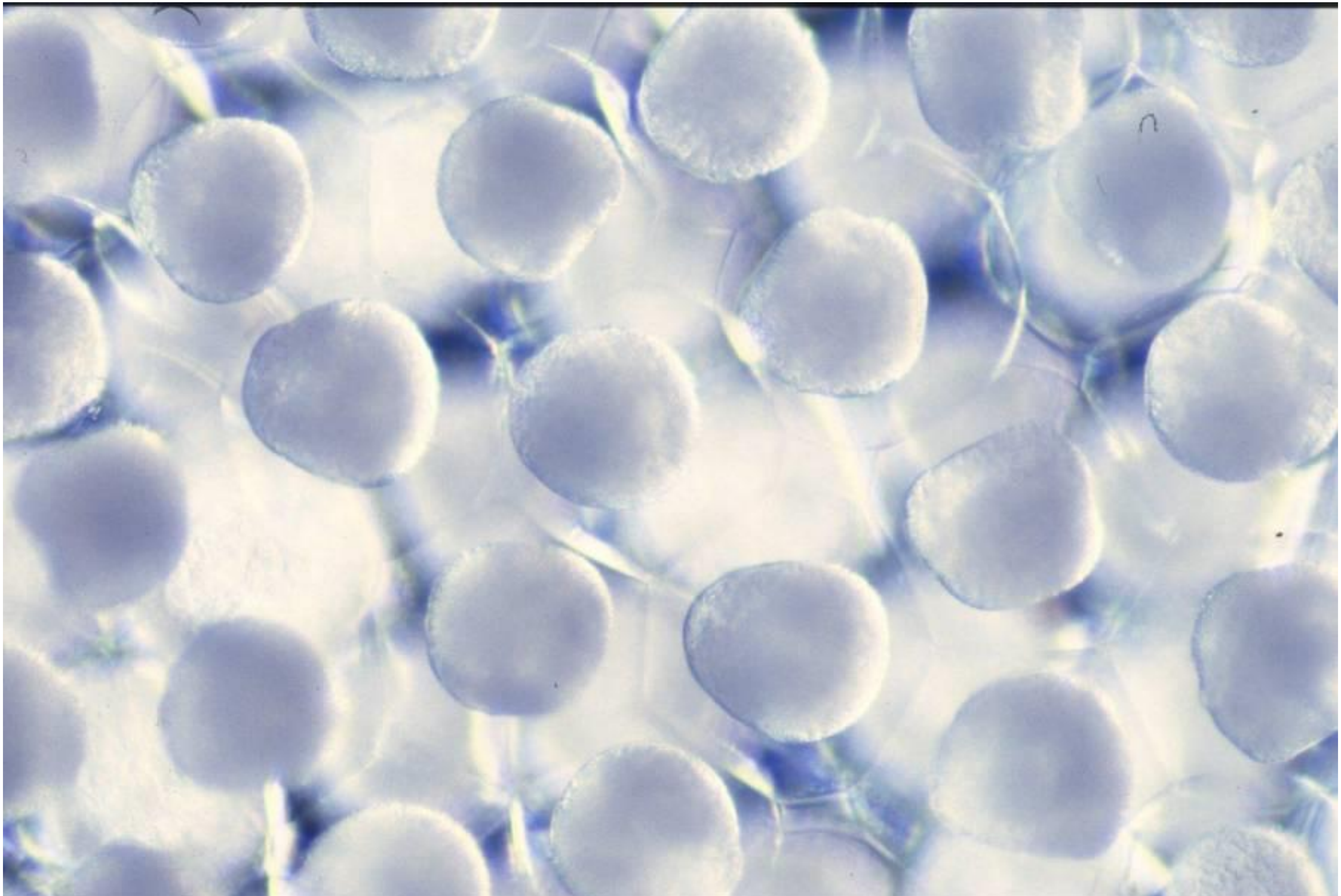


Blastulae *Elliptio dilatata*

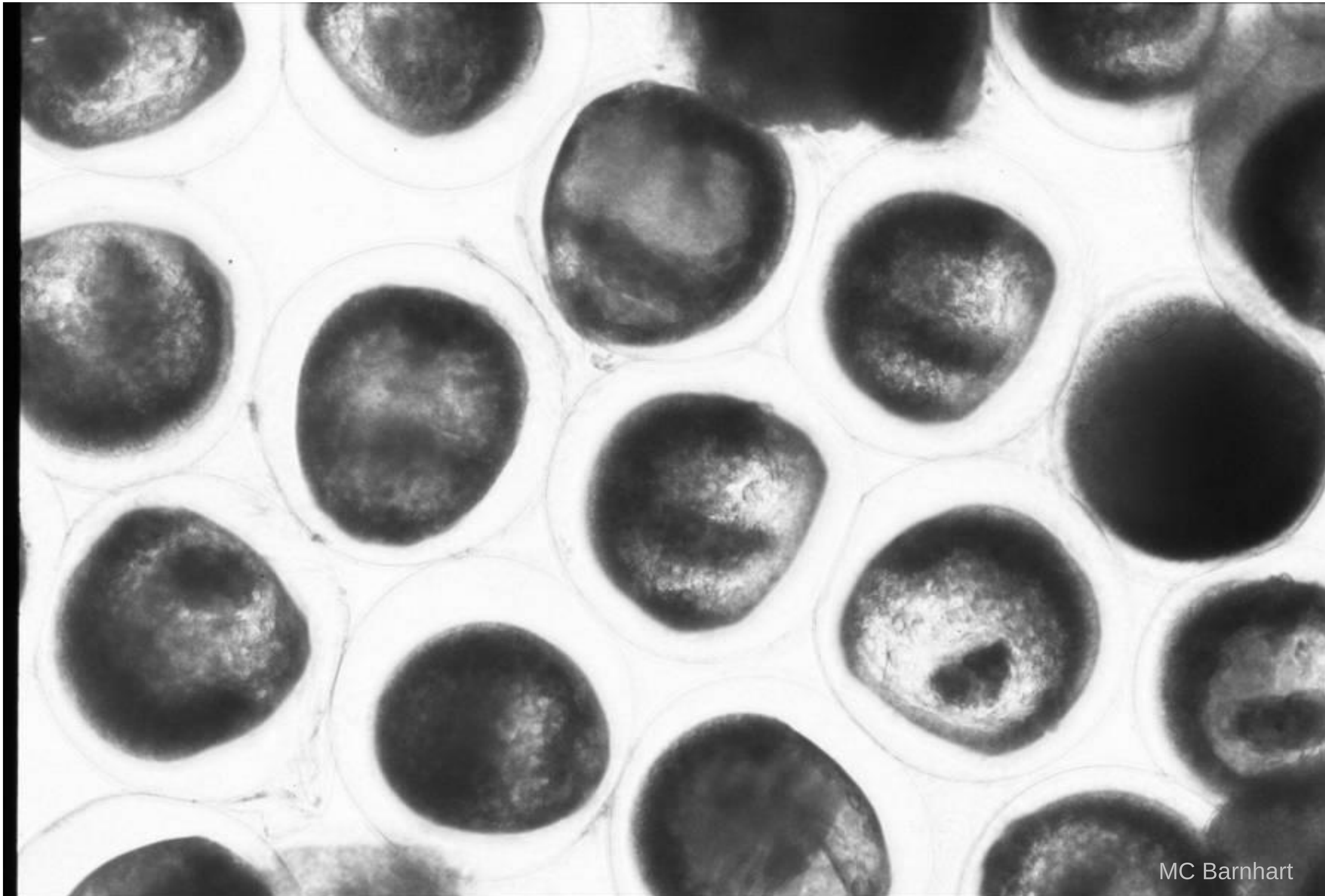


Gastrulae (~3 days)

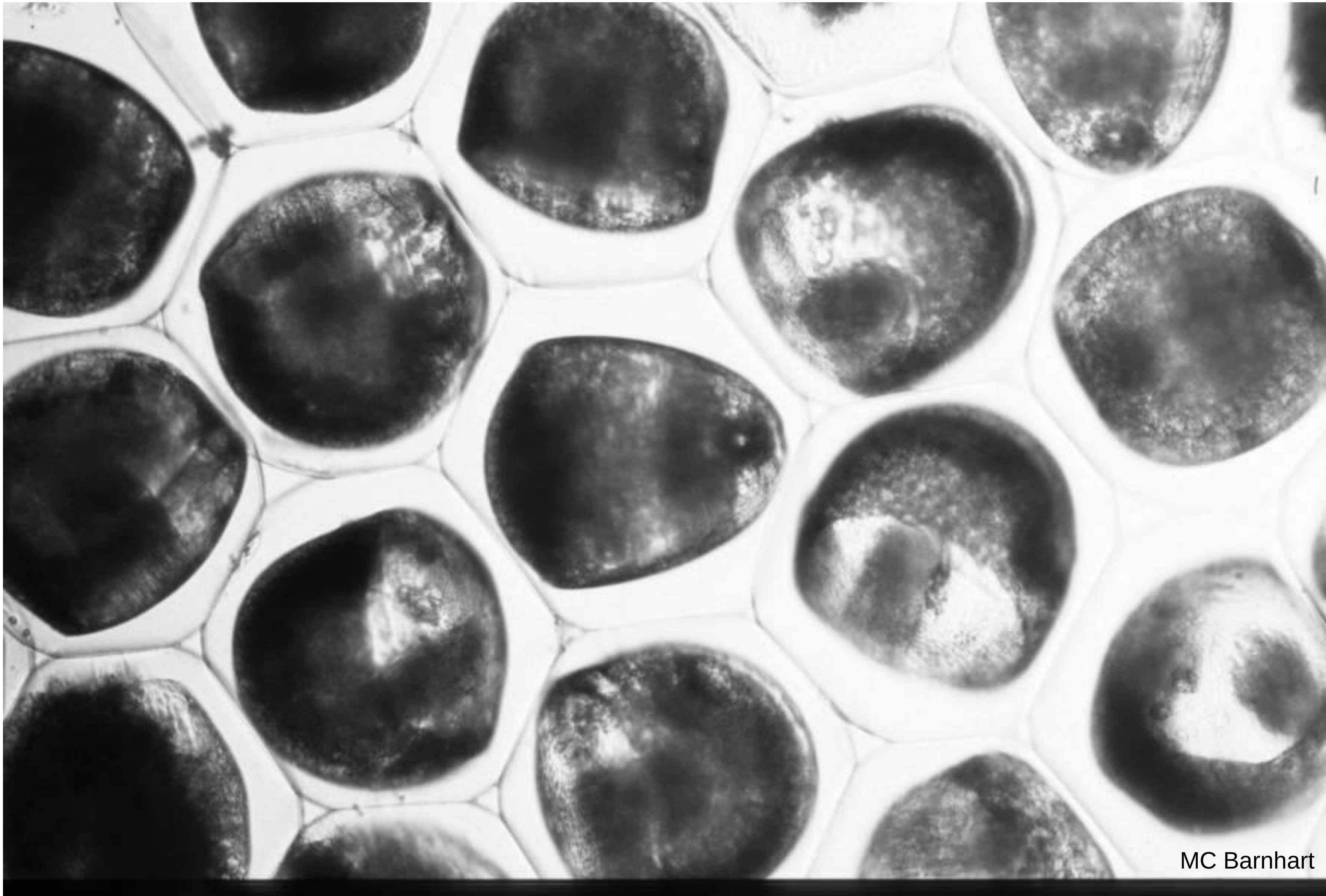
Elliptio dilatata



4. Adductor forming *Elliptio dilatata*



5. **Ventral cilia** rotate embryo, no gape yet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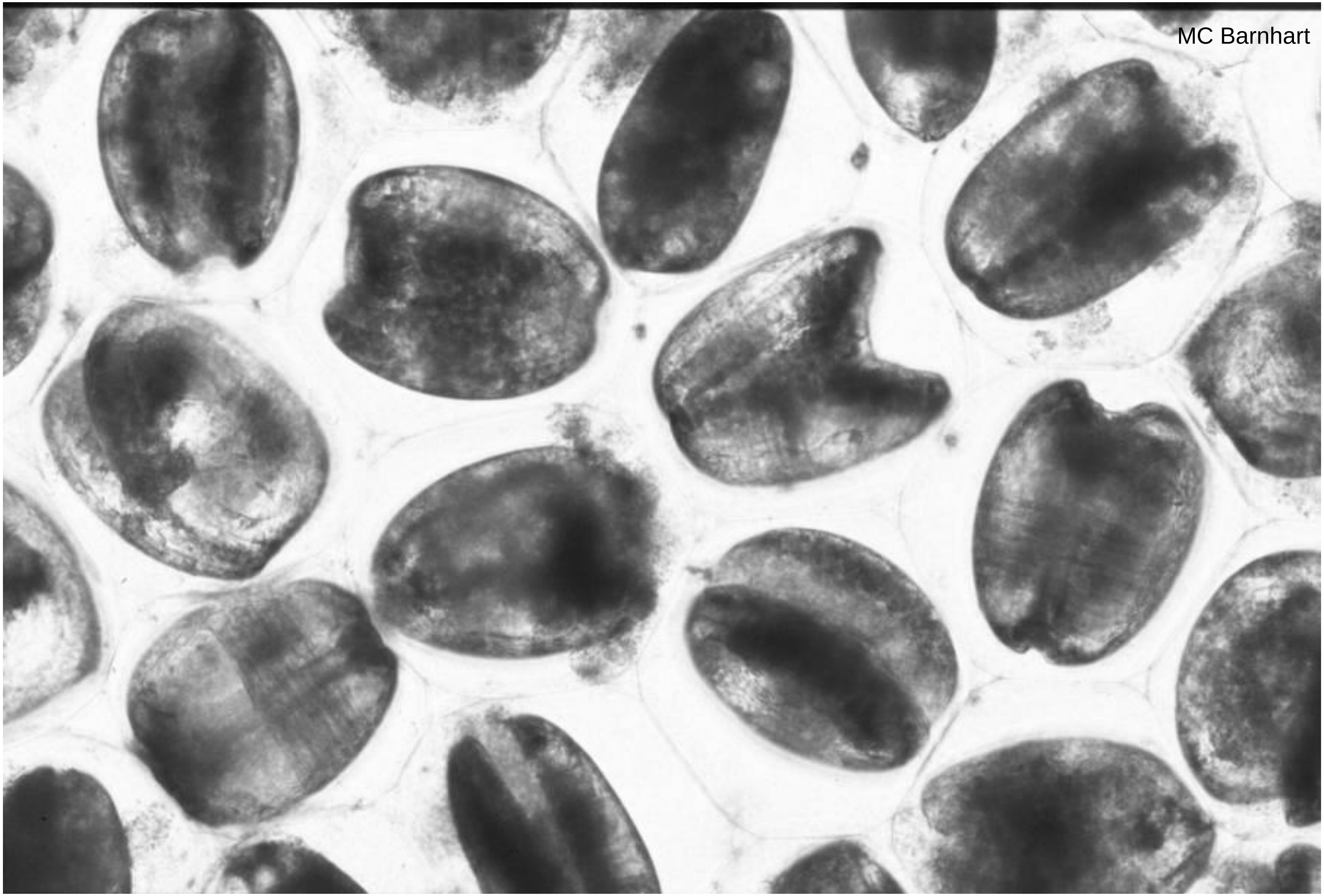
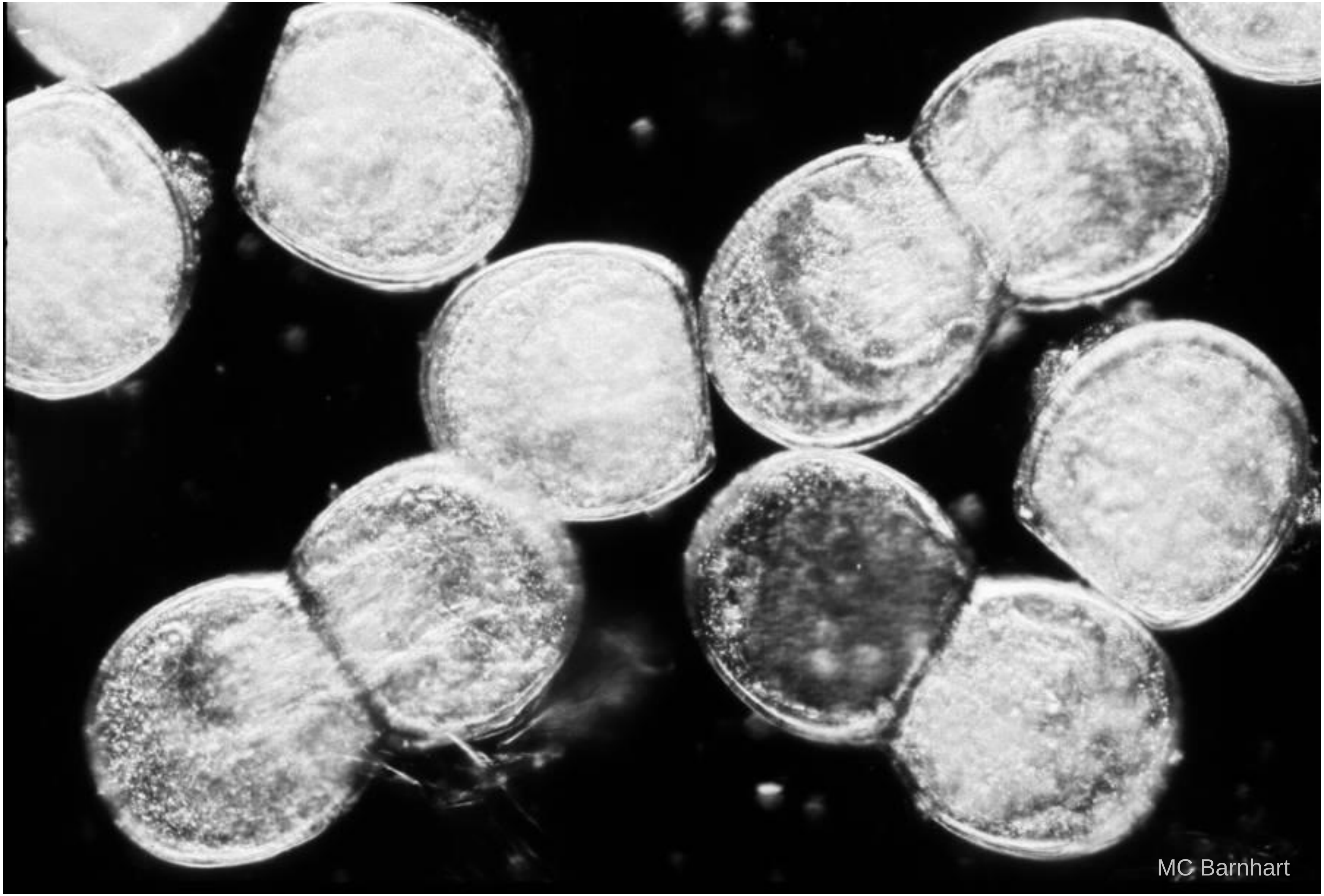
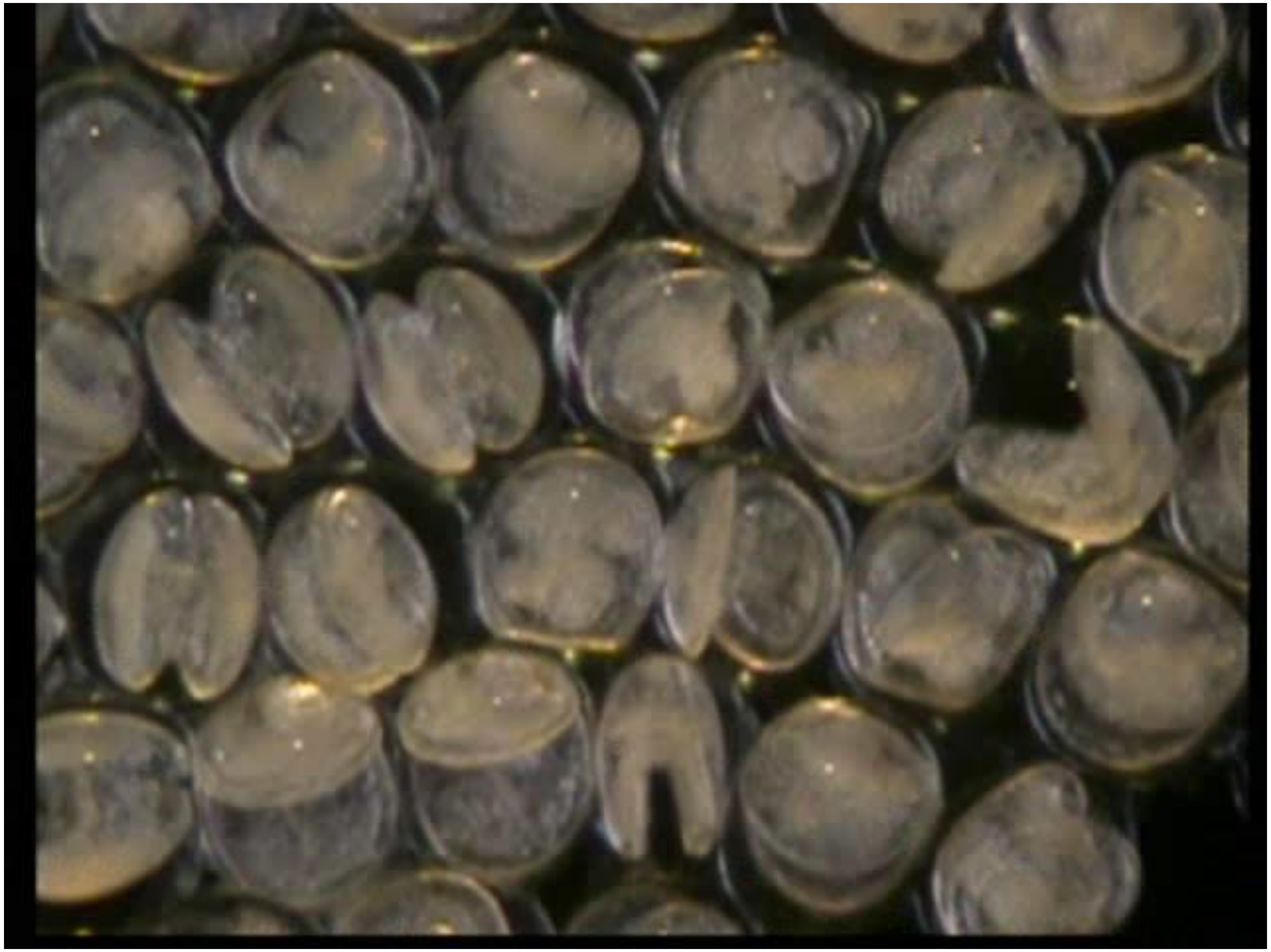
6. Ventral gape forms (~ 6-d) *Elliptio dilatata*



7. Mature glochidia (~2 wk) *Elliptio dilatata*



Important: use mature glochidia



http://youtu.be/GTEZuFChWTk

Avoiding premature release

- Particular issue for short-term brooders
  - “abortion” of immature conglutinates induced by disturbance
- Stabilize females during transport
  - Isolate in tall, narrow containers submerged within larger aerated volume of water
- Use native water for transport and holding
  - Avoid abrupt temperature change
  - Gradual water changes only

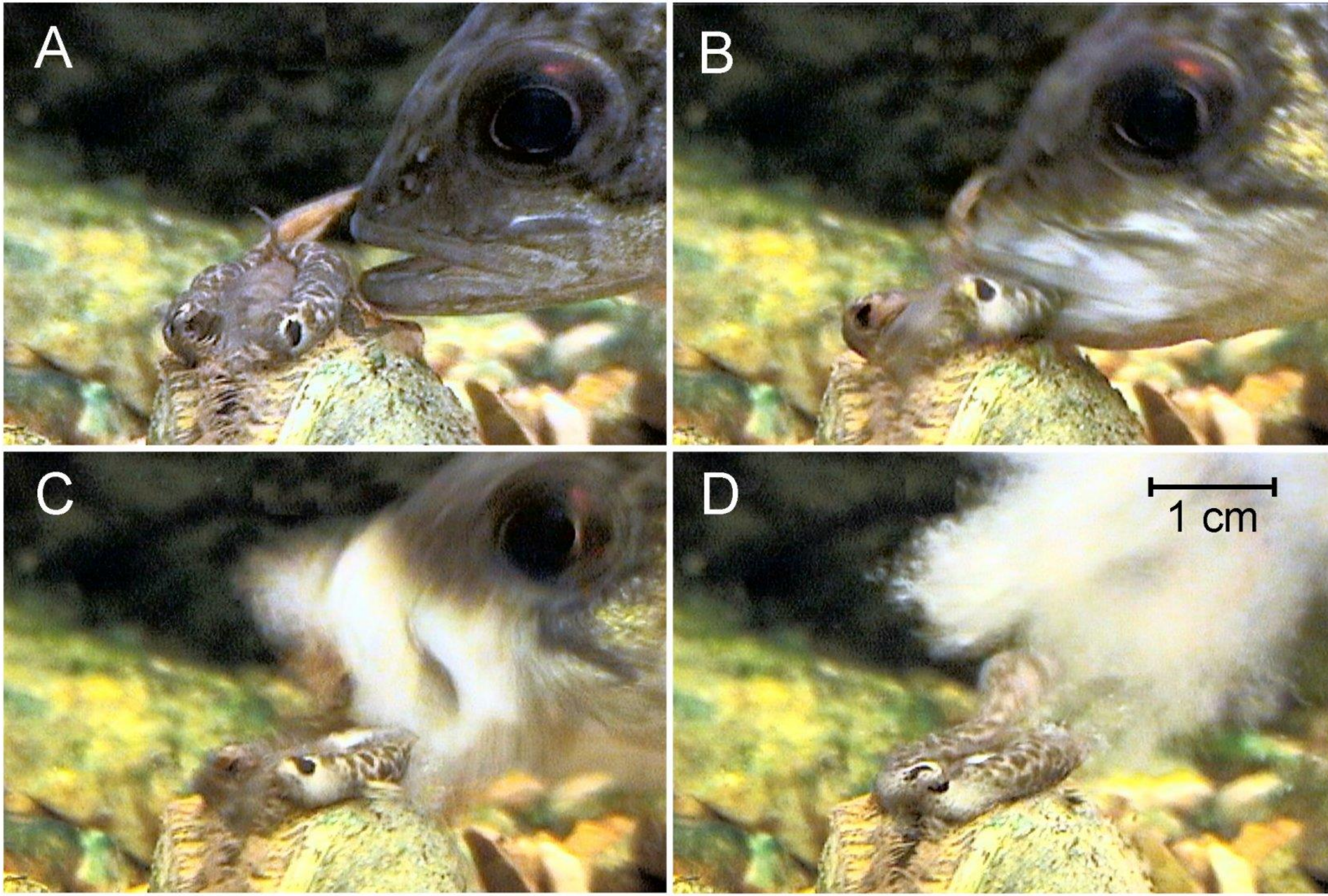
Transporting females

- Fill to submerge the inner containers- weight or restrain them so they do not float
- Aerate the larger volume of water so that it circulates slowly over the inner containers

Harvesting glochidia from gills

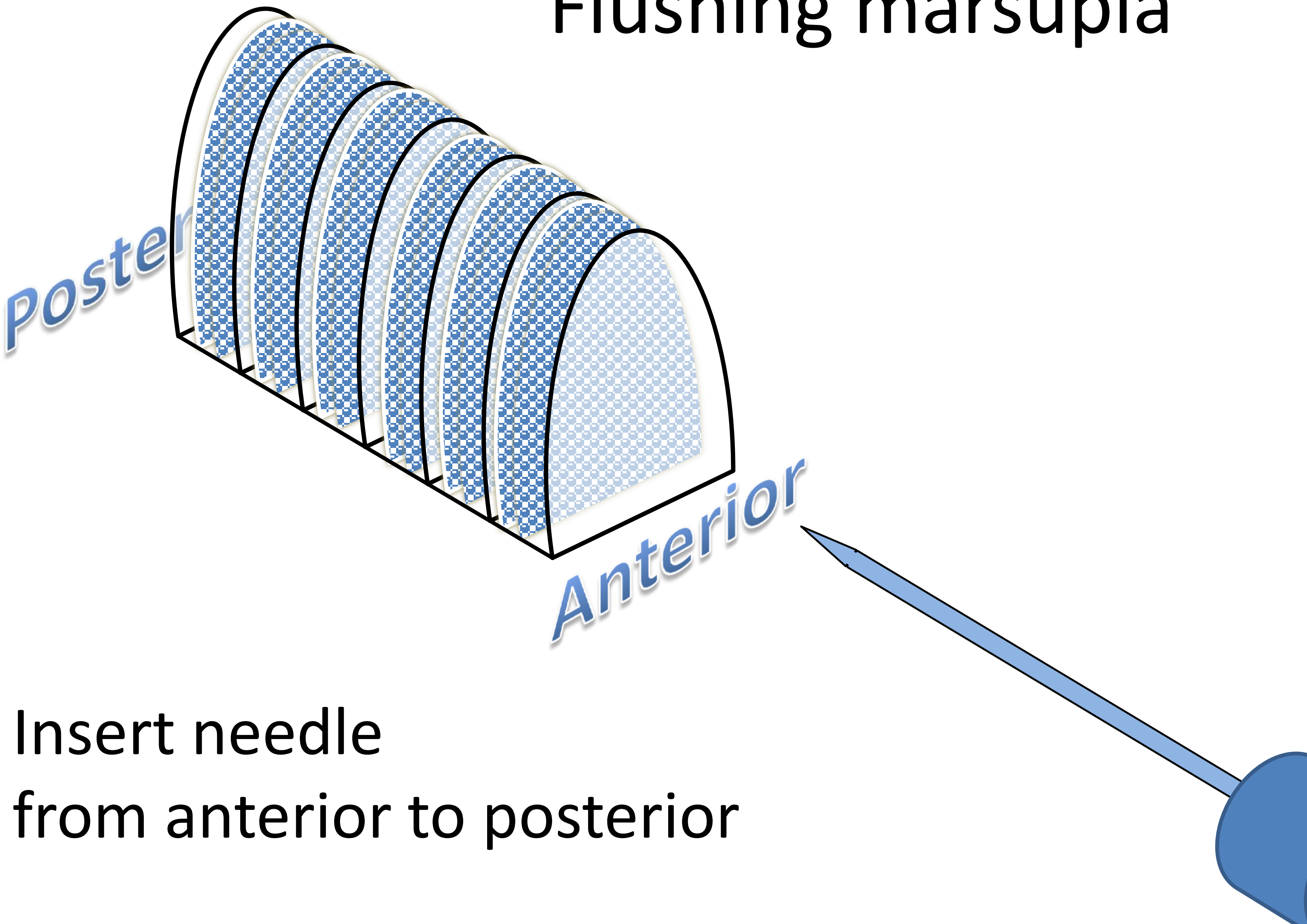
Harvesting glochidia

Lampsilis gills can take a lot of abuse



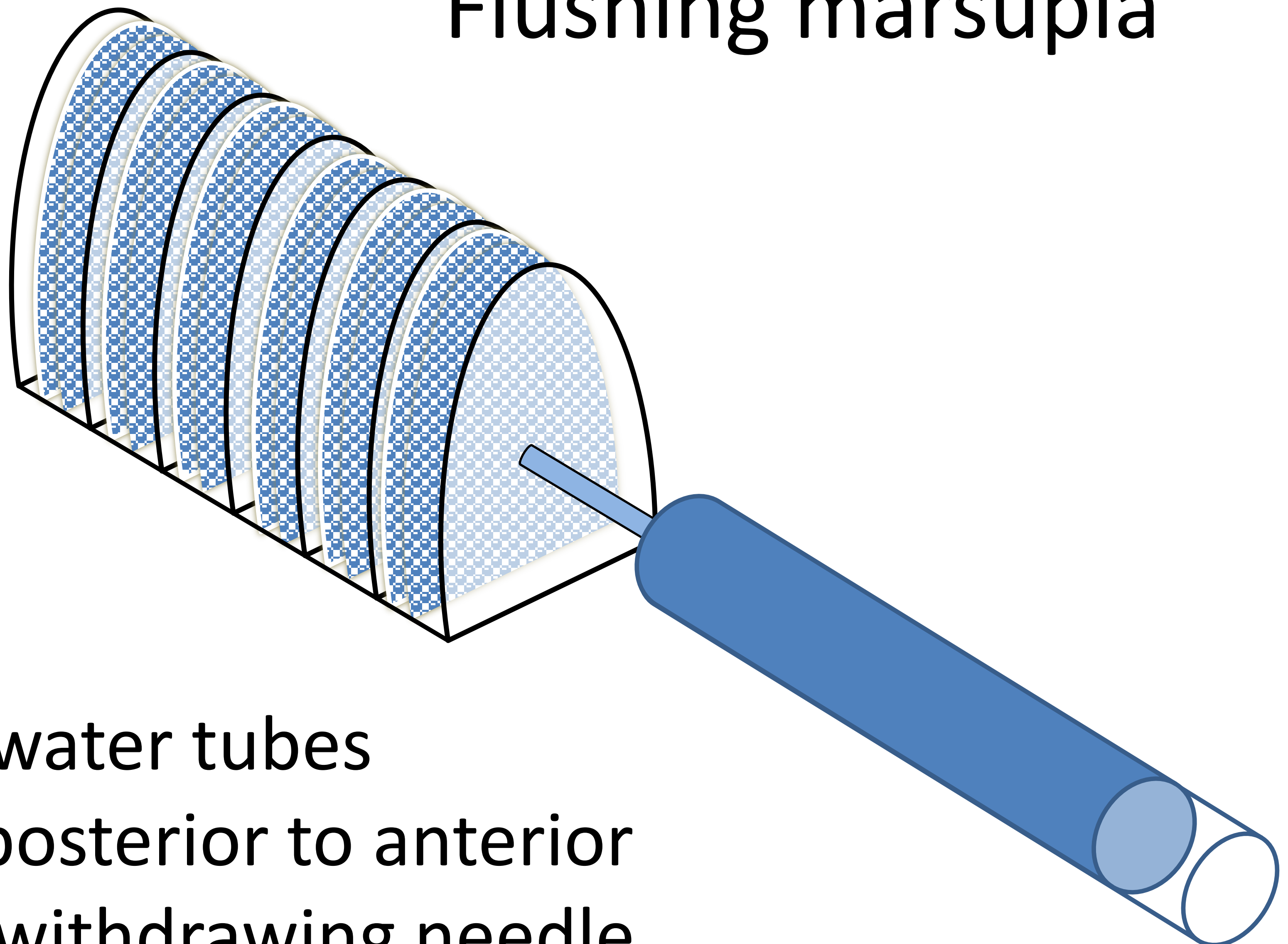
From Barnhart et al. 2008 JNABS

Flushing marsupia



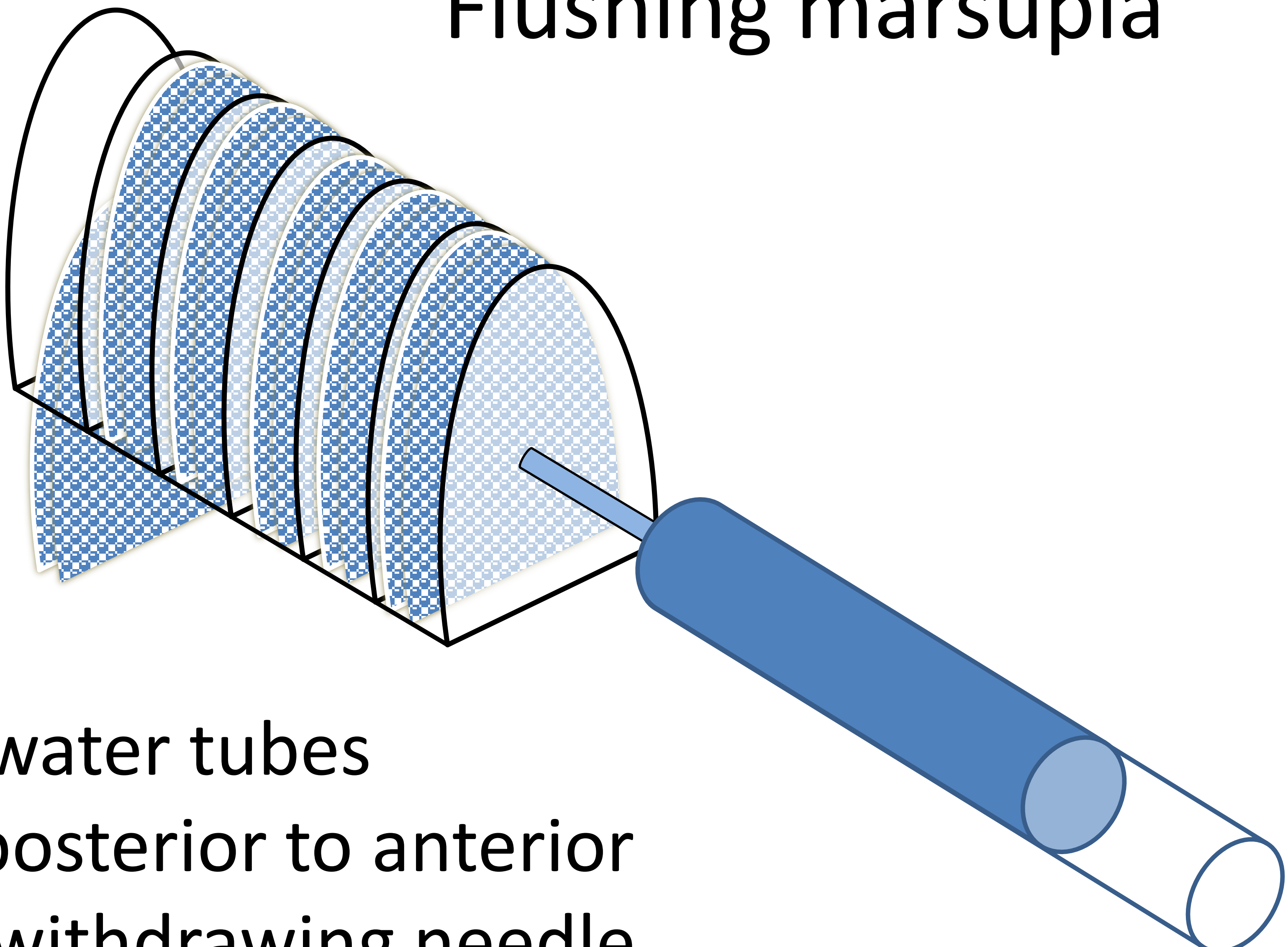
Insert needle
from anterior to posterior

Flushing marsupia



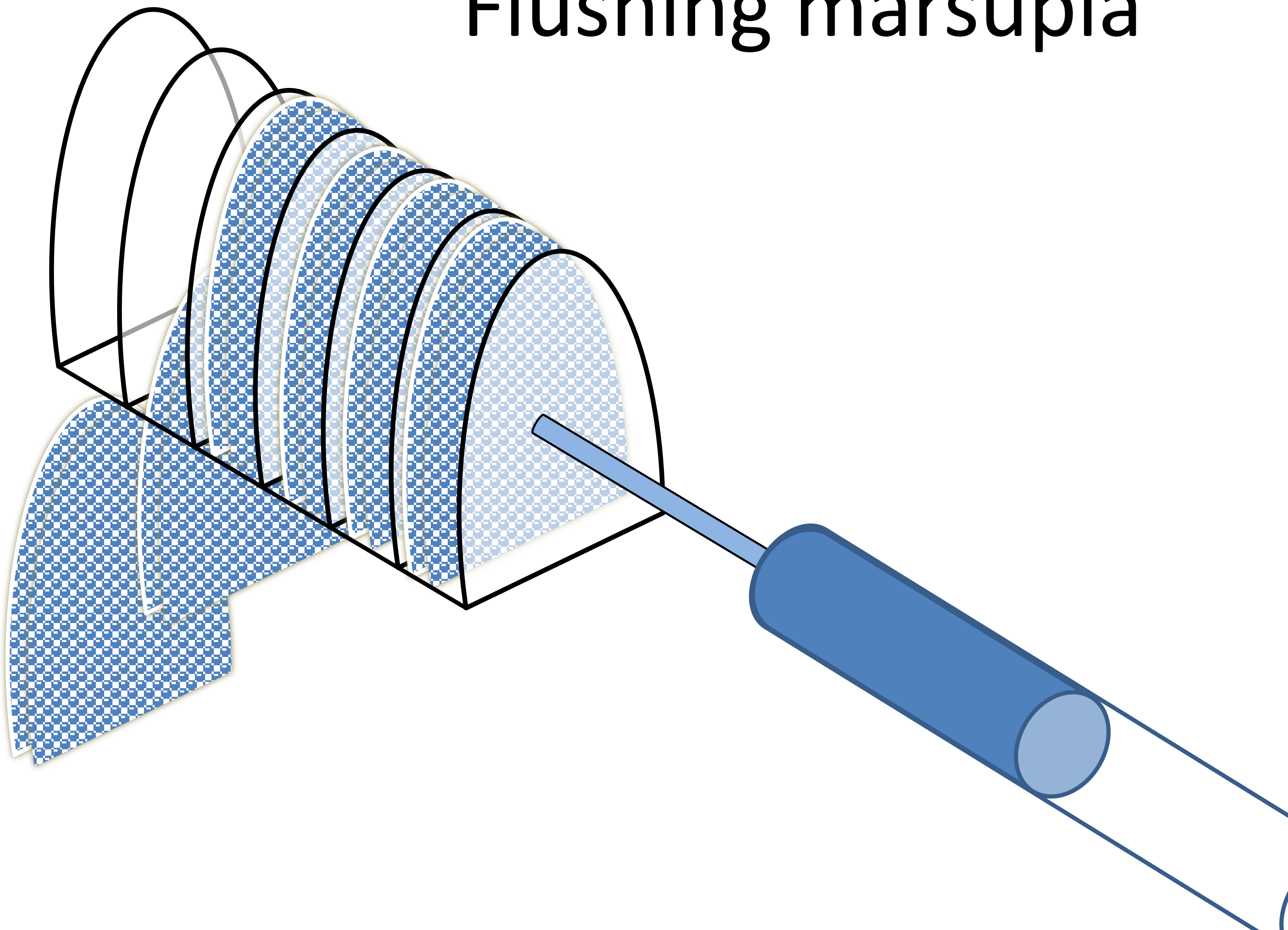
Flush water tubes
from posterior to anterior
while withdrawing needle

Flushing marsupia

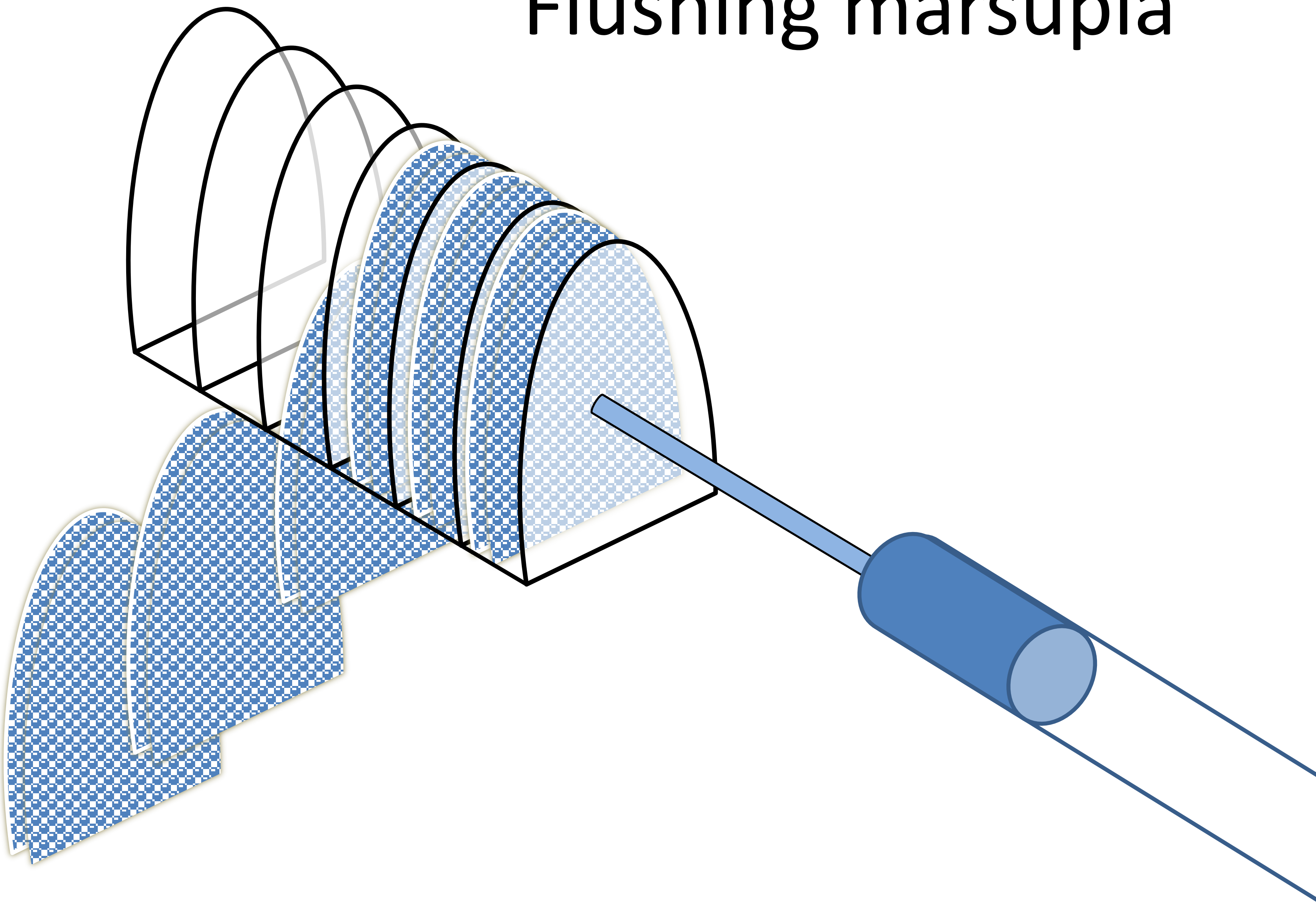


Flush water tubes
from posterior to anterior
while withdrawing needle

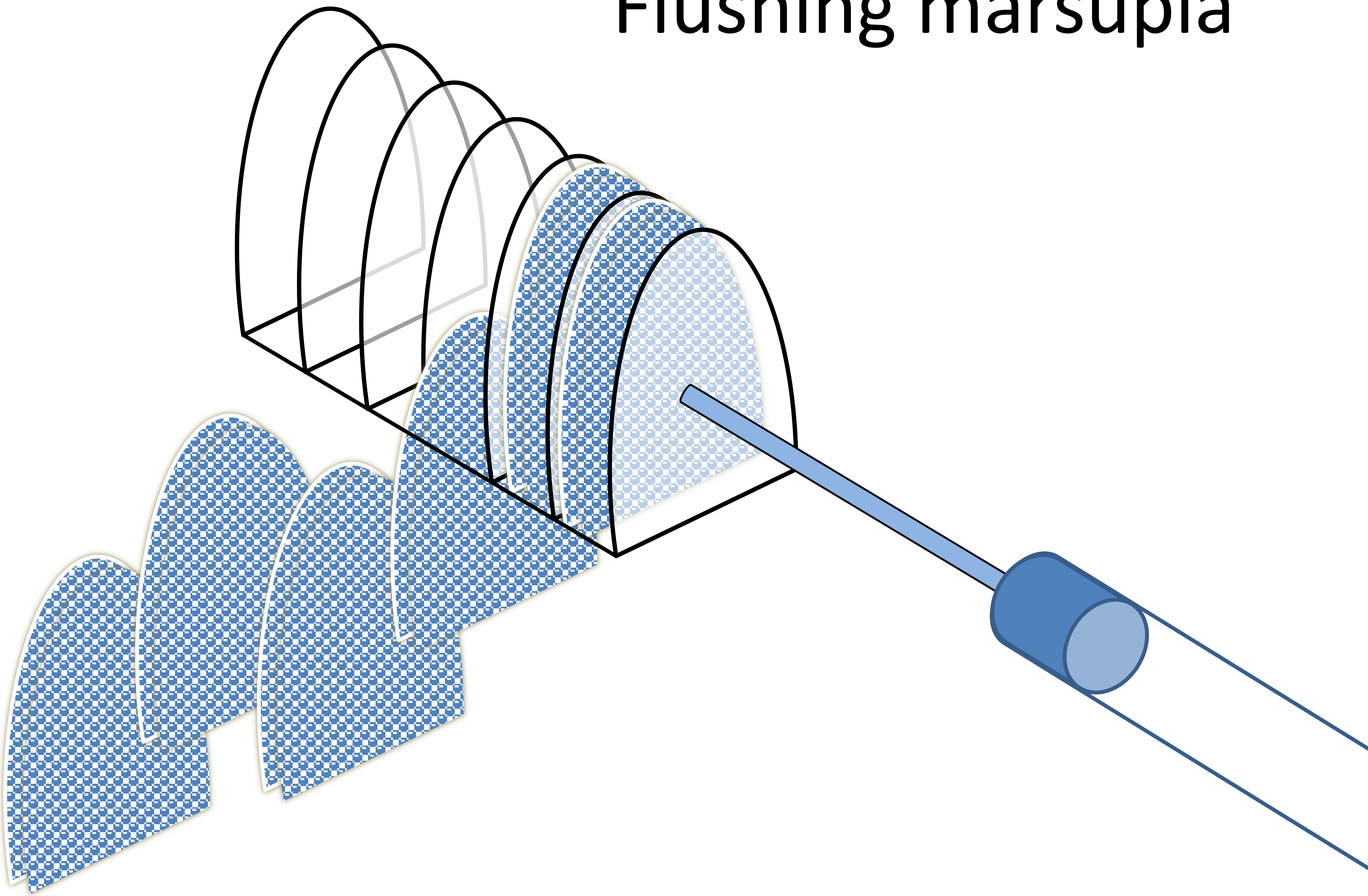
Flushing marsupia



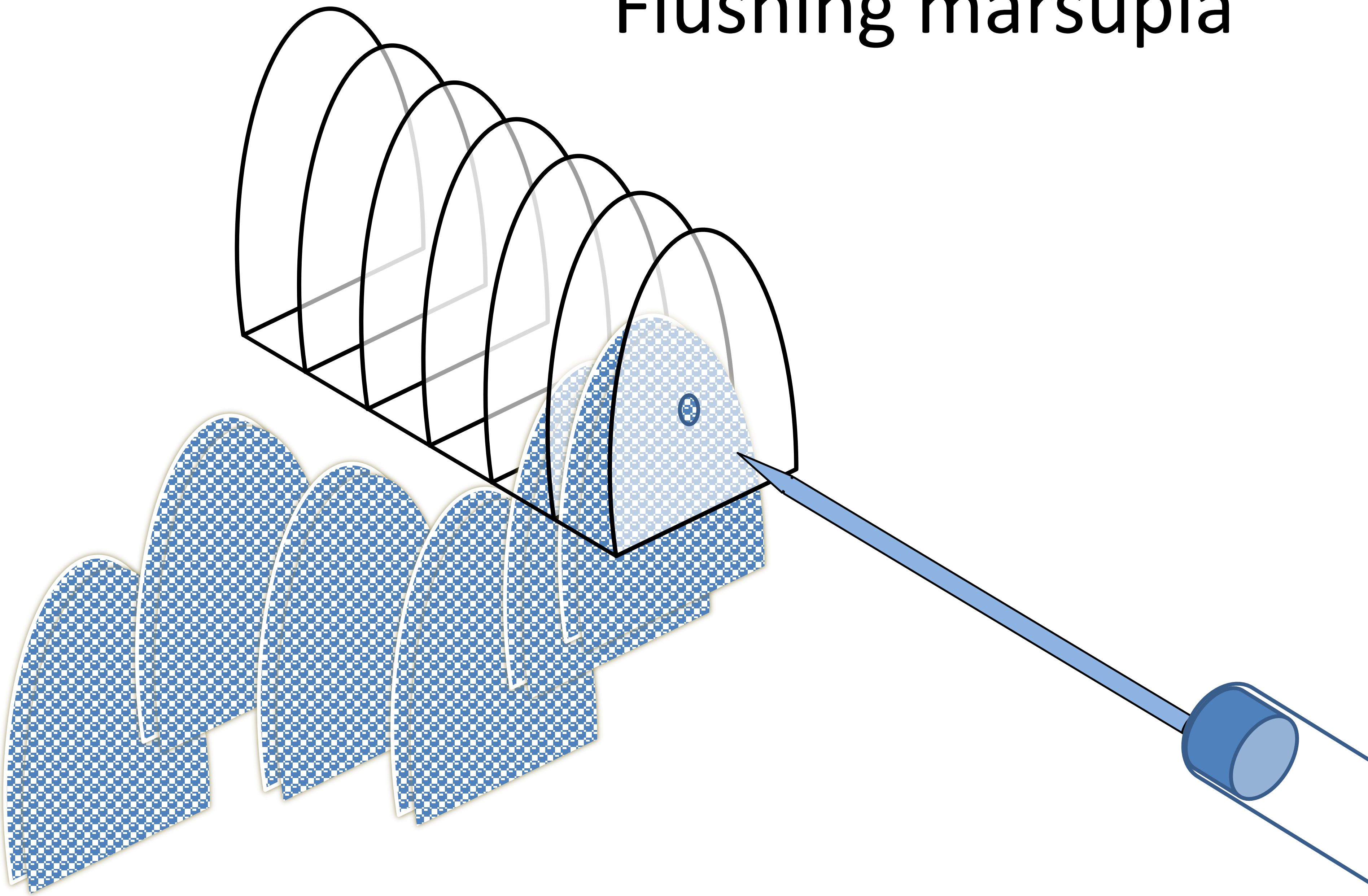
Flushing marsupia



Flushing marsupia



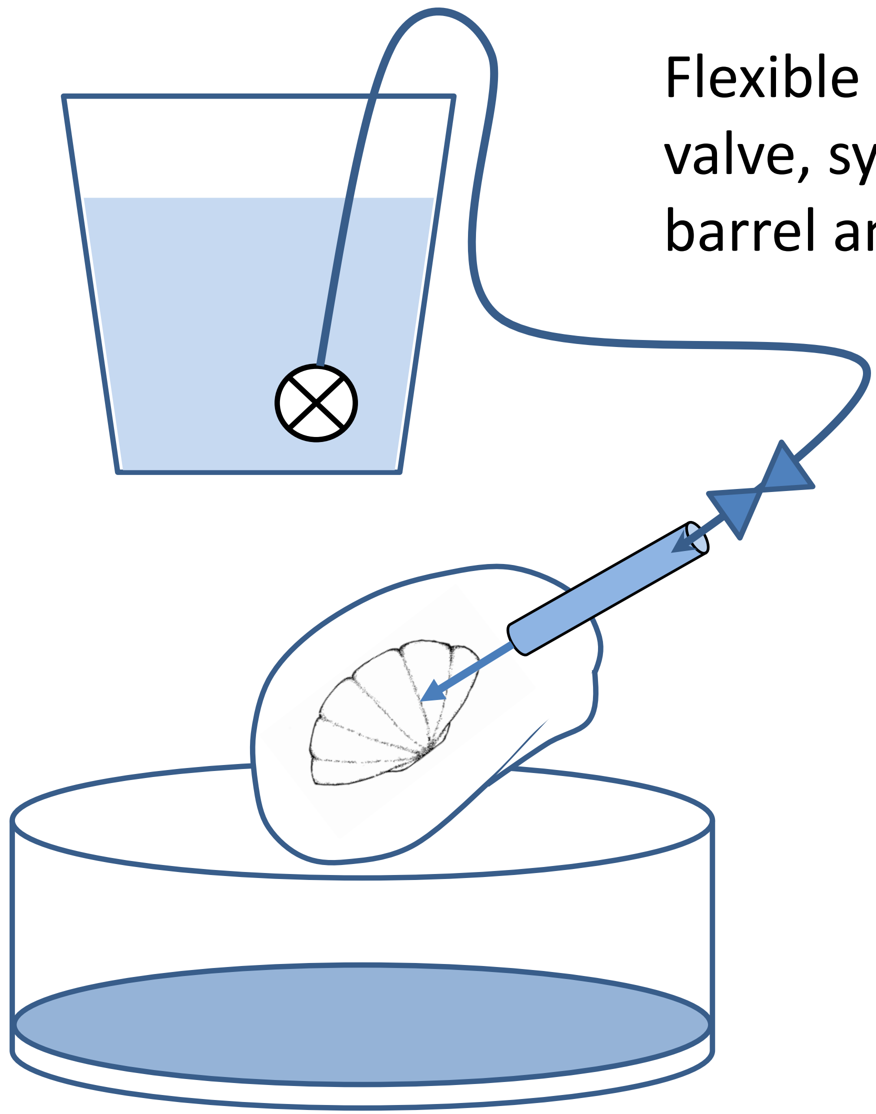
Flushing marsupia



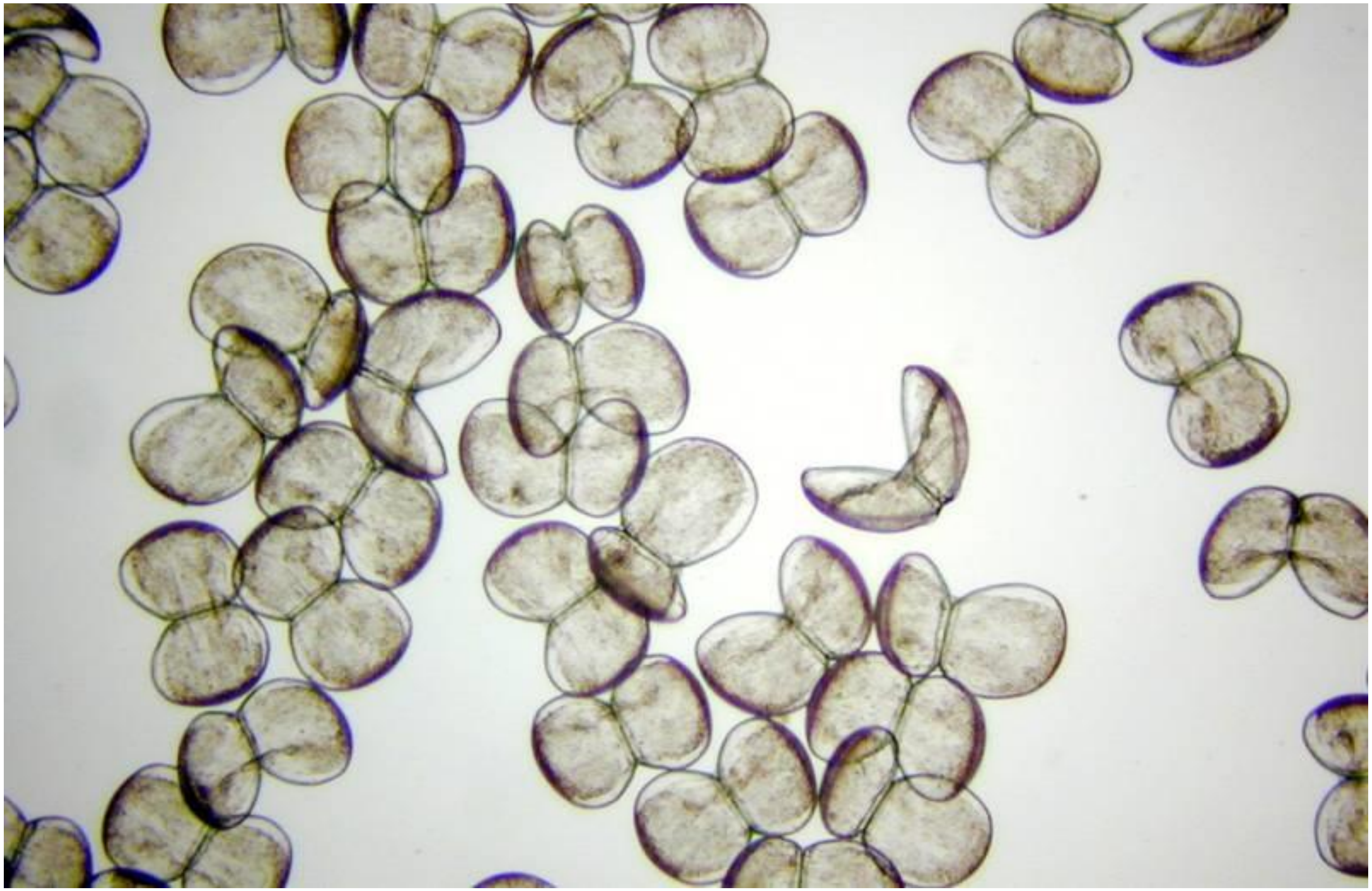
System for flushing marsupia

Bucket of water with powerhead pump

Flexible tube to valve, syringe barrel and needle

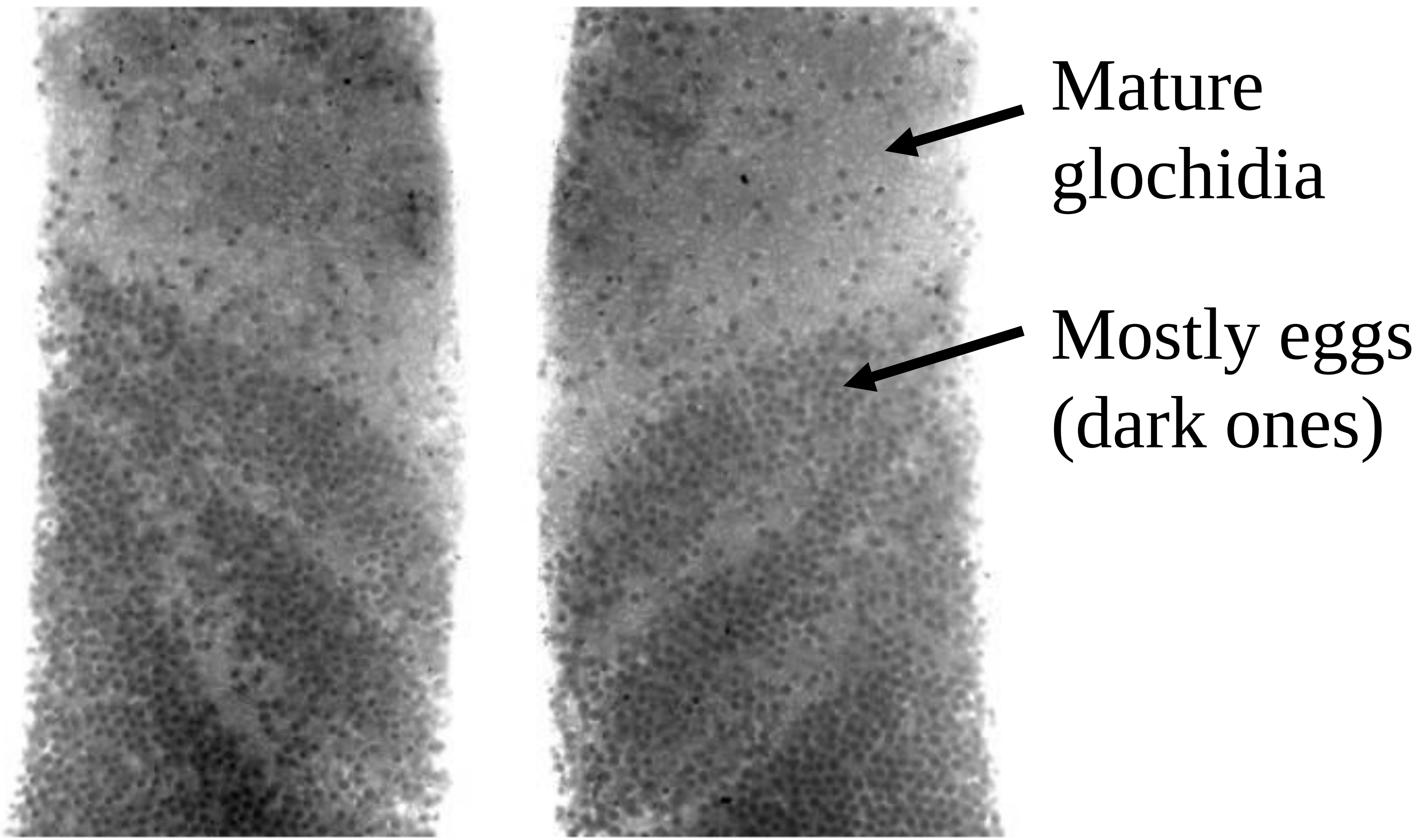


Mature brood from *Lampsilis* or other long-term brooders should separate easily from egg membranes-

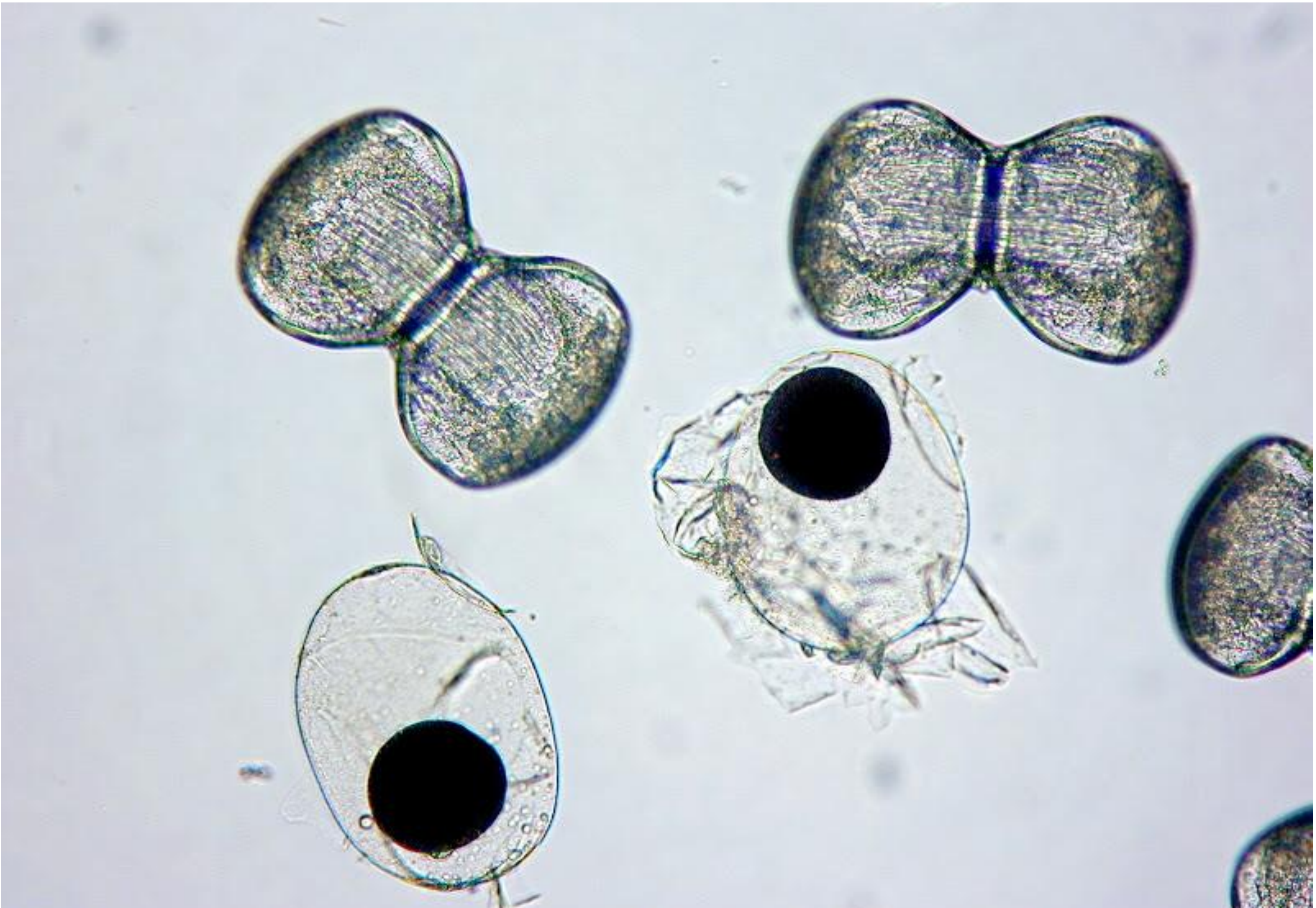


1 mm

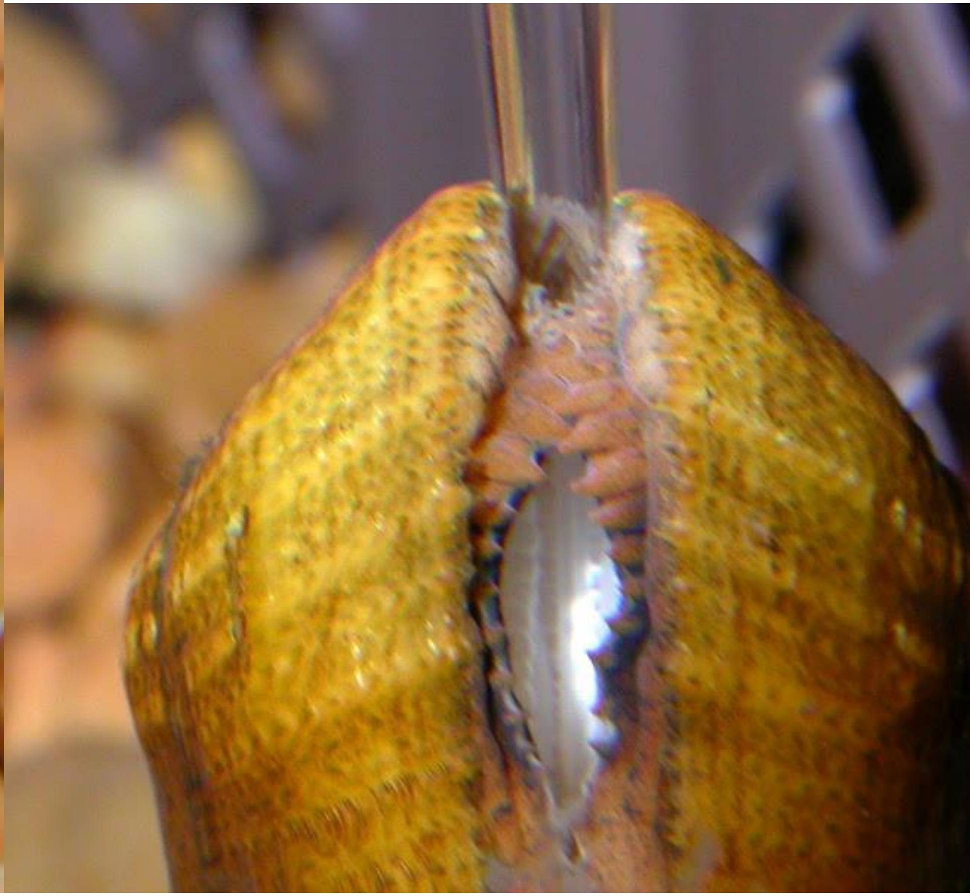
Glochidia and undeveloped eggs in a conglutinate of Neosho mucket (*Lampsilis rafinesqueana*).



Glochidia and undeveloped ova from scaleshell. Should always quantify this- useful information

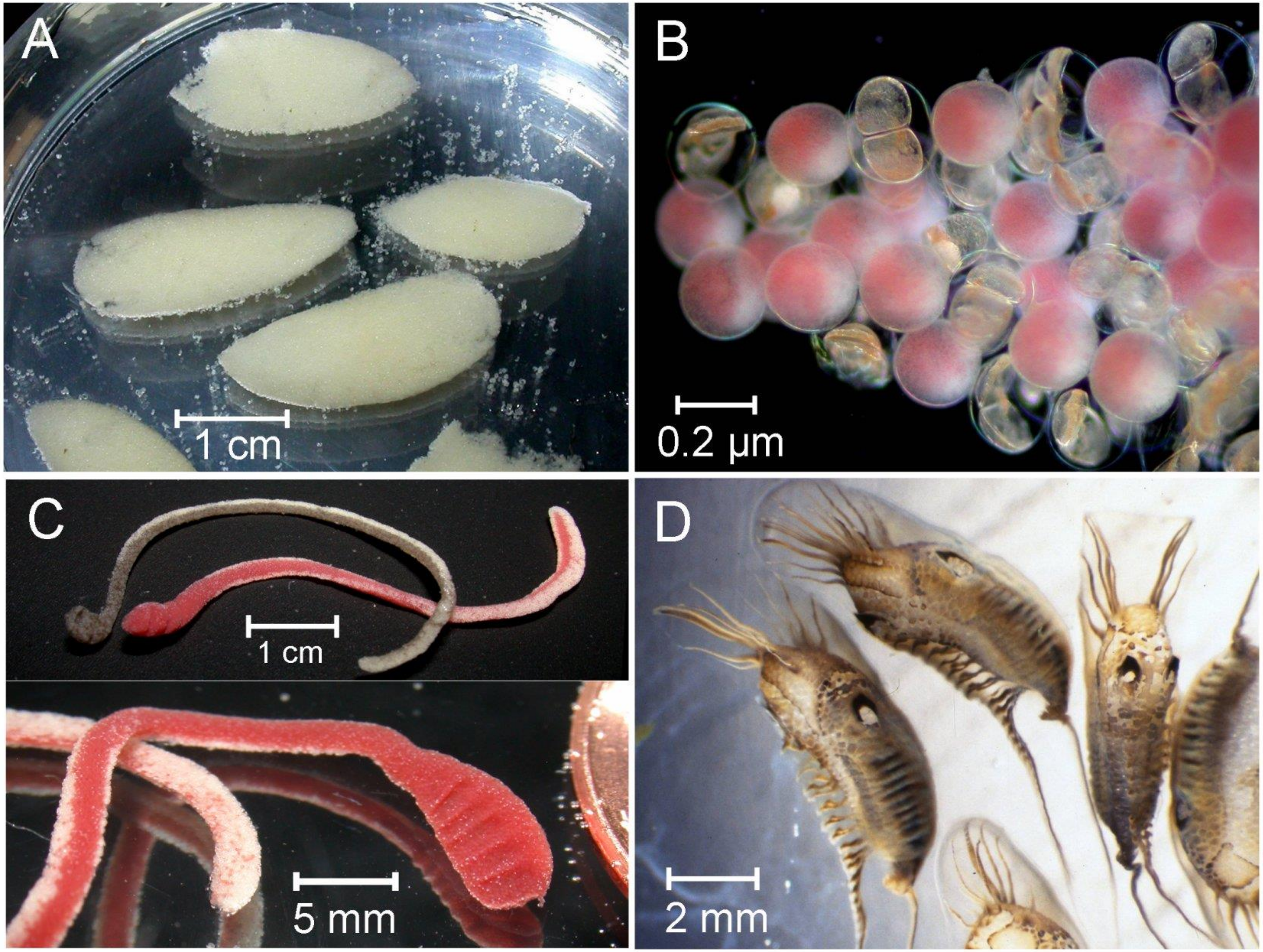


Snuffbox grasping water line that will be used to flush glochidia from the mantle



Working with conglutinates

- Most fragile conglutinates can be sprayed through a screen to free glochidia.
- Durable conglutinates such as *Ptychobranchus, Cyprogenia, Obliquaria,* can be drawn in and out of a plastic Pasture pipette (cut off the end to get proper diameter opening).



From Barnhart et al. 2008 JNABS

Gravid gill of *Obliquaria*

Care of glochidia

- Use glochidia within hours if possible- quality may decline rapidly. Keeping them cold helps.
- ‘Viability’ not always good indicator- M% declines much faster than ability to close (Fritts et al. 2013)
- Temperature shock has little effect on glochidia- OK to warm glochidia 10C/<15 min.
- Water quality is critical- use filtered native water if possible- test for premature closing

Counting glochidia

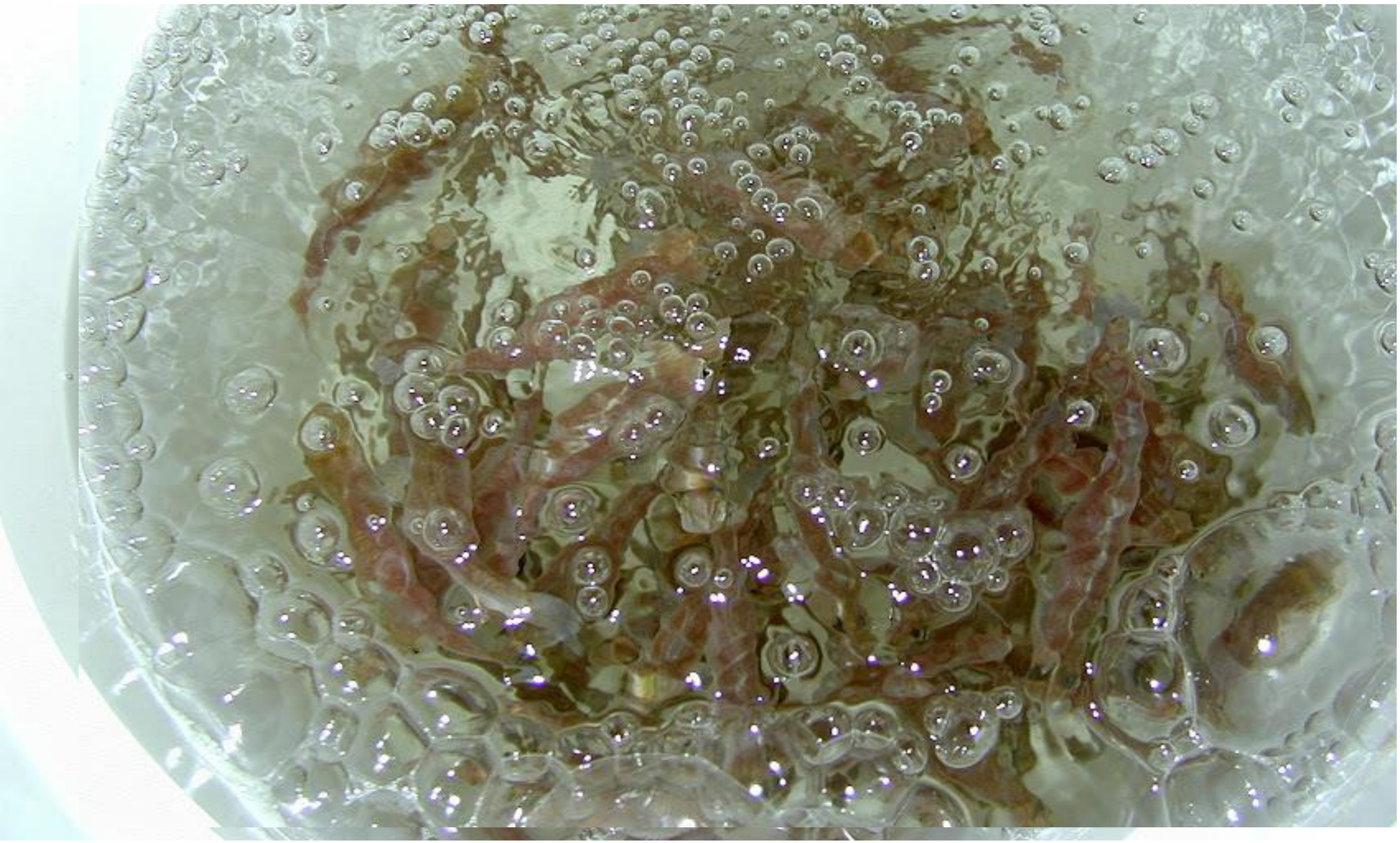
- Suspend glochidia in 1 liter of water, agitate with a rubber-bulb basting syringe
- Withdraw ten 200 ul samples using a volumetric pipette.
- Place the samples on a clean plastic petri plate and count the glochidia in each under a dissecting microscope.
- Adjusting concentration to about 10-30 per drop will keep you from going insane

Inoculation

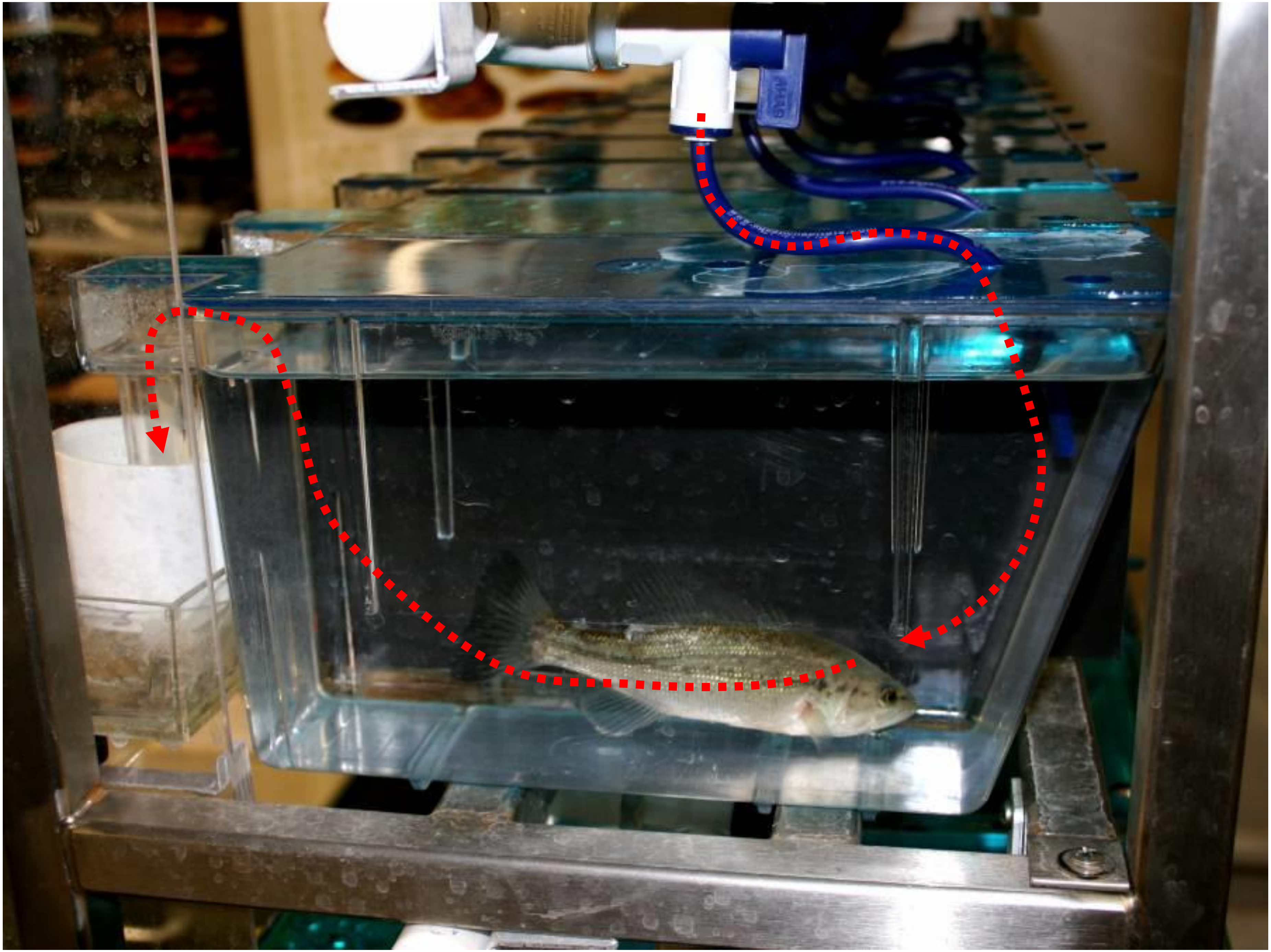
- Swim fish in glochidia suspension, typically ~4000 viable glochidia per liter.
- It is essential to keep the glochidia suspended- aeration is not enough because they will settle out. Scoop and pour water periodically
- 15-30 minutes- check for proportion of closed glochidia.

Inoculating hosts

Aeration is not enough! Also need to mix bottom water by dipping and pouring periodically



First use of AHAB for mussel work- 2002



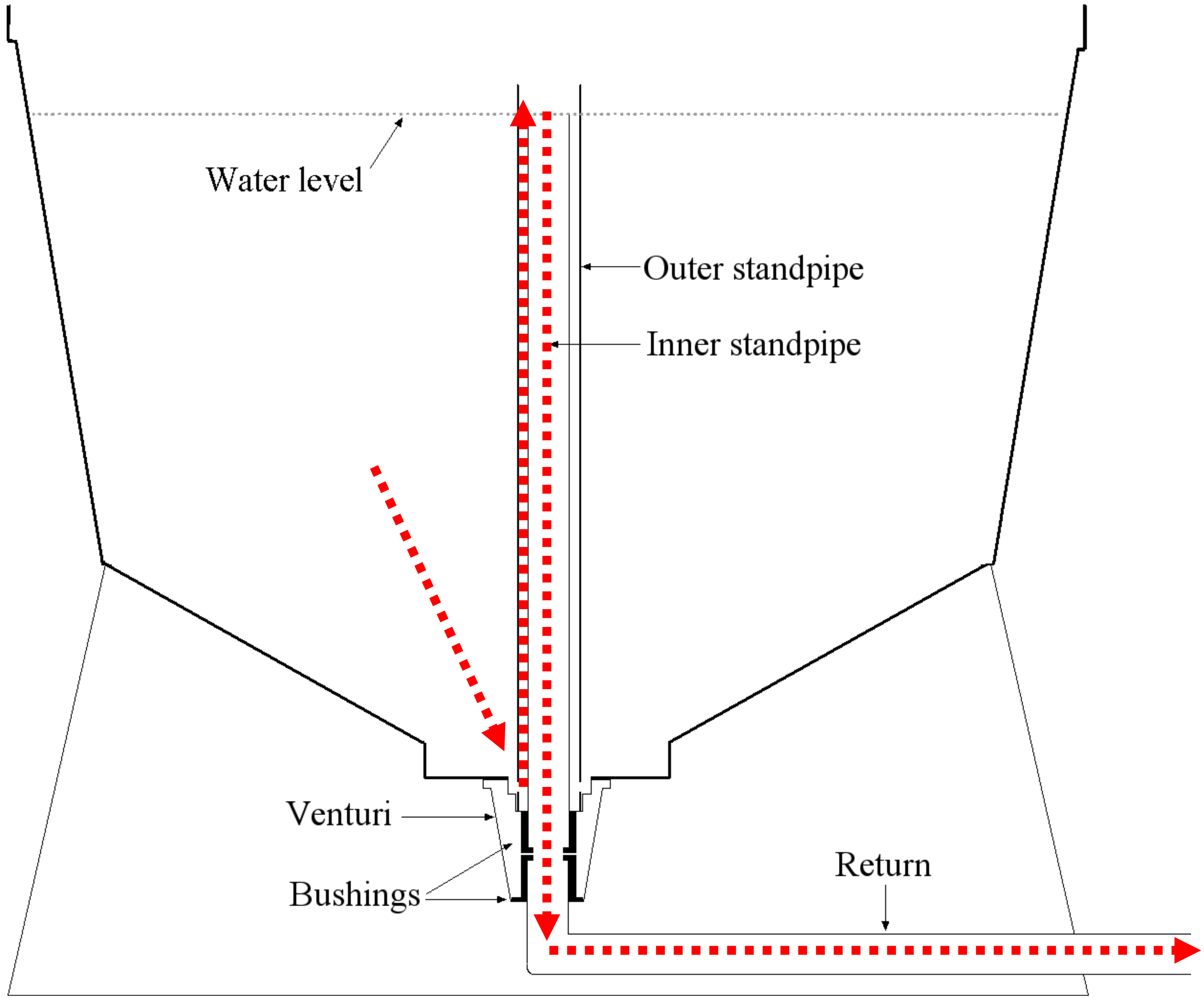
Filters for recovery of juveniles

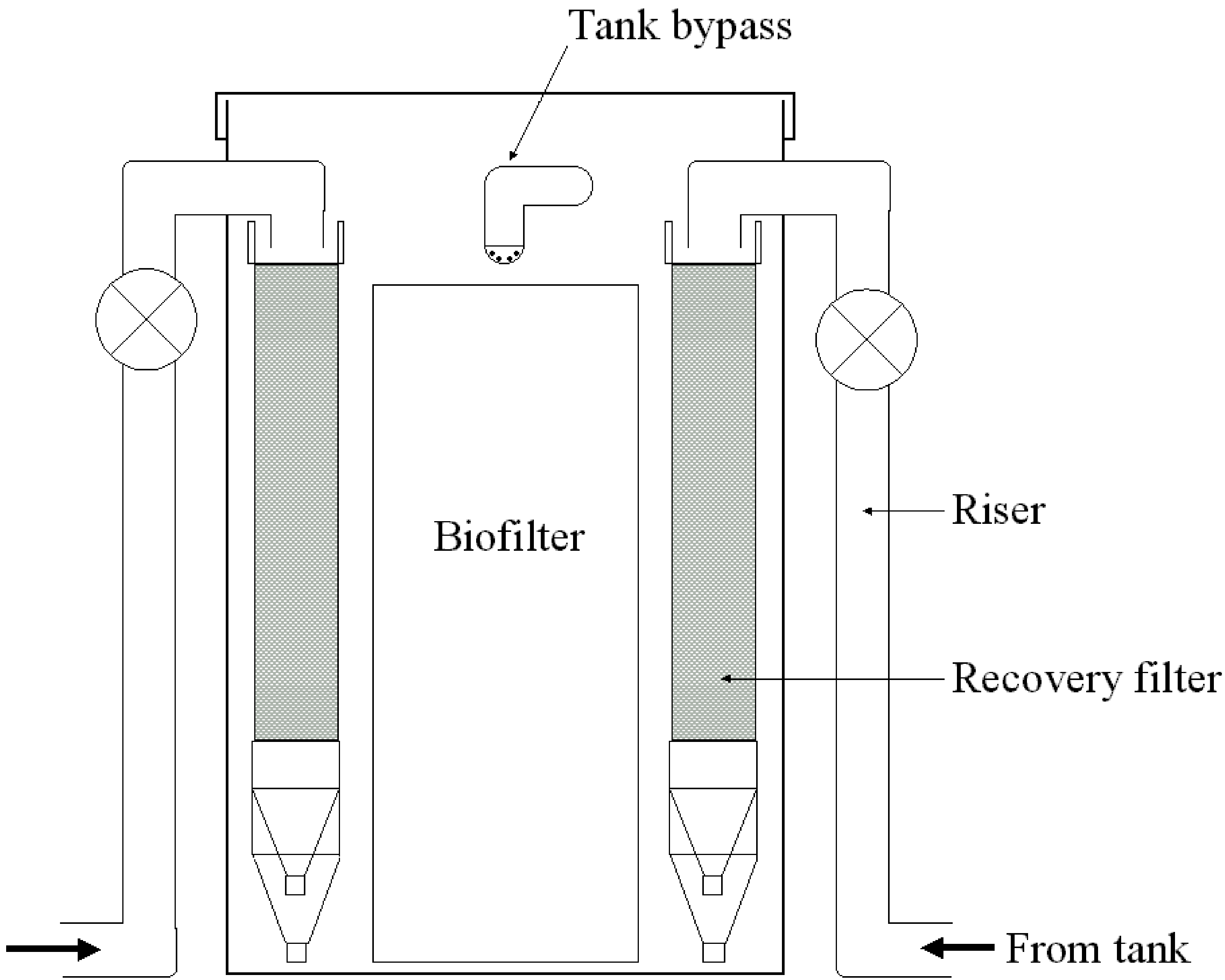
Custom AHAB gutters- note rails supporting 2-in filter cups

Using a header box for 9-liter AHAB tanks
Lost Valley Hatchery, Warsaw, MO

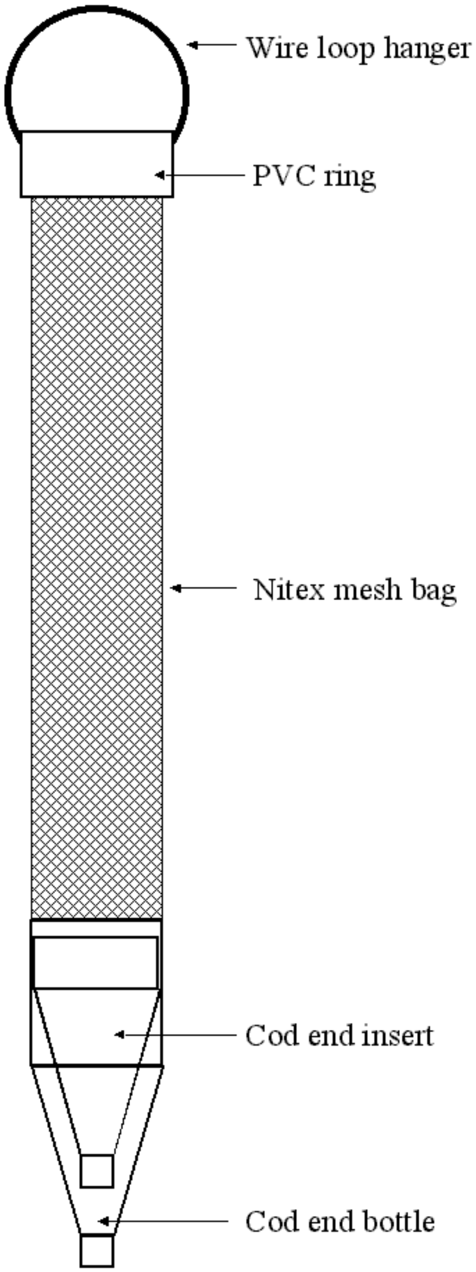
Tank outlets to filters in plastic boxes

RPS “recirculating propagation systems”


Water level
Outer standpipe
Inner standpipe
Venturi
Bushings
Return


Tank bypass
Biofilter
Riser
Recovery filter
From tank

Recovery filter



RPS of two sizes at MSU (100 and 400 gallon tanks). The smaller ones are insulated with closed cell foam and cooled by an in-line chiller

Lampsilis newly transformed