Juvenile Freshwater Mollusk Culture System Designs from Metamorphosis to Release

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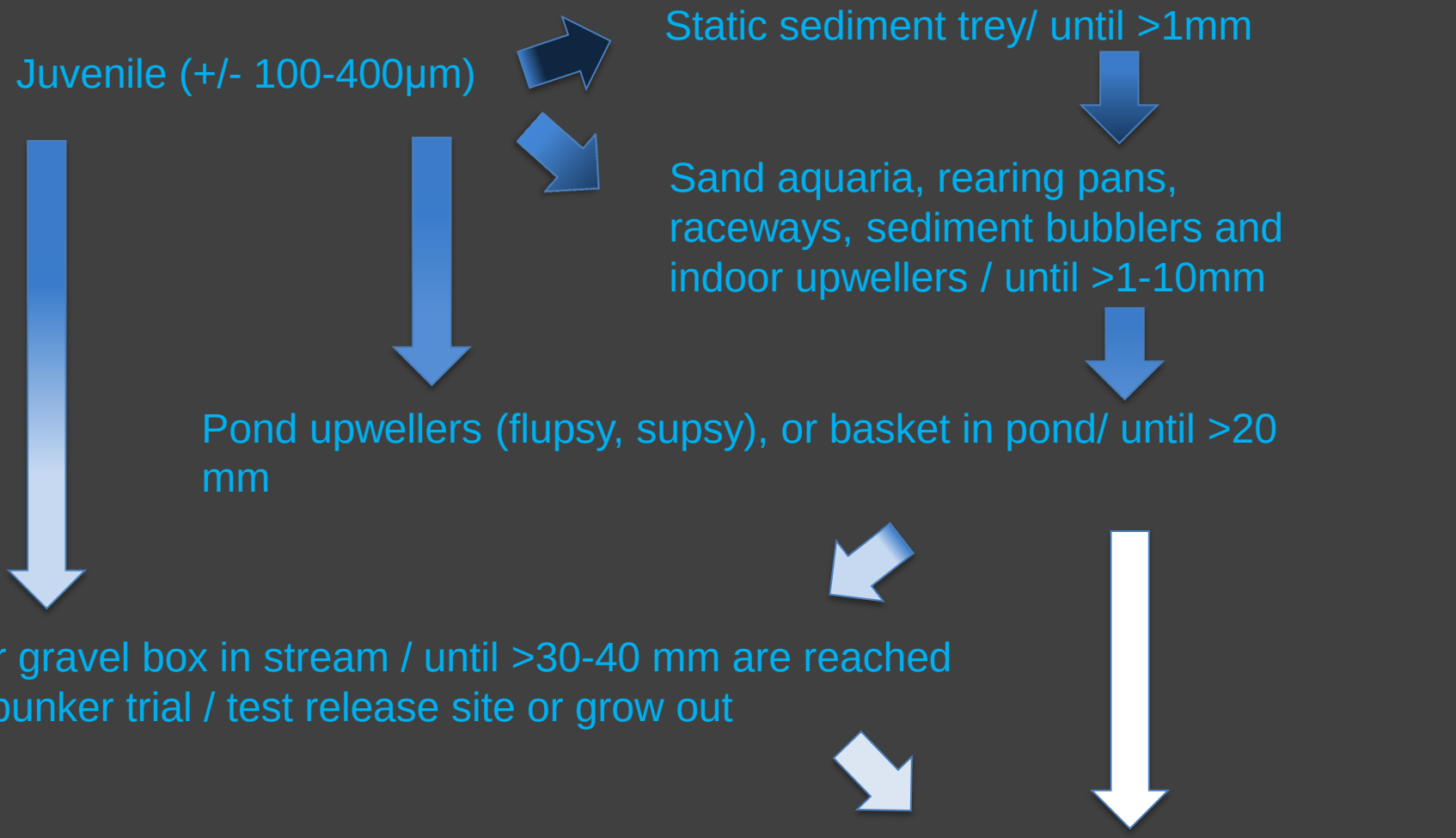
- Introduction- Outline
- Juvenile mussel culture systems from metamorphosis to release
  - Culture flow chart- Growth chart
  - Facility
  - Indoor culture systems
    - Culture of newly metamorphosed juveniles
      - Static aquaria with sediment (covered by Beth)
      - Mucket Buckets
    - Secondary indoor culture systems
      - Rearing pans, raceways and sediment bubblers
      - Flow-through bucket culture systems
      - Raceway upweller
      - Filtration

      BREAK
  - Outdoor pond culture systems
    - Pond upwellers (FLUPSYS and SUPSYS)
      - Water or air pump driven
    - Baskets
  - In stream culture systems
    - Silos and Bunkers
    - Cages (covered by Nathan)
  - Snail culture Systems
  - Facility containment devices
  - Mollusk culture facility list
- Discussion / Important variables
  - Feeding and nutrition (covered by Rachel), flow, temperature, sediment vs. no sediment, release size, number and size of stockings (covered by David), tagging (covered by Hua and Bryan)

Culture procedure
Growing juvenile mussels

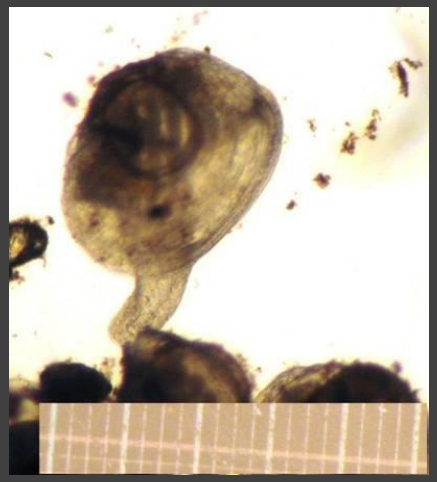


Modified slide from: Tanja Eybe et al. Freshwater Mussel Rearing Facility, Luxembourg

Hamiota altilis Growth Series

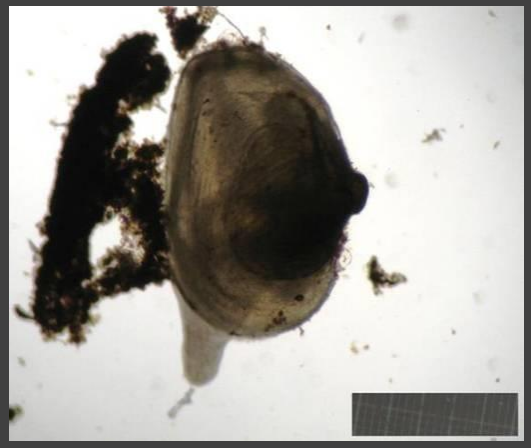
Day 1- Indoor Upweller

Day 14- Indoor Upweller

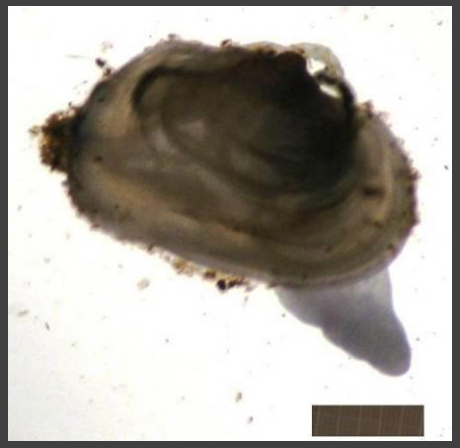


Day 56- Indoor Upweller

Day 63- Pond Supsy

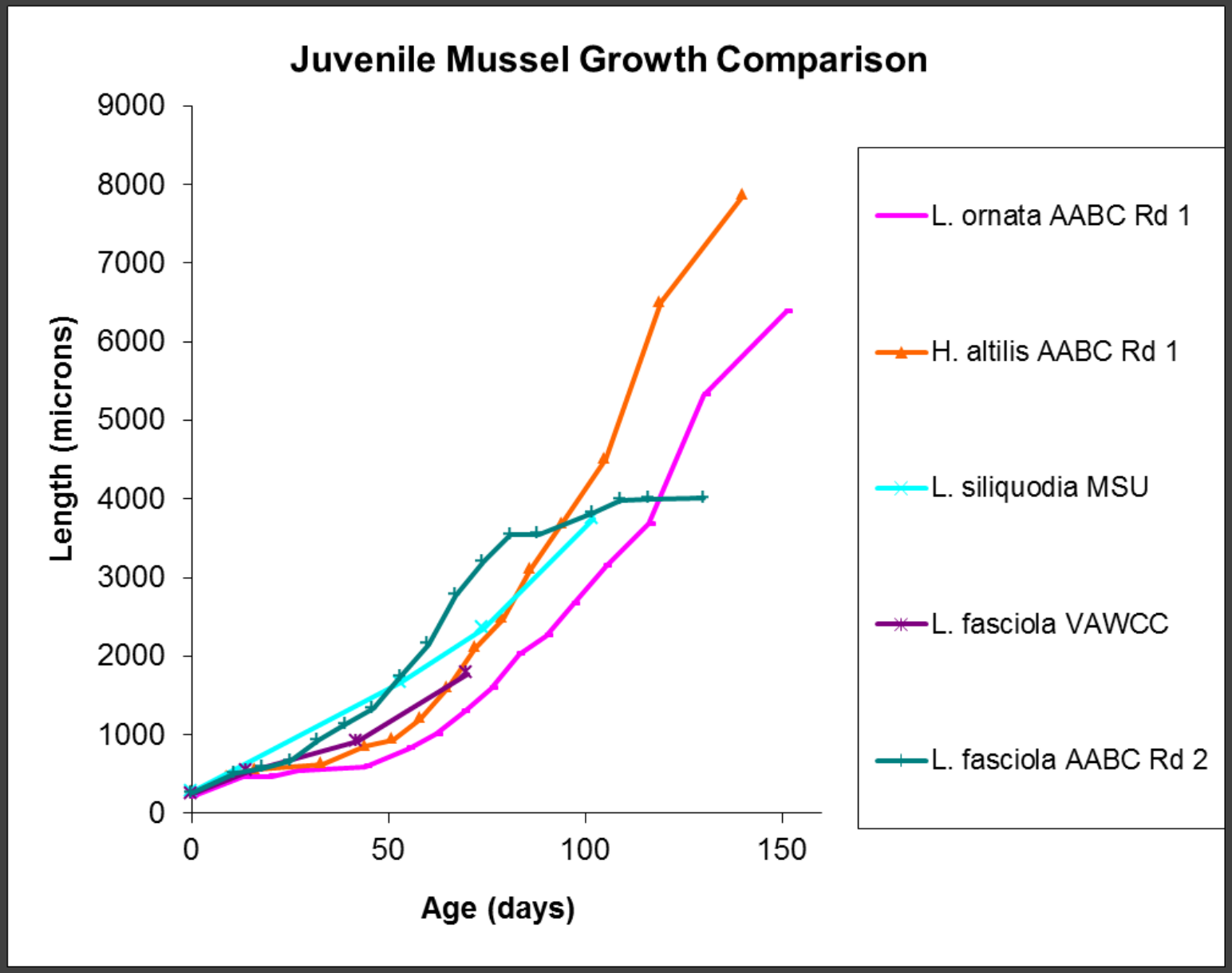


Day 91- Pond Supsy



Day 130- Pond Supsy

Day 730- Supsy 20mm day 365, Tagged, Released and Recaptured



Goal : 30-60 days post metamorphosis >1000µm

AABC Facilities

- Culture building, snail pad, and mussel pavilion
- Two water sources:
  125 gpm groundwater
  75 gpm surface water
- Maintenance & culture assembly building
- Administrative building
- Ponds for mussel and host fish culture

Compact and Mobile

Bryan Simmons USFWS, Mussel Popagation Building in Kansas (Top) Nathan Eckert, USFWS Genoa National Fish Hatchery

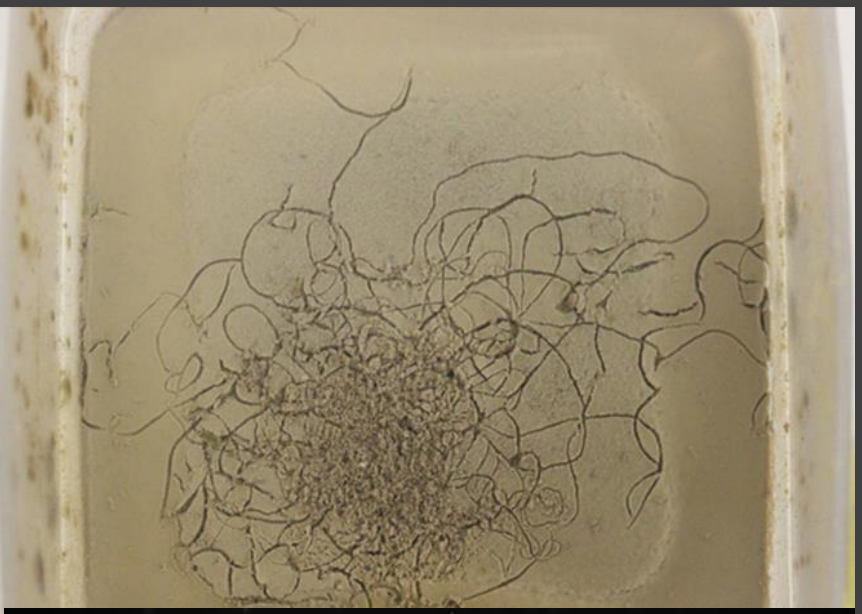
HruŠka boxes

Culture methods for *Margaritifera*

Hruška 1992 (reviewed in Gum et. al. 2011).

Chris Barnhart, Missouri State University, (Eybe et al. 2013) Freshwater Mussel Rearing Facility, Luxembourg

HruŠka boxes

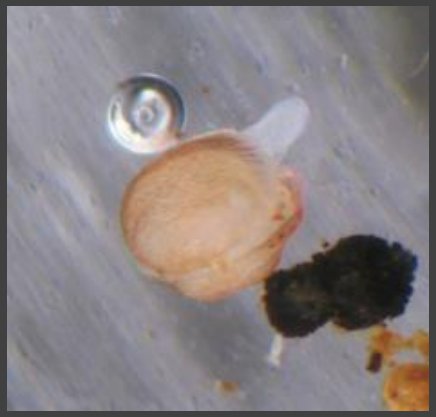


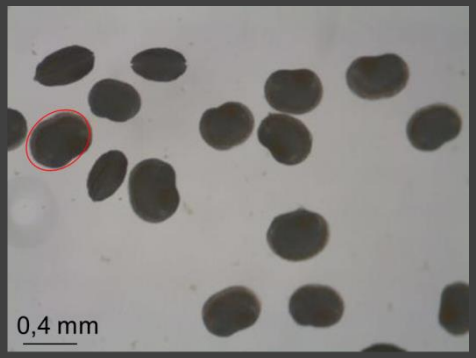
- 200 juveniles/box
- 500 mL static water
  - 18-20 ppm microalgae
  - Weekly/Biweekly renewal
  - 25 ml sediment sieved <150µm
- Dissolved oxygen
  - 75% saturation at sediment surface
  - Minimum 22% at depth

Species that prefer HruŠka boxes

Primarily Margaritiferidae and Unionidae/Anodontines

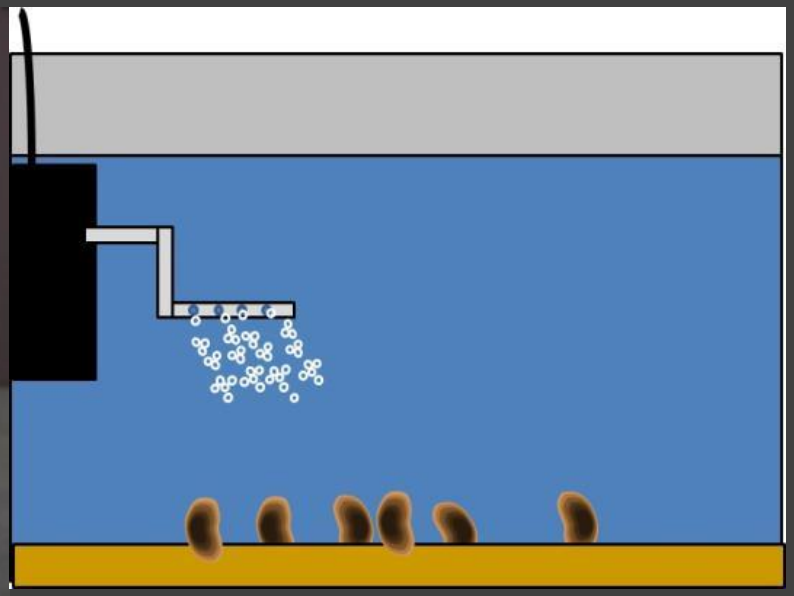
- Luxembourg
  - *Margaritifera margaritifera*
  - *Unio crassus*
- Missouri State
  - *Margaritifera falcata*
  - *Arkansia wheeleri*
  - *Arcidens confragosus*
  - *Anodonta californiensis*
  - *Anodontoides ferussacianus*
- AABC
  - *Margaritifera marrianae*
  - *Strophitus connasaugaensis*





Sand Aquaria

Culture of juvenile mussels >1mm



- 18 Liter river water
- Waterpump/filter removed (Swordfish Pump 200)
- Fed twice a day between 120-350 µl Shellfish and 2-4 drops of Nanno
- Water is changed weekly (using prefiltered river water)

Tanja Eybe, Frankie Thielen, Thierry Muller, Leo Klein, Sonja Heumann, Alexandra Arendt, Freshwater Mussel Rearing Facility, Luxembourg

Sand trays in Britany

- Sand size +/- 0.5-2 mm
- Small Pumps for flow
- Weekly water change using creek water by hatchery
- Supplemental fed microalgae

- Conclusion: 275 day trial
  - Plastic Boxes 130% growth
  - Sand Aquaria 450% growth

- Substrate (e.g. Sand) and flow / mussels grow faster
- Increased survival rates.
- Larger static systems with many mussels are possible

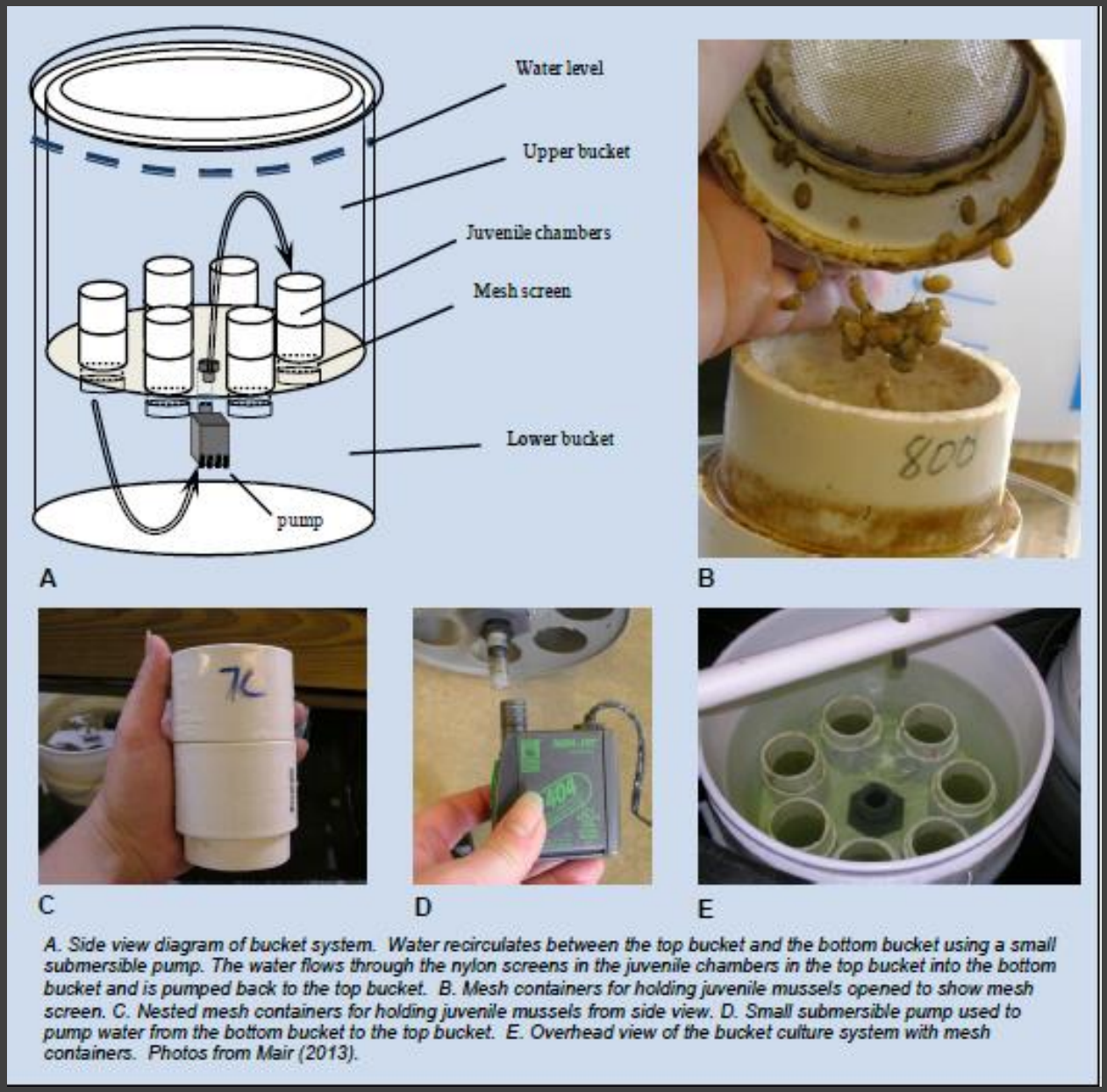
Pierrick , Frankie Thielen, Thierry Muller, Leo Klein, Sonja Heumann, Alexandra Arendt, Freshwater Mussel Rearing Facility, Luxembourg

AHAB tanks and sediment buckets-static substrate systems

- 2.8-L AHAB tanks / 5-gallon buckets
- Supplemental microalgae added, target concentration is 1 nl/ml
- 17-26 C
- <250 um sand, <125 um silt, mix of sand and silt, 50-100 ml
- 1-2 mm/month on average
- 5,000 in ATs and 10,000 in SBs
- Water is changed 1x weekly and sediment is changed 1x weekly to 1x monthly
- Calcium is added to increase hardness to about 125 ppm and 5 um filtered pond water is used

Brian Watson Virginia Fisheries & Aquatic Wildlife Center, VA, DGIF

Mucket Buckets (Barnhart, 2006)- Lampsilines, Quadrulines, & Amblemines

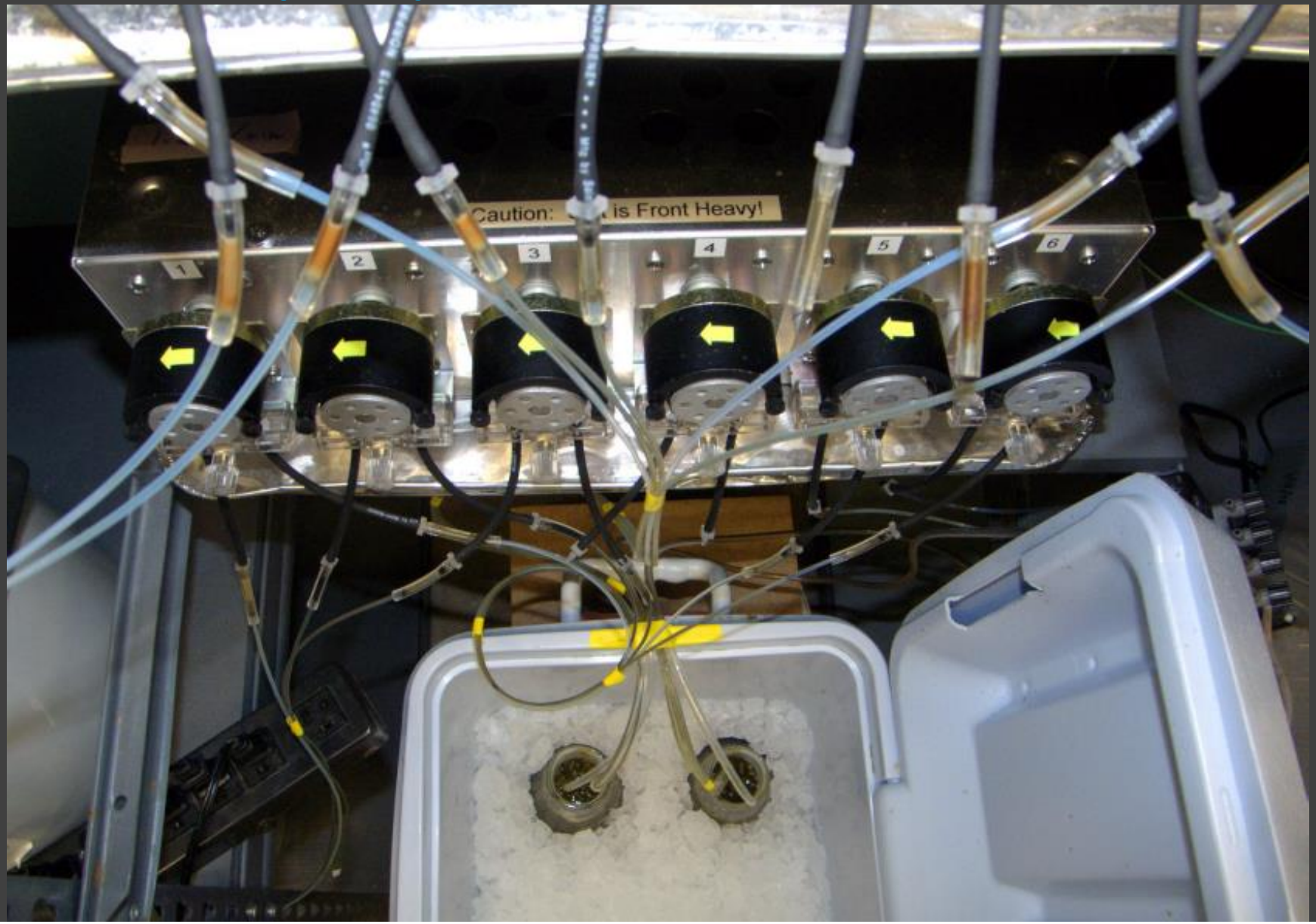


A. Side view diagram of bucket system. Water recirculates between the top bucket and the bottom bucket using a small submersible pump. The water flows through the nylon screens in the juvenile chambers in the top bucket into the bottom bucket and is pumped back to the top bucket. B. Mesh containers for holding juvenile mussels opened to show mesh screen. C. Nested mesh containers for holding juvenile mussels from side view. D. Small submersible pump used to pump water from the bottom bucket to the top bucket. E. Overhead view of the bucket culture system with mesh containers. Photos from Mair (2013).

- 18.9 L (5 gal) main bucket; 13.2 L (3.5 gal) upper bucket
- Pump- Aquarium Systems® Minijet model MN-404
- Nominal flow rate 400 L (106 gal) per hour (0.015 L/cup/sec)
- 7 cups- 5.1 cm (2 in.) diameter Schedule 40 polyvinyl chloride (PVC) plumbing pipe
- Nylon screen (Nitex®, 125 µm or larger mesh)
- Upwelling or downwelling

Chris Barnhart, Missouri State University

Peristaltic pumps deliver food to buckets



Chris Barnhart, Missouri State University

Bucket system for juvenile mussel culture (modified from Barnhart's bucket)

- Feeding system- water bottle (for dogs)
- Electronic auto-off Timer (H3CR-F8-300, OMRON Corporation)
- Solenoid valve (Evolutionary Concepts Inc.)
- Dispense rate is once per hour
- Fed daily- room temperature
- Bottle change- once/week

Hua Dan, Freshwater Mollusk Conservation Center, Department of Fish and Wildlife Conservation, Virginia Tech

Bucket system

- 5 chambers
- 18-25C
- 1,500 juveniles in each chamber
- 750 um to 1 mm/month
- Stunt at 7 mm if not moved
- Typically 1-3 mm then move
- Survival highly variable

Brian Watson Virginia Fisheries & Aquatic Wildlife Center, VA, DGIF

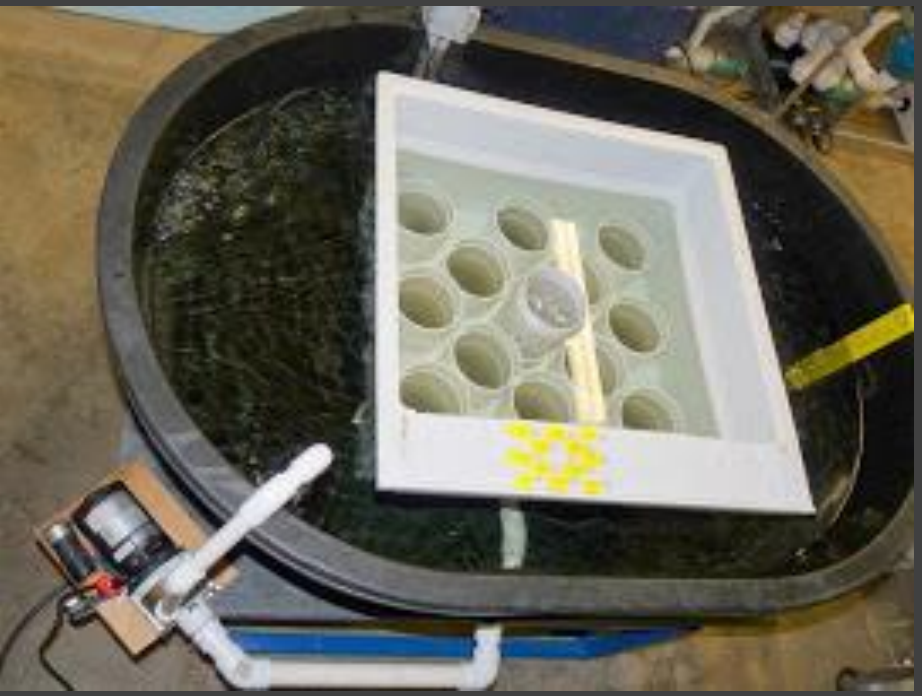
Potamilus capax (55 days old ~1.8 mm)

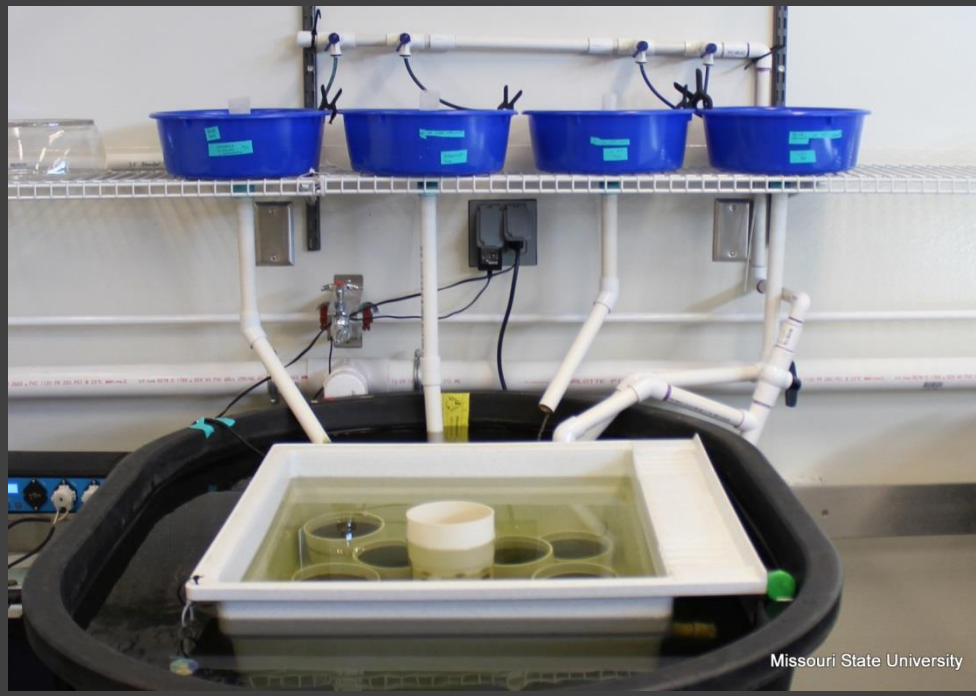
Chris Barnhart, Missouri State University

P. capax (5 months, ~5 mm)

Chris Barnhart, Missouri State University

Large Downwelling Bucket Systems





Chris Barnhart, Missouri State University

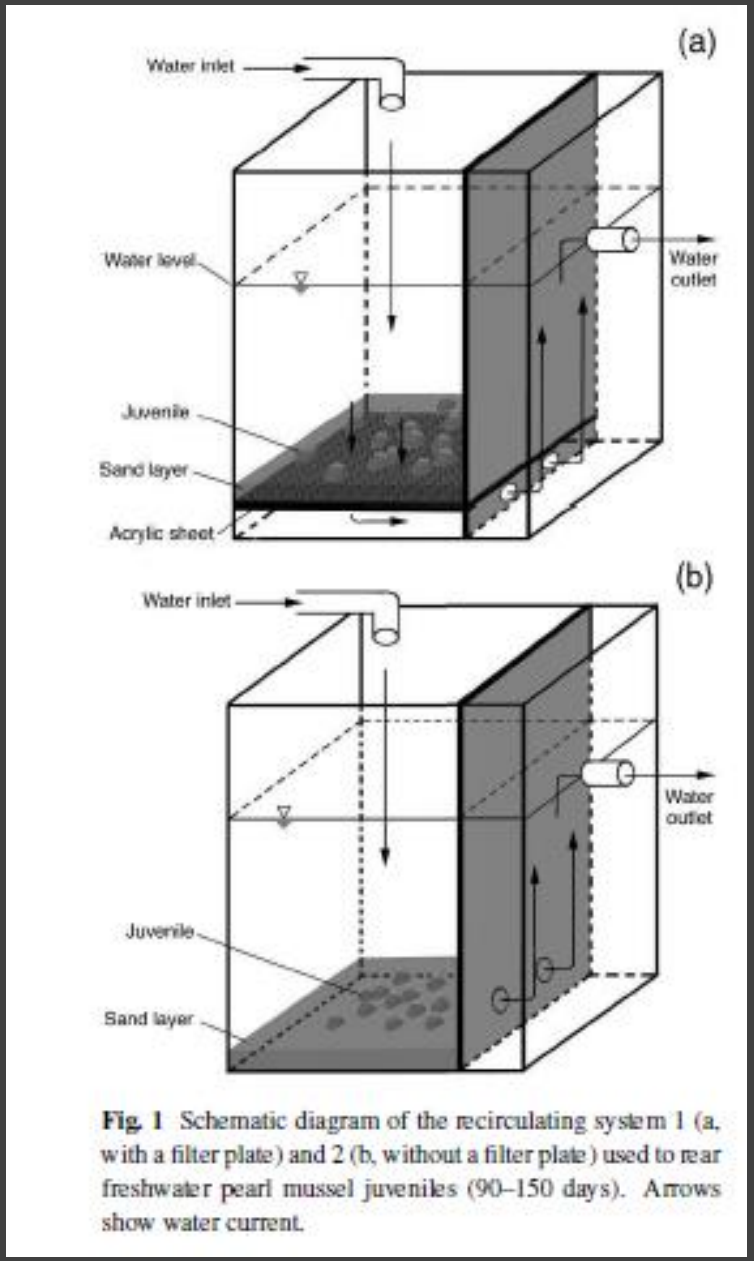
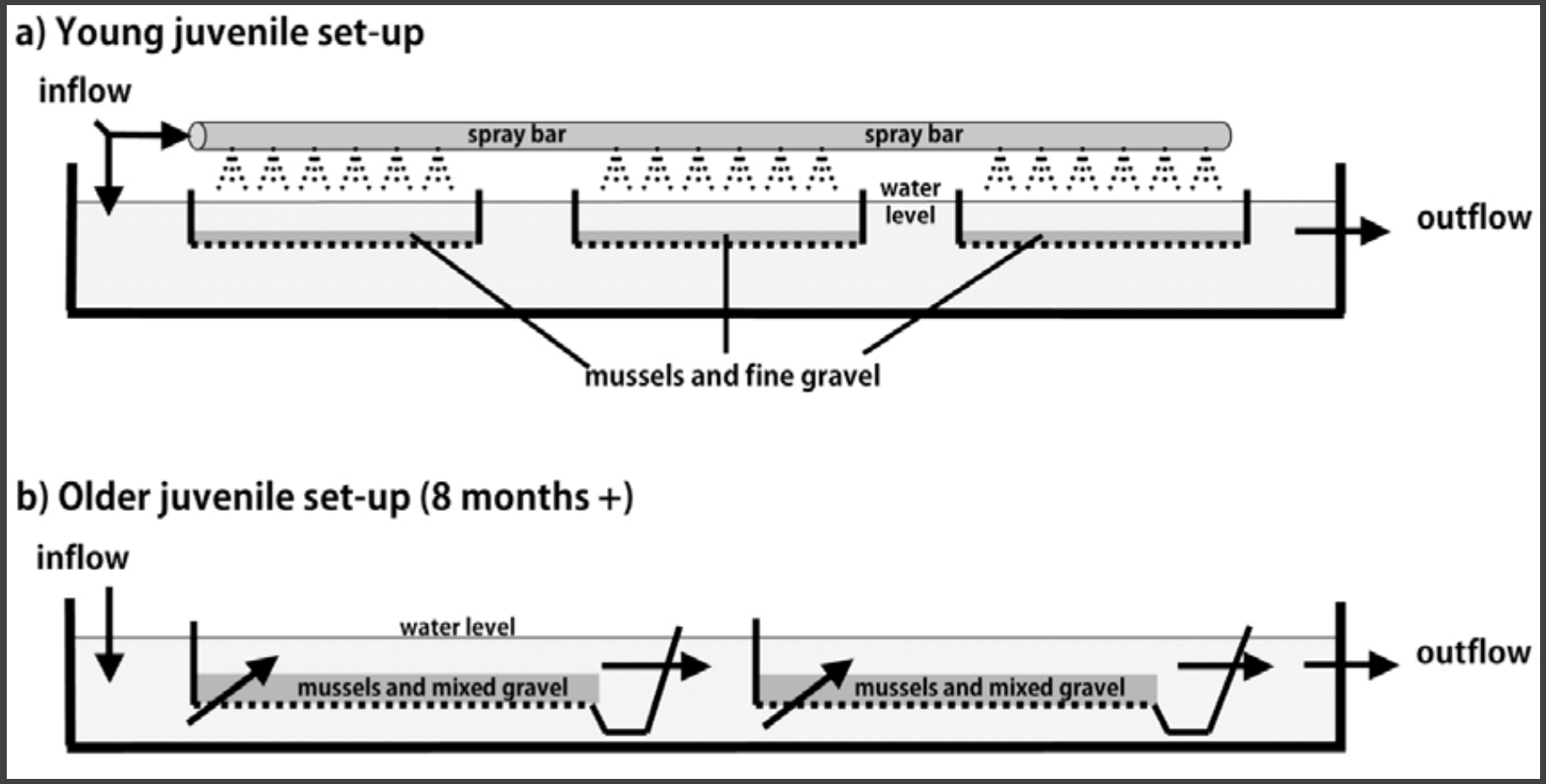


Fig. 1 Schematic diagram of the recirculating system 1 (a, with a filter plate) and 2 (b, without a filter plate) used to rear freshwater pearl mussel juveniles (90–150 days). Arrows show water current.

- *Chamberlainia hainesiana*
  - 0-90 days plastic culture units, closed recirculating
    - fed twice daily, Chlorella sp. and Kirchneriella incurvata
    - 20 g of sand (< 120 µm grain size)
    - sand 3 mm depth
    - three filter cabinets
  - 90-150 days (4mm + juveniles)
    - 5 cm depth with sand (> 4 mm grain size)
    - acrylic plate (6 mm thick w/ holes 3 mm in diameter throughout the plate) 10 cm above the cabinet floor
    - 3 L/min flow from 2 acre earthen pond
    - Juveniles cultured on filter plate had significantly higher shell growth ($p<0.05$), no effect on survival

Kovitvadhi and Kovitvadhi. 2013, Thailand

Rearing pans, raceways and sediment bubblers



(Scriven et al. 2011) Mawddach Fish Hatchery in, Wales, UK

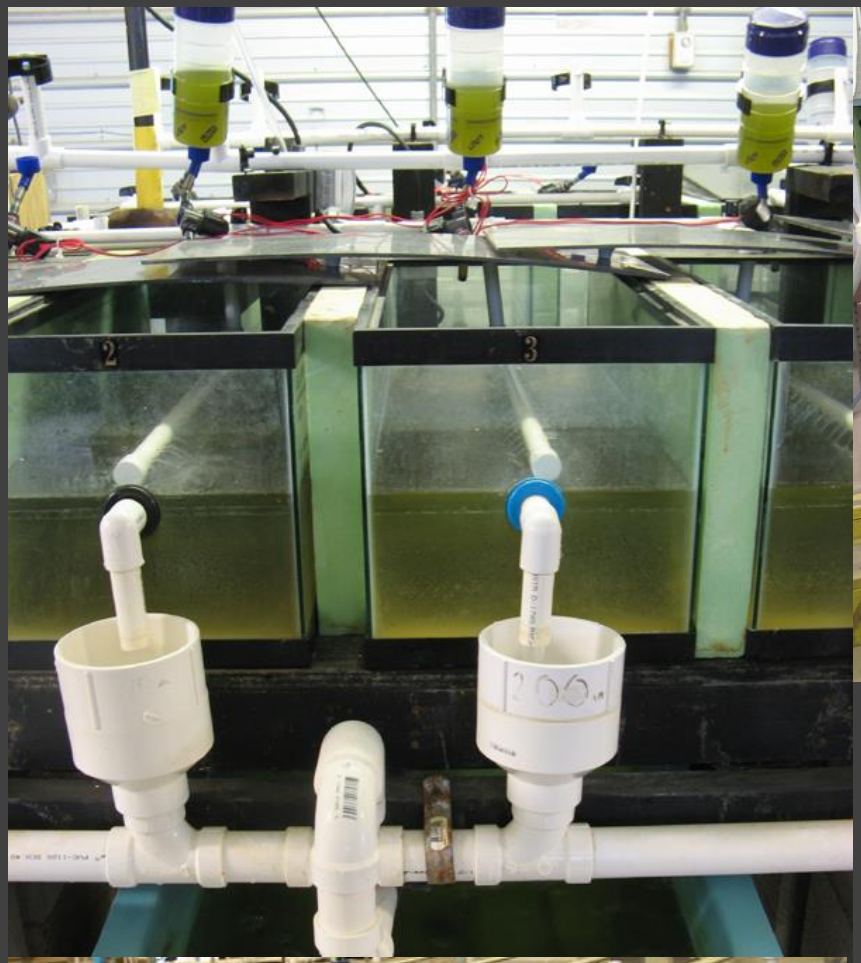
(Scriven et al. 2011) Mawddach Fish Hatchery in, Wales, UK

Trough system for juvenile mussels (recirculating with pond)

Hua Dan, Freshwater Mollusk Conservation Center, Department of Fish and Wildlife Conservation, Virginia Tech

Trough systems in grow-out building (supplied by pond water)

Hua Dan, Freshwater Mollusk Conservation Center, Department of Fish and Wildlife Conservation, Virginia Tech



Fine Substrate with continuous feeding device

Anodontines: *Alasmidonta raveneliana, Alasmidonta viridis*

Water: Filtered and UV treated surface water, recirculating.

Volume: 20 gal. tanks, 40 gal. sump, total=320 gals

Substrate: NC <125µm, frozen, aerated; VT <150µm, autoclaved, oxidized

Food: Reed Mariculture Shellfish 1800 and Nanno 3600

Hua Dan, Freshwater Mollusk Conservation Center, Department of Fish and Wildlife Conservation, Rachael Hoch, Marion Conservation Aquaculture Center

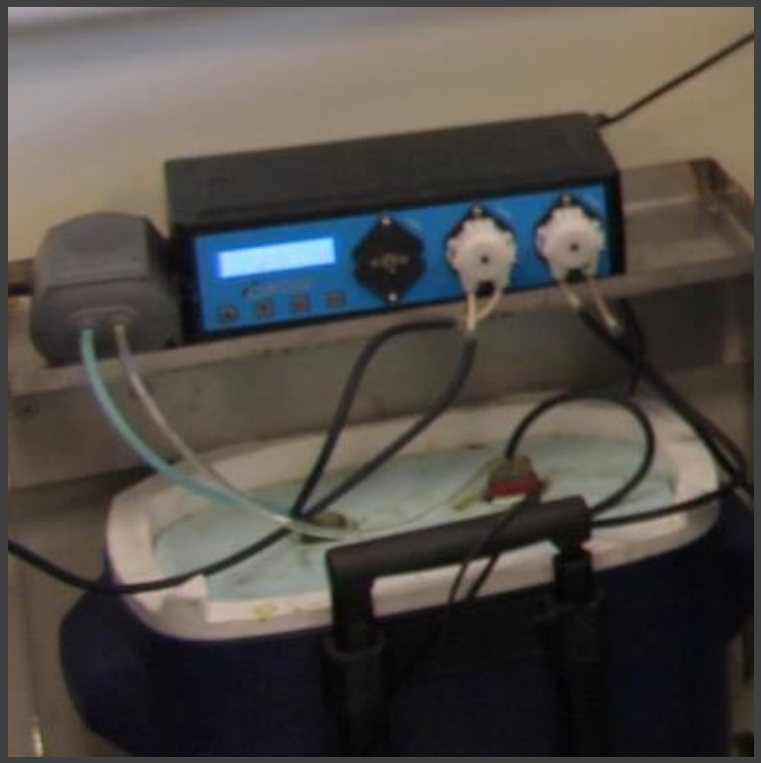
Top: Rearing pan system at Aquatic Wildlife Conservation Center, Marion, Virginia. Bottom: Close-up of the plastic culture pans, center drain, filter socks, and sump. Photo credit: Nathan Eckert, USFWS.

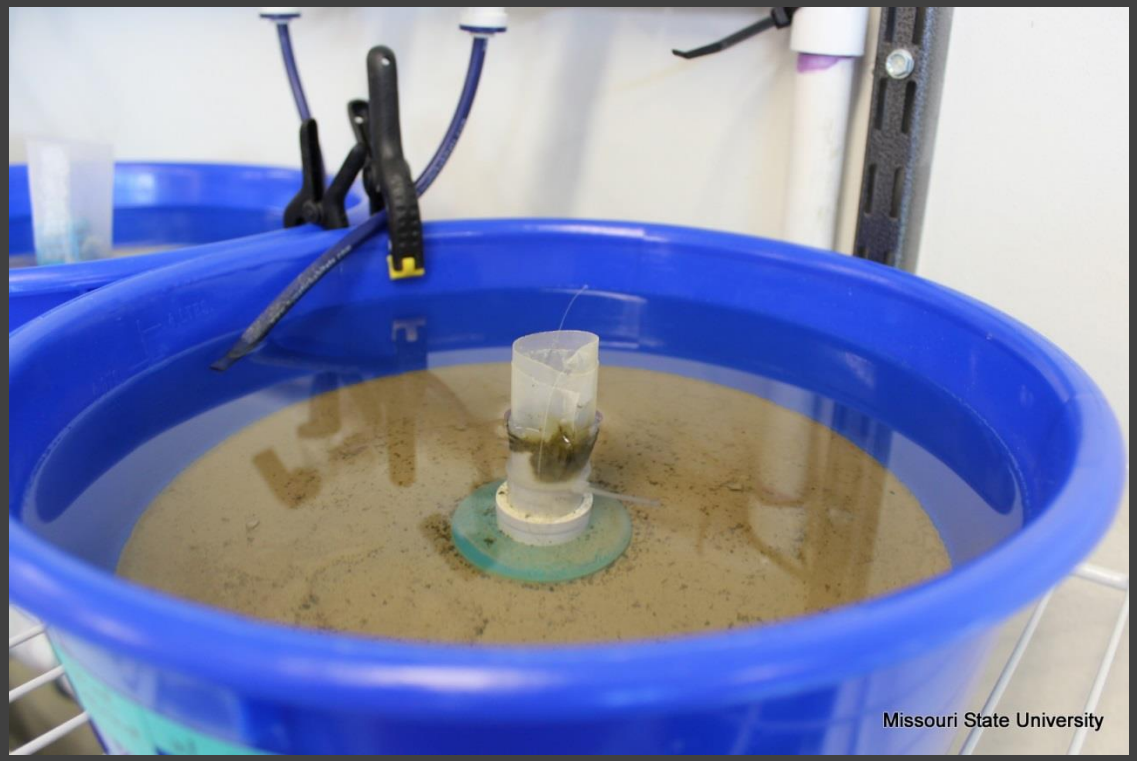
Lampsilines, Quadrulines, & Amblemines

- 35, 5-L dishes that sit over a 200-gal sump
- Recirculating or flow thru
- 1.25-acre pond water supply
- Temperature in recirculating mode runs about 20-30C, flow thru mode 4-33C
- 250 ml of substrate, which varies from < 1 mm sand, silt or a mix of sand and silt
- 750 um to 3 mm (4-6 mm/month)
- Max of 2,500/bowl
- Max size 12 mm
- Flow rate is about 1 L/min

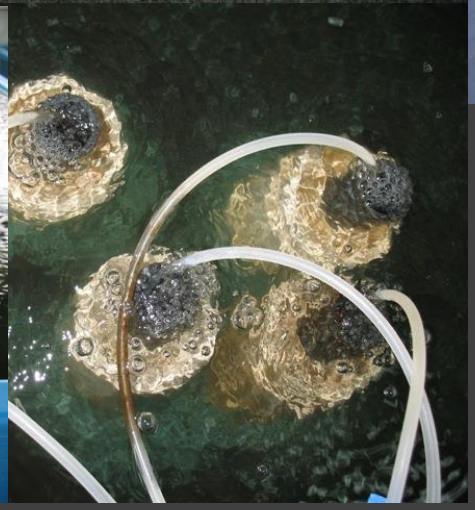
Nathan Eckert, Aquatic Wildlife Conservation Center, VA; Brian Watson VFAWC, DGIF

Dog Dish System -Kamoer dosing pump – cheap and reliable





Raceway Upwellers



Lampsilines & Amblemines:

Water: Filtered and UV treated surface water, recirculating; AABC Filtered only

Volume: 3 inch diameter upwellers, total= 624 L raceway; AABC 375 L raceway

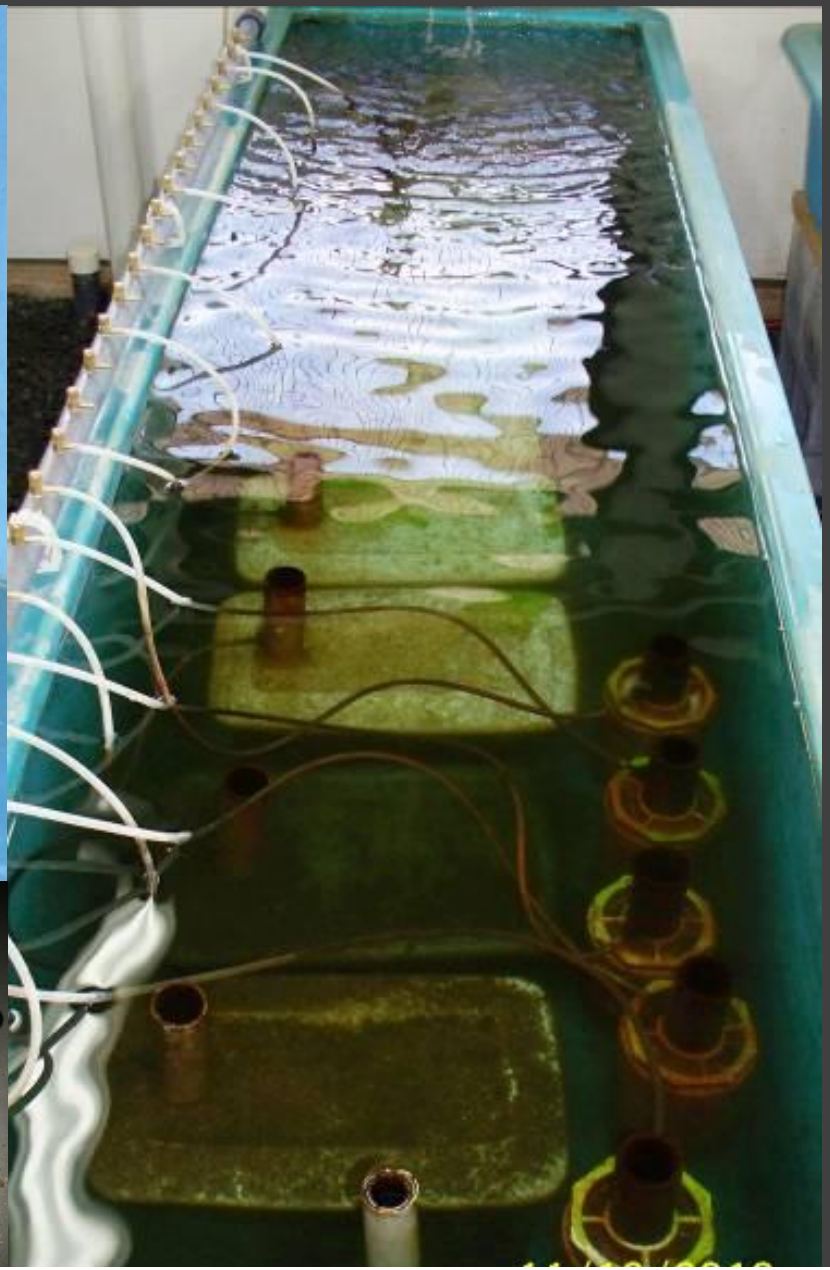
Screens: 105µm-1mm, stainless steel; AABC Nitex mesh 150µm-1mm

Food: Reed Mariculture Shellfish 1800 and Nanno 3600

Alabama Aquatic Biodiversity Center, ADCNR

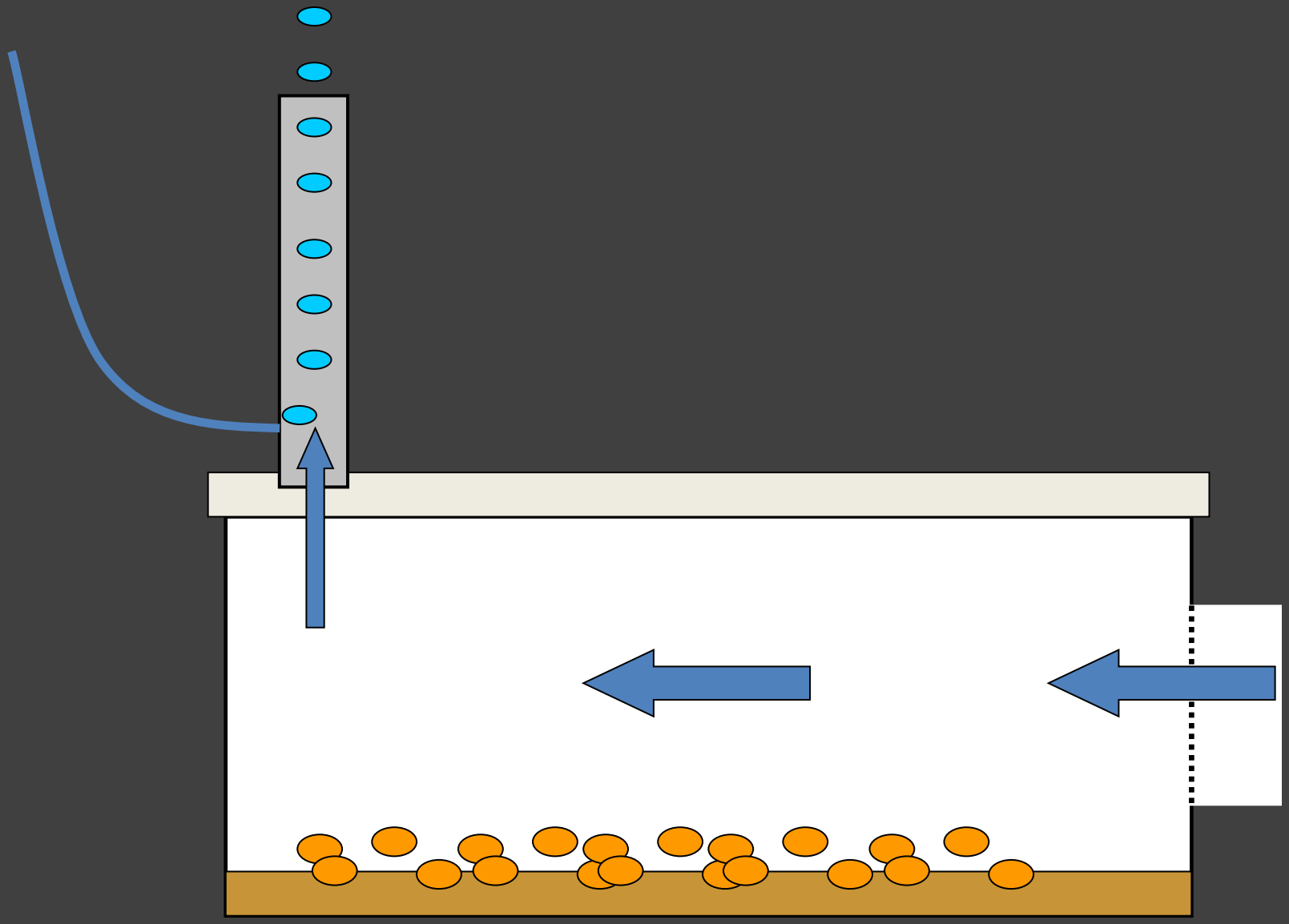
and Rachael Hoch, Marion Conservation Aquaculture Center, Marion, NC; Henley et al. 2001

Raceway Box culture- Anodontines (Aeration, regenerative blower 2hp)



Paul Johnson, Todd Fobian, Michael Buntin, Alabama Aquatic Biodiversity Center, ADCNR

Raceway Box culture- (Aeration, regenerative blower 2hp running two buildings)



Paul Johnson, Todd Fobian, Michael Buntin, Alabama Aquatic Biodiversity Center, ADCNR

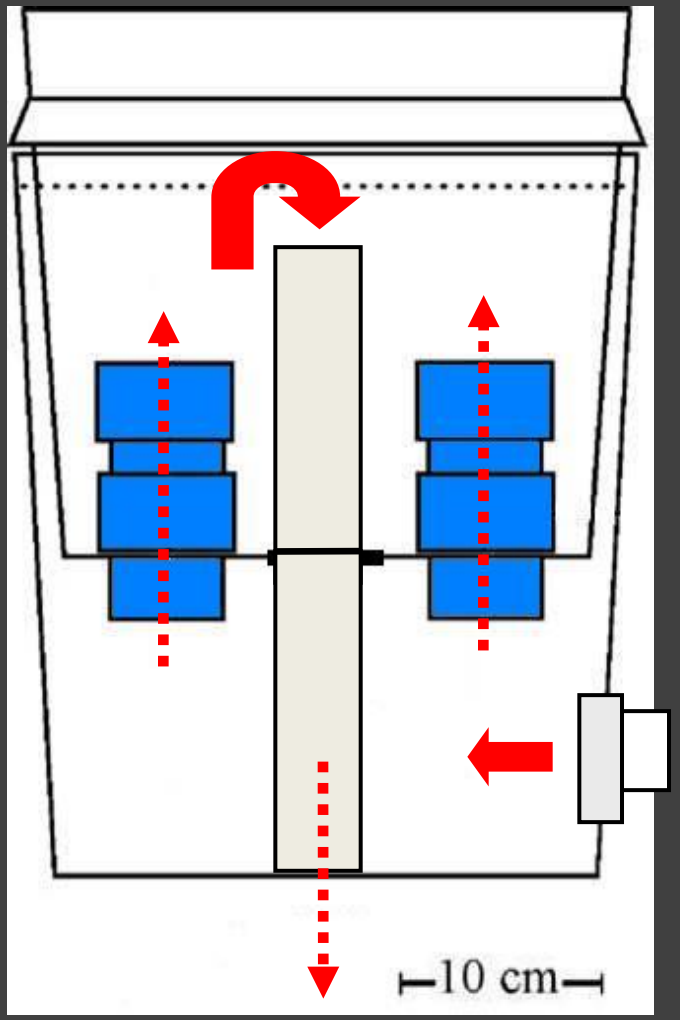
Flow-Thru Bucket Culture System: 454 L- 5-7 cups/bucket

Lampsilines, Quadrulines, & Amblemines

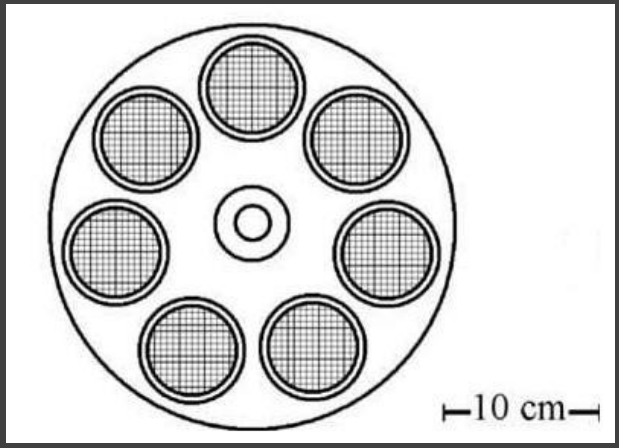
Paul Johnson, Todd Fobian, Michael Buntin, Alabama Aquatic Biodiversity Center, ADCNR

Upwelling Bucket System

Side View



Top View



Mesh Size:
Initially:150µm

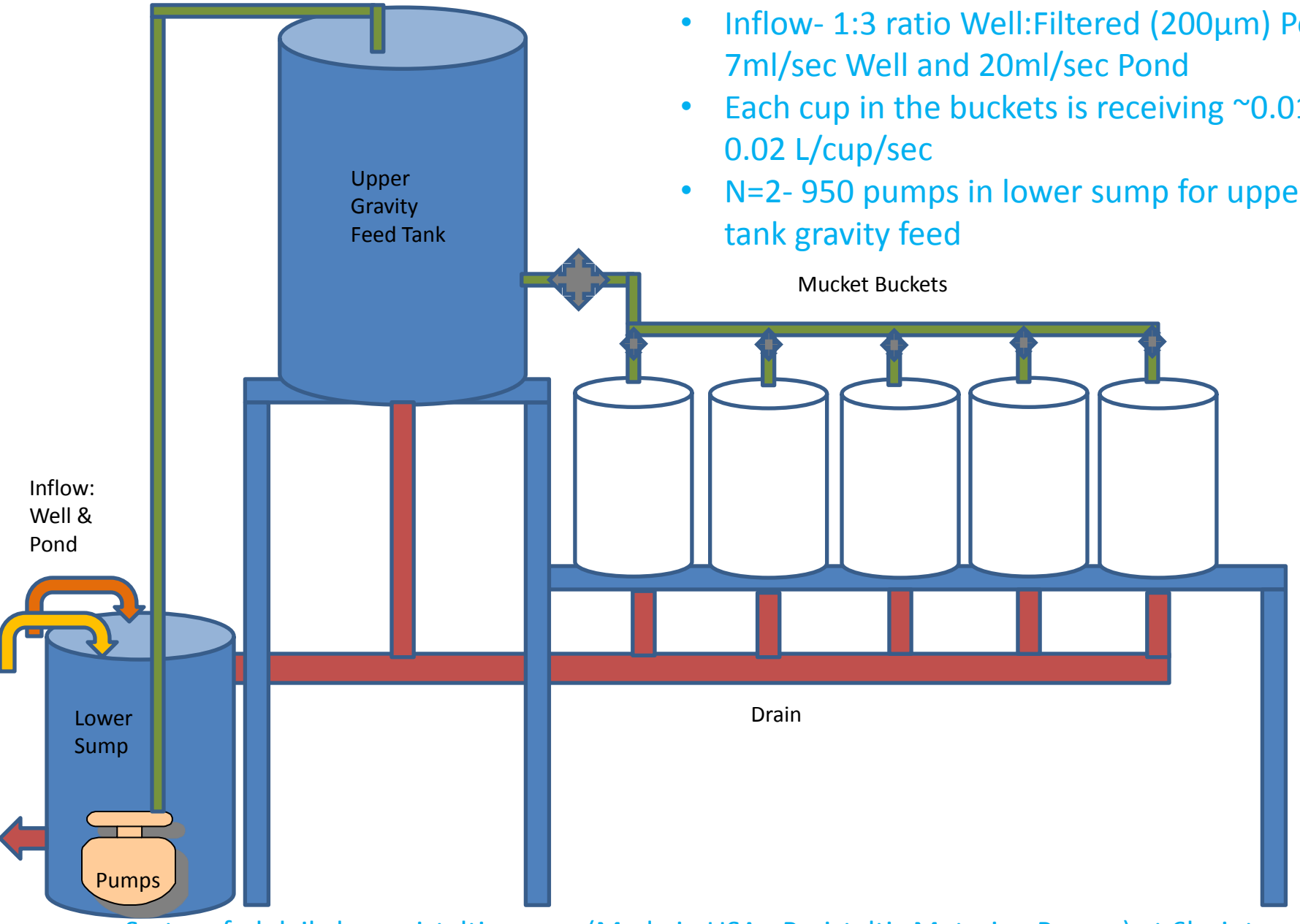
Move to: 250µm as soon as juveniles are large enough

Cup Screens Sprayed Weekly

500-1000 mussels/cup

Modified from figure in Barnhart et al 2005)

Paul Johnson, Todd Fobian, Michael Buntin, Alabama Aquatic Biodiversity Center, ADCNR



- Inflow- 1:3 ratio Well:Filtered (200µm) Pond 7ml/sec Well and 20ml/sec Pond
- Each cup in the buckets is receiving ~0.01-0.02 L/cup/sec
- N=2- 950 pumps in lower sump for upper tank gravity feed

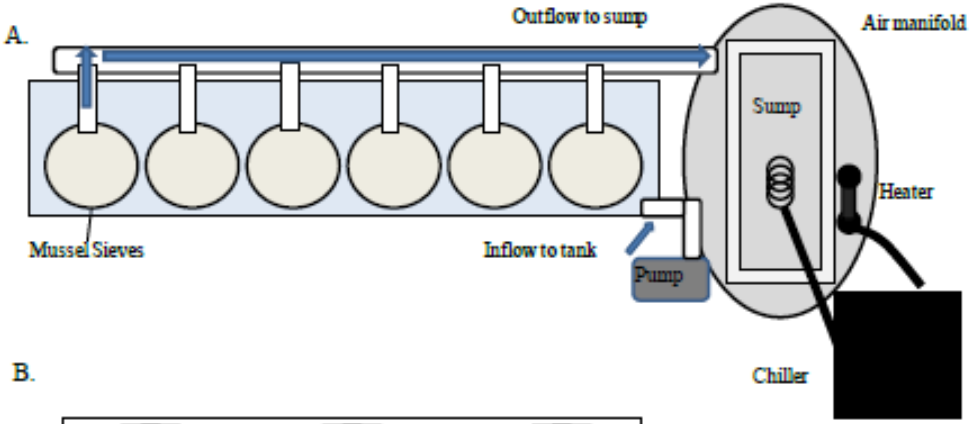
System fed daily by peristaltic pump (Made in USA - Peristaltic Metering Pumps) at 6hr intervals for 10min. each interval, feeding from a 2000mL beaker of stock solution (2000mL well water, 2ml nano, 17ml shellfish diet, kept refrigerated 10°C)

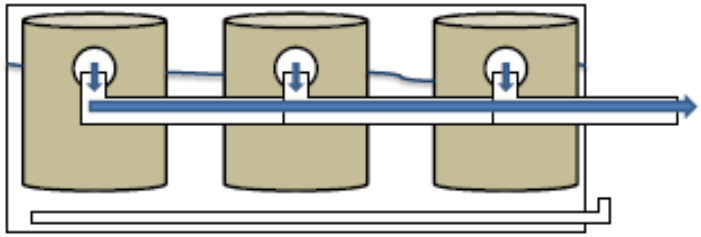
Juvenile Pond Water Filtration Supply

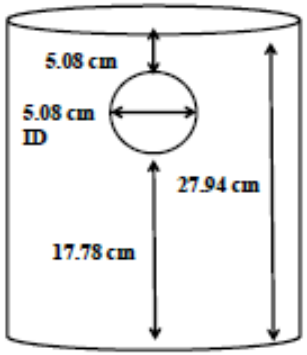
- MCAC
  - Filtered 100µm to 45µm to 30 µm
  - UV treated at 200 Watts
- AABC
  - 500 µm to 200µm

Rachael Hoch, Marion Conservation Aquaculture Center, Marion, NC

Raceway upweller- Sump







A. Top view of an indoor recirculating upweller system. Temperature-controlled water is recirculated from the sump into the rectangular tank. Each mussel sieve chamber drains back into the sump. An air manifold keeps the algae in suspension. B. Side view of the upweller system. Water is pushed up into the mussel chambers and then out the side of the chamber where is drains to the sump. C. Mussel chamber with dimensions. Source: Mair (2013).

Rachel Mair, Aquatic Resource Recovery Center, WV

Raceway upweller- Pond

Chris Barnhart, Missouri State University, Kansas City Zoo





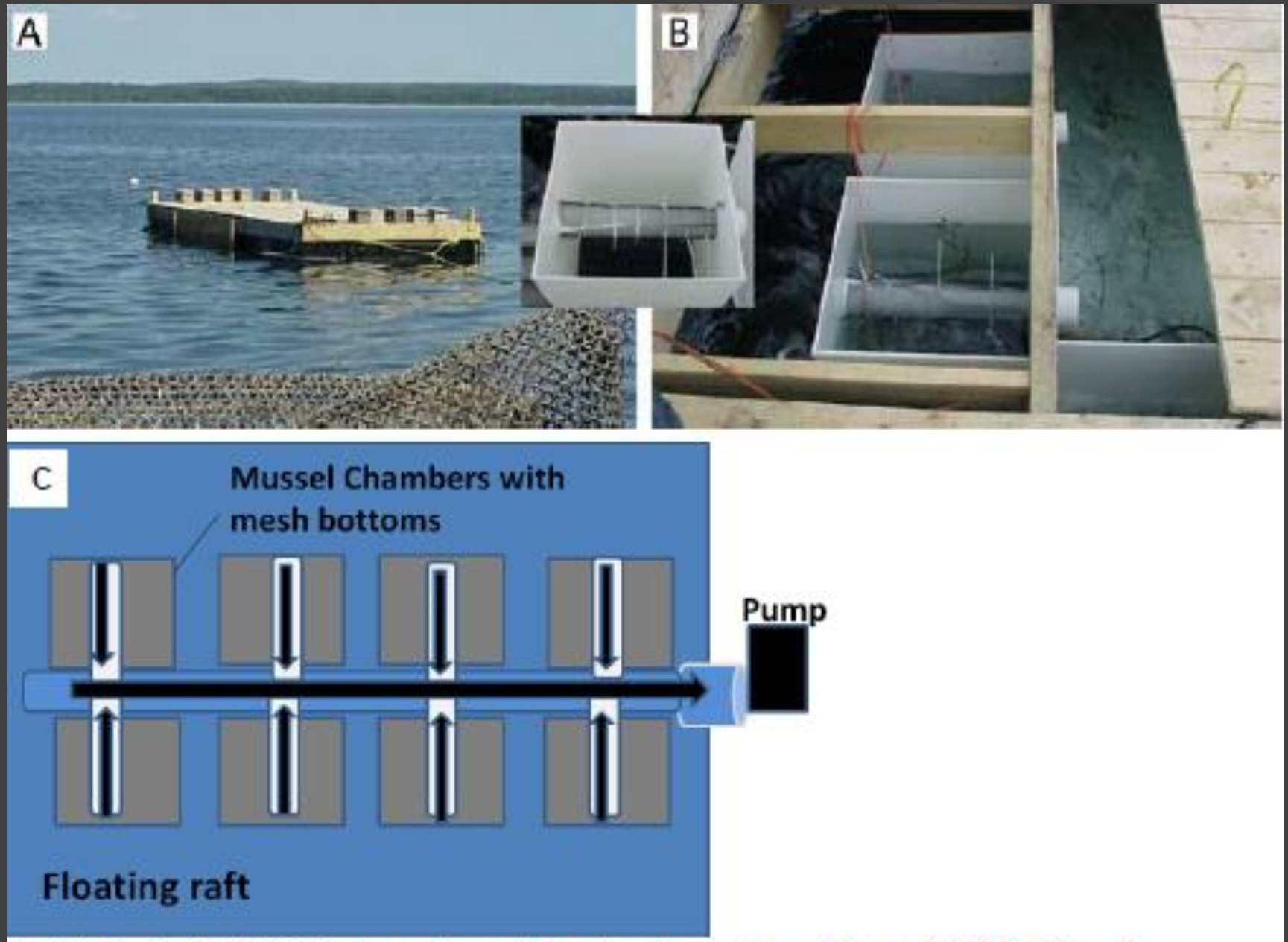
BREAK -

Stretch

Your

Mussels


Outdoor pond culture systems-Pond upwellers (FLUPSYS and SUPSYS)

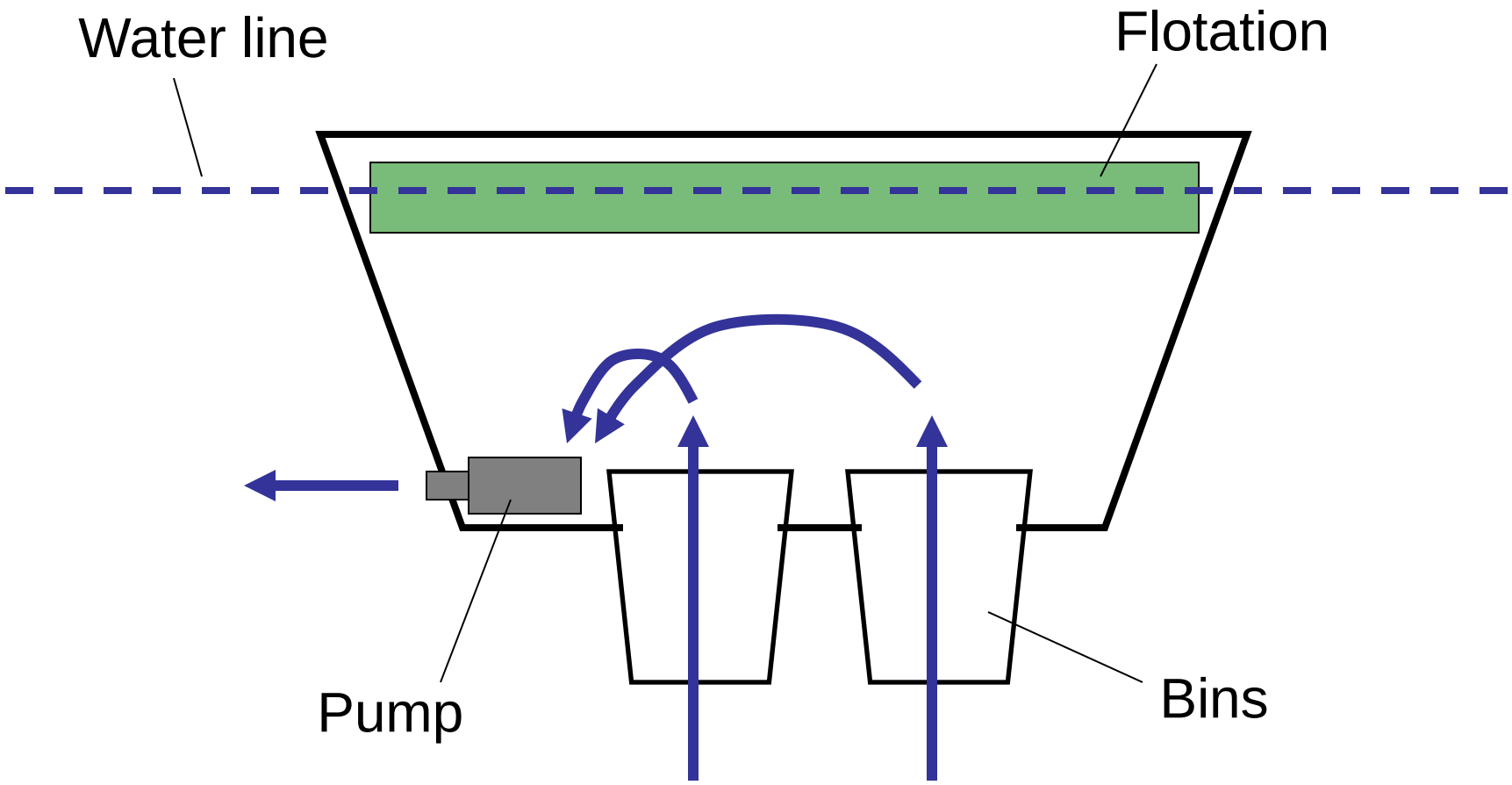


A and B. Marine FLUPSY, with close-up of mussel chambers. Source: Helm and Bourne (2004). C. Schematic of FLUPSY.

Tub-flupsy Four 2-gallon bins, stock tank

1200 GPH
Danner
Pondmaster
pump

Chris Barnhart, Missouri State University



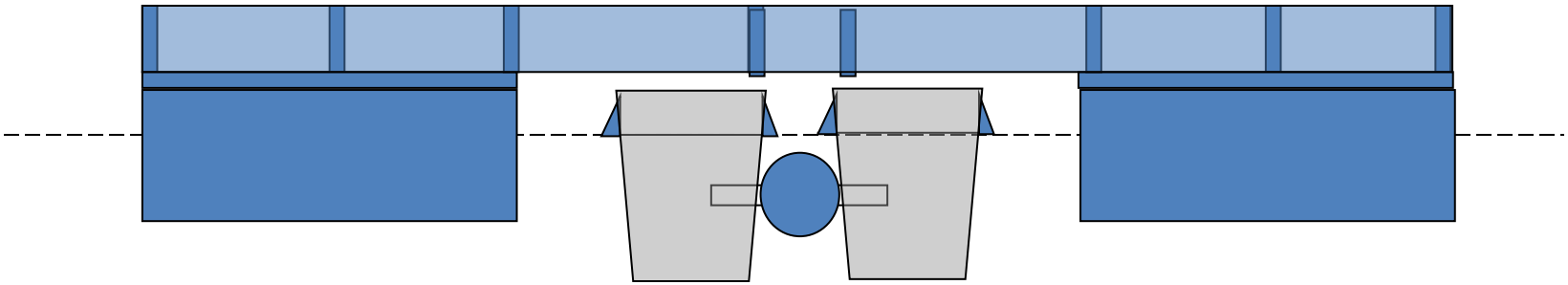
Chris Barnhart, Missouri State University

Chris Barnhart, Missouri State University

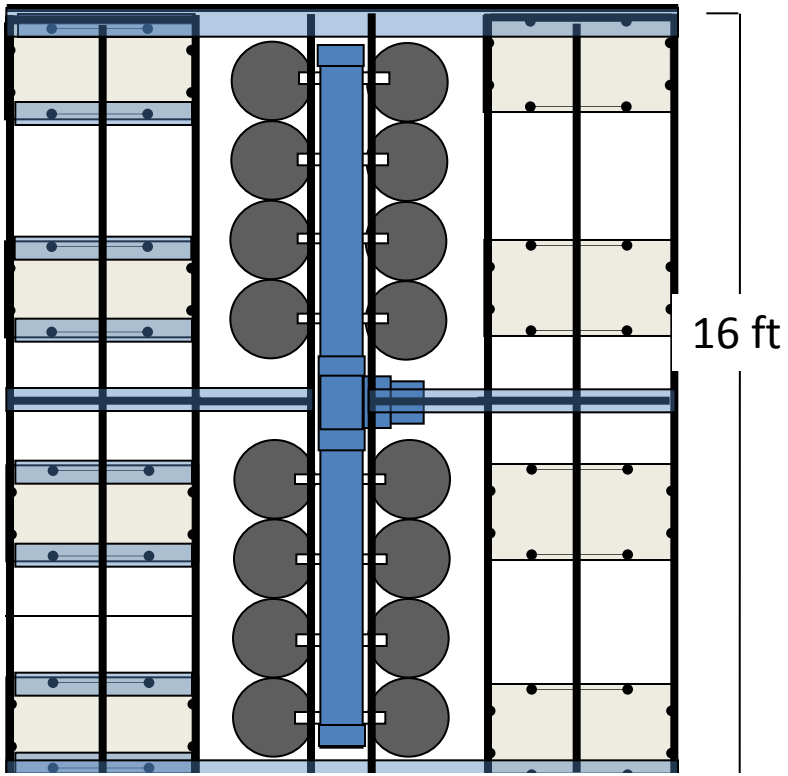
Mega Flupsy

Chris Barnhart, Missouri State University, Kansas City Zoo

Flupsy at Kansas City Zoo



- 14x16’ platform
- 16 34-gallon bins
- 10-inch manifold with ½ HP pump
- $3,800 materials



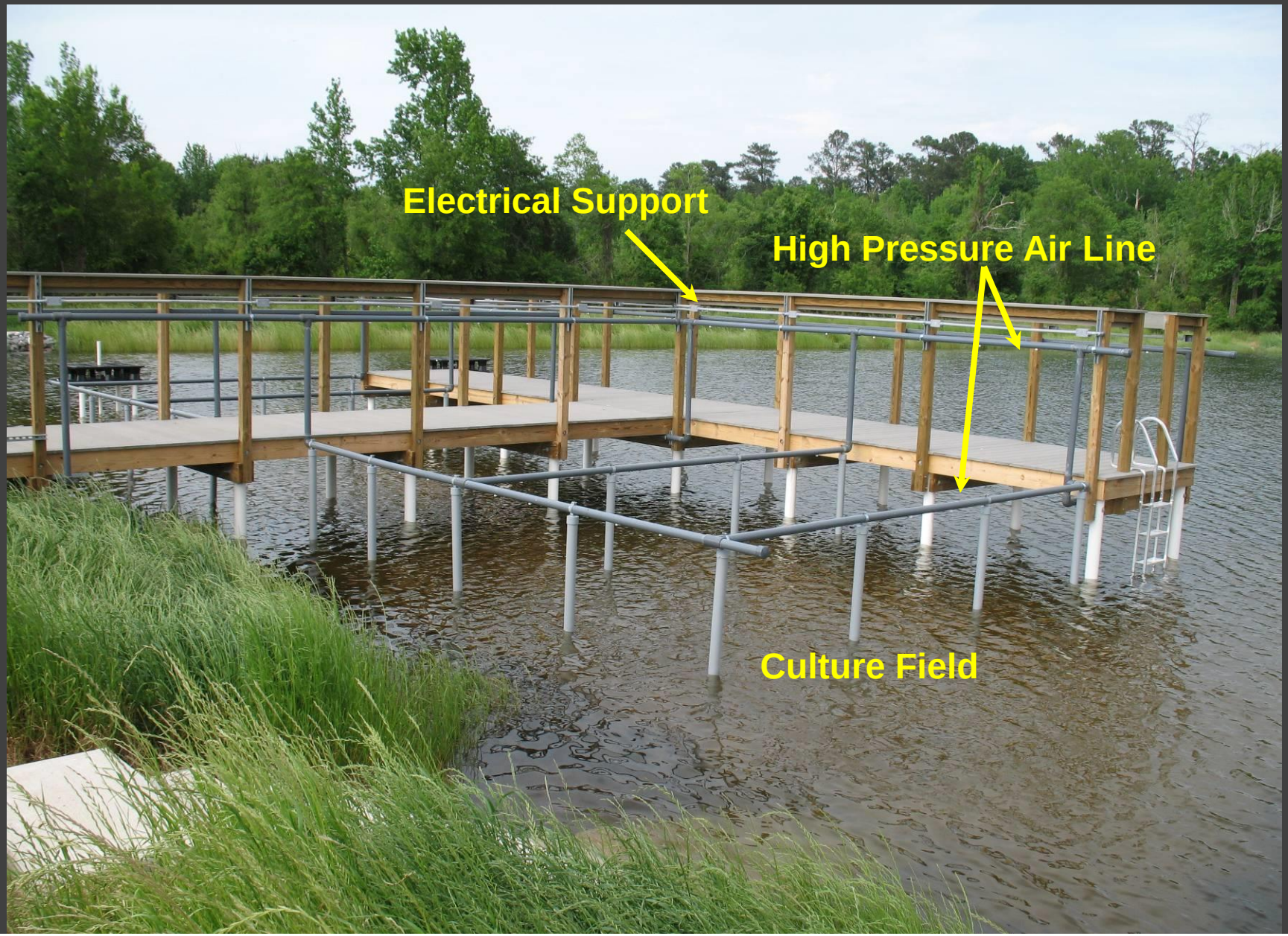
Chris Barnhart, Missouri State University

Two months of growth fatmucket

All tribes
Unionidae

Chris Barnhart, Missouri State University

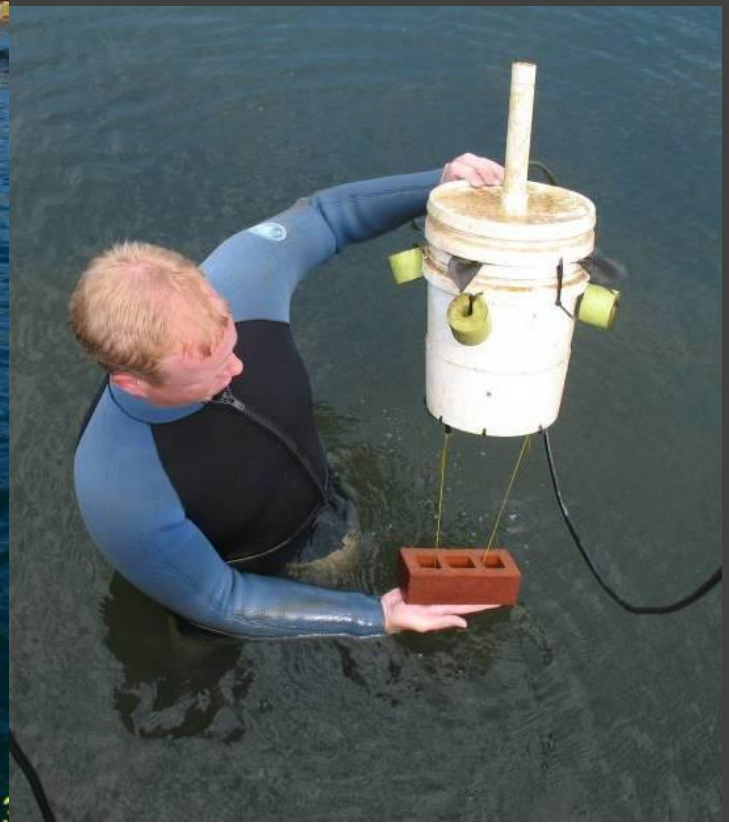
AABC Pond Culture Station – Pond 39 –

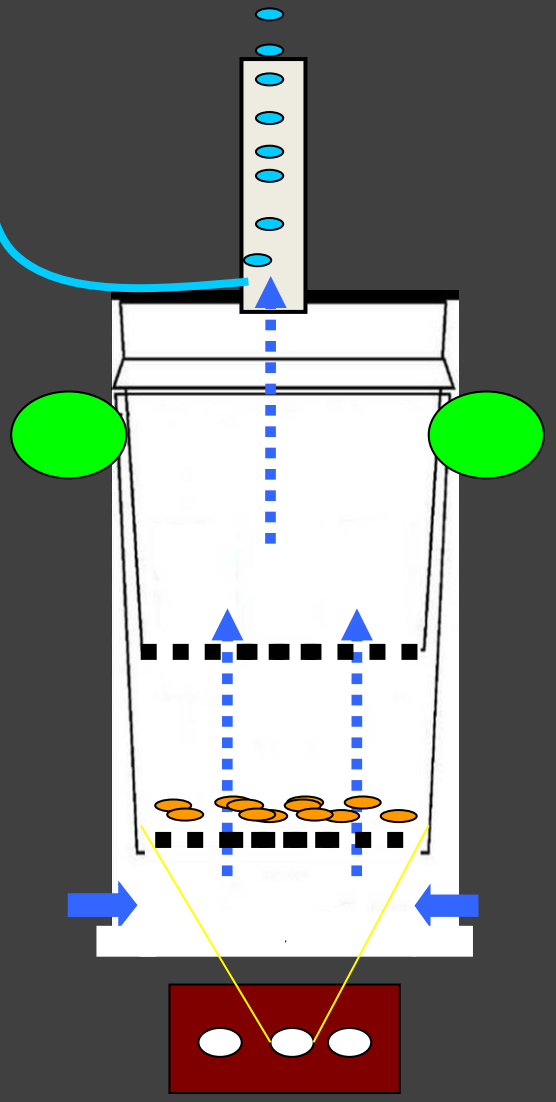


Paul Johnson, Todd Fobian, Michael Buntin, Alabama Aquatic Biodiversity Center, ADCNR

SUPSYS "Bubbler" – Mussel Culture Chamber

- 2 HP air blower, high pressure
- 2 gal buckets
- Window screen
- Pool noodles/net floats
- Brick/string
- Aluminum rivets



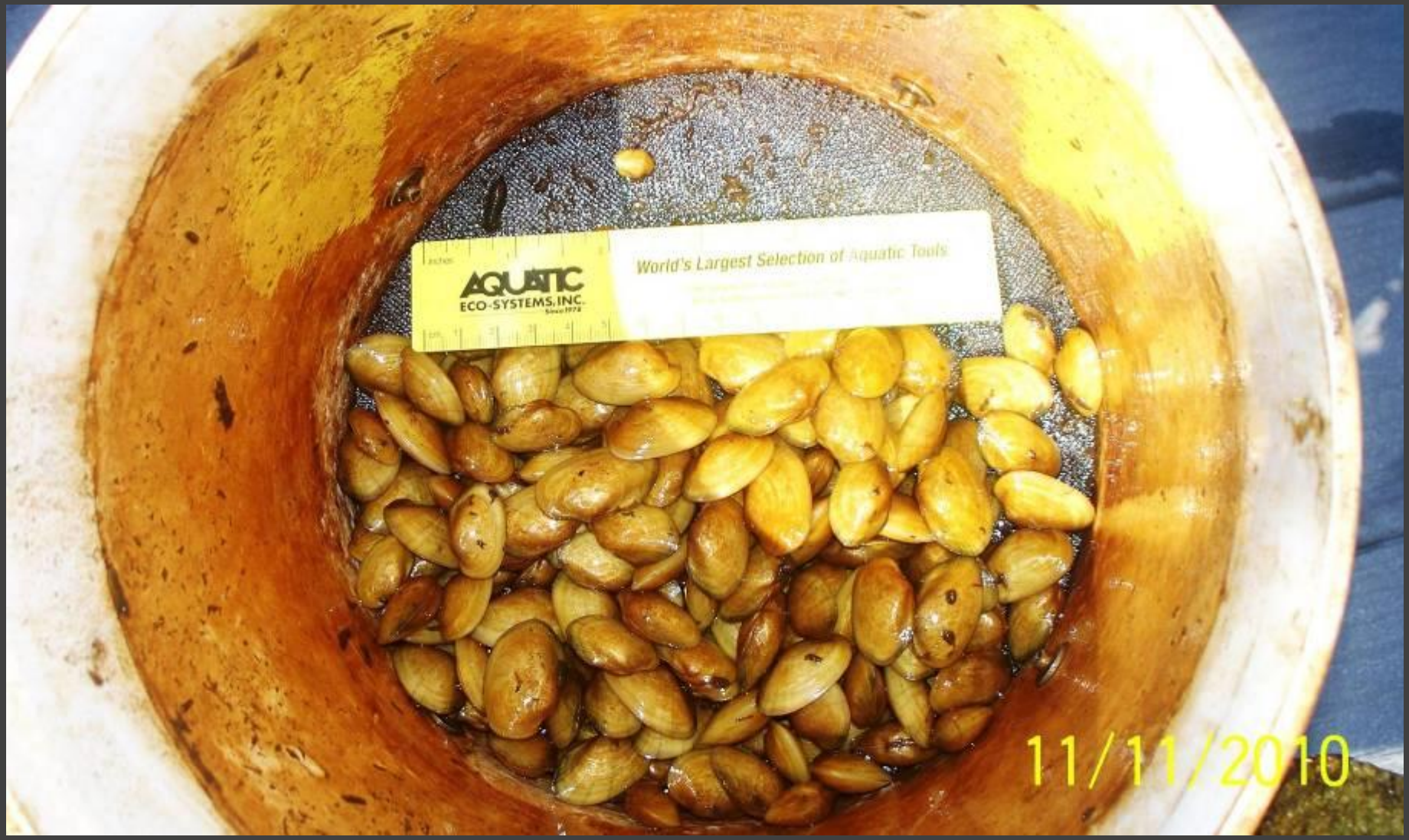


Paul Johnson, Todd Fobian, Michael Buntin, Alabama Aquatic Biodiversity Center, ADCNR

Alabama Lampmussel, *Lampsilis virescens* – May 2010 transformed

SUPSYS trial initiated 14 days previous

Photo taken July 23, 2010

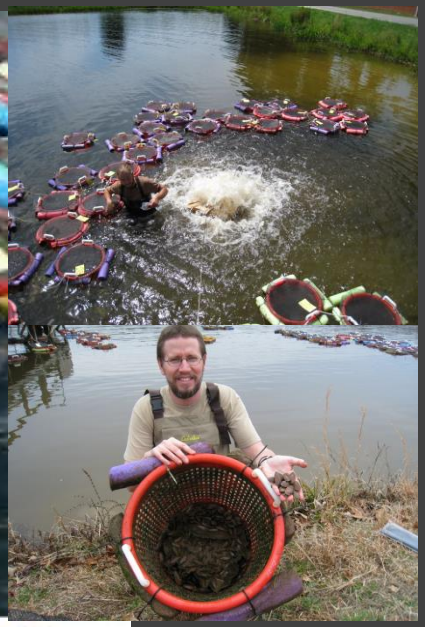


Alabama Lampmussel, *Lampsilis virescens* – May 2010 transformed

SUPSYS trial initiated ≈ 135 days previous

Photo taken November 11, 2010

Floating Pond Baskets



A and B. Floating cages currently in use at Harrison Lake National Fish Hatchery, Virginia Fish and Aquatic Wildlife Center..
Photo A: Brian Watson, Virginia Department of Game and Inland Fisheries. Photo B: Rachel Mair, USFWS

Primarily Lampsilines
125-150 screen on bottom siliconed and screwed with <1mm sand and silt.
On top of the 150 um mesh, add a loose piece of 200 um mesh / bowl for removing substrate and juveniles (4-33C)

Metamorphosis to 10mm
200-3,000 per basket
Growth can be 10 mm/mo

North Carolina State University, Harrison Lake National Fish Hatchery, Virginia Fish and Aquatic Wildlife Center, Marion Conservation Aquaculture Center

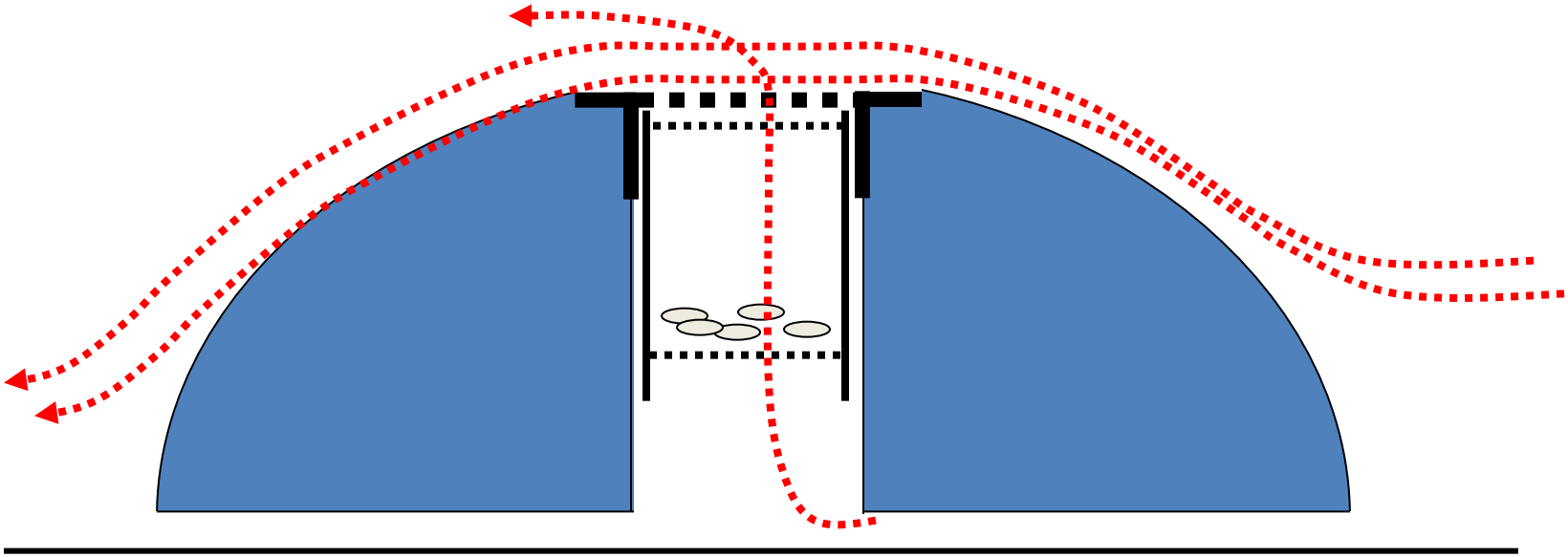
In stream culture systems- Silos and Bunkers

Photo of a single silo placed into the Cahaba River. Mussels are placed in a cup that's placed in the upweller chamber (vented top).

Upwelling cup containing juvenile mussels, placed into silo. Note the 1 mm mesh on the cup.

Silo systems developed by Dr. Chris Barnhart at Missouri State University

Bernoulli effect causes flow through chamber



Chris Barnhart, Missouri State University

Deployed Mussel Silo

Chris Barnhart, Missouri State University

9 Month old Southern Fatmucket placed into silo upwellers, May 11, 2010. sample split into 3 different silos. Silos placed in Cahaba River, Bibb Co., Alabama.

Photograph of Southern Fatmucket from Silo # 3 taken August 13, 2010 ≈ 90 days after trial initiation.

Mussel Bunkers

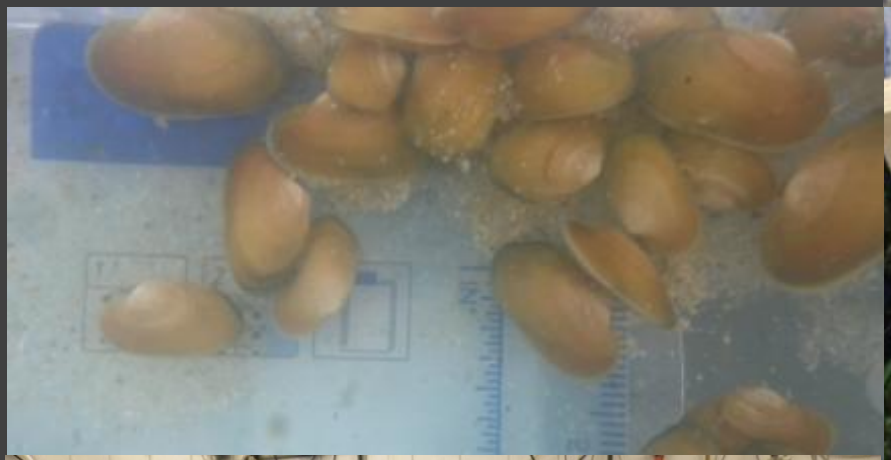







Chris Barnhart, Missouri State University

Release of mussels in gravel cages



- Mussel Size-Bayern 300 mm; Perlenbach 30 mm
- Small rearing channel on hatchery
- Screens of the cages are cleaned by hand 2-3 times a week
- Gravel mix (5-20mm) in size filling 30-40mm depth

Tanja Eybe, Frankie Thielen, Thierry Muller, Leo Klein, Sonja Heumann, Alexandra Arendt, Freshwater Mussel Rearing Facility, Luxembourg

AABC Gastropod Culture Production Pad – Interior View for mass culture of conservation targets

Paul Johnson, Todd Fobian, Michael Buntin, Alabama Aquatic Biodiversity Center, ADCNR

Snail species with spawning substrate/crevice preference

- Four suspended powerheads
- Solid bricks and terracotta tiles substrate

Pluerocera (fine sediment added)

- 60 gallon mortar style tanks
- Well or pond water supply (primarily well)
- Standpipe covers
- Shade top covers (algae control) (non treated wood shade cloth)
- Fine mesh nets (daily waste/excess algae removal)
- Snail densities (150-200 adults) (50 adults and 500-1000 juveniles)
- Heaters added for temperature control along with well water supply (500 Watt Titanium)
- Aeration, regenerative blower 2HP

Paul Johnson, Todd Fobian, Michael Buntin, Alabama Aquatic Biodiversity Center, ADCNR

Facility Containment Devices

- Central effluent capture sump
- Hydrotech drum filter 80 µm
- Gravel and sand chambers

Discussion

- Different system designs based on resources, goals and species
- Species culture variables/preferences-
  - Large river species culture gaps (ex. *Plethobasus sp.*, *Hemistena lata*)
  - Feeding and nutrition (covered by Rachel)
  - Flow
    - Barnhart unpublished silo study 2006 showed flow and food interaction/limitations at sites (high flow low food not growth limiting/ low flow high food somewhat growth limiting/ low flow low food growth limiting)
    - AABC ideal bucket cup flow ~0.01-0.02 L/cup/sec (Barnhart 2006, ~0.015 L/cup/sec)
  - Temperature
    - mean LT50 in 96-h juvenile tests was 34.7°C and ranged from 32.5 to 38.8°C (Pandolfo, et al. 2010)
    - mean LT50 35.6°C and ranged from 33.3 to 37.2°C (Archambault et al. 2013)
  - Sediment vs. no sediment
    - Survival and growth of juvenile mussels was significantly greater when cultured in a sediment substratum rather than sand or no substratum ($P < 0.001$)(Jones et al. 2005)
  - Release size
    - Release of larger (>20mm) individuals (Carey et al. 2015)
  - Number and size of stockings
    - ≥10,000 individuals (Ne=500) Clinch River, respectively, and ideally should be comprised of multiple smaller demes spread throughout a river (Jones et al. 2012)
  - Tagging (covered by Hua and Bryan)

Freshwater Mollusk Culture Facilities

Facility	Agency	Contact Person
Alabama Aquatic Biodiversity Center, AL	ADCNR	Dr. Paul Johnson
Aquatic Epidemiology and Conservation Laboratory, NC	NCSU	Chris Eads
Aquatic Resource Recovery Center, WV	USFWS	Rachel Mair
Aquatic Wildlife Conservation Center, VA	VDGIF	Megan Bradley
Aquatic Wildlife Conservation Center, VA	VDGIF	Amanda Duncan
Center for Mollusk Conservation, KY	KDFWR	Dr. Monte McGregor
Cumberland River Aquatic Center, TN	TWRA	David Sims
Freshwater Mollusk Conservation Center, VA	VT	Dan Hua
Freshwater Mussel Conservation & Research Center, OH	CZA	Dr. G. Thomas Watters
Freshwater Mussel Rearing facility , Luxembourg	Mill of Kalborn	Dr. Frankie Thielen
Genoa National Fish Hatchery, WI	USFWS	Nathan Eckert
Harrison Lake National Fish Hatchery, VA	USFWS	Michael Odom
Institute for Great Lakes Research, MI	CMU	Dr. Dave Zanatta
Institute for Great Lakes Research, MI	CMU	Dr. Daelyn Woolnough
Marion Conservation Aquaculture Center, NC	NCWRC	Peter J Lamb
Marion Conservation Aquaculture Center, NC	NCWRC	Rachael Hoch
Missouri State University (Barnhart Lab), MO	MSU	Dr. Chris Barnhart
Southeast Ecological Science Center, FL	USGS	Nathan Johnson
Virginia Fisheries & Aquatic Wildlife Center, VA	VDGIF	Brian Watson
South Auburn Fisheries Research Station, AL	AU	Jim Stoekel
Natchitoches National Fish Hatchery, LA	USFWS	Tony Brady

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- Scriven et al. 2011. A novel system for rearing freshwater pearl mussels, Margaritifera margaritifera (Bivalvia, Margaritiferidae), at Mawddach Fish Hatchery in Wales, UK. Ferrantia 64: 23–29.
- Hruška 1992 (reviewed in Gum et. al. 2011)
- Helm and Bourn 2004

2nd International Seminar Rearing of unionid mussels

Clervaux, Luxembourg, Tuesday 24th November – Thursday 26th November 2015

1st announcement

Restoration of Unio crassus rivers in the Luxemburgish Ardennes
LIFE11 NAT/LU/857

LE GOUVERNEMENT DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère du Développement durable et des Infrastructures
Département de l'environnement

LE GOUVERNEMENT DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Agriculture, de la Viticulture et de la Protection des consommateurs

CHAMBRE D'AGRICULTURE LUXEMBOURG

Questions?


BRAWNDO
THE THIRST MUTILATOR
IT'S GOT WHAT MOLLUSKS CRAVE!


BRAWNDO
THE THIRST MUTILATOR

AABC Water Quality

Well Water:

Temp. = 20° C

pH = 7.8

$CaCO_3$ = 120 mg/l

Ca = 39 mg/l

Bicarbonate = 129 mg/l

Specific Cond. = 220 µS/cm

DO = 6 mg/l – 65% Sat.

Interactions of flow, food density, and stocking density on food availability in silo