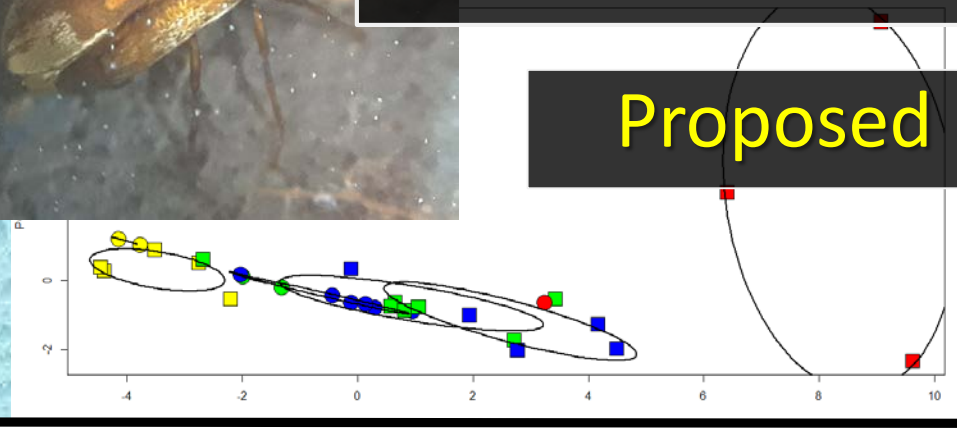
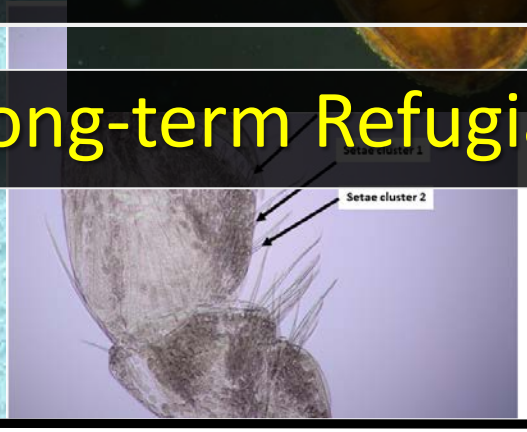
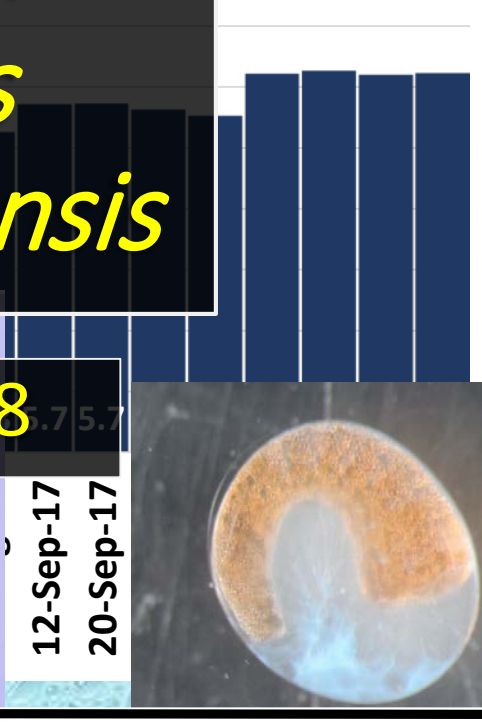
Macroinvertebrate Life Histories: *Stygobromus pecki, Stygoparnus comalensis,* and *Heterelmis comalensis*

Proposed Long-term Refugia Research 2018







U.S. Fish & Wildlife Service
San Marcos Aquatic Resources Center
Southwest Region

BIO-WEST

Stygobromus pecki – Background

- ~ 135 species within genus
- 3 sympatric species (*S. bifurcates*, *S. flagellatus*, and *S. russelli*)
  - May represent species complexes
- *Stygobromus pecki* is well supported as monophyletic (Ethridge et al. 2013)
- Consists of several subpopulations within Comal Springs with sufficient gene flow (Ethridge et al. 2013; Lucas et al. 2016)

Stygobromus pecki – Background

Amphipods in general:

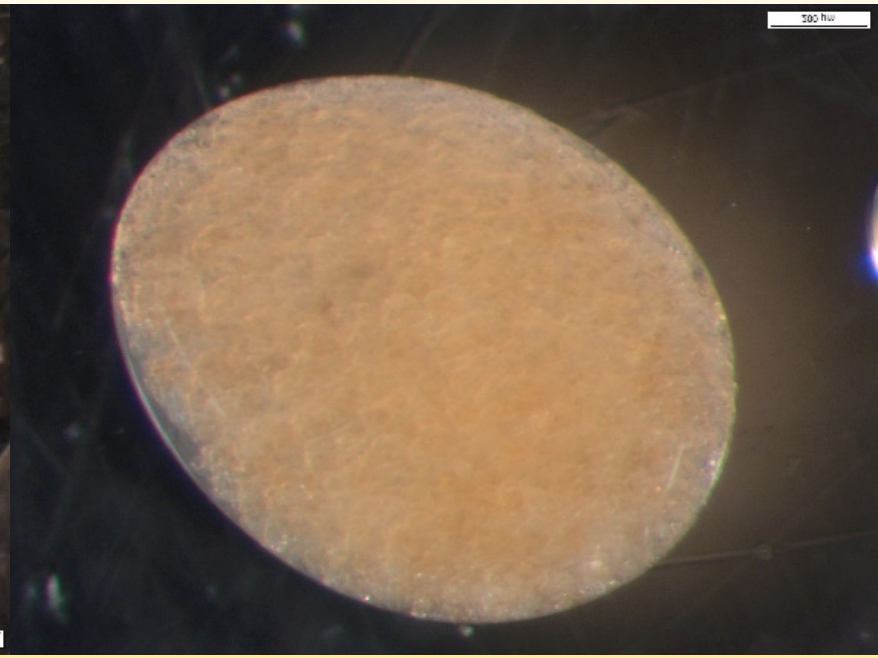
- Continue to molt throughout life
  - Set number of instars?
  - Asymmetrical (e.g. antennal segments, number of spines)
- Females receptive to mating after molt
- Males guard females via amplexus
- Males usually larger than females (Bollache and Cézilly 2004b; Franceschi et al. 2010; Worsham et al. 2017)
- Some amphipods subject to environmental sex determination (ESD) (Sutcliffe 1992; Watt and Adams 1993; McCabe and Dunn 1997)
  - Determined within weeks after leaving the marsupium

Stygobromus pecki – 2018 Objectives

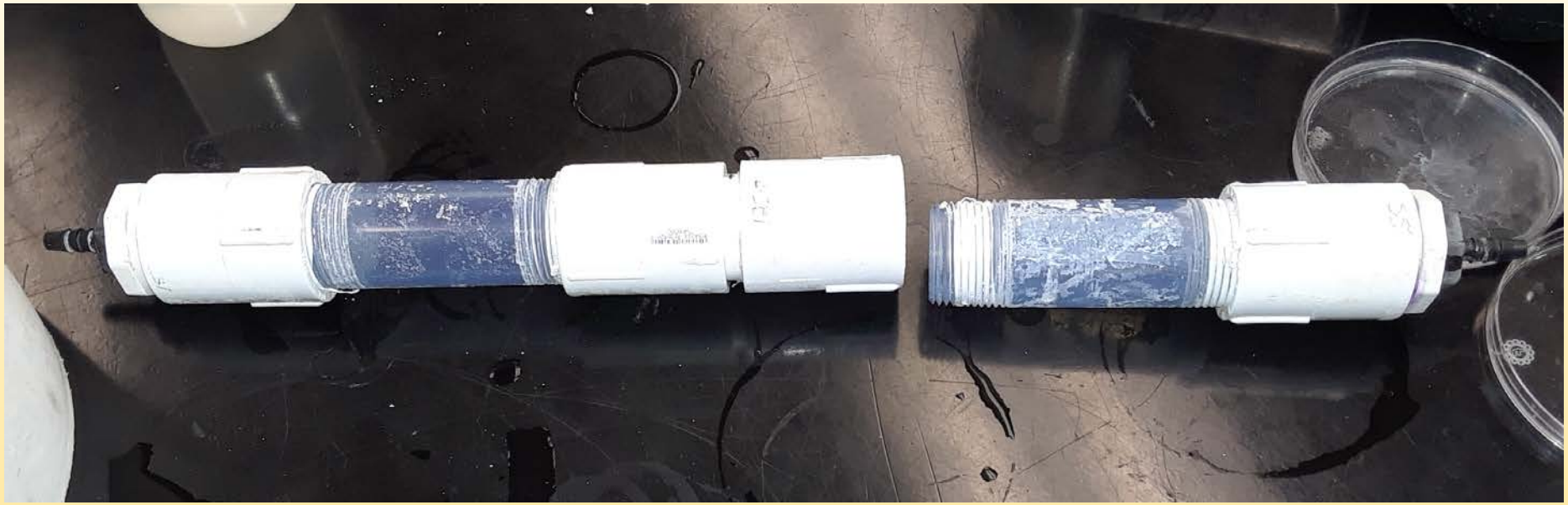
1. Estimate size class sexual maturity is reached
2. Estimate fecundity
3. Detect difference between immature sympatric congeners
4. Estimate growth rates
5. Investigate factors affecting sex ratios
6. Estimate egg incubation rates

Stygobromus spp. – Egg development

- Investigation initiated in 2017
- Brooding females obtained in coordination with SMARC personnel
- Traced 3 broods
- 58 ± 7.4 days to be released from the mother as free swimming



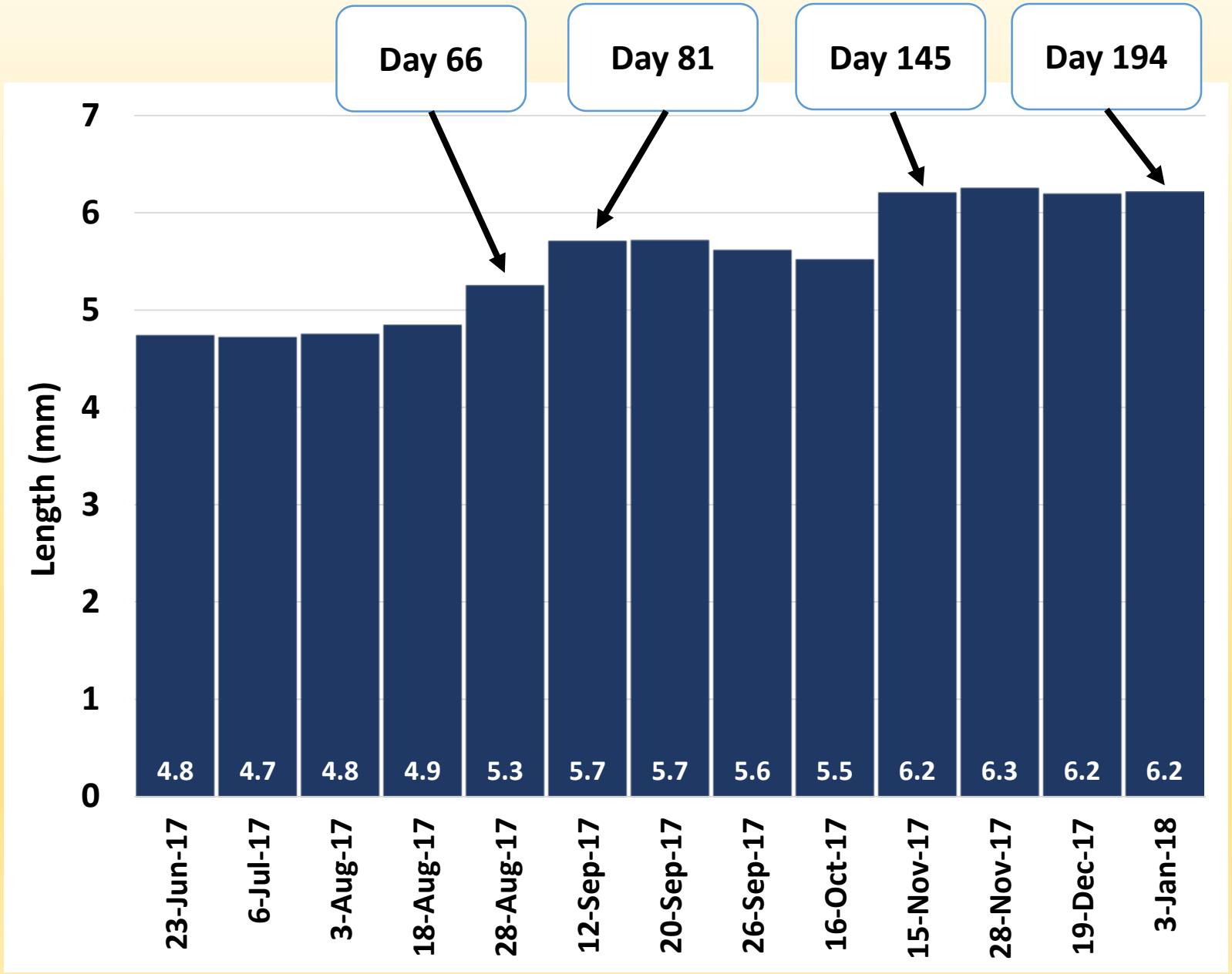
Stygobromus spp. – Egg development



- Brood chambers
- Neonates flow into tube through mesh female cannot pass
- Prevent cannibalism

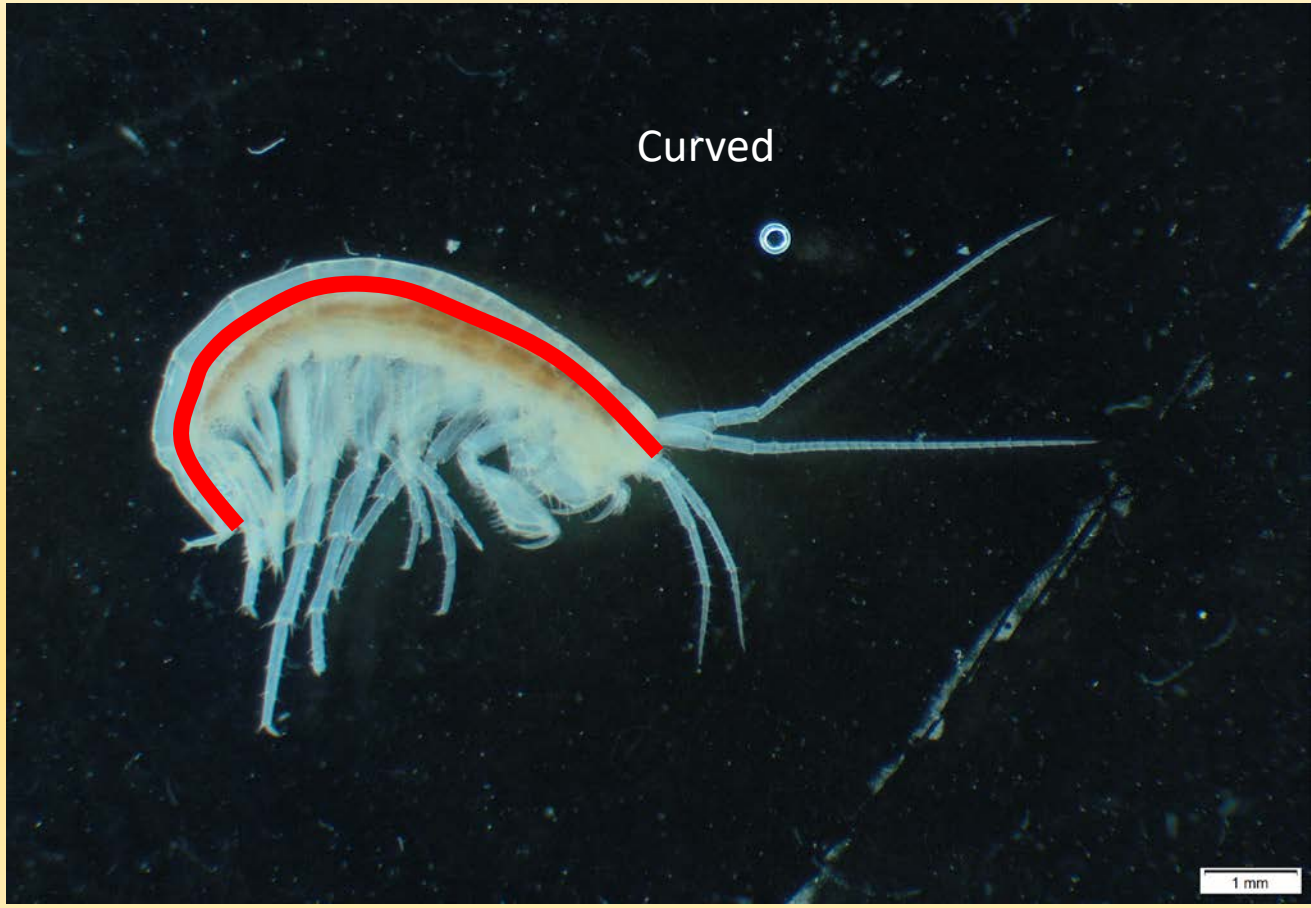
Stygobromus spp. – Immature Growth

- 2 individuals that have survived for ~ 200 days
- Grew ~1.5 mm
- ~ 2 months between molts



Stygobromus spp. – Immature Growth

- Does body position affect measurements?
- Straight vs. curved



Stygobromus spp. – Immature Growth

- 3-5 photos were taken of 6 individuals each in straight or curved positions at 2 time points
- Digimizer Image Analysis software used to measure body length
- t-test used to compare straight with curved measures
- Variability inspected

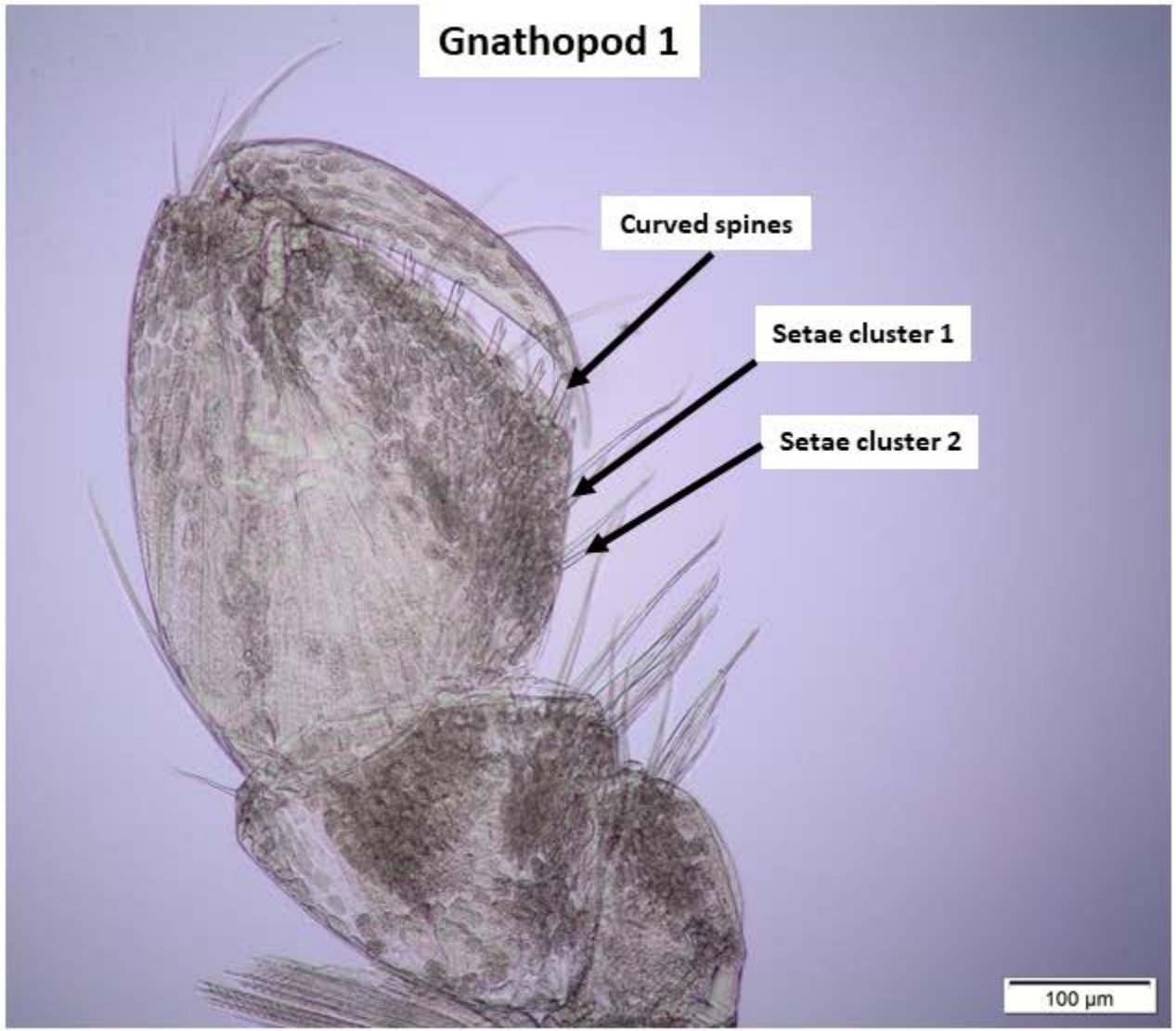
Stygobromus spp. – Immature Growth

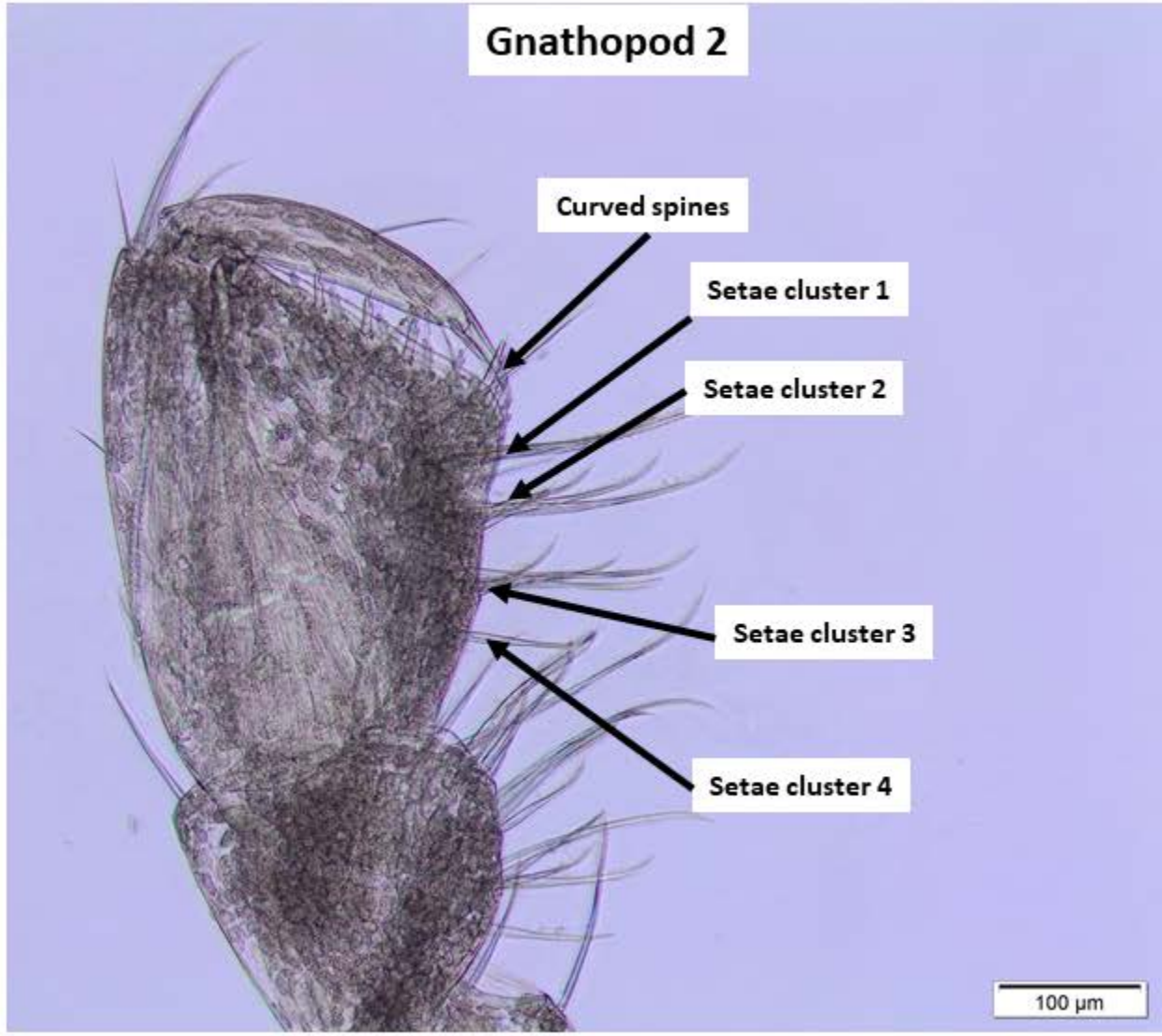
- In 4 of 11 (36%) comparisons straight and curved were different
  - 3 were longer in curved
- Curved position tends to be more natural
- ***Average 95% confidence interval of curved position was ±0.057 mm
- Combine other measures to determine successive instars (e.g. antennae)

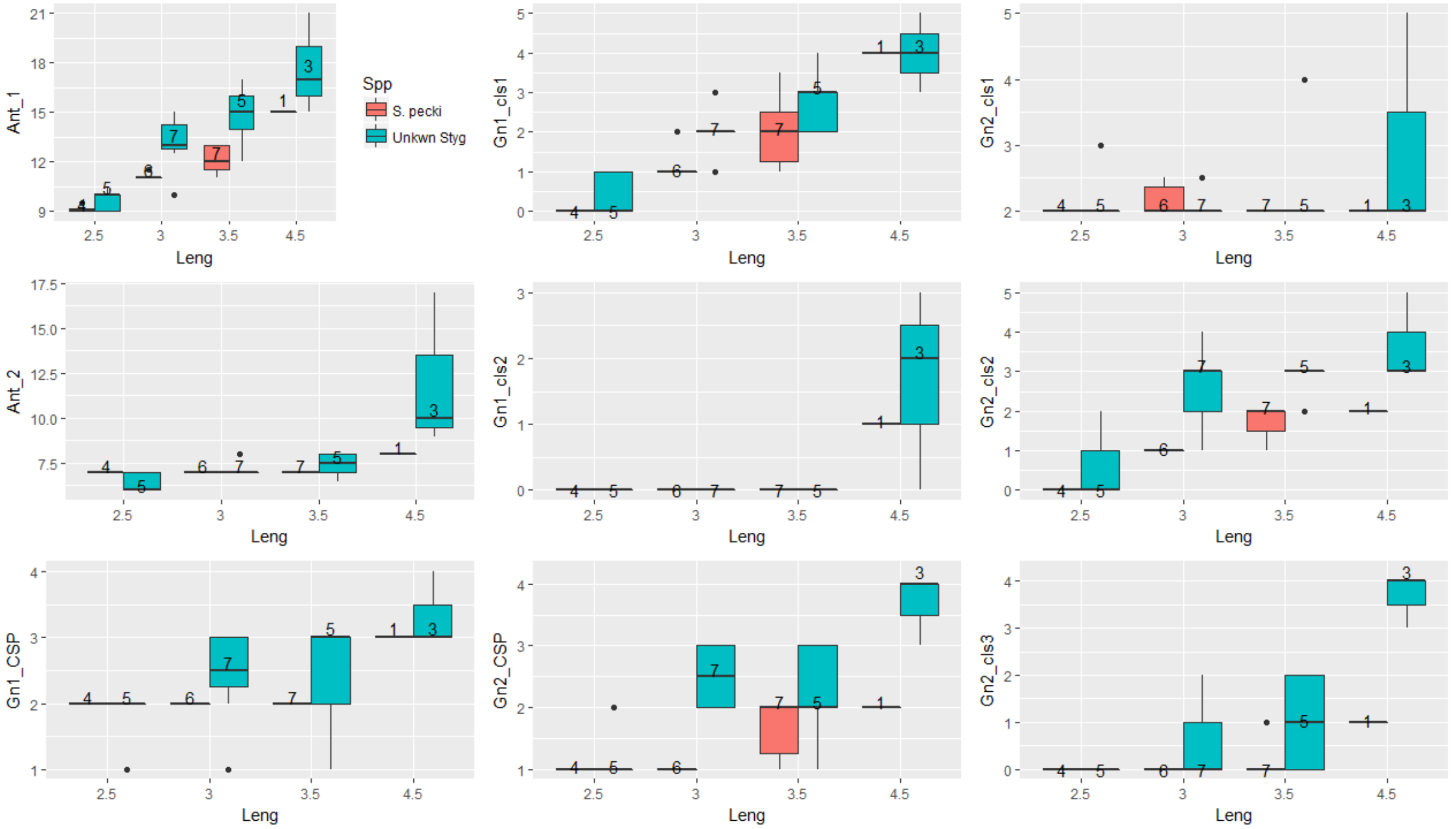
Stygobromus spp. – Immature Species Determination

- Pilot study conducted in 2017 with preserved specimens
- Comal Spring drift samples 2003
  - Presumed to be *S. pecki*
- Spring Lake samples 2008
  - Presumed to be *Stygobromus* spp. that are not *S. pecki*
- Looked at character states for different size classes

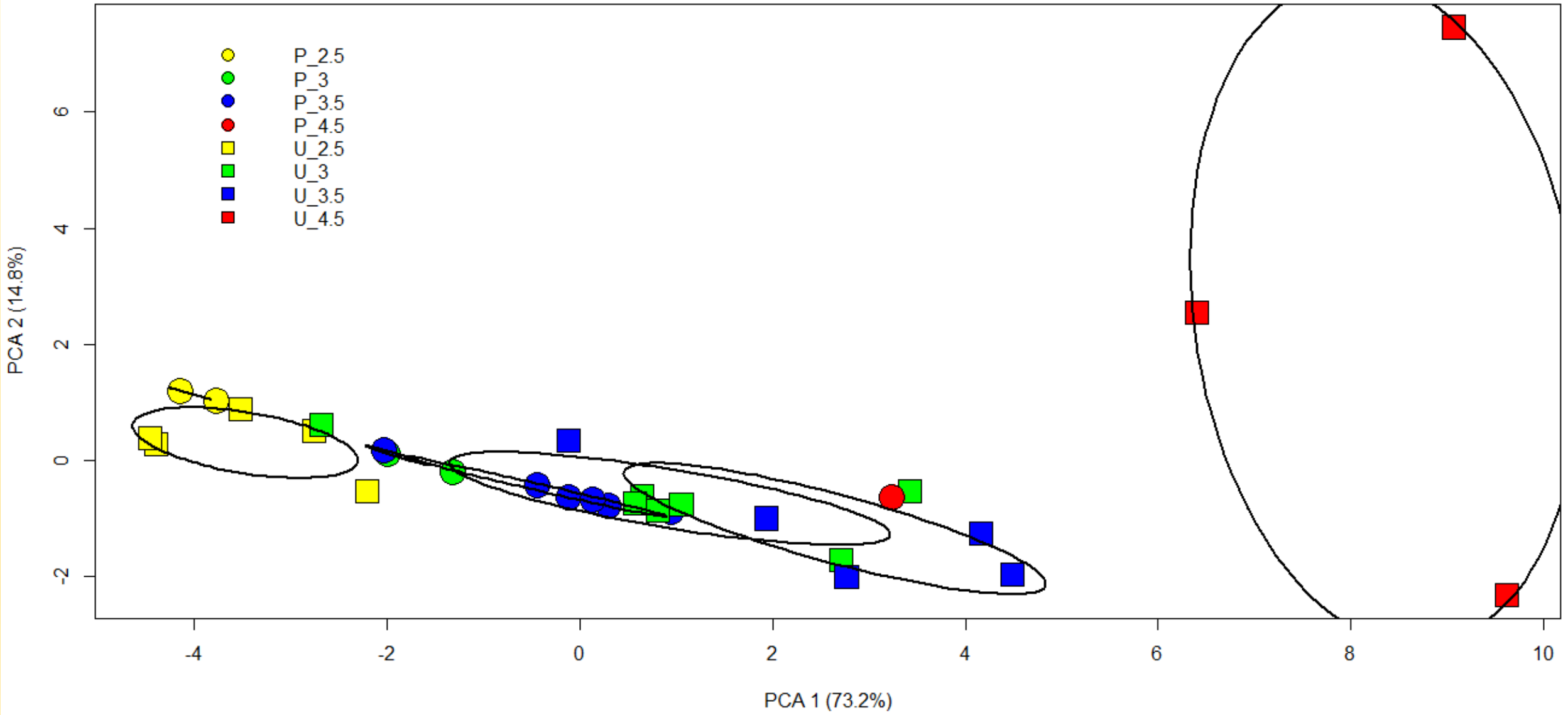
Example of characters used to distinguish stages of *Stygobromus sp.*






Ant_1
Leng
Spp
S. pecki
Unkwn Styg
Gn1_cls1
Gn2_cls1
Ant_2
Gn1_cls2
Gn2_cls2
Gn1_CSP
Gn2_CSP
Gn2_cls3

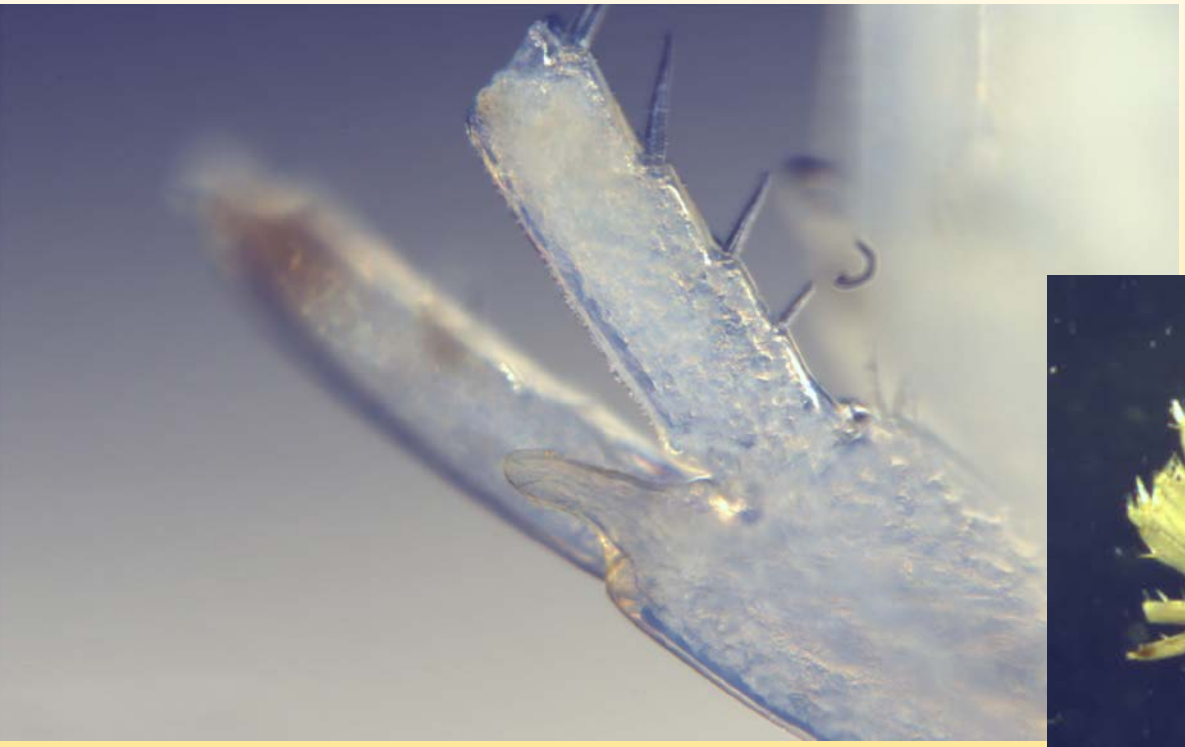
PCA – Specimen biplot



Stygobromus spp. – Immature Species Determination

- Obtain specimens from both localities
- Use AIC to determine best character for separating immature *S. pecki* from other species
  - Determined for each size class
  - More complicated models used if 1 character is not sufficient
- Individuals (all species) reared at SMARC used for validation

Stygobromus pecki – Sex Determination



- Uropod 1 of male with distal peduncular process

Stygobromus pecki – ESD Investigation

- Hypothesis:
  - Juveniles with more food achieve a larger body size and have a tendency to become males
- Rationale:
  - Other species display ESD to favor male development with larger body size
  - Environmental conditions may elicit greater food availability affecting size
  - Therefore, food availability may influence sex development

Stygobromus pecki – ESD Investigation

- Utilize *Stygobromus* other than *S. pecki* as surrogate species
- Setup 2 common gardens:
  - LOW – Feed less
  - HIGH – Feed more
  - Each contain 10 females and 10 males
- Gravid females removed
  - Released neonates placed into nurseries that reflect low and high feeding schedules
- Record:
  - Number of gravid females over time
  - Estimate fecundity (neonates released)
  - Compare sex ratios

Stygobromus pecki – Benefit to Refugia

- Egg Development and Immature Growth:
  - Determine how long eggs develop into breeding adults
- Immature Species Determination:
  - Help ensure refuge collections are correct species
  - Identify size class species can be deciphered
- ESD Investigation:
  - If feeding influences sex ratios, then an understanding of this mechanism should be of interest to captive propagation efforts

Stygoparnus comalensis – Background

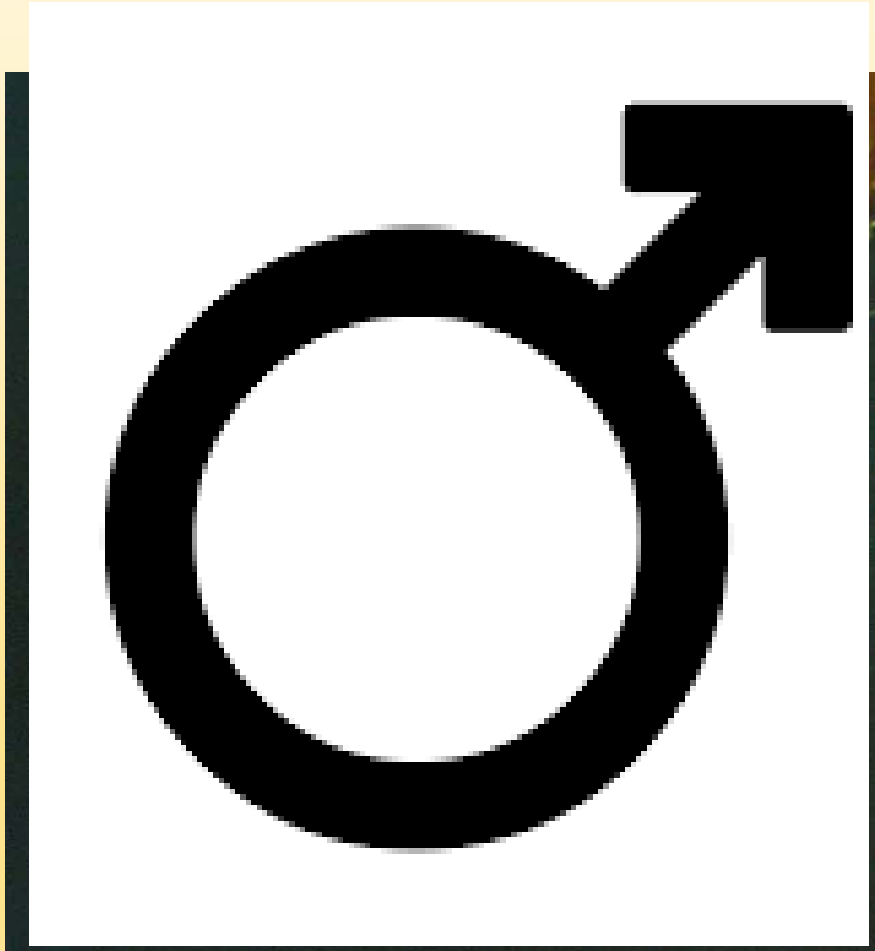
- Described from Comal Springs (Barr and Spangler 1992)
- Listed as federally endangered species (USFWS 1997)
  - Since < 150 specimens have been collected
- Known from Comal, Fern Bank, and Sessom Springs
- Adults fully aquatic and respire through plastron
- Larvae encountered less often
  - Habitat unknown
  - Proposed to live in subterranean air pockets (Barr and Spangler 1992)
  - Epigean species inhabit semi aquatic soil adjacent to streams (Brown 1987, Ulrich 1986)
- Female oviposition is unknown (aquatic or terrestrial)

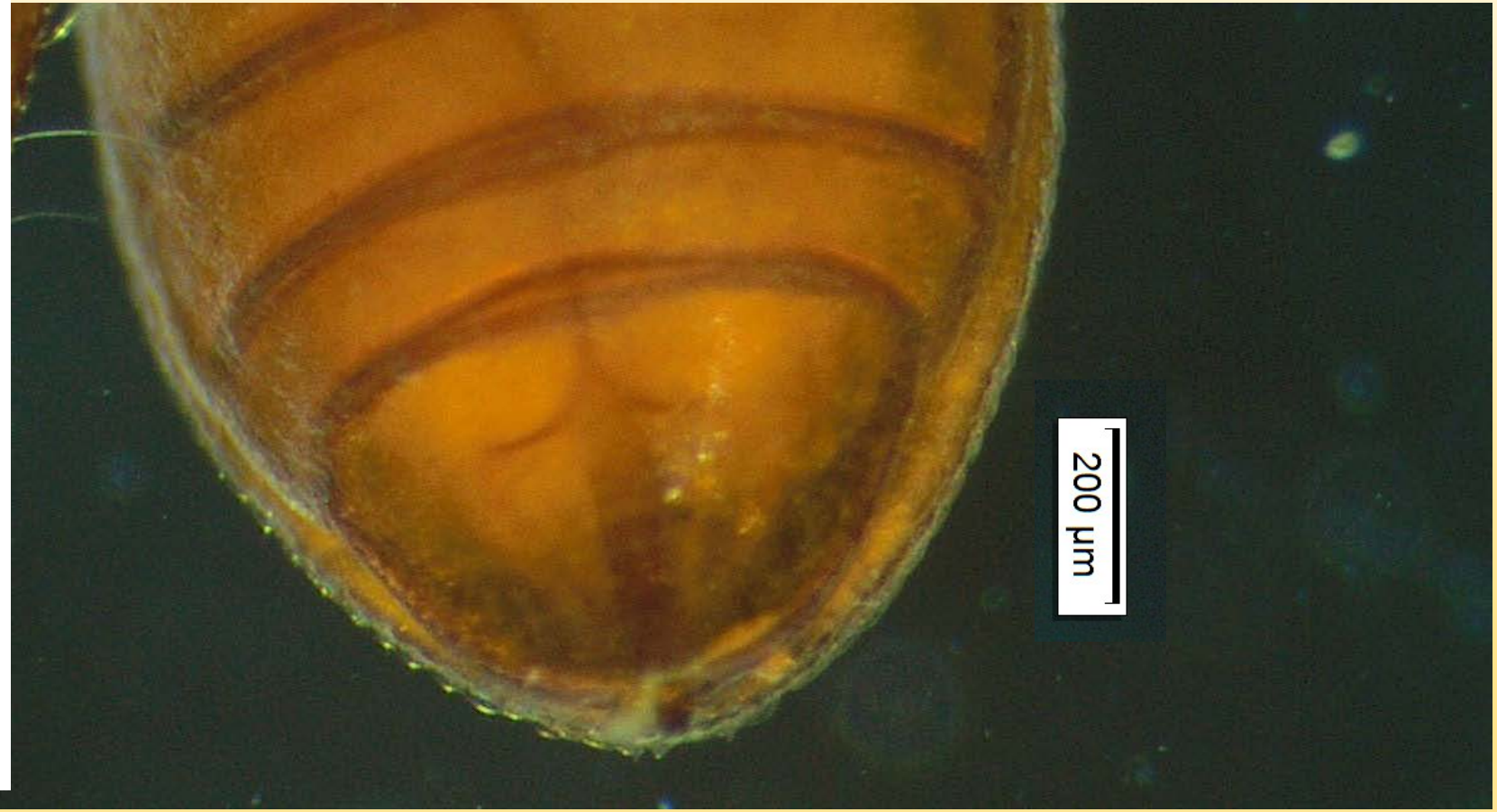
Stygoparnus comalensis – 2018 Objectives

1. Identify sexual dimorphic characters
2. Determine if eggs are oviposited above or below water
3. Estimate fecundity (number of eggs per clutch)
4. Egg incubation duration
5. Identify larval habitat (submerged or emergent)
6. Investigate larval growth rates
7. Identify adult response to flow

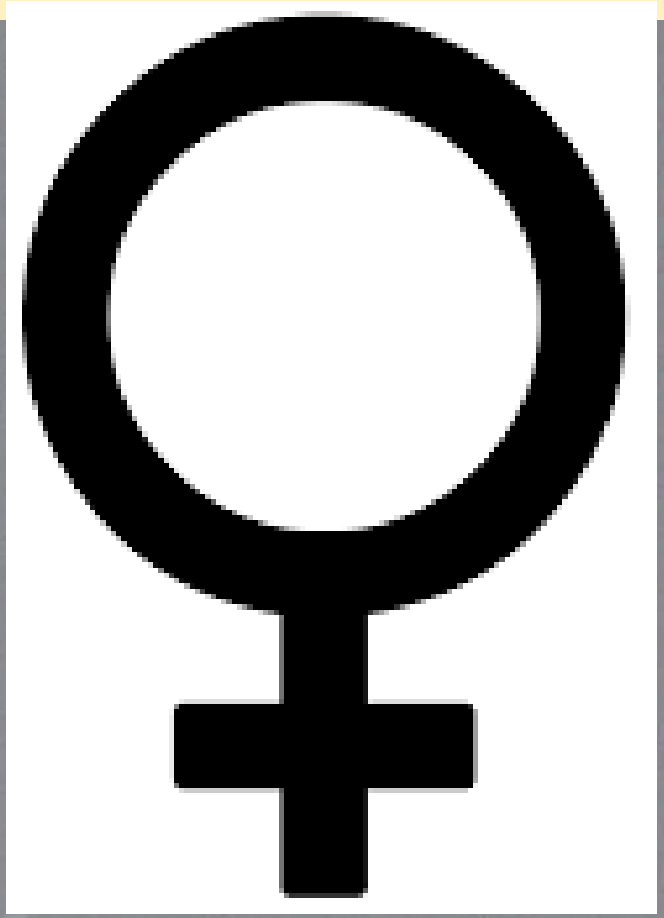
Stygoparnus comalensis – Sex Identification

Stygoparnus comalensis – Sex Identification



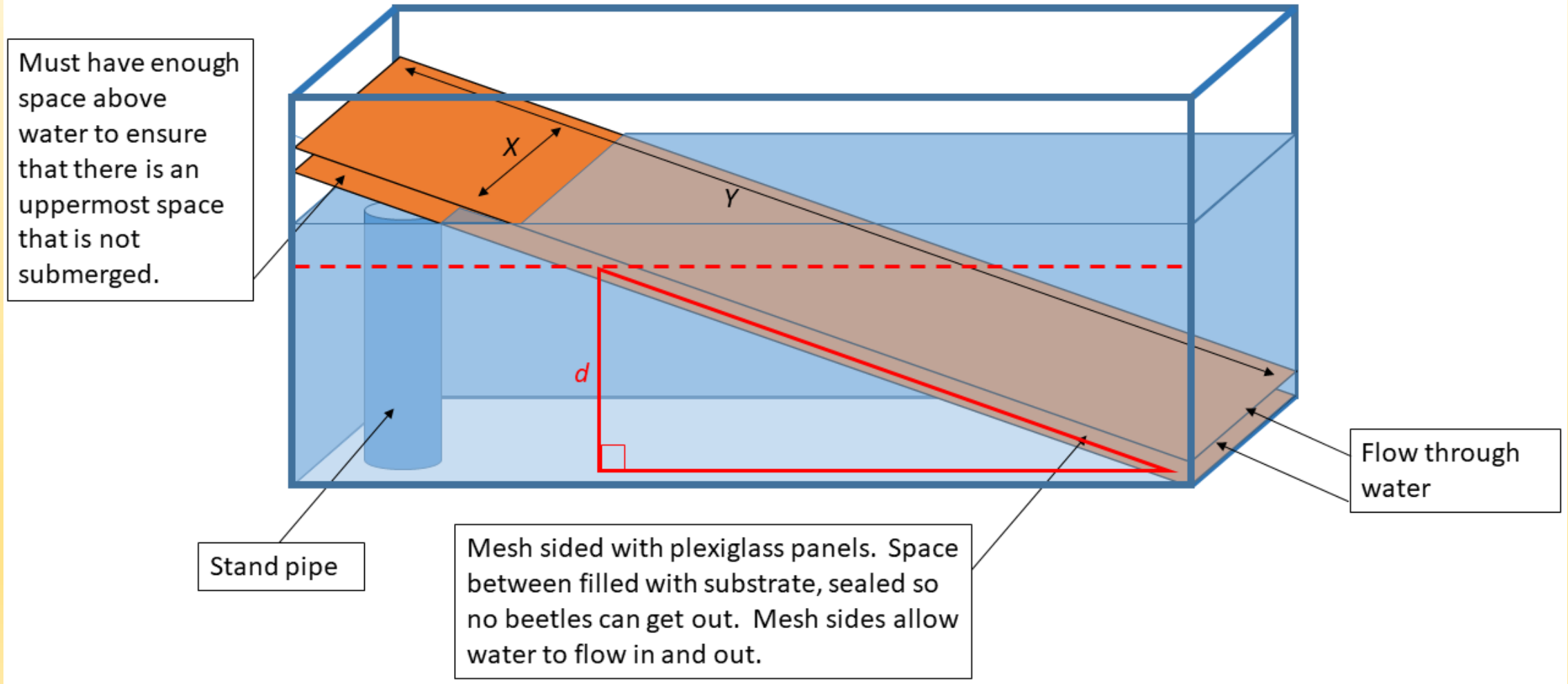


Stygoparnus comalensis – Sex Identification



Stygoparnus comalensis – Reproduction

Oblique Plane Apparatus (OPA)



Stygoparnus comalensis – Reproduction

- OPA pilot initiated 4 Dec, 2017
- Couple encourage to mate
- Migrated to bottom close to flow
- No eggs found
- May consider building smaller device

Stygoparnus comalensis – Flow Preference

- Understand response to flow
  - Adjust different intensities
  - Modular → can be modified with side chambers
- Test if response to flow is stronger than food availability
  - Place beetles in rich food source located in opposite direction of flow preference
- Determine flow maximum that could force them to surface
  - Are strong hydrologic events part of the life cycle?

Stygoparnus comalensis – Flow Preference

- Test run
  - *Macrelmis* sp. 6 each adults and larvae
  - 8 mins each at low, med, and max output

  - Low = 0.04 m/s; Q = 45 mL/s
  - Med = 0.18 m/s; Q = 170 mL/s
  - Max = 0.27 m/s; Q = 196 mL/s

  - 2 larvae and 1 adult in **Terrarium**
  - 1 larvae in **Chamber C**
  - 1 larvae and 1 adult in **Chamber B**
  - 4 adults and 2 larvae in **Chamber A**

Stygoparnus comalensis – Benefit to Refugia

- Reproduction:
  - Current knowledge is depauperate
  - Anything learned will be of value to understanding better husbandry and captive propagation techniques
- Flow preference:
  - Better understanding of environmental requirements

Heterelmis comalensis

Background:

- Many life-histories aspects have been conceptualized (Bowles et al. 2003, BIO-WEST 2015a, BIO-WEST 2015b, BIO-WEST 2017, Nowlin et al. 2017a, Nowlin et al. 2017b)
- Questions remain about behavior to flow variation and food supply

Objectives:

- Continued monitoring of ongoing pupation experiments from 2017
- Identify the behavioral response of adults and larvae to varying flow conditions with food resource effects

Heterelmis comalensis – Investigate Flow Preference

- Same flow apparatus as proposed for *Stygoparnus comalensis*
  - Same questions
- Use as a surrogate for response to various flow levels
  - Determine flow maxima
  - Establish flow preference
  - Determine if food availability is favored over flow preference

Benefit to Refuge:

- Develop better understanding of habitat and food preferences that contribute to captive propagation


Questions
and
comments?

PCA – Loadings