Factors Affecting Pupation in the Endangered Comal Springs Riffle Beetle

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TEXAS STATE BIOLOGY

Background

- *H. comalensis* listed in the EA-HCP
  - One of the organisms associated with flow requirements
- Great deal of previous work on CSRB
  - Life history, population and conservation genetics, habitat associations, diet and trophic ecology, environmental tolerances
- Establishment of refuge populations
  - Hold adults for extended periods, produce eggs and larvae
- Pupation rates are low, production of F1 adults
  - Need to explore factors affecting pupation and production of F1s

Life History

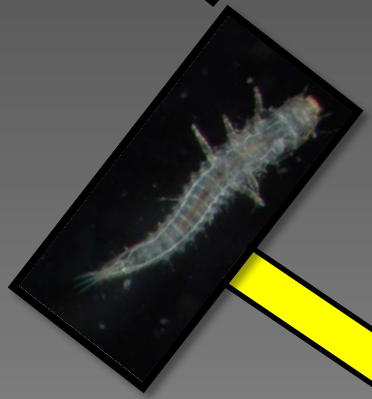
Life History

1 -3 clutches
3 – 18 eggs/clutch

Life History

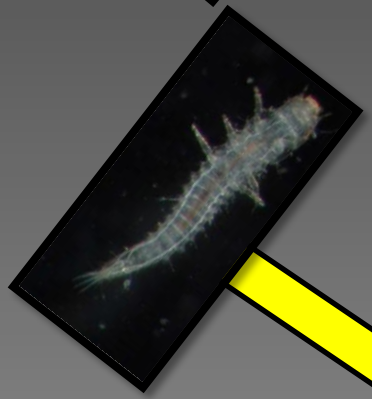
~3 weeks to hatch
~60% of eggs hatch

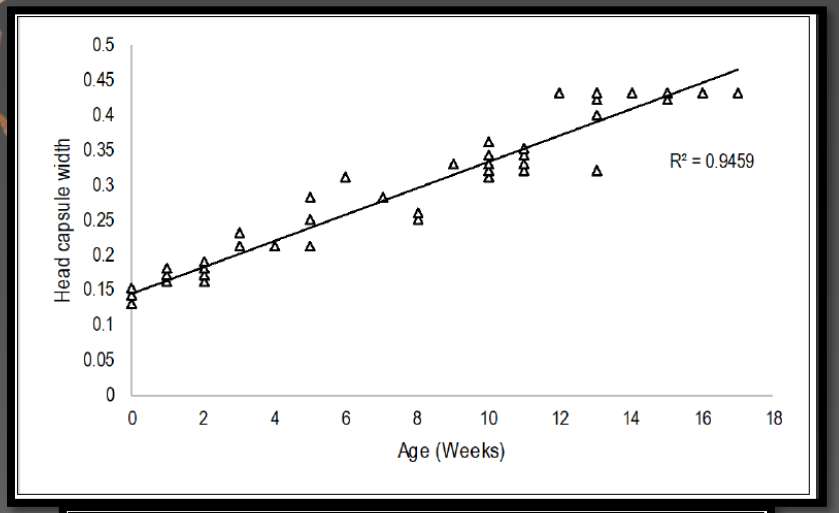
Life History

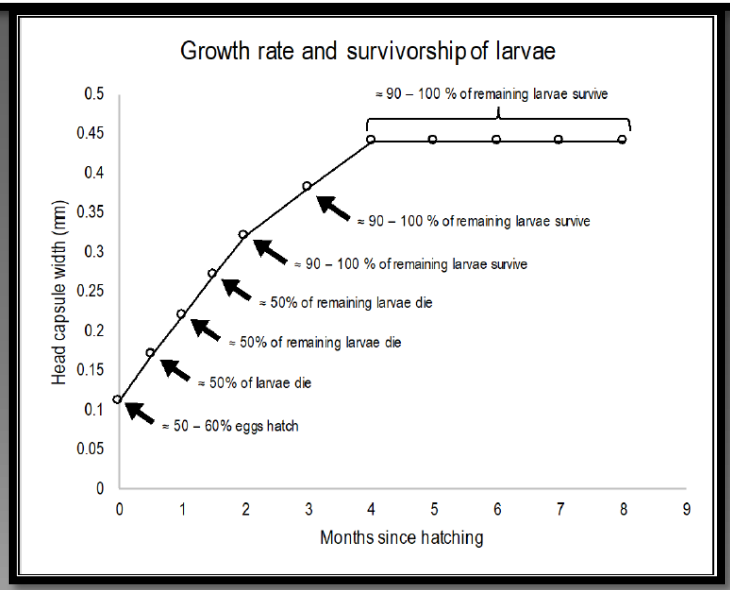


7 molts
Substantial mortality in early molts

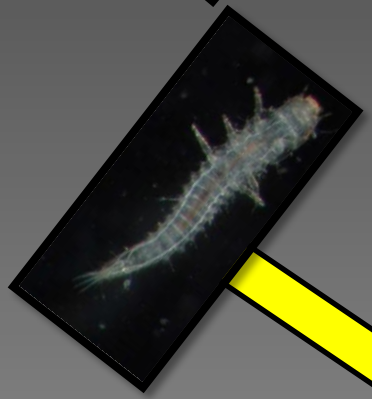
Life History





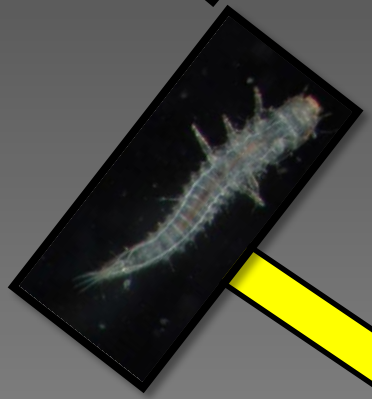


Life History



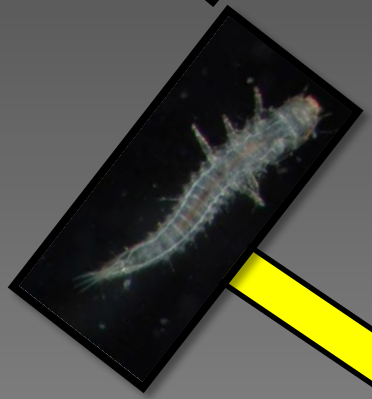
Persist as late-stage larvae
for 4 – 6 months

Life History



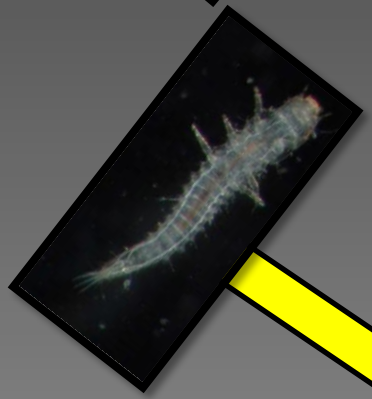
Persist as late-stage larvae
for 4 – 5 months

Life History



~ 1 month
Submerged
Potentially hydrophobic

Life History



Issues Surrounding Pupation

- High larval mortality not unusual in elmids, especially early instars[1]
  - Pre-pupation mortality ranges from 24 – 51%
  - ~80% mortality until pre-pupation in CSRB
- Rates of larval development and pupation in other may be dependent on food availability/food quality
- Biggest issues
  - Persistence in last-instar stage
  - Low rates of pupation and adult eclosion

Elliot 2006, FWBiol, 51:1527-1542

Proposed Research Goals

- Evaluate the role of diet/food resources in the development and pupation of late-instar CSRB in captivity
  - Main focus of the study

- Examine the influence on DO concentration on the development and pupation of late-instar CSRB in captivity
  - Reduce or eliminate this particular portion of the study?
  - Pupae, respiration, and "hydrophobicity"

CSRB and Food Resources

- Riffle beetles (elmids) often described as grazers/scrapers
  - Utilize biofilms on benthic surfaces
  - Detritus may be important for some species (*Stenelmis*, *H. vulnerata*)
- Lab animals maintained with well-conditioned leaf material and poly-cotton cloth material
- Often found in association with wood or leaves
- Diet reconstruction using SIs clearly indicate a reliance on CPOM
  - Derive EAAs from bacterial portion of these biofilms

Study Questions

- How does the composition and origin of biofilms on CPOM and poly-cotton lures differ between lab and field settings?
  - Captive beetles fed material grown in lab at SMARC/TxState
- Do biofilms of different origins (wild- versus lab-grown) affect development and pupation rates?
- Does the presence of CSRB themselves have a role in determining biofilm quality and does this affect development and pupation?

Rationale

- Nutritional quality can vary substantially with biofilm type and composition
  - Biochemical composition – lipds, carbohydrate, protein content
  - C:N
  - Biofilm microbial composition
- Presence of consumers affect their own food quality
  - Specialized and specific microbial gut flora
  - Grazing alters composition and quality of biofilms (facilitation)

Detailed Methods, Part 1

- Potential differences in biofilm quality and composition
  - Three substrate types
    - Leaves (*Platanus*) – condition for 4 weeks
    - Wood (dowels made of *Populus*) – condition for 3 months
    - Poly-cotton material – condition for 4-6 weeks
  - In the lab under "typical" conditions
  - In the field (Comal) close to spring openings
  - Characterize quality
    - C:N and protein, lipids, and total carbohydrates
    - Whole genome sequencing (WGS)
      - Up to species-level resolution

Detailed Methods, Part 2

- Effects of substrates from locations on CSRB development and pupation
  - Three substrate types from field and lab
  - Field materials will be examined under a scope to remove any invertebrates or obvious eggs
  - 3-5 late-instar larvae placed in PVC flow through chambers with one of two treatments
    - Wood and leaves
    - Wood, leaves, and poly-cotton material
  - Checked every 3-4 weeks to look for pupae or (teneral) adults
  - Two way ANOVA

Detailed Methods, Part 3

- Presence of CSRB necessary to condition OM material
  - Use the same OM sources as the previous two studies
    - Leaves, wood, poly-cotton cloth
  - Placed into plastic flow-through bins with same-sex groups of adults (preferably males) versus bins without beetles
    - After 4 weeks, material removed and processed for microbial biofilm composition, nutritional quality
  - Same types of material will be placed with 3-5 late-instar larvae in PVC flow through containers

Summary

- We will also collect adults, larvae from wild and captive populations and compare protein, lipid, and carbohydrate content
- Assess role of biofilm composition, quality on late-instar CSRB development and pupation
- Inform about types of materials and nutritional needs of CSRB for development and pupation