Ecology and Conservation of North American Crayfishes

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Highlands Biological Station
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Cover image (previous page): *Cambarus ludovicianus (Painted Devil Crayfish). Photo courtesy of Guenter Schuster.*

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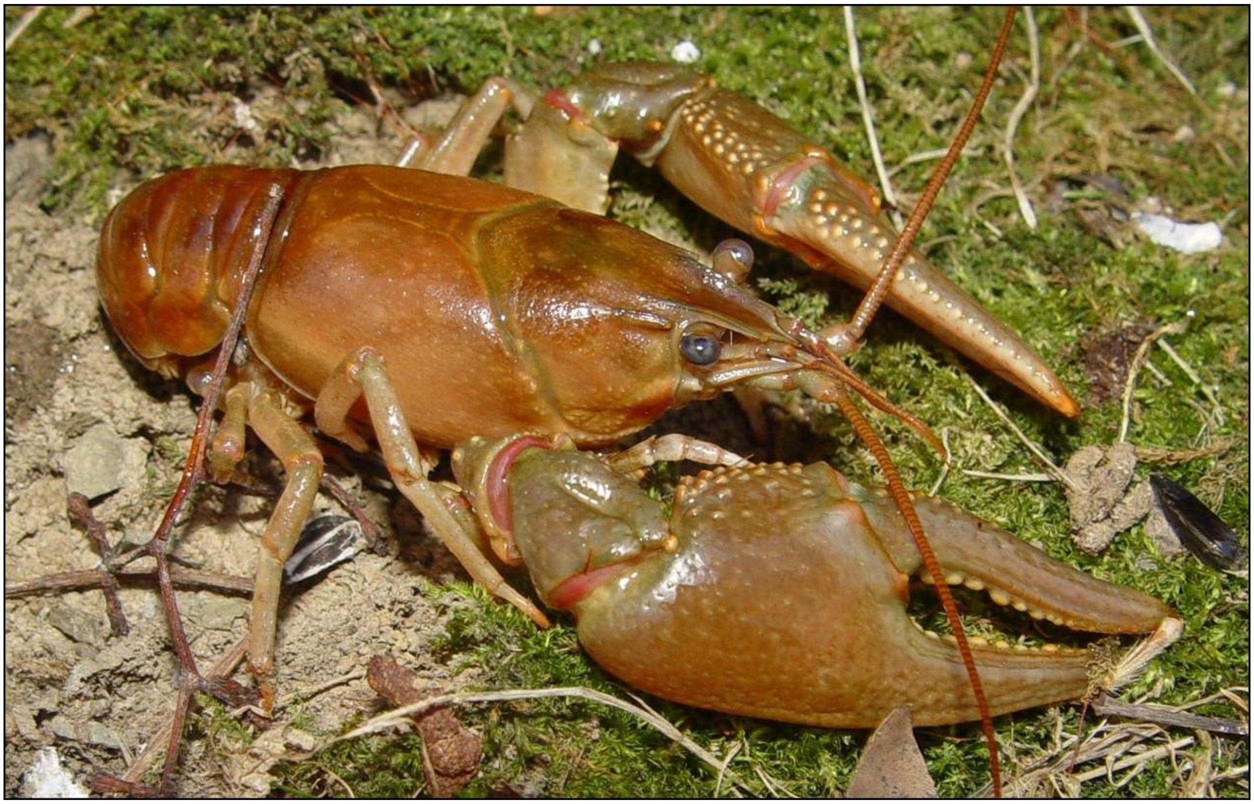
Chapter 1



Morphology

Key Morphological Features and Terms

This chapter defines the key morphologic parts of crayfishes needed to make a positive identification through use of keys. The chapter is setup in outline form, so you the student can make notes that aid you in understanding and defining crayfish morphology. we encourage you to take copious notes in the space provided. One of the most daunting aspects of crayfishes is understanding the technical jargon associated with their anatomy. The good news is the learning curve for crayfishes is a ninety degree angle; once you have mastered the vocab, they are relatively easy to identify.

Two terms in particular are very important to understand and defined early on in this chapter. Using the junction of the carapace and abdomen, defined below, as a common point of reference, all anatomic parts that are closer to the head are referred to as CEPAHLIC, and all parts oriented towards the abdomen are referred to as CAUDAL. These two terms were used extensively by Dr. Horton Hobbs Jr., the father of American astacology, and are still used today.

The large body part that all limbs are attached to is technically referred to as the CEPHALOTHORAX, but is often referred to as the CARAPACE. The tail is the ABDOMEN. The large pincers that are enigmatic of crayfishes are known as CHELAE, and are the 1st set of walking legs. Now that these key terms have been defined the following are the most important anatomical parts that an understanding of is needed in order to make a positive identification:

1) Cephalothorax = Carapace

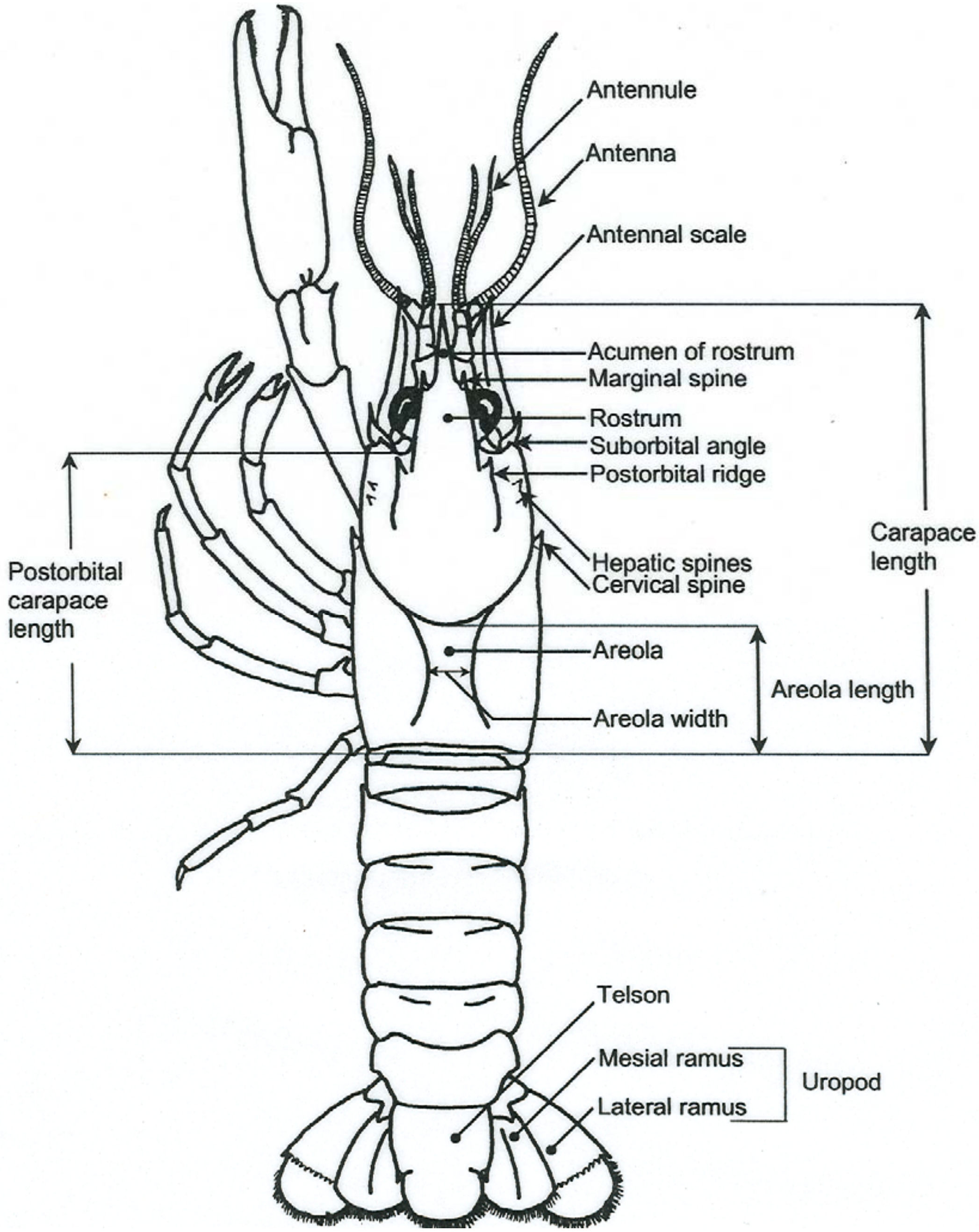
- Dorsal
- Lateral
- Rostrum

2) Rostrum

3) Chelae

4) Gonopods – Form I and Form II = Very important for identification in some species.

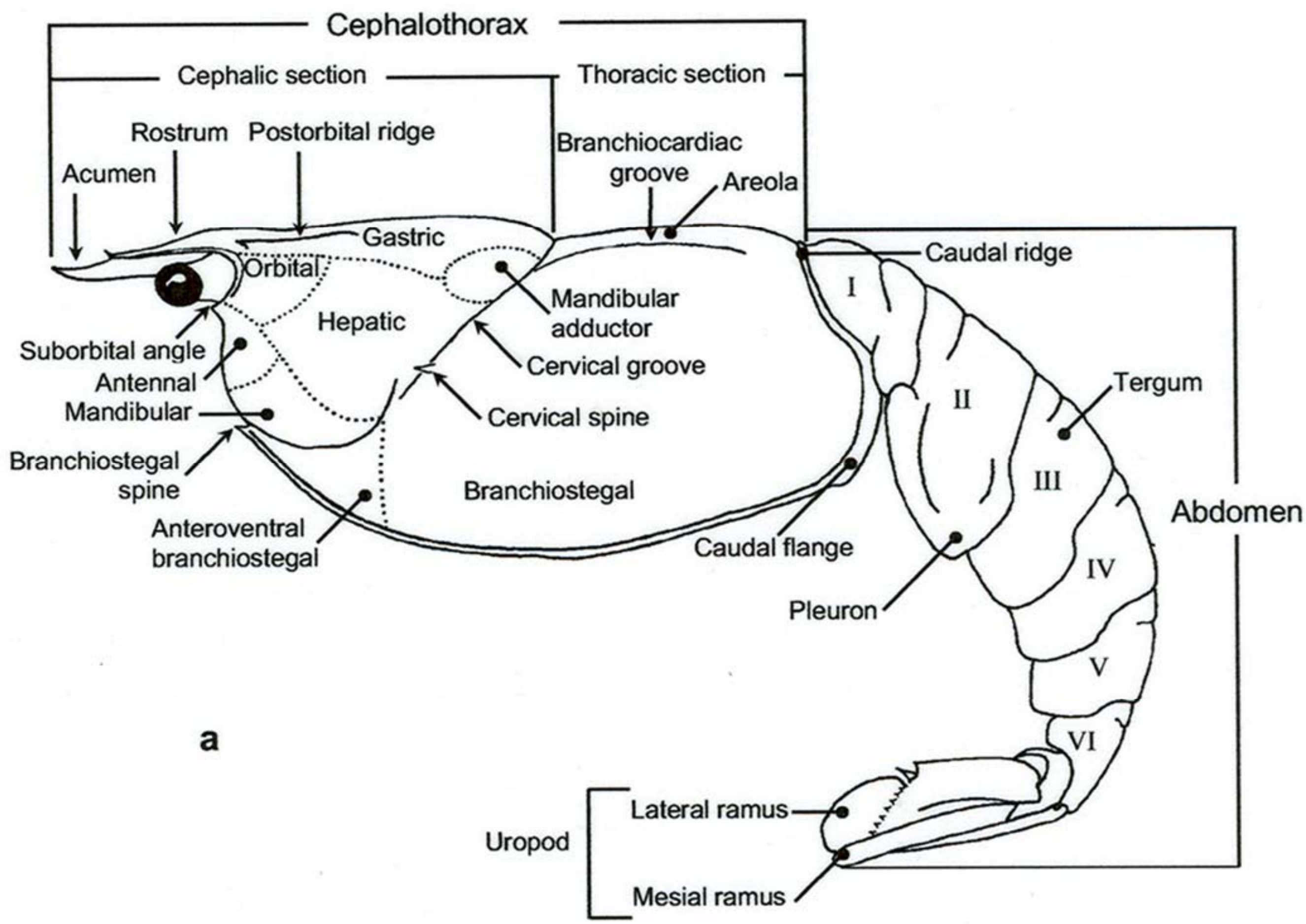
Dorsal View Diagram



Important anatomical features include:

- Rostrum shape
- Hepatic spines
- Cervical spines
- Chelae shape
- Overall body size

Lateral View Diagram

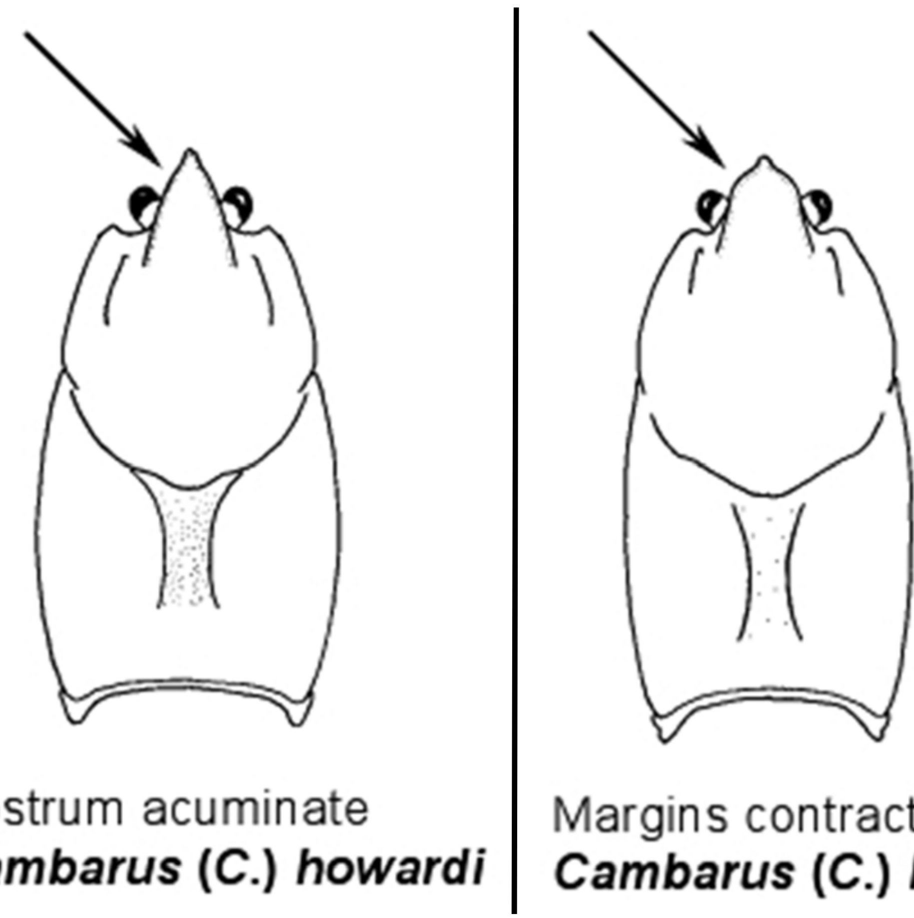


Important anatomical features include:

- Suborbital angle
- Cervical spine
- Acumen shape
- Rostrum Shape
- Postorbital tubercle

Rostrum

Acuminate vs. Convergent

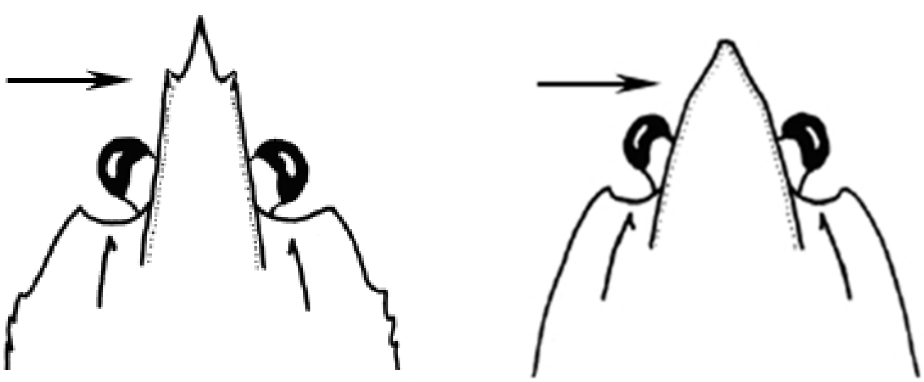


Rostrum acuminate
Cambarus* (*C.*) *howardi

Margins contracted
Cambarus* (*C.*) *bartonii

Rostrum

Marginal Spines



With marginal spines

Without marginal spines

Rostrum Morphology

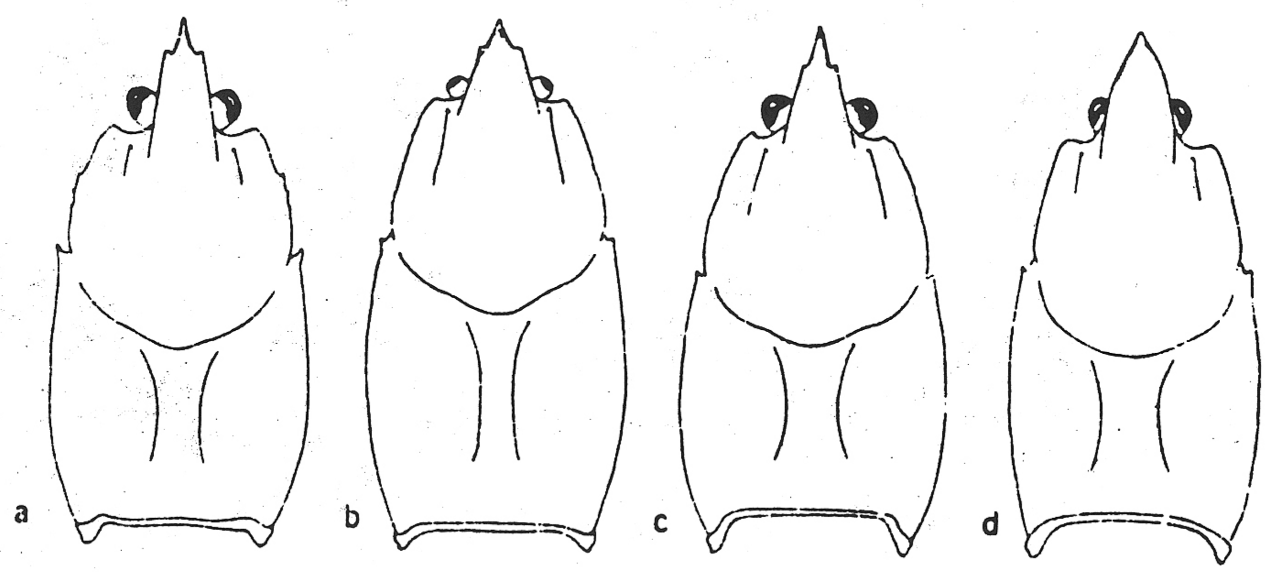
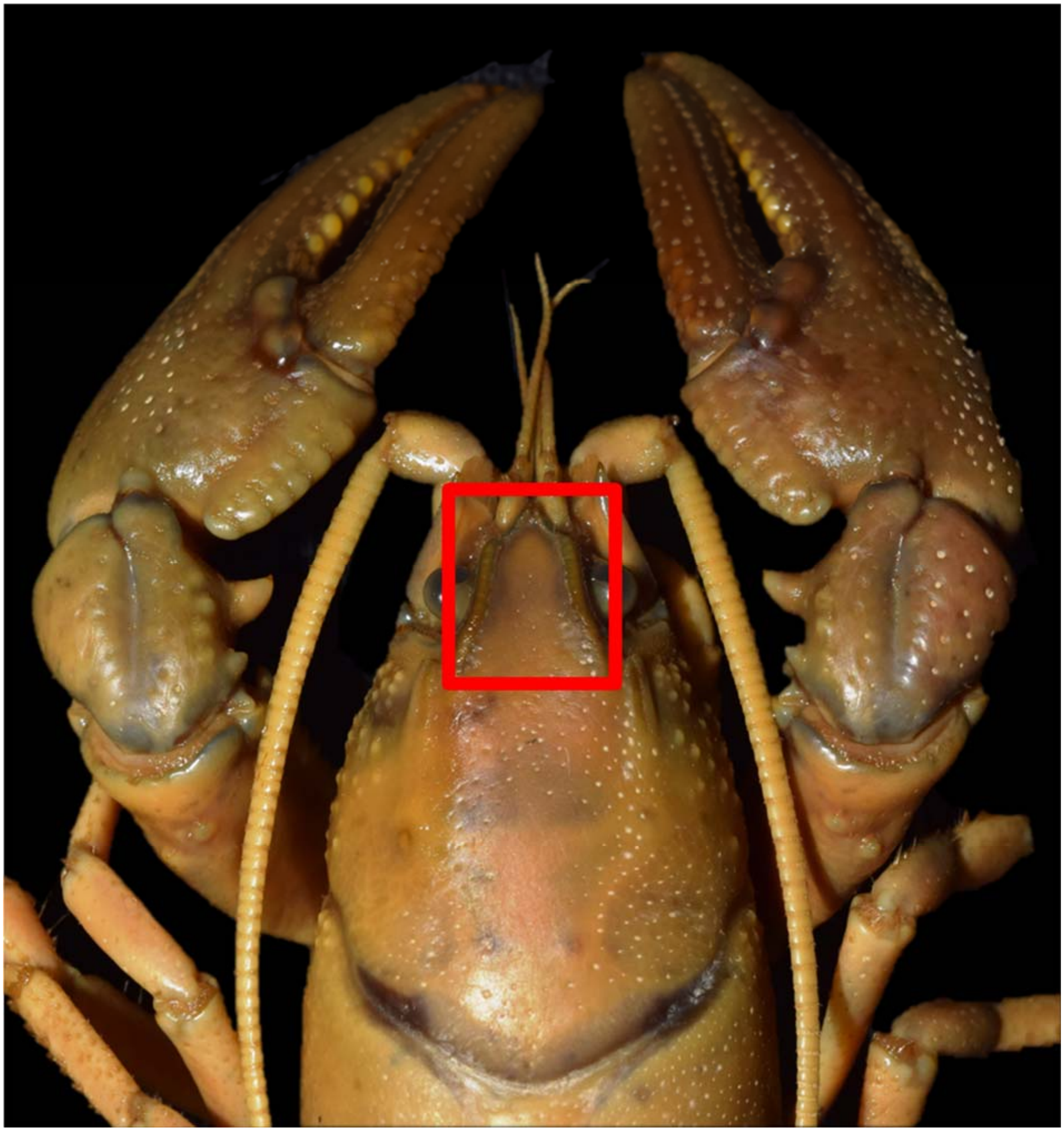


Figure depicting various rostrum types

A. Narrow rostrum with rostral spines
B. Broad rostrum with rostral spines
C. Acuminate rostrum with rostrum spines
D. Acuminate rostrum lacking rostral spines

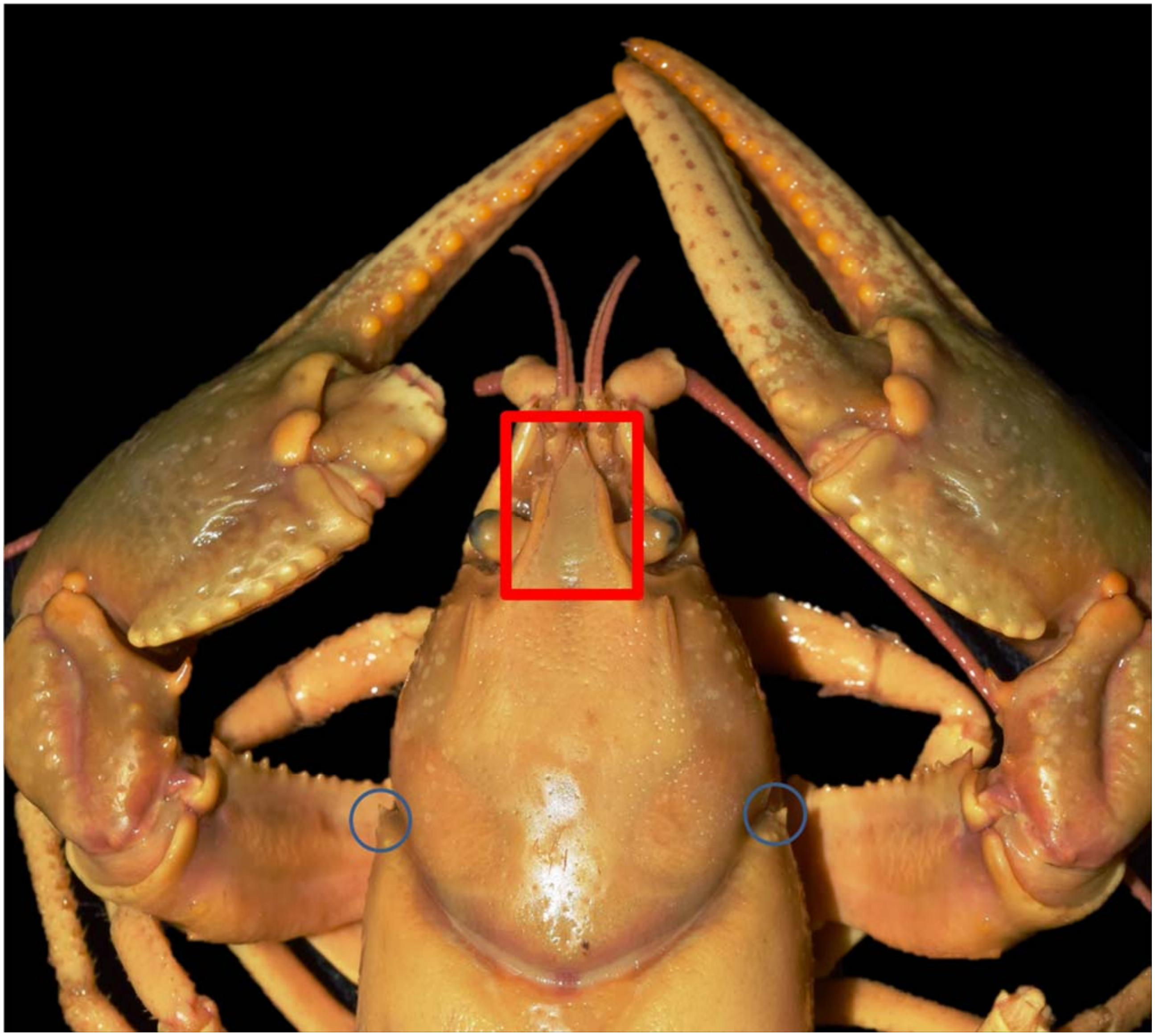
Rostrum

Cambarus theepiensis



- Polymorphic
- “Subacute”
- Defined acumen

Cervical Spine absent

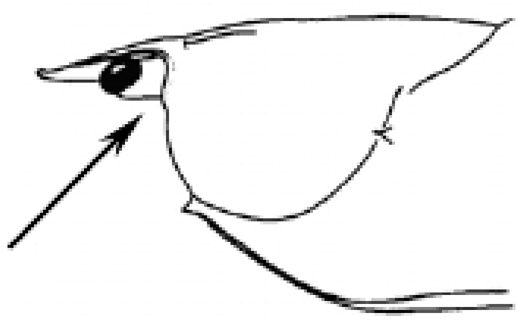


Rostrum of *Cambarus callainus*; cephalothorax with cervical spines.

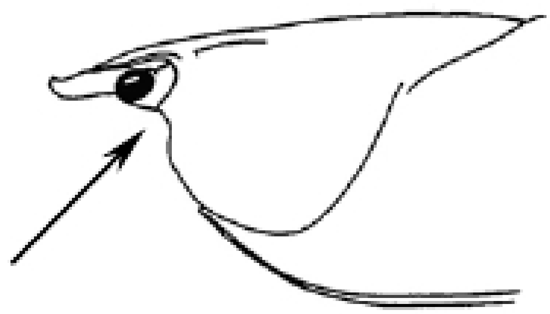
Rostral carina on *Faxonisu cristavarius*. Blue box.

Rostrum

Suborbital Angle



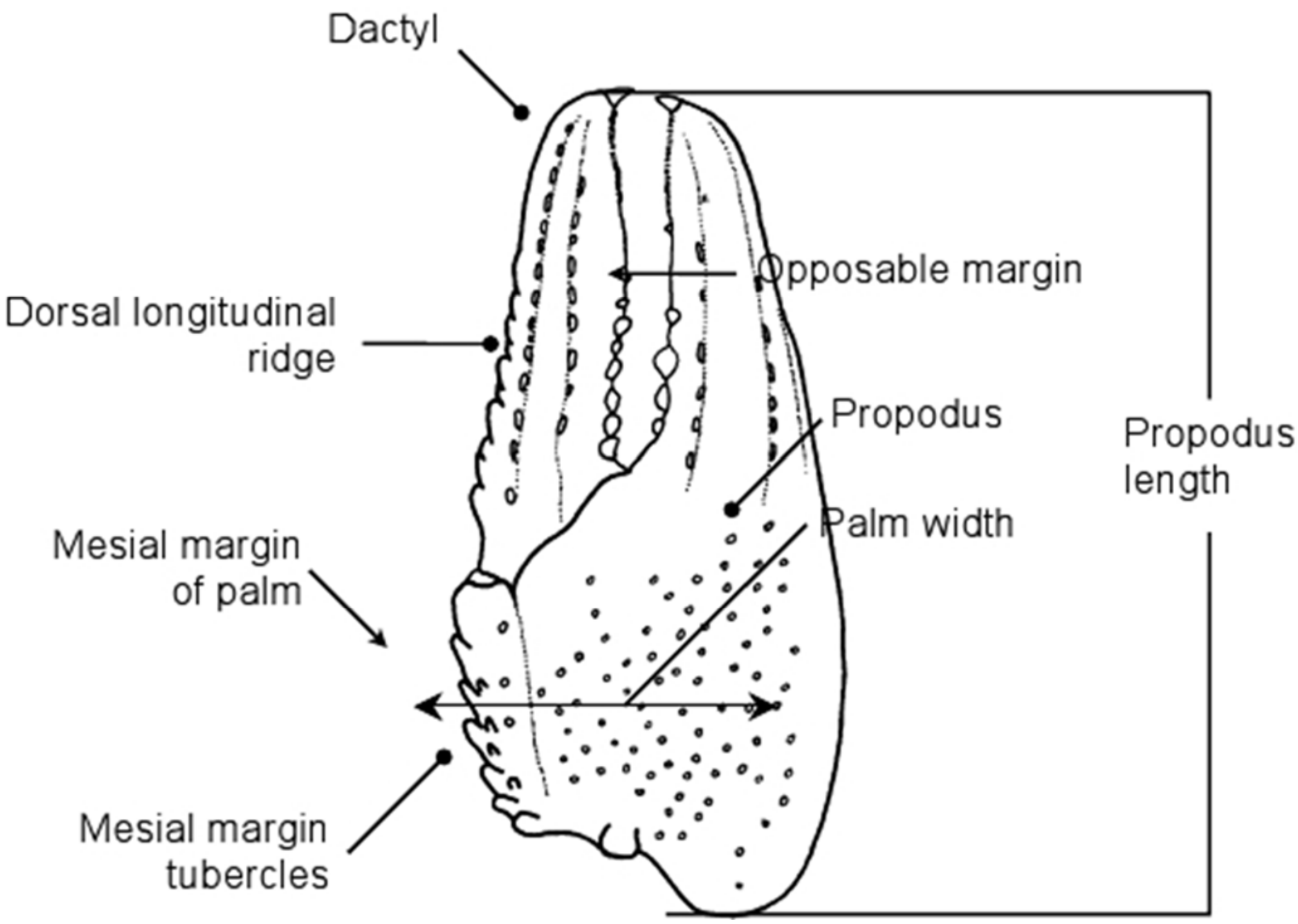
Suborbital angle absent



Suborbital angle present

This character can oftentimes be ambiguous and difficult to observe. It should only be used with confidence to identify *Creaserinus* and *Fallicambarus* species.

Chelae Morphology



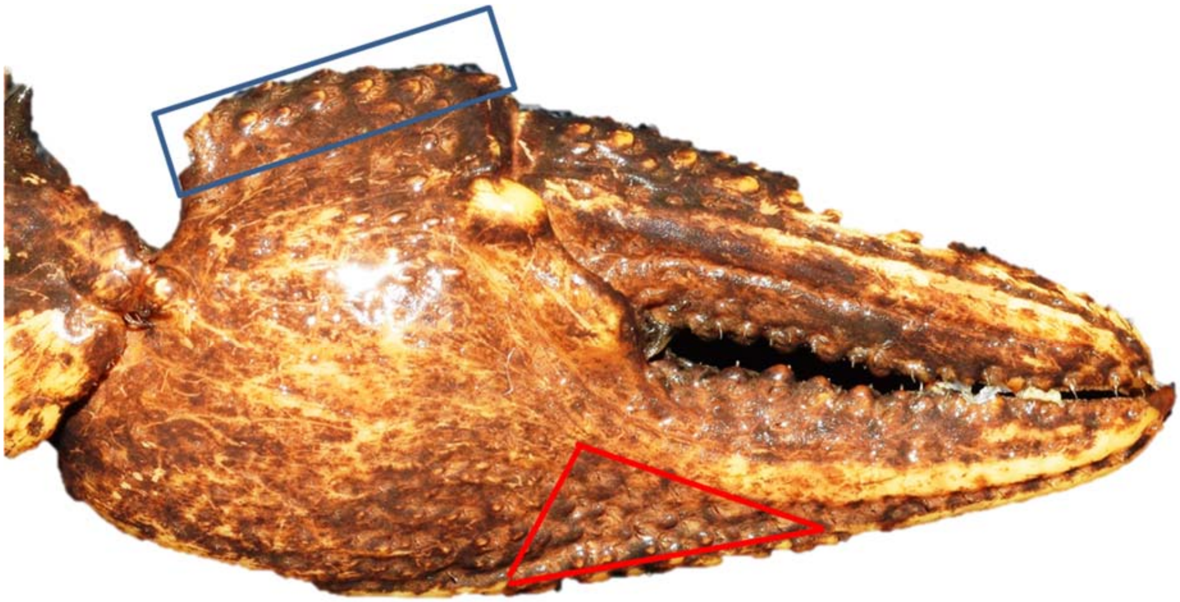
Regenerated chelae


An understanding of whether the crayfish in hand has regenerated chelae or original chelae is important if a positive identification is going to occur. A good rule of thumb is if the chelae's palm length is equal to or greater than the palm width of the chelae, the chelae you are looking at is regenerated. If the palm length is less than the palm width, then you either have an original chelae, or a regenerated chelae that was regenerated early in the life of the crayfish. Another way to tell if a chelae is regenerated is to look at the architecture and sculpturing of the chelae. IF the key anatomical parts are muted or incomplete, there is an good chance that the chelae was is regenerated

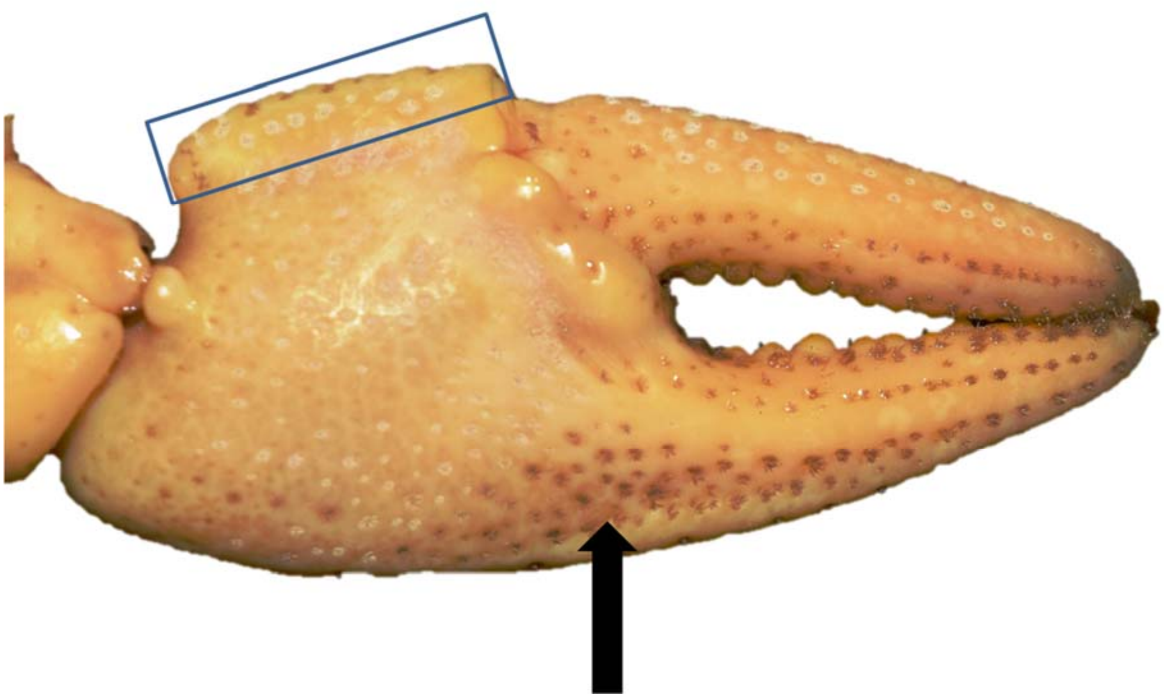
The crayfish on the right has regenerated chelae; the one on the left has two original chelae.

Identifying key characters on a chelae

Box highlights two rows of tubercles on the mesial margin of the palm; red triangle highlights a lateral impression.



Blue box highlights a single row of tubercles on the mesial margin of the palm; black arrow highlights the lack of a lateral impression.



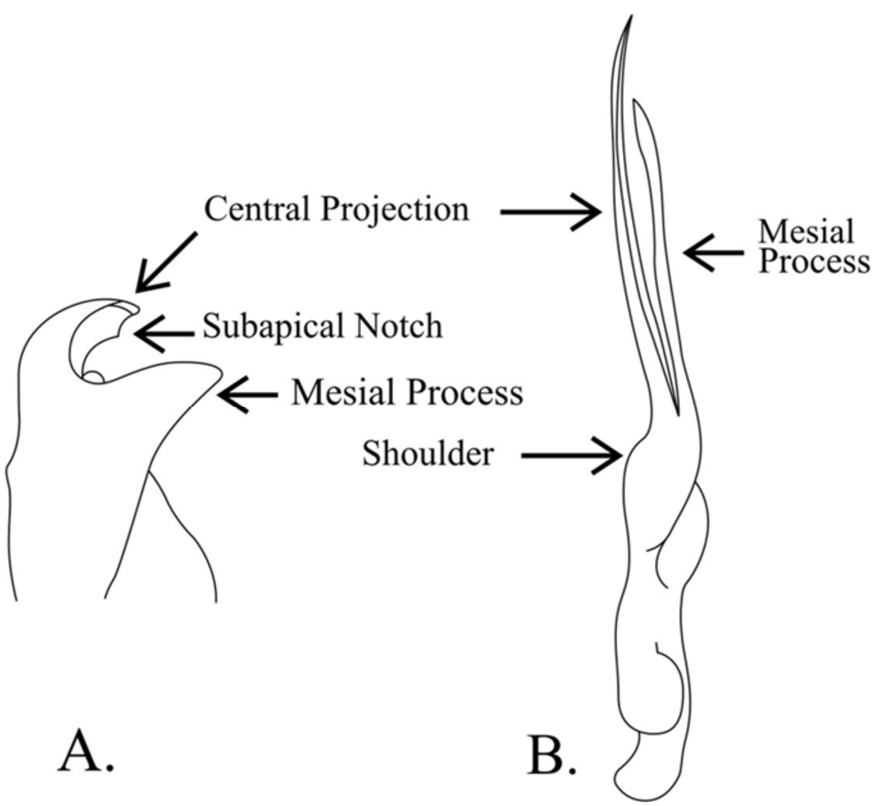
Gonopods

Crayfish gonopods are important for species level identification, especially in *Faxonius* and *Procambarus*, as well as several *Cambarus* species. The CENTRAL PROJECTION (CP) is the projection that is cephalically oriented when the gonopod is erected. The MESIAL PROJECTION (MP) is caudally oriented, and usually smaller than the cephalic projection.

Cephalic and Mesial Projections = In *Cambarus,* it's important to note if the distal end of the CP extends past, to, or before the MP. Also, the presence of a subapical notch is important. In Faxonius species the length of the CP relative to the entire length of the gonopod, as well as the curvature is important. The thickness of the MP at the distal end can be important as well

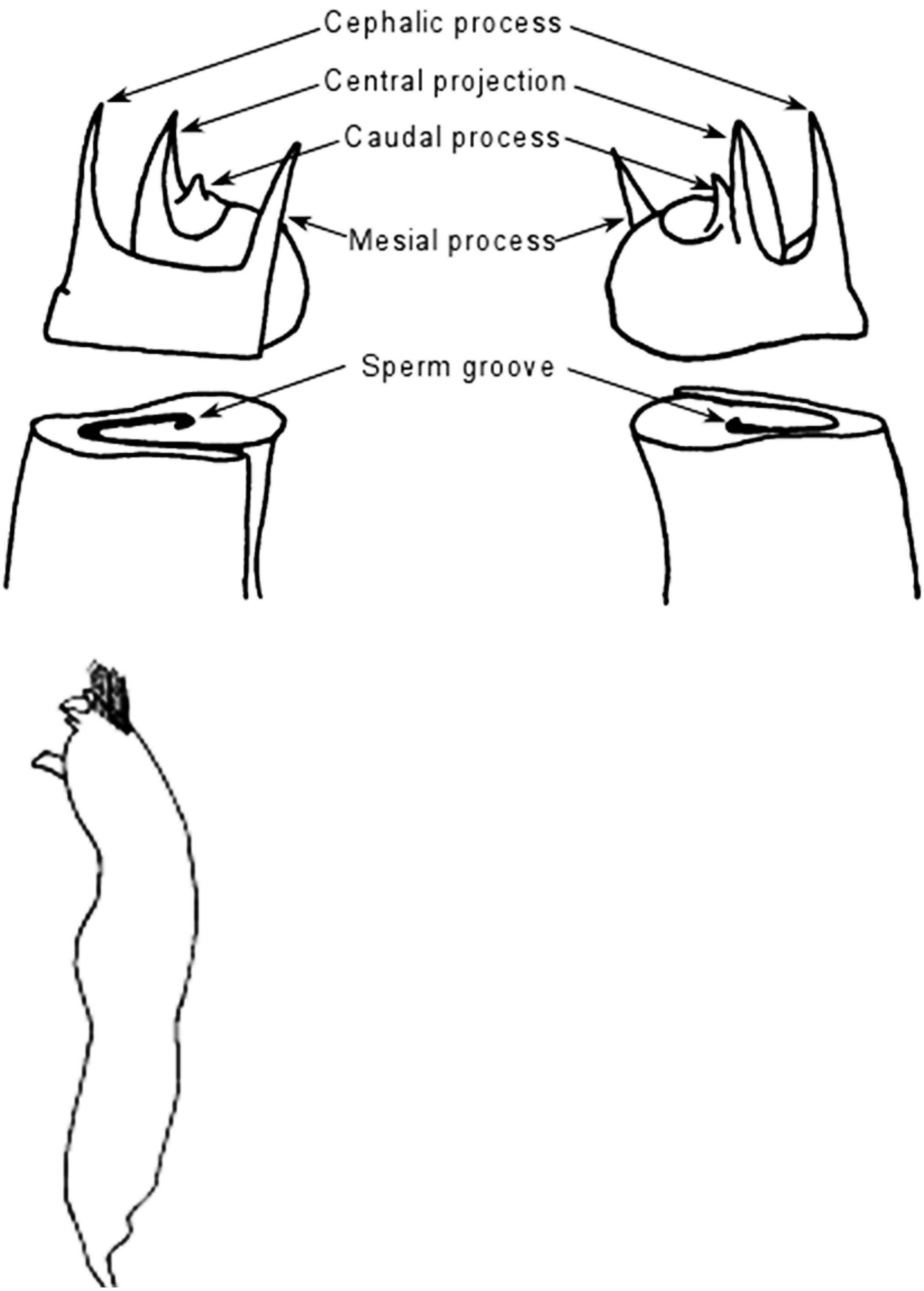
The *Procambarus* gonopod is…."special".

Cambarus (A.) and *Faxonius* (B.)



Gonopods

Procambarus



Procambarus gonopods.

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Chapter 2

Taxonomy

Crayfish Taxonomy

Note: In addition to traditional taxonomy, crayfishes are classified based on behavior

Classification

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malocastraca

Order: Decapoda (Crayfish, crabs, lobsters, shrimp)

Suborder: Pleocyemata (All members carry eggs on pleopods)

Infraorder: Astacidea (Composed of lobsters, holarctic crayfishes, & australasian crayfishes)

Superfamily: Parastacoidea (Australasian crayfishes; Southern hemisphere)

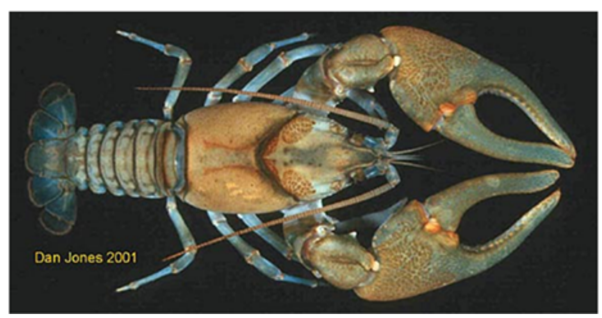
Superfamily: Astacidea (Holarctic crayfishes; Northern hemisphere

Family: Astacidae

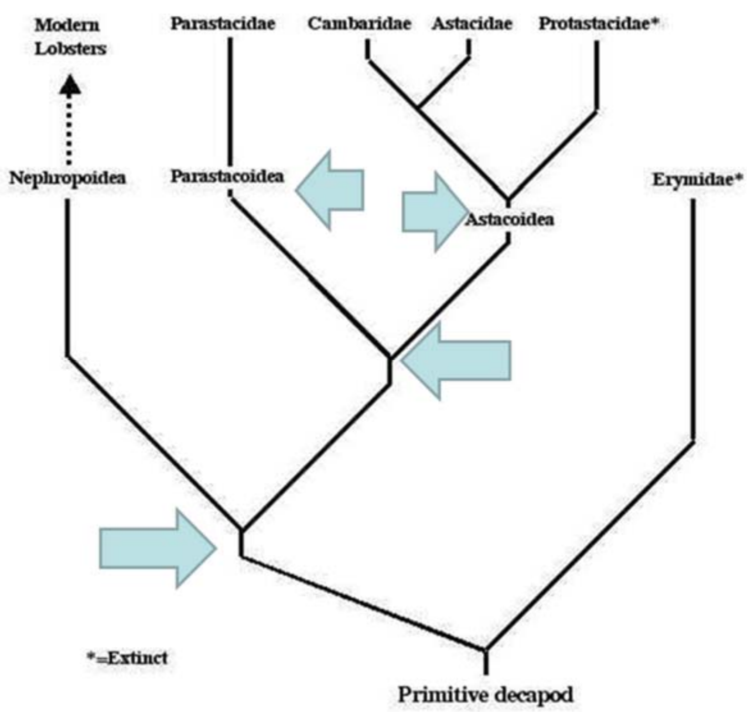
- Genus *Pacifasticus*

Family: Cambaridae

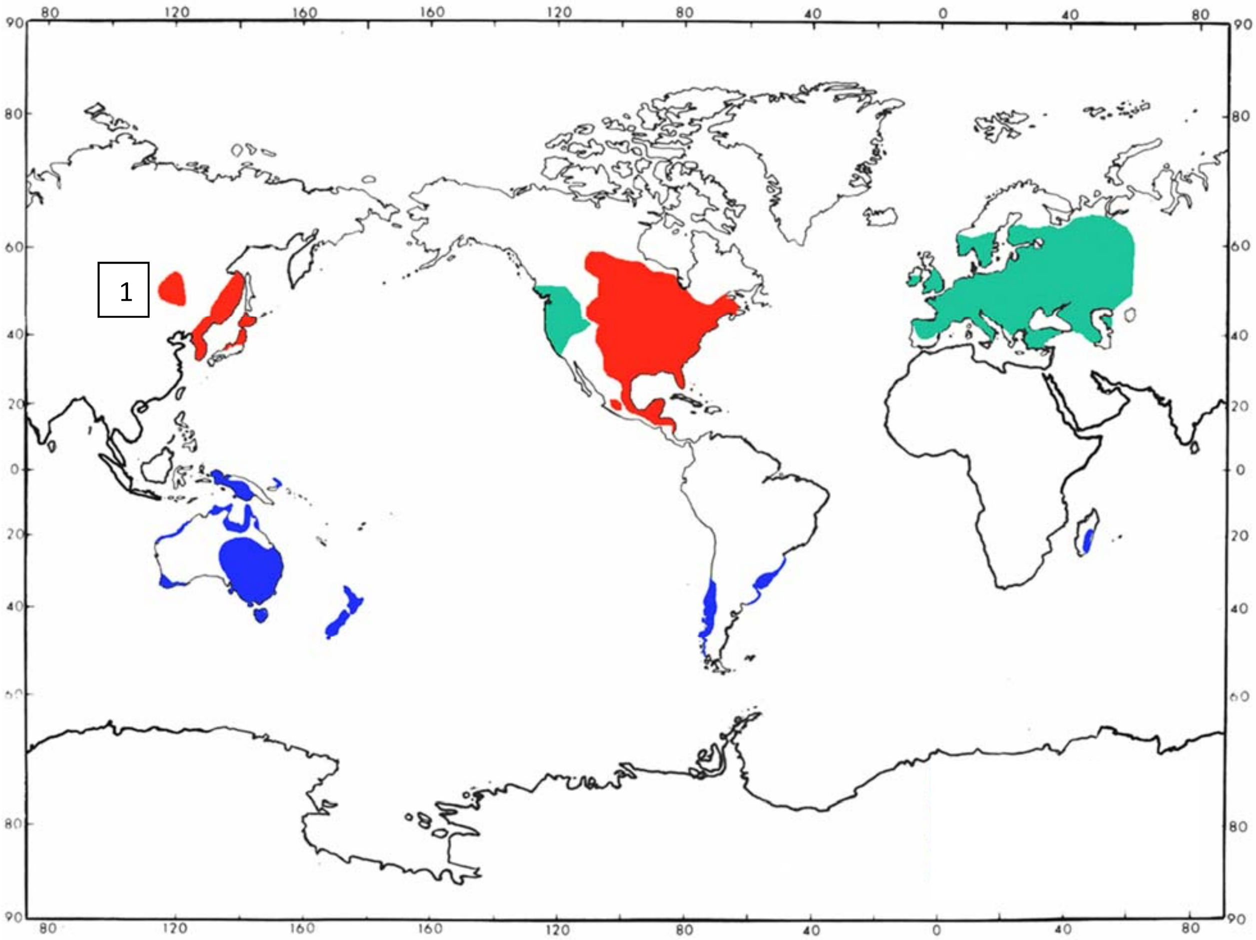
- Genus *Cambarus*
- *Genus Faxonius*
- *Genus Creaserinus*
- Genus *Orconectes*
- Genus *Procambarus*
- Genus *Fallicambarus*
- Genus *Barbicambarus*
- Genus *Distocambarus*
- Genus *Cambarrellus*
- Genus *Faxonella*
- Genus *Hobbseus*
- Genus *Troglocambarus*



Crayfish origins



Astacologist theorize that crayfishes had a marine ancestor that invaded freshwater environments in the early to Mid Triassic period, 250-225 million years ago. Most carcinologist agree that the likely ancestor of modern day crayfishes was a member of or morphologically similar to the extinct crustacean family Erymidae. These animals occurred in estuarine environments, and it is likely some of their ranks invaded freshwater, and evolved the ability to persist in said environments. Crayfishes are not lobsters! They differ from lobster in the way they rear their young, in gross morphology, and in the physiological way they deal with salt.



Crayfish Families

Until the fall of 2017 and the publication of Crandall and Degrave (2107), all crayfishes were limited to three families, the cambaridae (red), the astacidae (Green), and the parastacidae (Blue). Crandall and Degrave (2017) designated a new family, the cambaroididae for the former Asiatic cambarids (Red with 1). Crayfish reproductive strategy, as well as male reproductive anatomy is what is used to determine which family a species fo crayfish belongs to. Cambarid and cambaroidid crayfishes under go form changes in male gonopods, with Form I males being able to pass on spermatophores to females, and form II males lacking this ability. We will discuss this in great detail in the life history chapter. Astacid and parastacid crayfishes lack specialized form I gonopods, and as such, guide female crayfishes to spermatophores that are delivered to the substrate, where they are received by the female.

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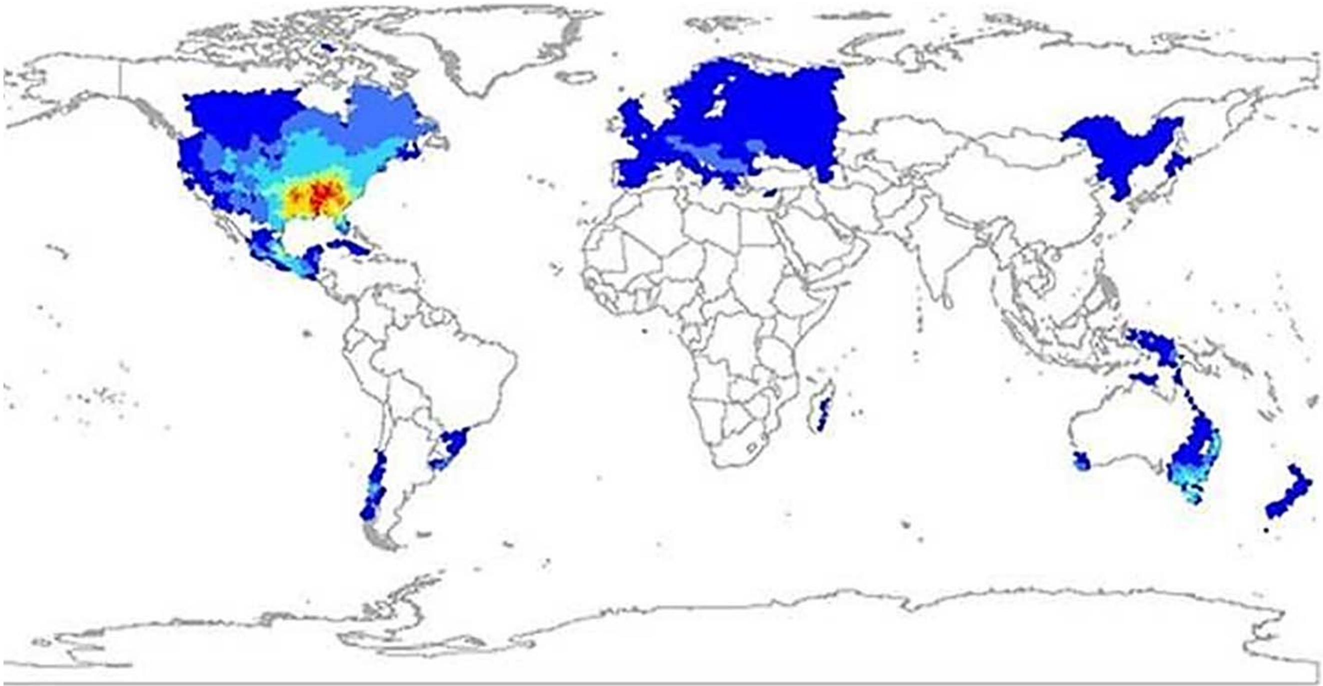


North American Crayfish Diversity

North America harbors more crayfish diversity than any other continent on the planet. The family Cambaridae is exclusively North American, and the most diverse crayfish family, harboring 423 species as of fall of 2017 (Crandall and Degrave, 2017). Both the Pacific North west and the northern Rocky Mountains harbor a single genus, *Pacifastacus*, which is North America’s sole member of the Astacidae, a family of crayfishes that ranges across Eurasia.

Three ecological regions harbor unique crayfish faunas composed primarily of three speciose genera, and a smattering of less diverse genera. The Appalachian Mountain region is the center of diversity for the genus *Cambarus,* the Coastal plain is the center of diversity for the genus *Procambarus,* and the Ozark and Ouachita mountains is the center of diversity for *Faxonius*

Crayfish Families

1) Parastacidae

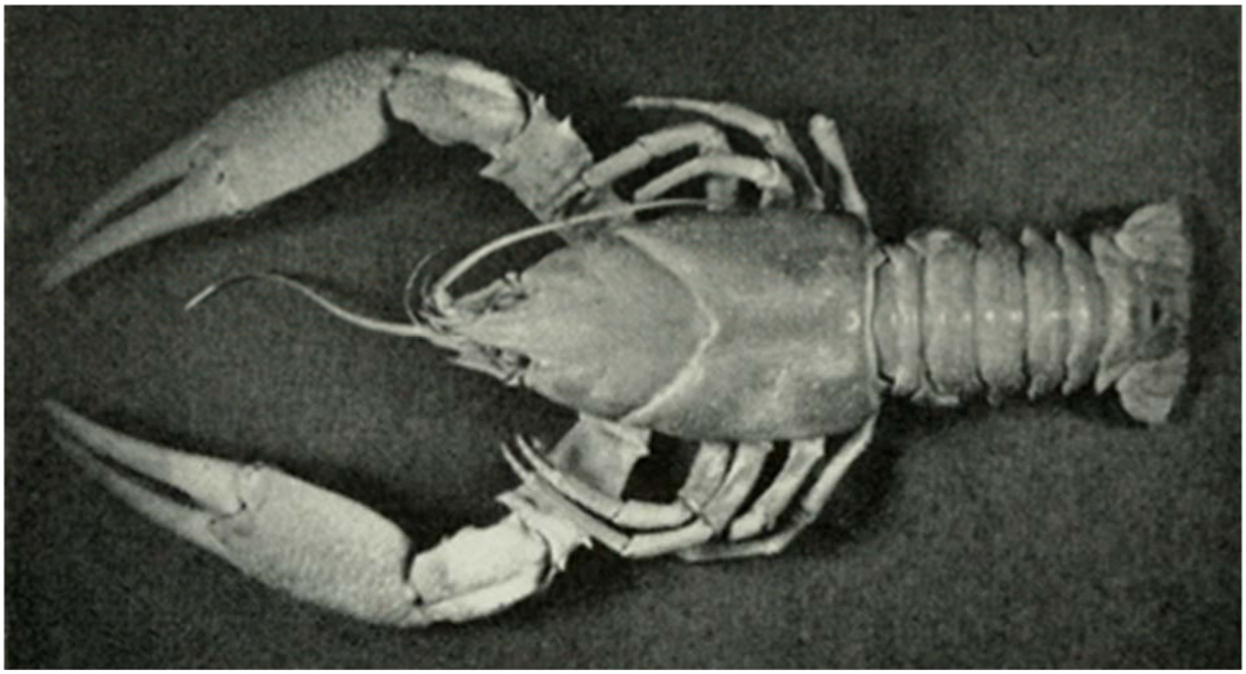
No parasstacid crayfishes occur in North America, though introduced populations now exist in Costa Rica, and are present in the Americas. Next to Cambaridae, this is the most diverse crayfish family, with genera spread throughuot the southern hemisphere except for both continental Africa and Antarticta. Unique faunas are present in South American, Madagascar, and Australasia. The largest benthic macroinvertebrates on the planet are members of this family, with *Astacopsis gouldi* being the largest crayfish on Earth. Australia is home to more parastacid diversity than anyhwere else, with the genera *Euastacus, Cherax,* and *Engaeus* habroing the majoiring of species. Interestingly, there are ecological paralleles between parastacids and camabarids. Euastacus are ecological equivalents to *Cambarus*, *Cherax* represent the same with *Faxonius* and Procambarus, and the genus Engaues has paralleless to *Lacunicambarus* in North America.

2) Cambaroididae

This family is the least diverse and the most limited geographically. All ## species occur in temperate Asia and Japan, and like cambarids undergo form change from molt to molt once adulthood is achieved. All *Cambaroides* species are small, brown crayfishes that occur in the exact same habitats as North American secondary and tertiary burrowing species. Among the worlds crayfishes, this family is the least studied, and the least understood ecologically.

3. Astacidae

Astacids reach their highest diversity in Eurasia, with the highest diversity isolated to mainland Europe. OF the ## genera, *Pacifastacus*, North Americas only genus is one of the most diverse. Pacifastacus species are readily differentiates from all other North American crayfishes by the males less developed gonopods, and the structure of the chelae, whichis very unique combared to cambarids. Two



species of *Pacifastacus* are of conservation concern. *Pacificastcus nigricens* is presumed extinct, and *Pacifastacus fortis* currently is listed as Endangered by the USFWS. Pacifastacus leniusculus is a common species throughout much of the Pacific Northwest, and likely represent a species complex in need of revision. Outside of North America, P. leniusculus is an important invasive species.

3) Cambaridae

Cambarid crayfishes are globally the most diverse family of crayfishes and occur throughout central and eastern North America, with a few species occurring in Central America and Cuba. All cambarids undergo form change, and historically their taxonomy relied heavily on an understanding of male gonopod anatomy. Several subgenera were proposed by Hobbs for *Cambarus* and *Procambarus*, and *Faxonius* by Fitzpatrick which recent molecular work have proven to be erroneous. The following section is sertup more like a handbook than a tradition book chapter in an effort to focus on what makes each genera unique, and more specifically, what key characters you as a biologist should que in on to make an identification. In addition to classic Linean taxonomy, crayfish also have a practical “behavioral” taxonomic regime that is quite helpful in both understanding the anatomy needed for a taxonomic identification, as well as understanding the possible species that occur in your region. Some general attribtues to cambarids are:

- Taxonomy in this family is moderately involved and technical
- Taxonomy is determined by male gonopod anatomy, tubercles, and chelae ornamentations
- Distribution matters – several endemic genera

On with the show!

1.) *Cambarus* Characteristics

Rostrum

- Blunt, shelf-like rostrum
- Terminates in single point

IM Gonopod:

- Club shaped

Body:

- Stocky, chunky build

Distribution:

- Appalachia
- Piedmont
- Coastal Plain
- Midwest – less diverse

- Mesial process bent 90°

2) *Faxonius* Characteristics



Rostrum:

- 3 distinct tubercles
- Makes a “V”

IM Gonopod:

- 2 terminal elements
- Terminal elements vertical

Body:

- Sleek build
- Dorsal laterally flattened
- “Red Cheeks”

Distribution:

- Ozarks
- Ouachita’s
- Midwest
- Piedmont

3) *Procambarus* Characteristics

Rostrum:

- Acuminate rostrum
- Usually end in single point

IM Gonopod:

- 3 or more terminal elements
- Terminal elements reduced

Body:

- Dorsal ventrally flattened
- Chelae narrow and elongate

Distribution:

- Coastal Plain
- Piedmont
- Midwest
- Ozarks & Ouachita

4) *Creaserinus* Characteristics

Rostrum:

- Convergent rostrum
- Ends in single point

IM Gonopod:

- Club shaped
- Mesial process bent 90°

Body and Chelae

- Dorsal laterally flattened
- Chelae distinct notch
- Chelae setiferous

Distribution:

- Midwest
- Coastal Plain
- Piedmont
- Appalachia

5) *Fallicambarus* Characteristics

Rostrum:

- Convergent rostrum
- Ventrally deflected
- Ends in single point

IM Gonopod:

- Club shaped
- Mesial process bent 90°
- Angled sharply ventrally

Body and Chelae

- Dorsal laterally flattened
- Chelae distinct notch
- Chelae lack setae

Distribution:

- Gulf Coastal Plain
- Ozarks
- Ouachitas

6) *Orconectes* Characteristics

Body and Chelae

- Dorsal laterally flattened
- Chelae distinct notch

Rostrum:

- Convergent rostrum
- Ventrally deflected
- Ends in single point

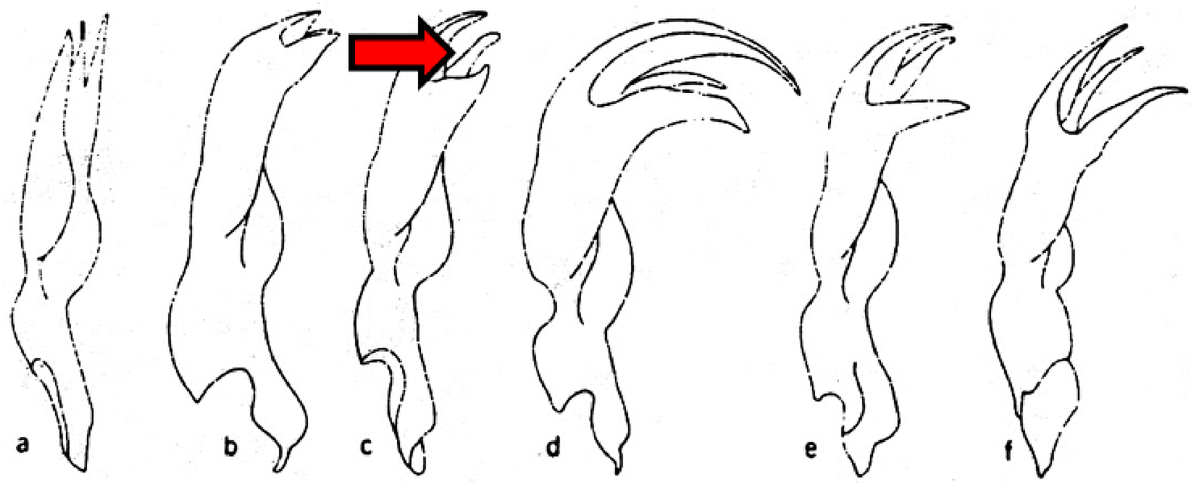
IM Gonopod:

terminating in two short elements

Distribution

- Widespread

7) *Cambarellus* Characteristics



Rostrum:

- Broad rostrum
- Ends in single point

IM Gonopod:

- Intermediate between Fax. and Camb.
- Ischial hooks on 2 and 3

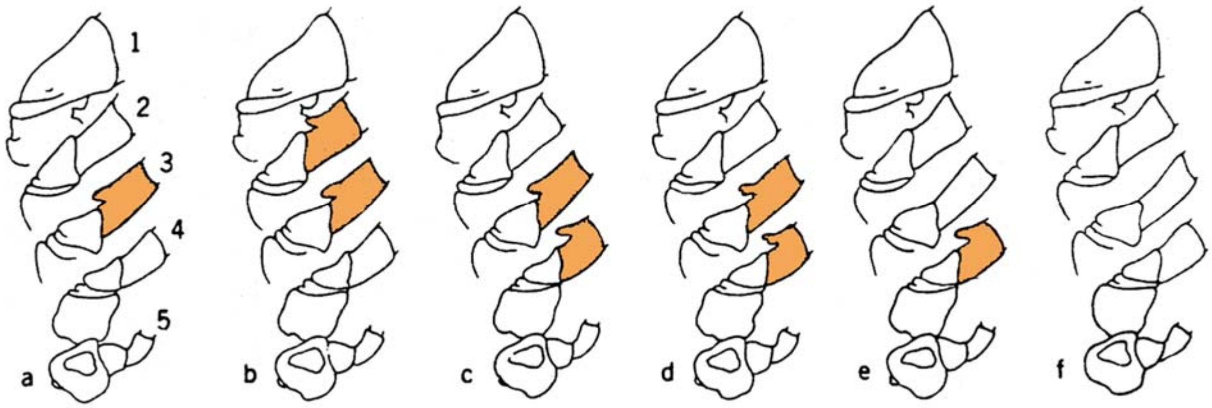
Body and Chelae

- Weakly dorsal laterally flattened
- Chelae palms swollen
- Wide areola
- Small!!!!

Distribution

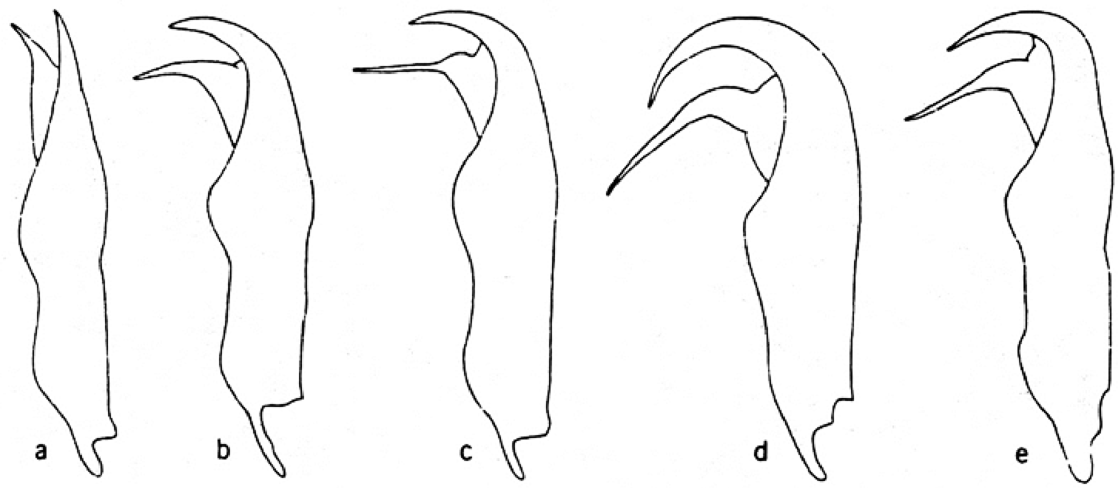
- Gulf Coastal Plain
- Midwest

Ischial hooks



Ischial hooks can be used to identify various cambarid genera

8) *Hobbseus* Characteristics



Rostrum:

- Convergent rostrum
- Ends in single point

IM Gonopod:

- Multi shaped
- Mesial process “bent”

Body and Chelae

- Small = TBL – 2”
- Chelae thick
- Chelae nondescript

Distribution

- Gulf Coastal Plain
- Mississippi

9) *Distocambarus* Characteristics

Rostrum:

- *Cambarus* esque
- Ends in single point

IM Gonopod:

- *Cambarus* esque

Body and Chelae

- Dorsal-laterally compressed carapace
- Chelae palms inflated
- "Gentle" notch in chelae
- BURROWERS

Distribution

- Midwest
- Coastal Plain
- Ozarks
- Ouachitas
- Appalachians

10.) *Distocambarus* Characteristics

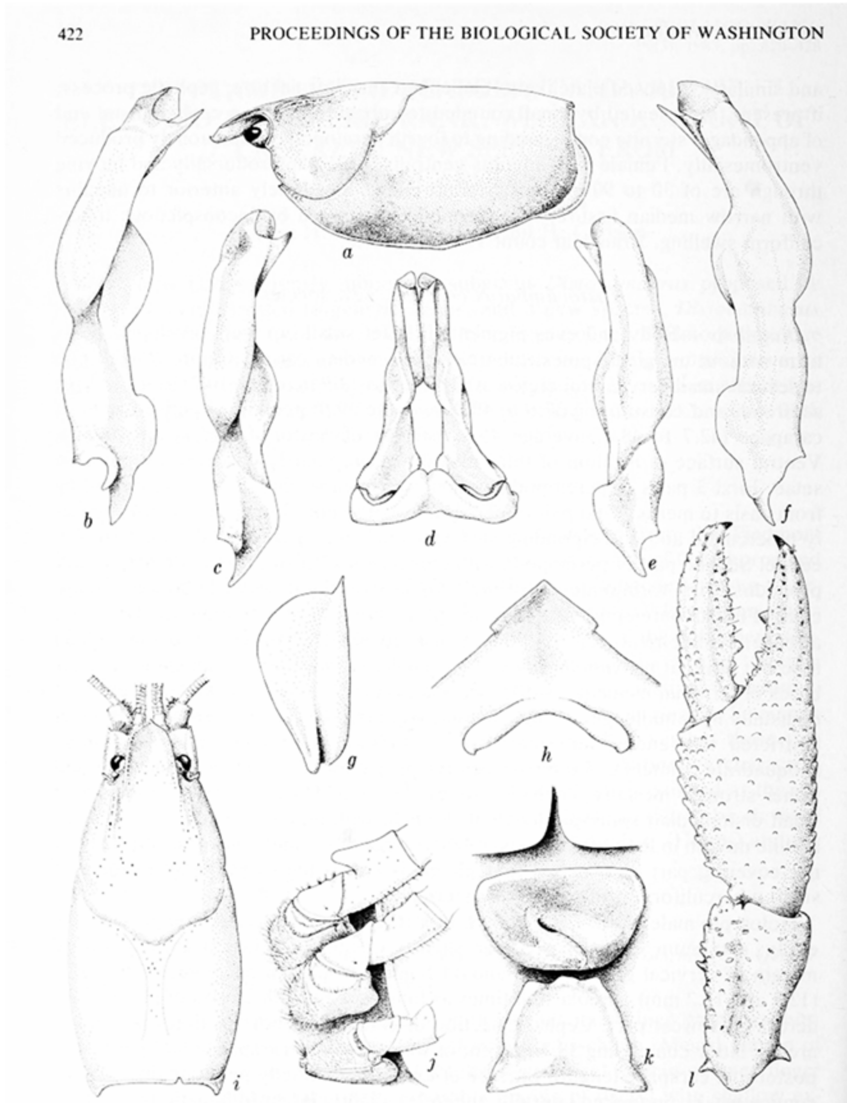


Fig. 1. *Distocambarus crockeri* (all illustrations from holotype except c, e from morphotype, and k from allotype): a, Lateral view of carapace; b, c, Mesial view of first pleopod; d, Caudal view of first pleopods; e, f, Lateral view of first pleopod; g, Antennal scale; h, Epistome; i, Dorsal view of carapace; j, Proximal podomeres of third, fourth, and fifth pereiopods; k, Annulus ventralis; l, Dorsal view of distal podomeres of cheliped.

Rostrum:

- Broad and short rostrum
- Ends in single point

IM Gonopod:

- Two short terminal elements

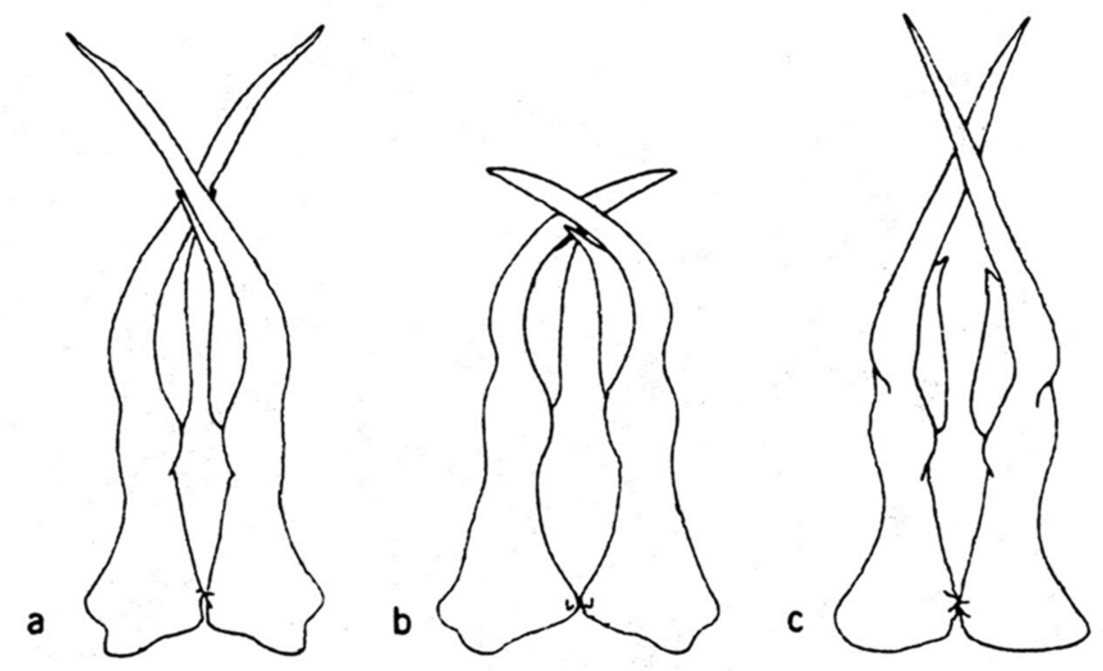
Body and Chelae

- Long palm
- Palm length > Palm width
- BURROWERS !!!

Distribution

- Piedmont
- South Carolina

11) *Faxonella* Characteristics



Rostrum:

- Acuminate rostrum
- Ends in single point

IM Gonopod:

- Terminal elements cross

Body and Chelae

- SMALL !!
- Palms elongate
- Nondescript…

Distribution

- Midwest
- Coastal Plain

12) *Barbicambarus* Characteristics

Rostrum:

- Convergent rostrum
- Ends in single point

IM Gonopod:

- Club shaped
- Mesial process bent 90°

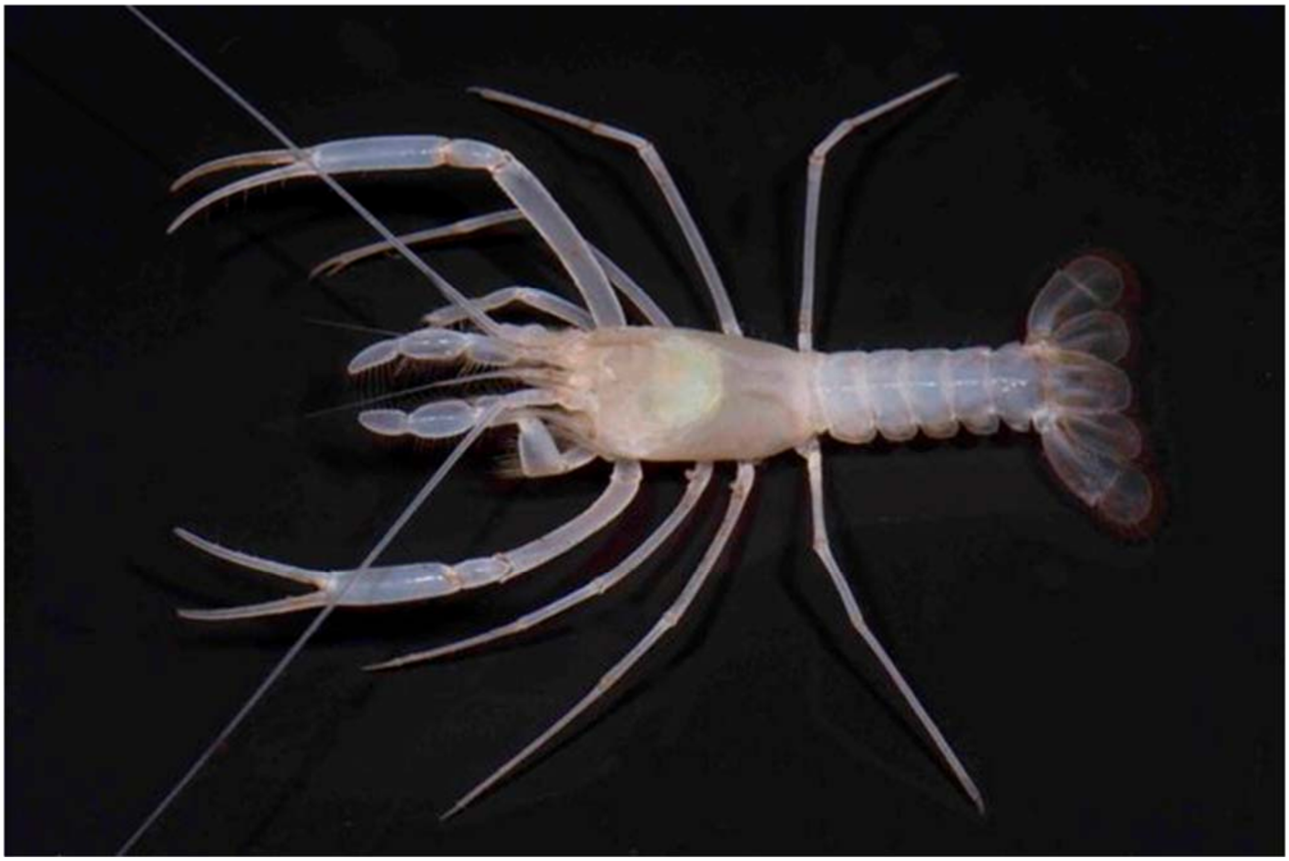
Body and Chelae

- Dorsal laterally flattened
- Chelae distinct notch
- Setiferous antennae

Distribution

- Green River
- Tennessee River
- Kentucky
- Tennessee
- Alabama

13) *Troglocambarus* Characteristics



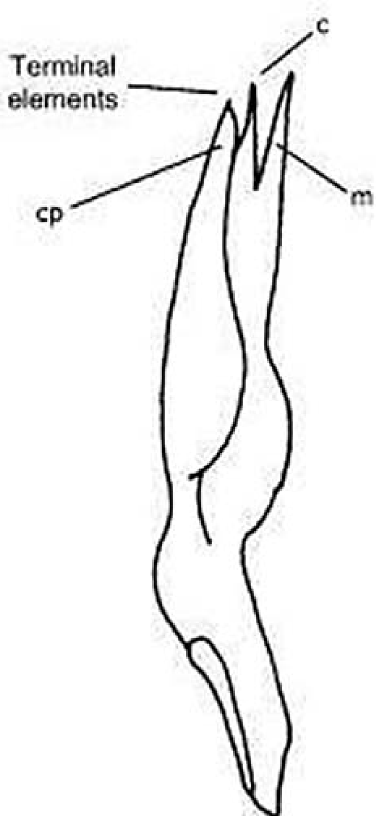
Nothing on God's green earth looks like *Troglocambarus*….except *Troglocambarus*

- Cave adapted
- MAXILLIPEDS

Distribution

- Florida caves

14) *Bouchardina robinsoni* Characteristics



Rostrum:

- Convergent rostrum
- Ends in single point

IM Gonopod:

- Terminal elements vertical
- Three terminal elements

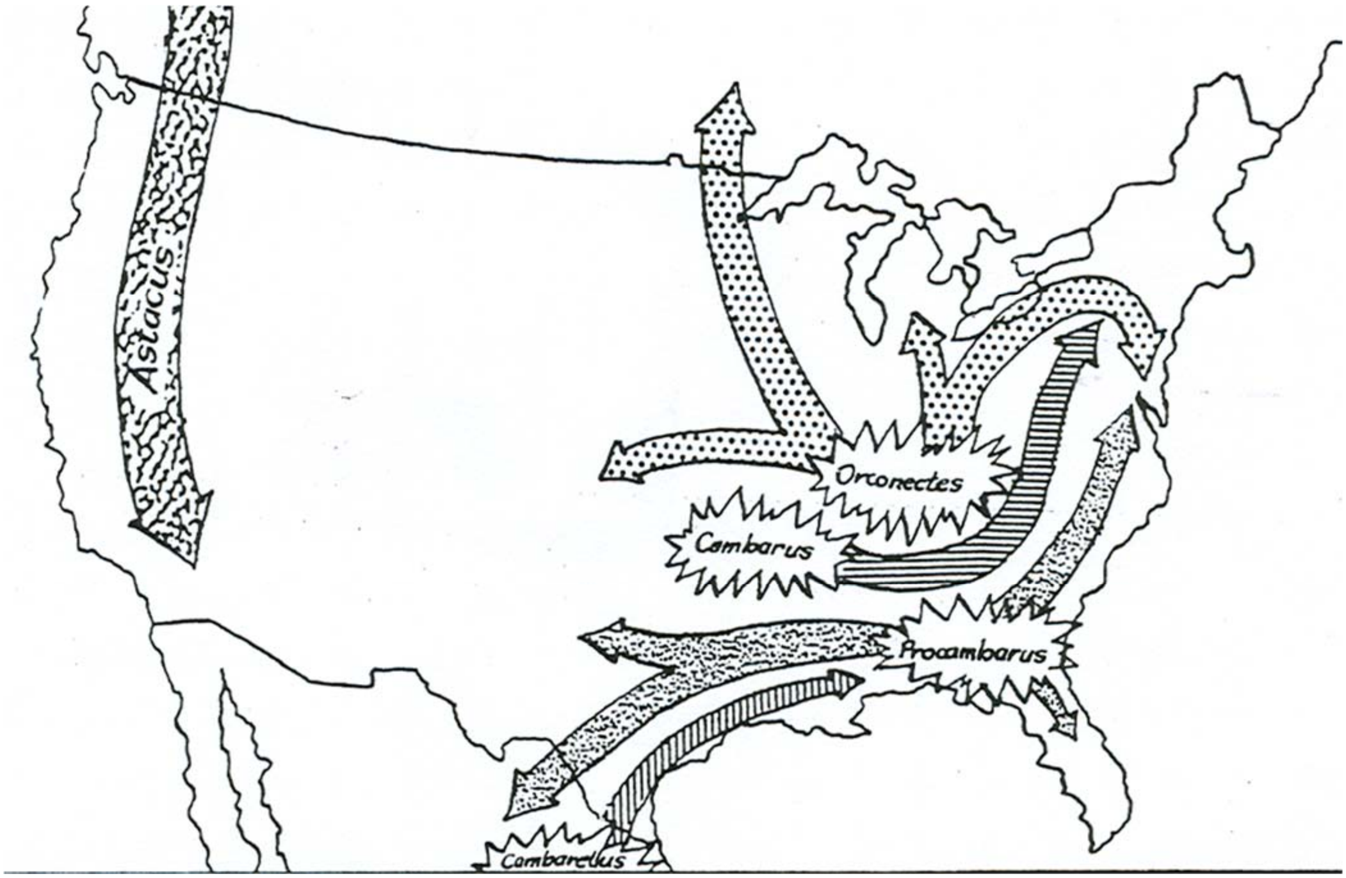
Body and Chelae

- Small
- Elongate palm
- Swamp adapted…

Distribution

- Arkansas

American Crayfish Evolutionary History



Map depicting hypothesized evolutionary routes of the most diverse crayfish genera.

Cambarus = Primarily Appalachian radiation

Orconectes = *Faxonius* = Ozarkian radiation

Procambarus = Coastal plain radiation

Cambarellus = Mexican radiation

Astacus = *Pacifastacus* = Asia to North American radiation

Cambarid list of genera

- 12 genera compose the Cambaridae

  - *Procambarus* dominant along coastal plain
  - *Cambarus* prevalent throughout the piedmont and Appalachians
  - *Orconectes* diversity peaks in the Ozark Mountains

Genera with 50+ species include:

- *Procambarus* = 177 species
- *Cambarus* = 96 species
- *Faxonius* = 89 species

Genera with 2 - 18 species include:

- *Fallicambarus* = 18
- *Cambarellus* = 17
- *Hobbseus* = 7
- *Lacunicambarus* = 6
- *Distocambarus* = 5
- *Faxonella* = 4
- *Troglocambarus* = 2
- *Barbicambarus* = 2

Monotypic genera include:

- *Bouchardina* = 1

Chapter 3

Life History

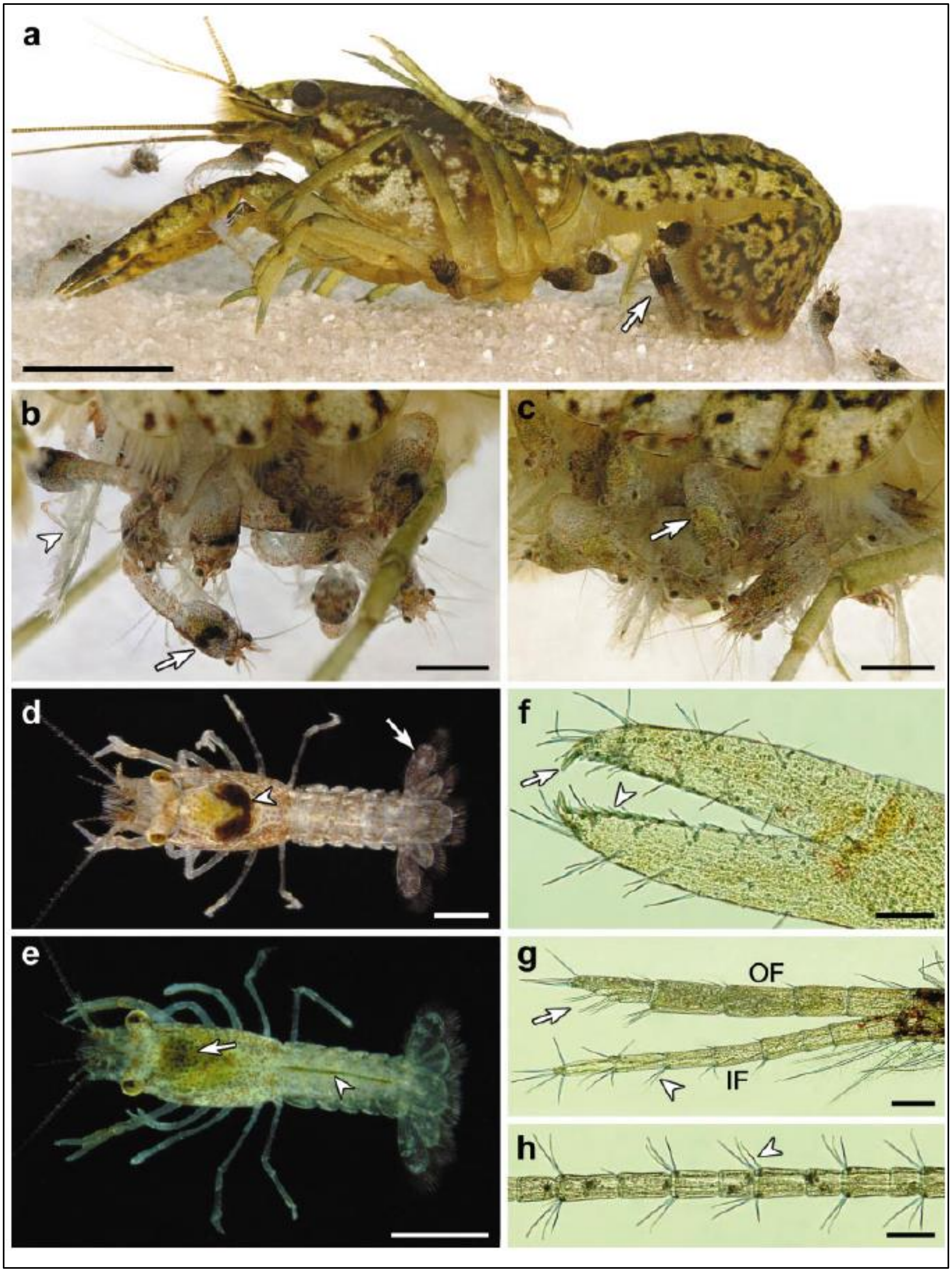
Crayfish life history

An understanding of life history is paramount to effective crayfish conservation. Most biologists are unaware that cambarid crayfish actually exhibit a unique life history relative to most other animals. In both sexes, sexual maturity is reached, though effective mating may be impossible as adults. The following is a brief description of the life history of cambarid crayfishes, followed by a description of methods that anyone in this class should be able to utilize for completing annual life history studies of crayfish species.

Egg to adult stages

In the proceeding paragraphs a detailed assessment of crayfish life history is described specific to adult males and females. The first section focuses on juvenile crayfish regardless of sex. All crayfish begin life as fertilized eggs attached to their mother's pleopods (pleopods are also referred to as swimmerets). Unique to freshwater crayfish, post-hatch juvenile crayfish remained attached to their mother's abdomens, and several behavioral studies indicate that female crayfish do become noticeably more territorial when both eggs and juveniles are in their care. Females normally seclude themselves away in burrows which they back fill closing them off from the outside environment. Within these domiciles crayfish rear their young. Juvenile crayfish undergo a series of four molts, with stages I-IV associated with each molt. Stages are determined by the presence of chelate walking legs (pereiopods), the digestion of yolk stores, and the shortening of telson threads. Telson threads emanate from the telson of juvenile crayfish and are used to attach juveniles to their mother's pereopods. Following the fourth molt, crayfish can become free living, though studies with captive animals indicate that fourth stage juveniles are often reluctant to leave their mothers care. In Cambarus species, fourth stage juveniles are often found clinging to their mother's abdomen in the spring and early summer, though egg extrusion for these species usually occurs in June, July, or August.

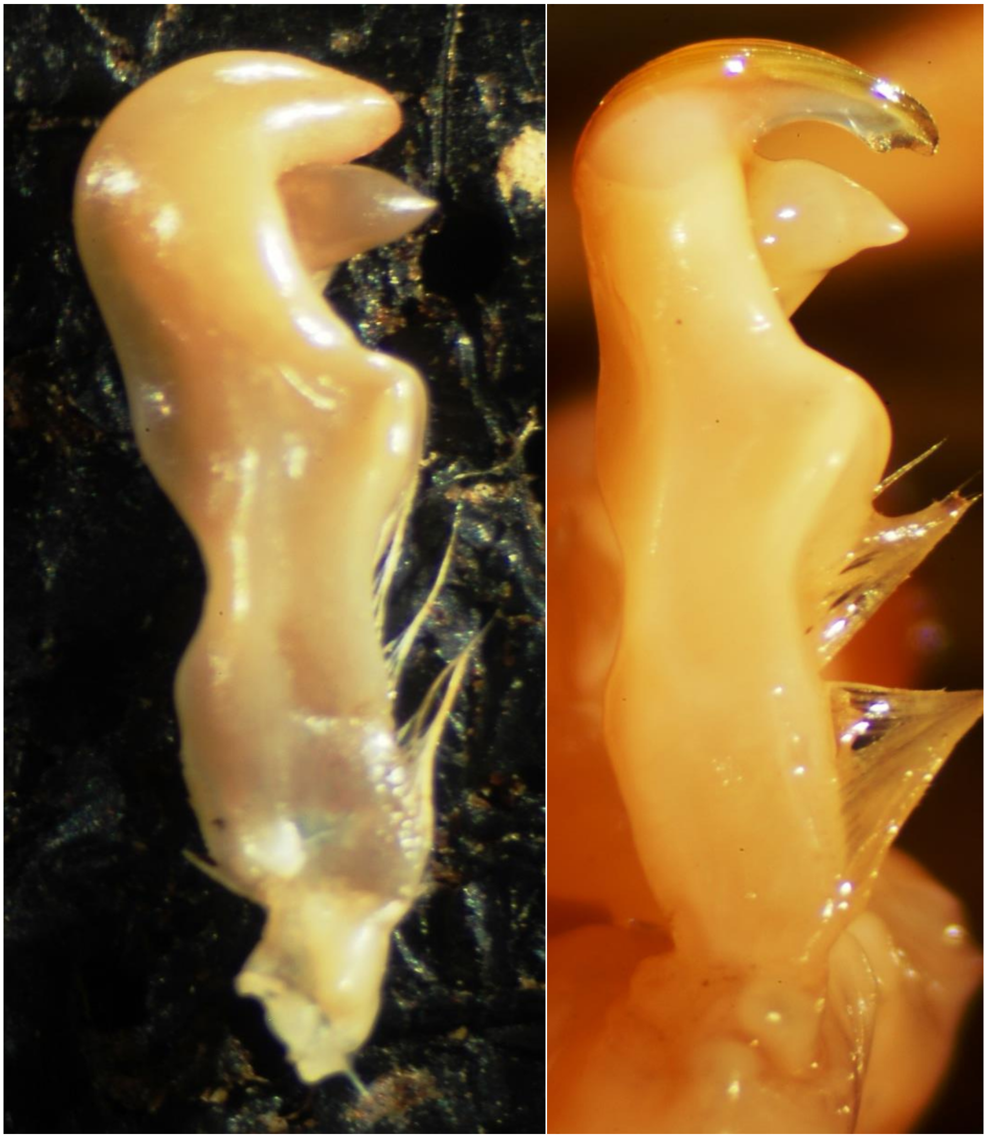
Once crayfish become free living, they are extremely susceptible to predation. In response to this, many species grow rapidly, especially those that do not readily use burrows and slab rock retreats. With each successive molt, a crayfish gets closer to sexual maturity, and Form I condition, as explained in the proceeding section.



Female Procambarus fallax brooding her young. *(a.) Adult female with juveniles; (b. & c.) Juvenile crayfish attached to female pleopod; (D.) Stage III juvenile showing remnants of yolk; (E.) Stage IV juvenile showing obliteration of yolk; (E.-G.) Juvenile morphology of the pereopods and antennules. Taken from Vogt and Tolley 2004.*

Males

Horton Hobbs Jr. was the first person to describe the morphological differences between two forms of adult male crayfish, i.e., males that can and cannot pass sperm onto females. Hobbs used the term Form I for males that could mate, and Form II for males that were not capable of mating. The structure and development of male gonopods (male reproductive organs) is the primary means of determination of Form,



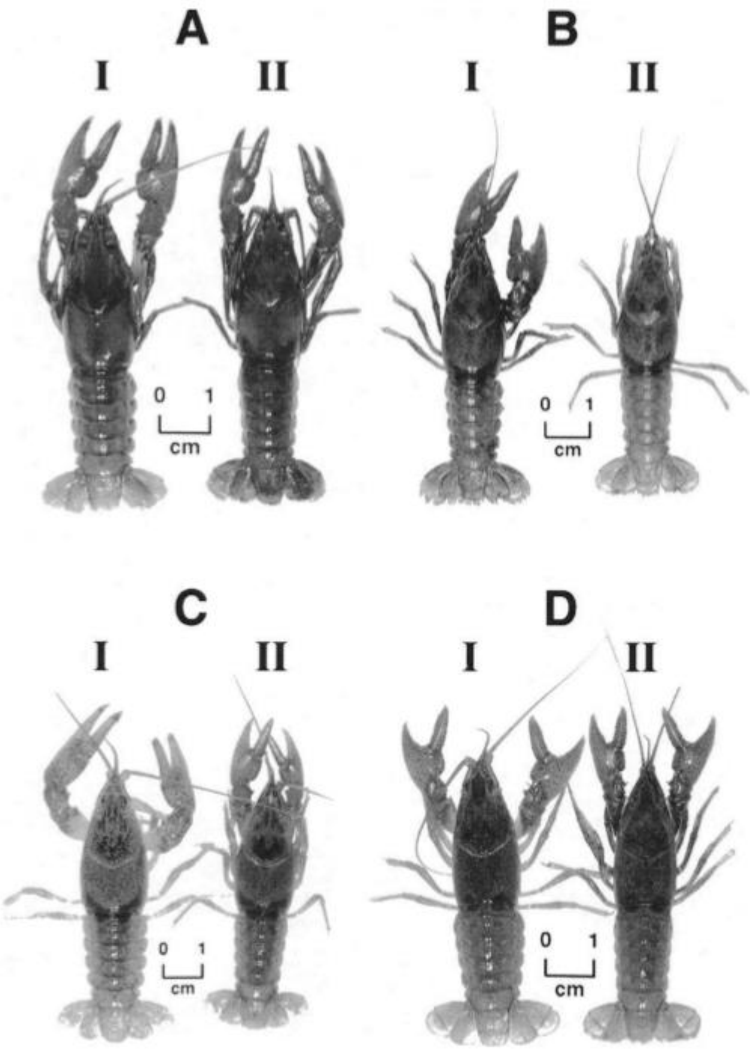
Form II (Left) and Form I (Right) gonopod

though other morphological characteristics can also be used to demarcate between the different forms sparingly.

The central projections of Form I gonopods are both flexible and malleable, and often maintain an amber hue in coloration. Form II gonopods are not flexible, are club like in shape, and lack an amber coloration. Male crayfish change from Form II to Form I following molts, so a Form II male following a molt would be Form I, and a Form I male following a molt would be a Form II. Recent observations have indicated that this dynamic is not always maintained, and it is possible that males can molt from Form I to Form I, and vice versa (Loughman personal observation). The chelae of Form I males can be noticeably larger than those of Form II males. Hormone levels also are noticeably higher in Form I males, leading to more aggressive interactions and a stronger territorial imperative. Juvenile male crayfishes are considered Form II until they reach their first molt to Form I.

Females

Until the early 2000's, most astacologists (all six of them) did not believe that female cambarid crayfishes underwent any appreciable morphological demarcation of form states. Wetzel (2002) using multivariate statistics and morphometrics clearly demonstrated in several *Faxonius* species that female crayfish abdomen width vs abdomen length ratios differed seasonally in association with population wide molts. Adult female crayfishes had wider abdomens relative to abdomen length following autumnal molts, and narrower abdomens relative to abdomen length following summer molts.



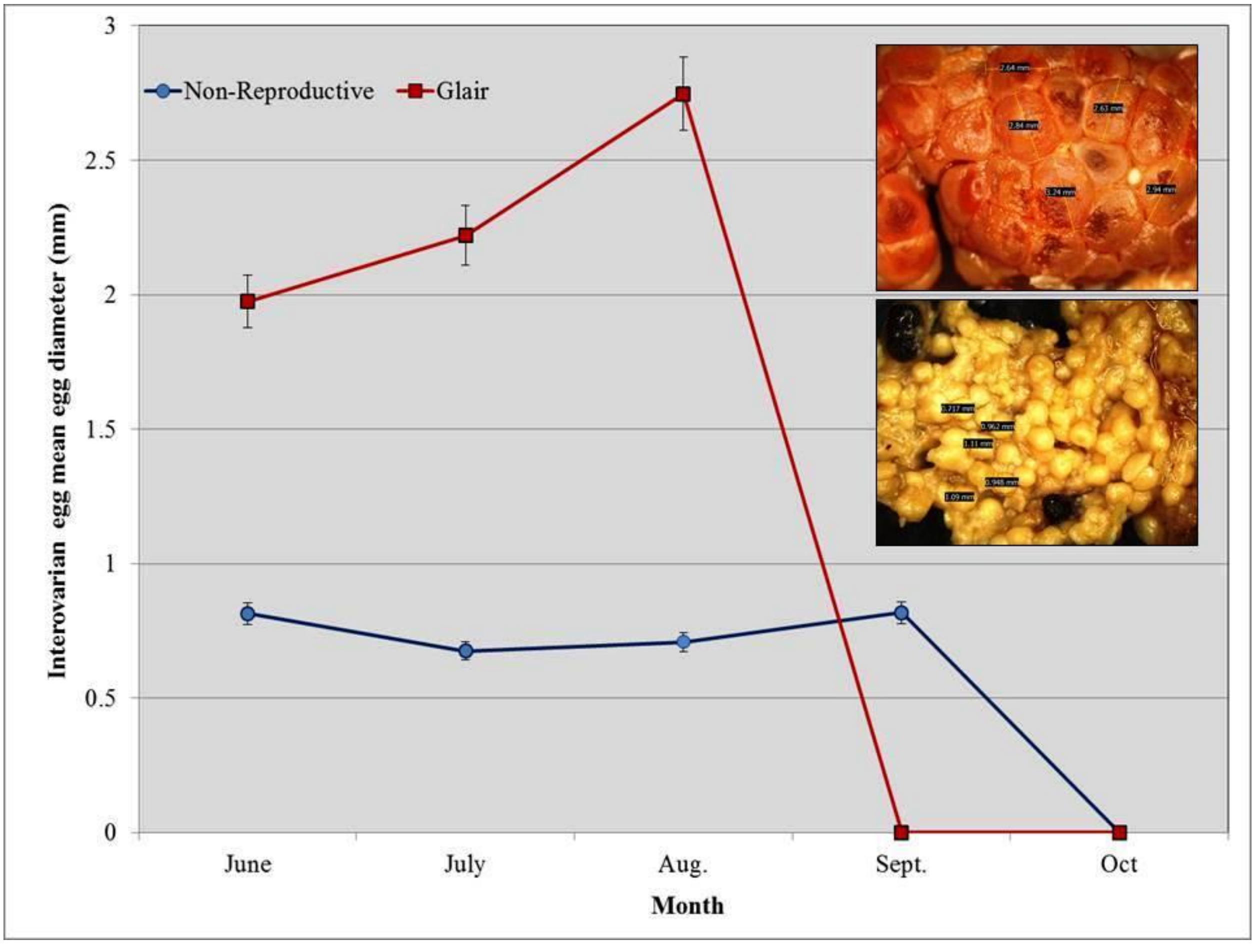
Form I (I) and Form II (II) female Faxonius species. *(A.) F. illinoiensis; (B.) F. indianiensis; (C.) F. propinquus; (D.) F. virilis. Image taken from Wetzel (2002)*

During the spring months following winter aestivation females extrude eggs, which are cemented to their swimmerets/pleopods in a process detailed later in this section. Wetzel's results clearly demonstrate that wider abdomens are present in females during this period of the year and narrower in months when females are not brooding young. Wetzel also found that female crayfishes had smaller chelae when they were not brooding young, and larger chelae when young were present. Though Wetzel demonstrated that female morphology differs in response to the extrusion of young and a “non-reproductive” period, the adoption of the terms Form I and Form II female has been slow. Recent life history publications employed Wetzel’s approach, and indicated that in both *Cambarus* and *Procambarus*, females do indeed undergo an alteration in Form, and I implore you to use these terms in any life history work you complete.

A possible explanation as to why Form I and Form II where not readily used to describe female reproductive state is the lack of an obvious biological character indicative of the Form I condition in female cambarids. Recent work by Foltz et al. (2015) and the Loughman laboratory has at least in *Cambarus* demonstrated that glare glands, and their activity can be used as an indicator of form. Glare glands, also known as cement glands, line the outside of each abdominal pleon, as well as the junctions of each leg boss of the cephalothorax. Glare is produced in these glands, and is excreted by female crayfish prior to and during oviposition and fertilization. Recent work with both *Cambarus chasmodactylus* and *Cambarus theepiensis* clearly demonstrate that females exhibiting glare glands, have ovarian oocytes that are noticeably larger, and undergoing maturation in spring and summer months, and had Form I abdomen length/width ratios. Females within the population that did not have active glare glands did not have maturing oocytes, and had abdomen width/length ratios indicative of Form II females.

Female Cambarus thomai with inactive (Left) and Active (Right) glare glands. *The glare glands are the globular round white structures.*

During oviposition, females lie on their sides, and rock gently in a lateral motion. As they rock, glare is extruded, which combines with water and quickly expands, as it rolls down



Monthly mean interovarian egg/oocyte diameter for glared (red) and nonglared (blue) female Cambarus chasmodactylus. Inset pictures of oocytes from glared (top) and nonreproductive females (bottom) in August just prior to egg extrusion in September.

and out of the open space in the females curled abdomen. The glare in effect fills all the spaces to prevent eggs from escaping to the environment. Once all spaces are filled, females extrude unfertilized eggs from oviducts located on the bosses of the 3rd set of walking legs. Extruded eggs roll along the ventral surface of the cephalothorax and over or in the vicinity of the annulis ventralis. Males deposit spermatophores into the annulis ventralis, which can lie dormant for months, and in extreme instances years. As the egg moves over the annulis ventralis, rehydrated sperm is released by the female, and the egg is fertilized. Eggs continue rolling within the confines of the “abdominal basket”, and then come in contact with extruded glare and the females swimmerets. Now fertilized, the eggs bond with glare on the swimmerets, and ultimately become glued to the females pleopods, where they will remain until they hatch.

The number of eggs a female extrudes depends on burrower type as well as female size. In both *Faxonius* and *Procambarus*, larger females almost always produce more eggs than smaller females. *Cambarus* species do not always demonstrate this trend, though most tertiary burrowing species do have size/egg count relationships. The noted exception to this rule is primary burrowing species, which lay fewer eggs than both secondary and tertiary burrowers, and also lay eggs that are noticeably larger than both tertiary and secondary burrowing species of similar size.

These conditions lend credence to the application of r and K selection life history strategies to crayfishes. Both *Faxonius* and *Procambarus* species seem to produce larger numbers of offspring, which grow rapidly, and are not long lived. *Cambarus*, *Fallicambarus*, *Distocambarus*, and *Creaserinus* species all exhibit K selected life history attributes. All of these genera produce fewer, larger eggs, and in the case of burrowing *Cambarus* species, tolerate their young's presence in their burrows, and even cohabitate with them well beyond the fourth juvenile stage of development.

Crayfish Life History Studies

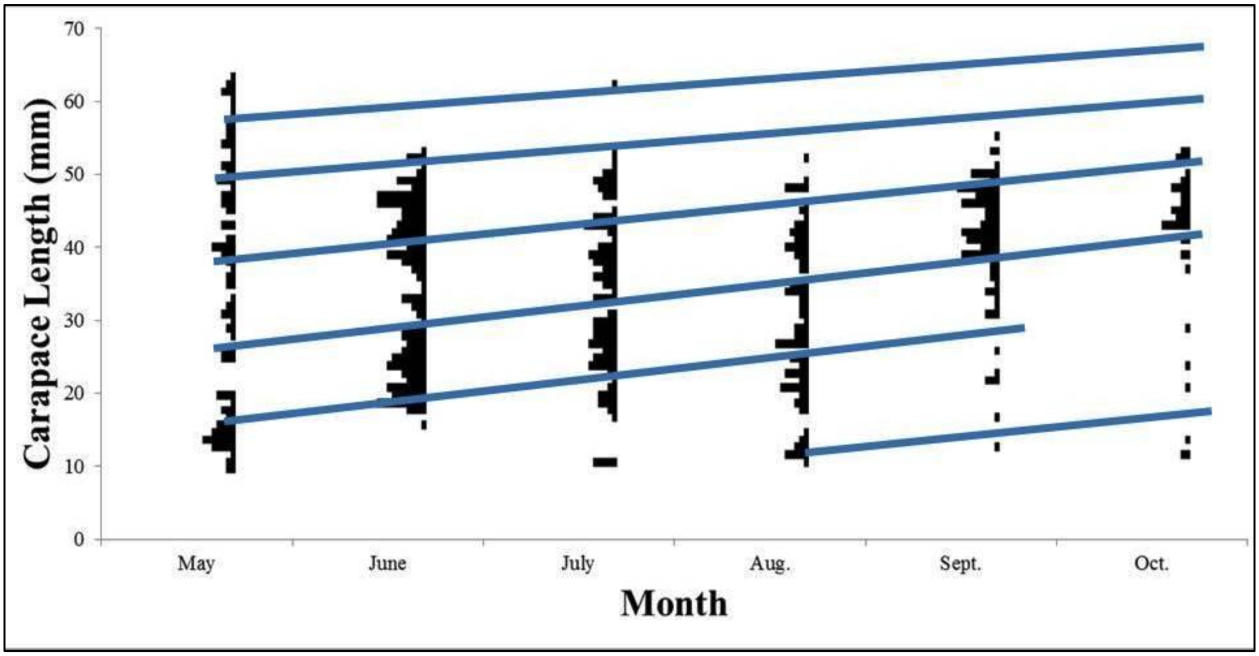
Crayfish life history studies are in dire need of completion for most North American taxa, and incredibly easy to complete. In essence, all that is needed to complete a life history study is (1.) a robust, stable population of crayfish at (2.) an accessible location that (3.) can be sampled monthly. Moore et al. (2012) provides a review of the need and justification of this science; even common species life histories are needed to be studied just as much as endangered taxa. The following is a basic protocol to complete a life history study.

1. Discovery of multiple sites within a watershed where the focal species occurs
2. Development of a monthly protocol for collections of animals and abiotic data

Sites selection can be limited to 3-5 stream reaches on a single stream, or sites where the focal species occurs throughout a given watershed. Once site selection has been determined, monthly sampling needs to occur. A good practice is to ensure that animals are collected within the same window of time each month. In the Loughman lab, we collected data between the 10^{th} and 20^{th} of each month. During each period of collection, at least 30 individuals are captured if possible, with the hopes of collecting at least 100 individuals. Data collected on each animal captured include but are not limited to:

1. MOLT STATE: Intermolt; Fresh Molt, Pre Molt, Molted
2. FORM STATE - Males: Form I and Form II males
3. FORM STATE – Females: Nonreproductive, Preglare, Glare, Eggs, Juveniles
4. MORPHOMETRICS – Total Carapace Length, Palm Length, Abdomen width and length
5. NOTES – Regenerated chelae, Missing chelae, damaged rostrum, other….

Collected data is maintained in Excel or Google spreadsheet, and used to determine form change in females (Abdomen length vs width ratios, TCL, and chelae measurements run in an ANCOVA), pooled number of individuals present in a given size cohort can be plotted in histograms or graphs and used to determine size cohorts present in the populations. The smallest female with glare, and Form I male can be used to derivate size at sexual maturity. If each month's cohorts are plotted together,



Example size cohort histogram for Cambarus chasmodactylus. Lines indicate proposed size/age cohorts within the population based off of when Young of the Year are recruited into the population.

and the period of molting is noted, it is possible to estimate age of various cohorts, and see when recruitment to older cohorts occurs over the course of a year. The number of eggs extruded by females or the number of young attached to female abdomens can be counted to determine recruitment rates per female.

Chapter 4

Ecology

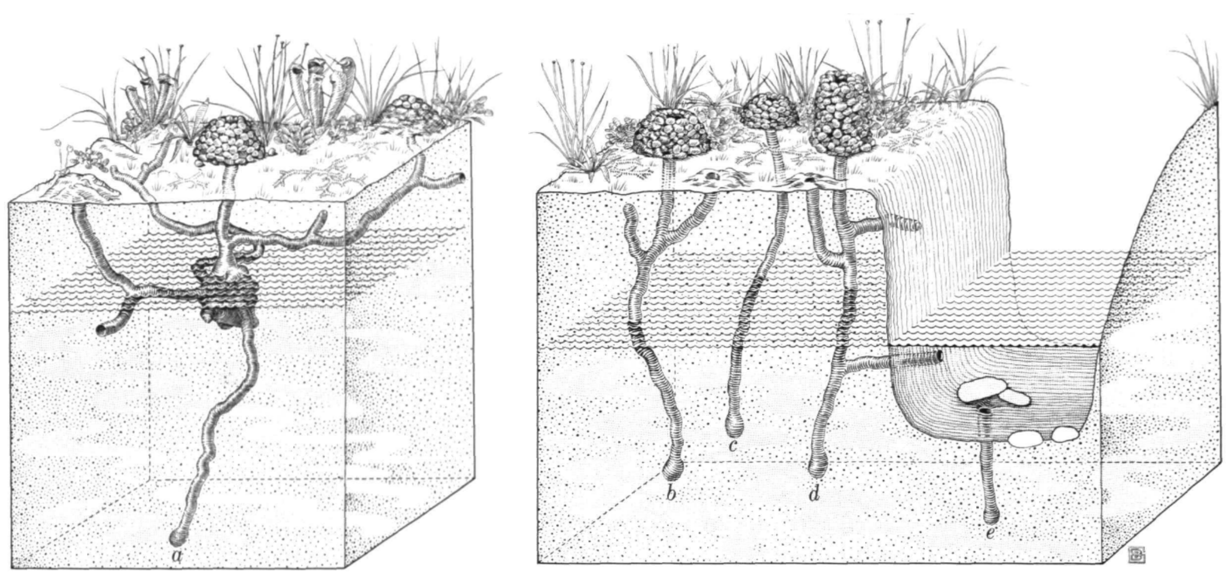
Crayfish Ecology

Within a wide range of habitats, such as wetlands, streams, rivers, or forested seeps, crayfishes are incredibly important players in trophic dynamics, manipulation of substrates, and creation of new microhabitats. From a trophic perspective, crayfishes are an important food source for many aquatic and terrestrial animals. Additionally, through manipulation of substrates or creation of burrows, crayfishes have been labeled as ecosystem engineers, providing important microhabitats and resources for many animals. In some cases, crayfishes play a role as keystone species, because they create critical microhabitats for certain species, In the following section we will explore the general ecology of crayfishes, focus specifically on three behavioral guilds, and provide examples of the important roles that crayfishes play within ecosystems.

Tertiary, Secondary, and Primary Burrowers

In the front piece of the monograph "The Crayfishes of Georgia," Hobbs (1981) was the first to described and define tertiary, secondary, and primary burrowing crayfish. In essence, when sampling across multiple habitats of eastern North America, Hobbs noticed an interesting pattern of crayfish occurrence, where crayfish species were found in three general habitat categories; 1) perennial waters, 2) ephemeral waters, or 3) ground water supplies that were often distant from surface waters. In addition to this perceived habitat partitioning, Hobbs also noticed that crayfishes had key anatomical structures that enabled them to persist in the aforementioned habitats.

In response to these observations, Hobbs described three ecological categories for crayfishes, all of which were dependent on a given species' propensity to burrow. This categorical scheme has held up over 35+ years of scrutiny with little exception. These ecological categories are paramount for understanding species composition and geographic distribution of local crayfish faunas.



Generalized examples of crayfish burrows from Hobbs (1981), including those of primary burrowers (a, d), secondary burrowers (b), and tertiary burrowers (c, e).

Tertiary Burrowers

All tertiary burrowers occur in perennial waters, such as creeks, rivers, lakes, or swamps. Tertiary burrowing crayfishes, in order to carry out their life cycle, are dependent on permanent waters. All tertiary burrowing crayfishes are capable of burrow construction, though several species do not create elaborate burrows. The complexity of the burrow is species specific, with the majority of tertiary burrowers utilizing some permanent benthic object to construct a simplistic burrow underneath. Utilization of the term burrow may be generous for some species, because some species at best construct nothing more than an excavated cavity, though other species can and do construct elaborate tunnels leading to enlarged chambers. Interstitial spaces are also used frequently as refugia by tertiary burrowers.

Among tertiary burrowing species, selection seems to result in dorsal-ventrally flattened large bodies and the presence of spines. The majority of North America's largest crayfishes are tertiary burrowing species. Given that tertiary burrowers are a common prey species, particularly for predatory fishes, their large body size and spines likely aid survival. The evolutionary equation here is simple; predation of large crayfish usually requires a large-mouthed predator, making North American crayfish excellent players in examples of gape limitation in predator-prey interactions.

Several tertiary burrowing species have a large gap between the dactyl and propodus when the chela is closed. Most of these taxa live in streams with fast moving riffles and runs, where the chela gap may be used to create and maintain hydrofoils when the crayfish is moving in fast water currents. Hydrofoils produced by the gaps in chelae may assist crayfish with station holding on the stream bed in high water current velocities. Crayfish species with large chelae gaps also interestingly lack spination, which many tertiary burrowers possess.

Cambarus chasmodactylus, a tertiary burrowing crayfish with large gapes in closed chelae.

Cervical, rostral, and hepatic spines all appear in greatest frequency on tertiary burrowing crayfish. Specifically, species that inhabit moderate to slow moving perennial waters often possess spines. Spines are a deterrent to predators, decreasing the palatability of crayfish. Both *Faxonius limosus* and *Cambarus spicatus* are incredibly spinose, particularly around their heads.

Cambarus aldermanorum, a tertiary burrowing crayfish with excessive spination.

Tertiary burrowers serve as important ecosystem engineers in perennial waters. All crayfishes are busy, spending a fair amount of the 24-hour activity cycle excavating and maintaining their burrows. As crayfish excavate and move benthic substrates, they immobilize and release nutrients to the stream or lake bed. Important microhabitats, created via burrow construction and maintenance, are utilized by a multitude of species. Sculpins, darters, and madtoms all utilize crayfish burrows to complete their life cycles. Benthic macroinvertebrates that need access to the undersides of rocks freely take advantage of crayfish burrows.

Secondary Burrowers

Secondary burrowing crayfish occur in ephemeral lotic and lentic systems. A common habitat condition experienced by secondary burrowers is the complete drawdown of water at some point over the course of the year. When present, secondary burrowers readily utilize surface waters, and burrow in response to these drawdown conditions. Burrow construction and architecture of secondary burrowing crayfish are more involved and elaborate than those of tertiary burrowers. The actions of secondary burrowers are also more readily observable, given their burrows occur in stream banks and high walls, wetland floodplains, and other terrestrial systems

Cambarus b. cavatus, an example of a secondary burrowing crayfish.

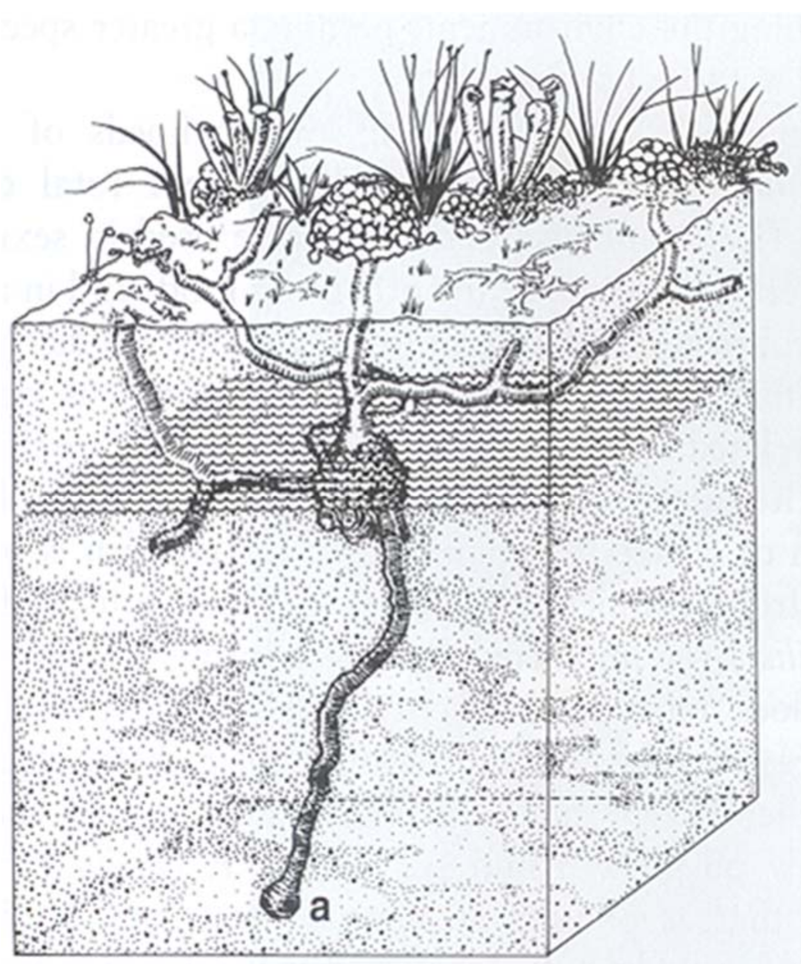
Anatomically, secondary burrowers are more circular in cross-section, and in the case of *Cambarus* species, have broad, powerfully-built spade-like chelae which are used in the excavation process. Given the absence of fish predators, secondary burrowing species lack excessive spination, and are moderate sized crayfishes compared to tertiary burrowers.

The burrows of most secondary burrowers consist of an entrance portal leading to a tunnel that terminates in an enlarged domicile called the resting chamber. Resting chambers almost always are inundated with water, and often are constructed where vadose water supplies normally occur. Burrows are not always highly consolidated structures. In gravel-cobble dominant headwater streams, burrow construction may simply follow the path of least resistance to the groundwater. On floodplains and in dried wetlands where silts and loams are the dominant substrates, secondary burrowers construct legitimate burrows. Burrow chimneys are constructed by these animals, created from the spoil of their excavations, which often dot the Earth's surface following drawdown.

In headwater streams, secondary burrowing crayfishes are frequently the largest benthic macroinvertebrates present. Crayfish are both important predators of benthic macroinvertebrates and important players in the assimilation of autochthonous energy sources in these ecological systems. In wetland and lake ecosystems, especially in the coastal plain, secondary burrowing crayfish (*Procambarus* spp.) often reach incredibly high densities and represent a disproportionate amount of invertebrate biomass. Many terrestrial vertebrates rely heavily on secondary burrowing crayfishes as forage. Even more so than tertiary burrowers, burrows created by these animals are utilized by hundreds of animals, both aquatic and terrestrial, making them important members of their ecological communities.

Primary Burrowers

The burrows of primary burrowing crayfish are quite elaborate relative to those of the tertiary and secondary groups. For most species of primary burrowing crayfish, the burrow is utilized for all aspects of their biology. With the exception of *Distocambarus* species which rely on dew and rainwater, most primary burrowing crayfish are associated with shallow water tables. Shallow is a relative term here, given that individuals have in several instances been excavated at depths in excess of 3 meters. Groundwater is used to inundate the burrow, and as such it is possible to have populations occurring in environments that do not maintain surface waters. Compared to tertiary and secondary burrowers, primary burrowing crayfishes are without question the least understood ecologically.



Schematic view of a burrowing crayfish burrow.

Burrows of primary burrowing crayfish share several features with those of secondary burrowers, but also have unique features. Like secondary burrowers, primary burrowers often have a primary tunnel leading directly to the resting chamber, and this tunnel is utilized more often than other burrow tunnels. Several ancillary tunnels often radiate from the resting chamber. Most ancillary tunnels run laterally out of the resting chamber, and can return to the Earth surface creating another portal, or simply terminate underground. Most burrow tunnels ramify and bifurcate, resulting in several tunnels associated with a single burrow. It is not uncommon for burrows to have multiple portals all associated with a single crayfish's burrow complex.

Cambarus dubius hunting at the burrow portal. Cambarus dubius is part of a large complex of undescribed Appalachian burrowing crayfishes, and an example of a primary burrowing crayfish.

Given reliance on excavation and the importance of burrow maintenance, primary burrowing crayfishes have a unique morphology. Carapaces are vaulted, and ovoid in cross-section, causing the obliteration of the areola. This vaulted condition results in the gills having greater surface area, aiding water vapor absorption for respiration. Chelae are vertically inclined when resting, compared to the horizontal condition observed in most tertiary and secondary burrowing species. This condition enables them to manipulate soils and other objects more readily within the confines of their burrows. Abdomen widths are greatly reduced in primary burrowers compared to those of secondary and tertiary burrowing species. Reduced abdomens are associated with lower egg counts compared to stream dwelling species.

Primary burrowing crayfish are keystone species, supporting higher levels of biodiversity where they occur. Several animals are crayfish burrow specialists, and occur only where large colonies of primary burrowing crayfish persist. Eastern Massassauga Rattlesnakes which were recently granted federal protection, and Kirtland’s Snakes, which have been petitioned for federal protection are known crayfish burrow specialists. Crawfish frogs are state protected everywhere they occur, and are dependent on the presence of crayfish burrows for both winter and summer aestivation sites. The federally protected Hines Emerald Dragonfly utilizes burrowing crayfish colonies as ovipositioning sites and larval habitat, and experience immediate extirpation following the destruction of burrowing crayfish colonies. To date over 300 species have been noted utilizing crayfish burrows. It is easy to see how the destruction of burrowing crayfish colonies leads to an immediate reduction in overall biodiversity at the local level.

Most ecological and behavioral studies of primary burrowing crayfish have been conducted recently. It was believed prior to recent work that individuals spent the majority of their lives within their burrows, and utilized surface environments rarely. Recent research has shown that primary burrowing crayfishes are nocturnal. When conditions are conducive, individuals are active at their burrow portals at night. Most primary burrowing crayfish spend a significant amount of time maintaining the integrity of their burrows. Burrow maintenance behaviors increase after periods of precipitation when loose substrates flood burrows. In addition to burrow maintenance behaviors, individuals graze vegetation near their burrows portals, and readily engage and consume invertebrates and small vertebrates that venture near their portals. Burrowing crayfish also cache food in their resting chambers during periods when resources are high.

Montane North American species, occurring primarily in forested seeps, often tolerate the presence of their young or other individuals in their burrows. Several biologists have noted finding multiple individuals from different size and age cohorts co-occurring in the same burrow. Gregarious behaviors seem to be limited to environments where space is limited. In large river floodplain and bottomland forests, gregarious behaviors are rarely noted, indicating that not all burrowing crayfish are the same. Investigating the ecological plasticity these animals demonstrate is an area of burgeoning crayfish research.

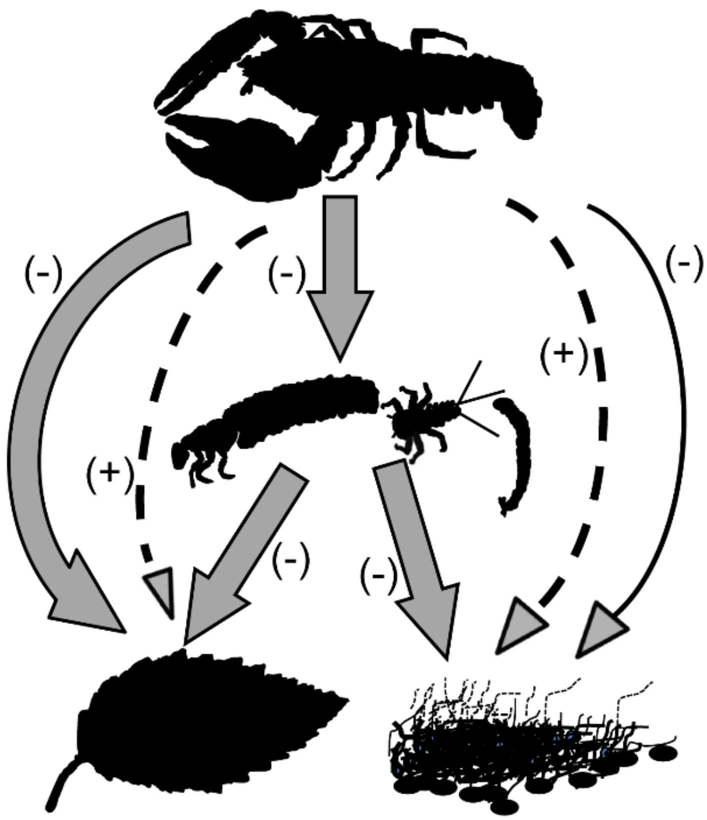
Stygobitic Crayfish

Several species of crayfish, including all *Orconectes* and *Troglocambarus* species, are cave obligates. Cave dwelling species lack pigments and eyes, have elongated chelae, and persist in the dark zones of caves. Four of North America’s seven federally protected species are cave obligates. All cave dwelling crayfishes are omnivorous scavengers that occur in cave pools and slow moving waters. These animals are long lived, reproduce rarely, and susceptible to any environmental perturbations that occur within their caves.

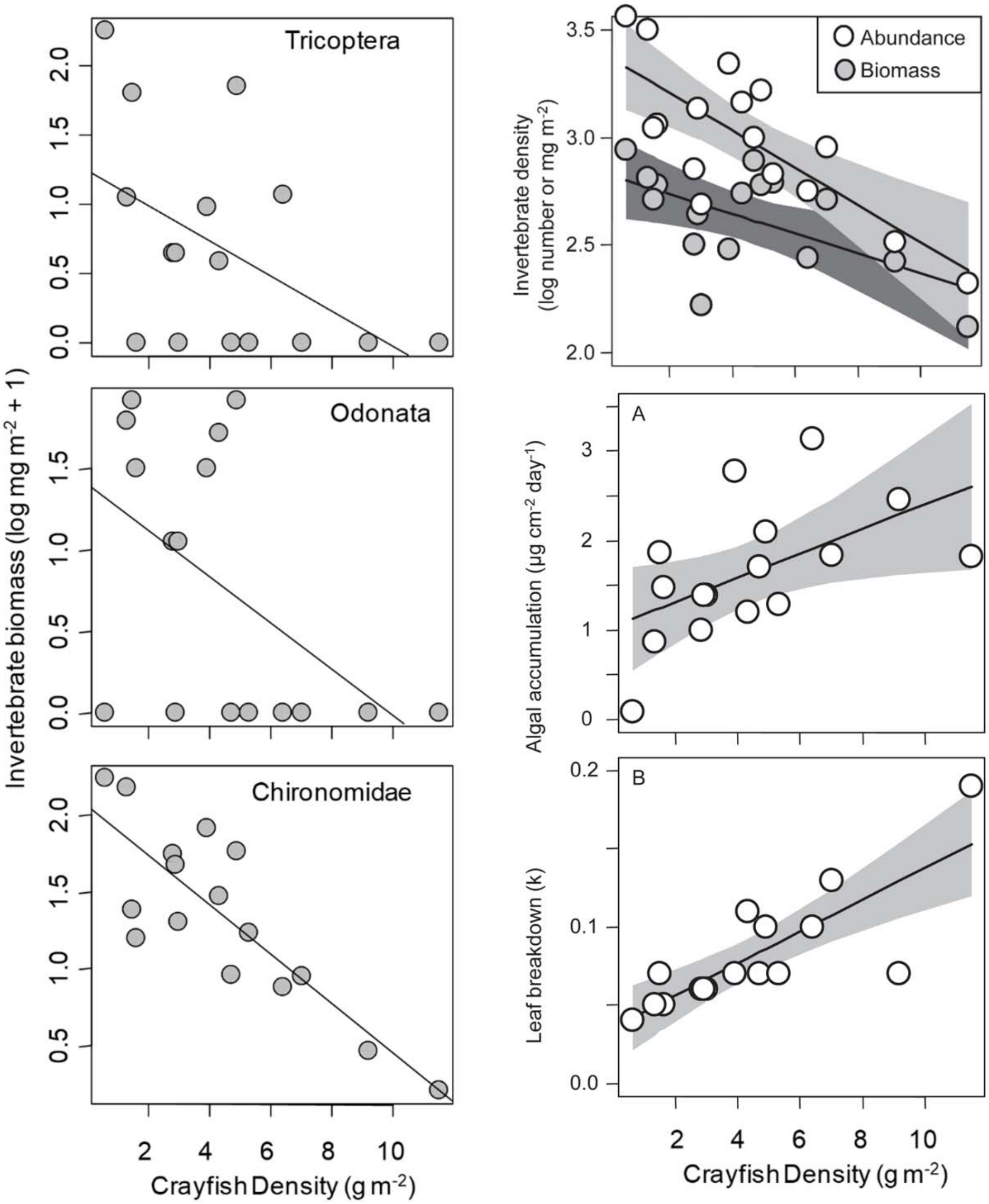
A stygobitic crayfish.

Crayfish trophic effects on ecosystem processes

Research by Moore et al. (2012) provides some examples of how the signal crayfish directly and indirectly effected trophic levels within a forested high gradient coastal stream ecosystem of central California. Consider the following relationships presented by Moore et al. (2012):



Note that the invertebrates in the middle are detrivores. Crayfish, commonly considered as omnivores, were primarily eating (having a direct negative effect on) leaves and aquatic insect larvae, and occasionally algae. The insect larvae were also foraging on leaf bits and algae. The crayfish reduced the population of insect larvae, and thus reduced consumption of leaves and algae (demonstrated by the indirect “positive effect” dashed lines). Thus, we have an example of a classic trophic cascade, where crayfish predation on grazing benthic insects can indirectly increase algal density.



Figures adapted from Moore et al. (2012). The left column depicts a negative relationship between crayfish density and biomass of aquatic insect taxa. The upper right figure demonstrates the overall relationship between densities of a crayfish and aquatic insect larvae. The middle right figure demonstrates the end result of the trophic cascade effect, where an increase in crayfish density is related to higher accrual of algae. The lower right figure shows the expected relationship of crayfish density and leaf breakdown.

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Chapter 5

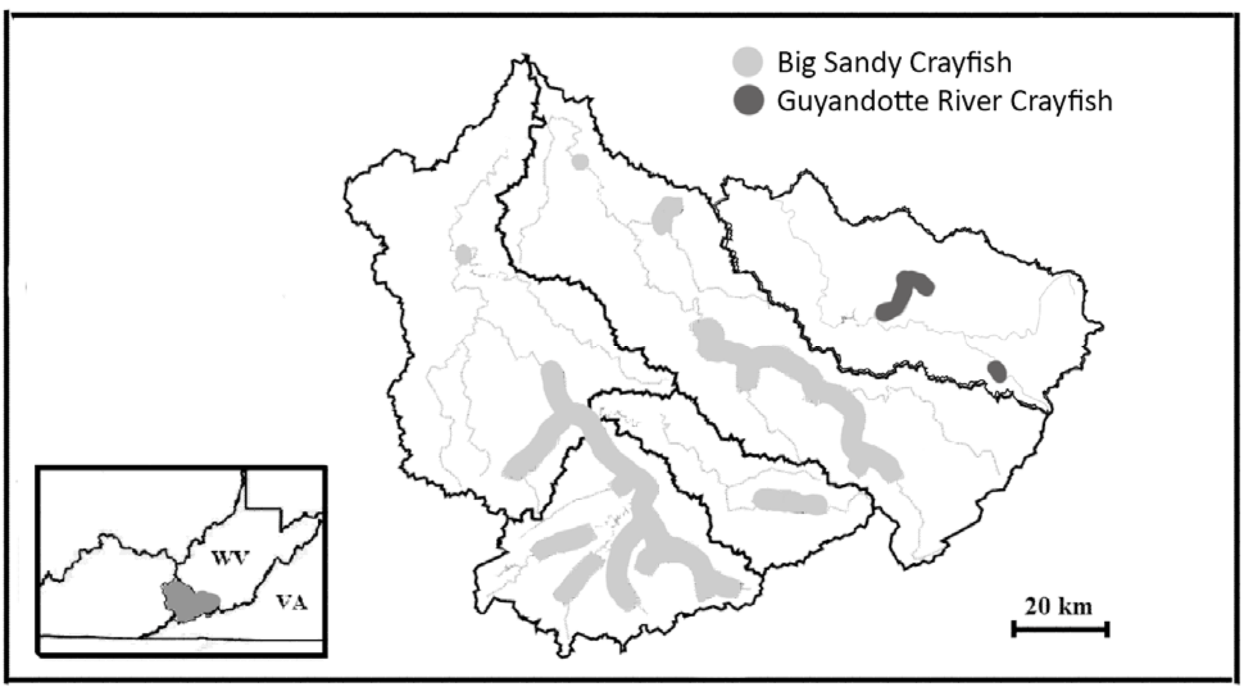
Conservation

Crayfish Conservation

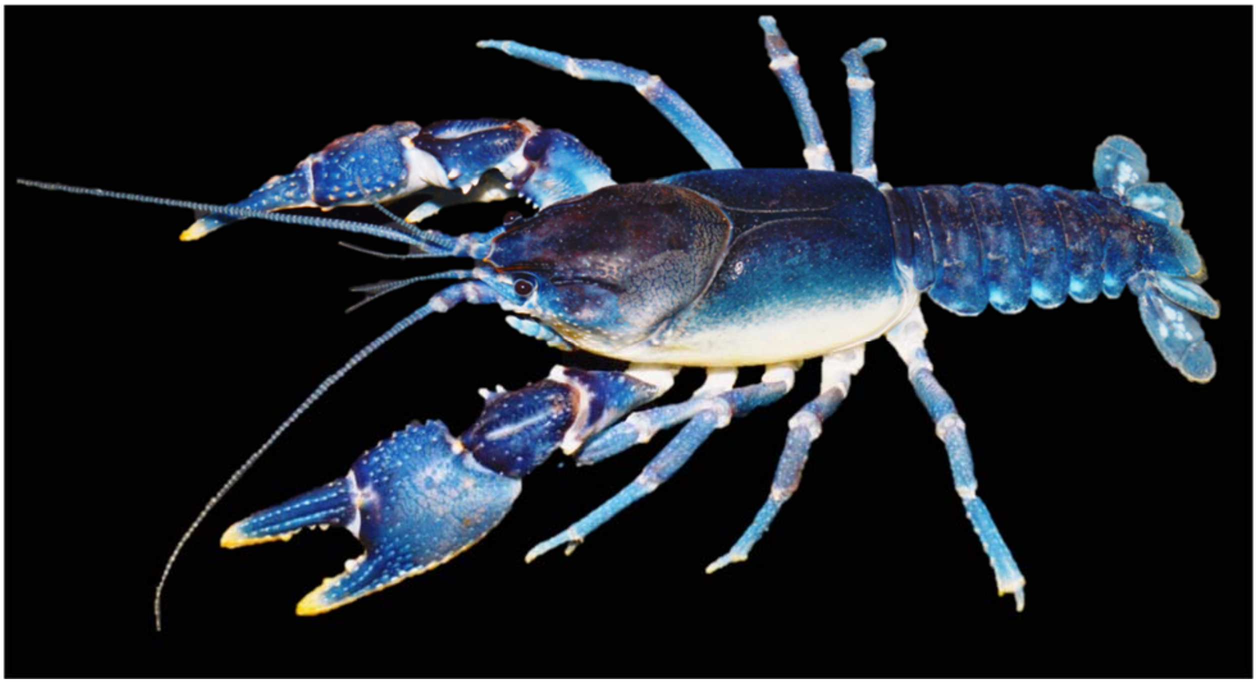
About 10 years ago, Taylor et al. (2007) published an important paper toward recognizing crayfish conservation concerns, titled as “A reassessment of the Conservation Status of Crayfishes of the United States and Canada after 10+ Years of Increased Awareness.” This 2007 paper was a follow up to Taylor et al. (1996), a paper titled as “Conservation status of crayfishes of the United States and Canada.” During the approximate 10-year period between Taylor et al. (1996) and Taylor et al. (2007), the awareness of conservation concerns for crayfishes were elevated. More recently, Richman et al. (2015) examined crayfish conservation on a global level. However, as indicated by Loughman et al. (2017), crayfishes are still often treated as a biological conglomerate, where biologists often lump all crayfish species as “decapoda” during aquatic surveys. Given a current emphasis on recognizing biodiversity, we believe that regional workshops and classes on crayfish identification are needed, so that distributions and diversity of crayfish species can be recognized, and species conservation concerns can be addressed.

In 2007, Taylor et al. compiled a list of 363 native crayfishes, including 2 possibly extinct taxa (< 1%), 66 endangered taxa (18.2%), 52 threatened taxa (14.3%), 54 vulnerable taxa (14.9%), and 189 currently stable taxa (52.1%). Although these numbers have changed in the last 10 years, the separation of these conservation categories highlight the overall conservation concern for North American crayfishes.

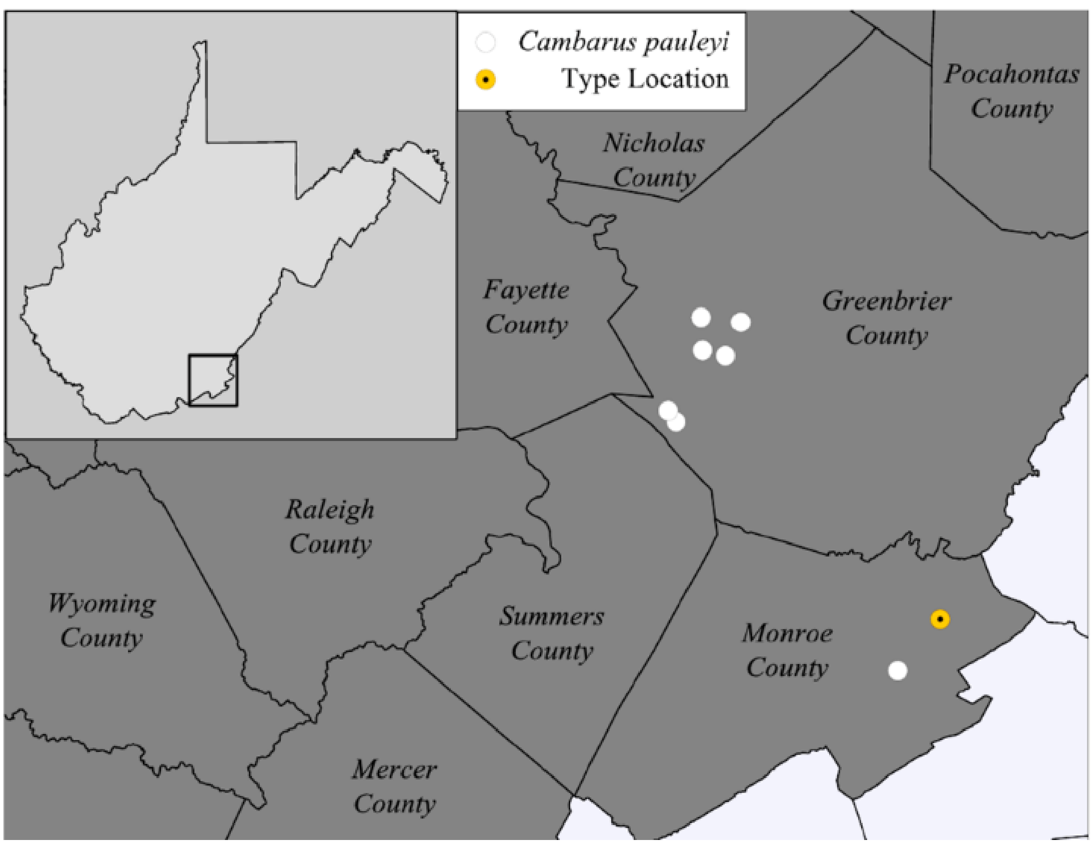
One major conservation issue is the “taxonomic impediment,” where large gaps in taxonomic information hinder management and conservation of crayfish taxa, an issue owing in part to a lack of trained astacologists. Conservation concerns cannot be addressed for undescribed species. As example, consider *Cambarus veteranus*, a species once thought to be extinct. Upon rediscovery, it was determined that two species were masquerading as *C. veteranus*, which led to the description of *Cambarus callainus*. Conservation concerns of these two species are now being addressed, as recently *C. veteranus* and *C. callainus* were federally listed under ESA as endangered and threatened species, respectively.



Expected distributions of Big Sandy and Guyandotte Crayfishes (adapted from USFWS 2016)



Cambarus pauleyi, is a species that was described in 2016. This species prior to description was part of a wide ranging species complex. Following description, it was determined that C. pauleyi's entire range was limited to 2 counties in West Virginia



From a conservation perspective, there are many possible explanations for declines and extirpation of crayfish populations. Four major categories listed by Taylor et al. (2007) are as follows:

1. Loss, degradation, or alteration of habitat
2. Chemical pollution
3. Introduction of nonindigenous organisms
4. Overexploitation

The conservation importance of each of these four categories is often species dependent. Conservation threats for endemic crayfish species with narrow distribution ranges often exceed those for species with wide geographic ranges.

Loss, degradation, or alteration of habitat

Crayfishes and their habitats in permanent and ephemeral streams, as well as ground waters, are vulnerable to natural and anthropogenic disturbances. Most conservation concerns involving habitat are linked in some way to anthropogenic impacts.

In streams, excess sedimentation is a relatively common type of habitat alteration, where fine sediments enter the stream owing to run off from landscape disturbance. Excess sedimentation often results in substrate embeddedness, and reduces the ability for crayfish to burrow or seek refuge under rocks.

Stream channelization is also a common type of habitat alteration, where the stream channel is typically widened and large rocks and woody debris are removed. For many crayfish species, adult individuals prefer large rocks as cover, thus the loss of this habitat component is detrimental to crayfish populations.

In some cases, habitat loss can be extreme, such as with impervious surfaces associated with urbanization or mountain top removal and valley fills associated with coal mining.

Most anthropogenic impacts to habitat are relatively localized, but narrow endemic crayfishes are vulnerable to population decline, extirpation, or extinction from local events. Crayfish species with relatively wide distributions are less vulnerable to conservation threats, but habitat fragmentation can result in geographically-isolated populations. Population isolates, similar to narrow endemic species, have an increased vulnerability to conservation threats, where the extirpation of each population isolate takes the species one step closer to extinction, a process known as the extinction ratchet.

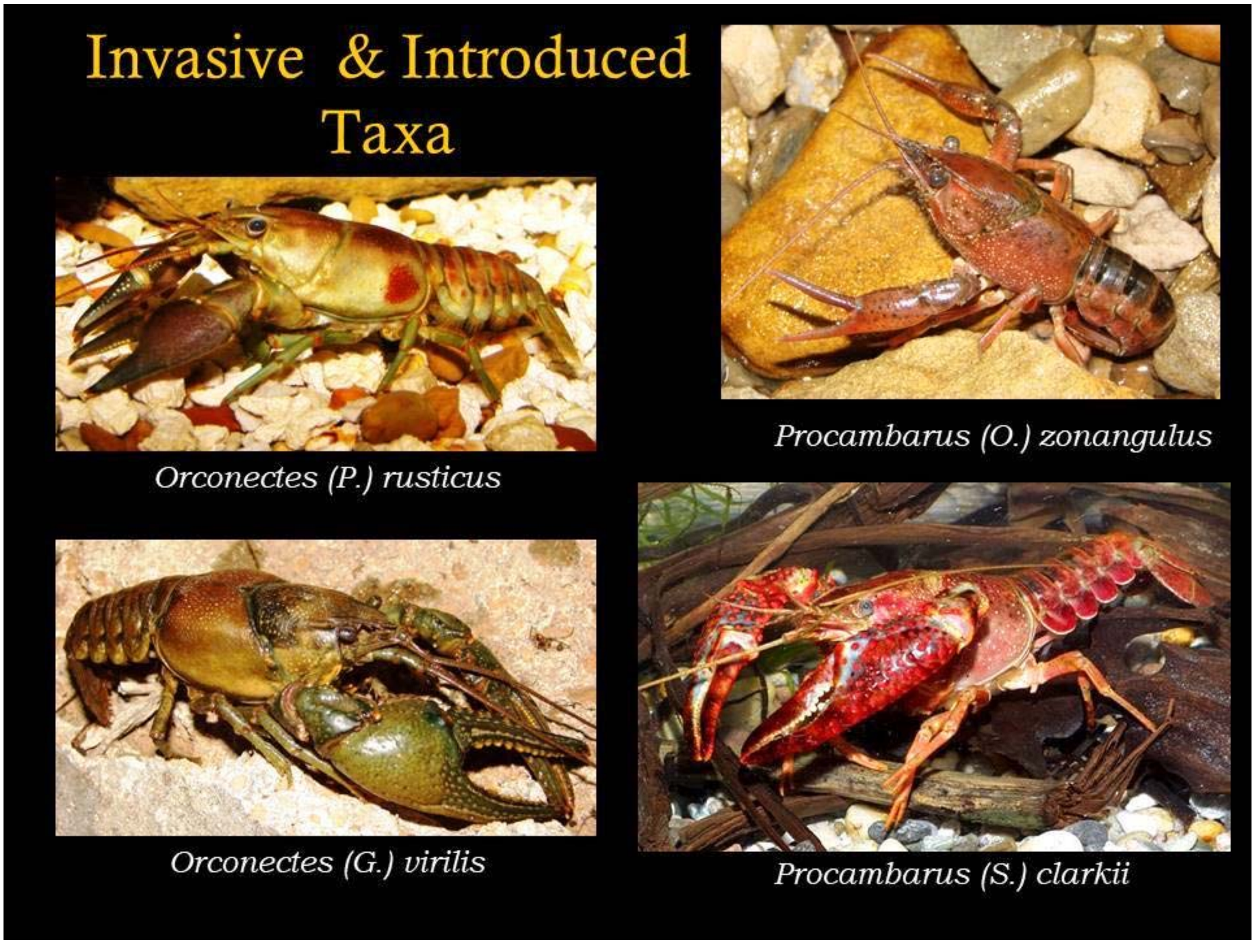
Photos of Sauerkraut Run adjacent to road construction indicating changes in streambed. The white dot indicates the same tree in each photo. Photo 7-5-02 shows large alluvial material; photo 4-5-03 reveals removal of that material following high flow; photo 10-12-03 depicts deposition of gravel bed; and photo 12-21-03 shows removal of gravel bed following high flow (Hedrick 2008).

Chemical pollution

Chemical pollution of aquatic systems is widespread. Levels of tolerance or intolerance to chemicals differ among crayfish species. Intolerant species are useful as biological indicators. In an interesting example, a Czech brewing company uses crayfish to monitor the quality of water used in making beer.

Previously, pollution concerns of aquatic systems have included a wide range of players, such as metals and solvents, fertilizers, pesticides, and petroleum. Recent concerns have including estrogens or estrogen mimicking compounds, and endocrine disruptors.

In some cases, chemicals are known to effect molting and reproduction. Endocrine disruptors may inhibit molting. Several types of estrogen and androgen compounds, some pesticides, and some heavy metals can effect molting. Several organic compounds have been shown to affect sex ratios and the development of male and female secondary sexual characters.



Four commonly invasive or introduced crayfishes in North America

Introduction of nonindigenous organisms

Many aquatic and terrestrial animals forage on crayfishes, thus predatory nonindigenous species may impact native crayfish species. Nonindigenous species may competitively exclude native crayfish species, or alter crayfish habitat. Also, nonindigenous species may introduce disease and parasites. In rare cases, hybridization and introgression may occur between nonindigenous and native crayfish species.

Much of the literature on impacts of nonindigenous species on native crayfish involves nonindigenous crayfish species. There are many possible explanations for the introduction of crayfishes outside of their native ranges:

1. Angler bait bucket releases, or escapees from bait shop

2. “Hitchhikers” in shipments or stocking tanks of bait fish or sport fish

3. Intentional release (aquarium pet or food for an aquarium pet).

4. “Hitchhikers” in earth fill material used for construction or land development.

In extreme cases, introduced crayfish species can extirpate populations of native species. In other cases, the native species may suffer population decline or be competitively excluded from preferred habitats.

Characteristics to consider for invasive crayfish:

1. Aggressive and territorial behavior
2. Competitor for space and food. Large-bodied individuals (or individuals with large chelae) may have a competitive advantage over smaller-sized crayfish.
3. Large body size may also reduce predation risk (i.e. predator gape-limitation)
4. Behavior that reduces predation risk, e.g. YOY seek shelter
5. Fast growth rate, high metabolism
6. High fecundity or level of parental protection (i.e. things that increase recruitment or survival)
7. Generalist/specialist relationship of invasive species relative to that of native species
8. Colonizing ability
9. Environmental tolerance
10. Reproductive interference, e.g. native males may prefer to mate with nonnative females
11. Introgressive hybridization

As examples, *Faxonius rusticus*, *Faxonius virilis*, and *Procambarus clarkii* are three nonindigenous crayfish species that are commonly considered as invasive and detrimental to native species. *Faxonius rusticus* (native to the Ohio River drainage) and *Faxonius virilis* (Native to north-central US) have been introduced widely within the US, likely owing to use as fish bait. *Procambarus clarkii* is native to southeastern states and had been introduced widely in the U.S. and in Europe, possibly owing to its importance as human food. Two other *Procambarus* species (*P. zonangulus* and *P. acutus*) are also commonly introduced outside of their native ranges.

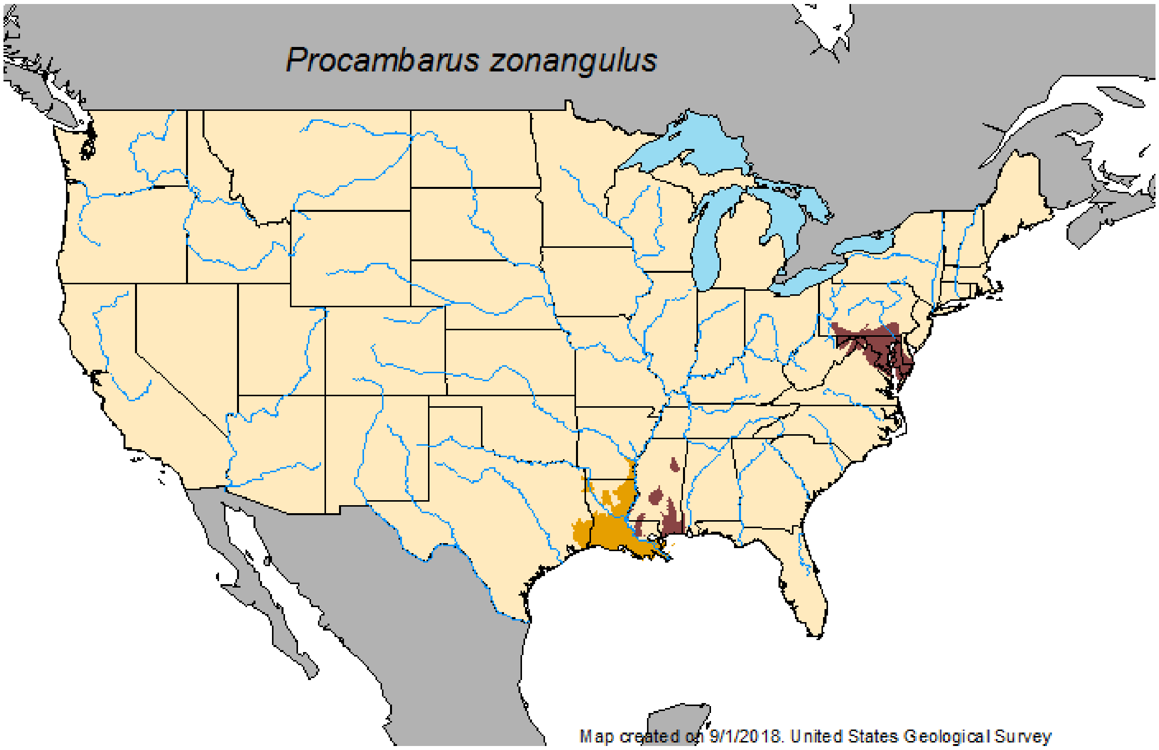
In the western US, the signal crayfish (*Pacifastacus leniusculus*) is an invasive species in some areas, and may have contributed to the range reduction of the shasta crayfish (*Pacifastacus fortis*). Also, *P. leniusculus* and *P. clarkii* may have contributed to the extinction of the Sooty crayfish (*Pacifastacus nigrescens*).

Procambarus acutus (photo from Loughman and Simon 2011)

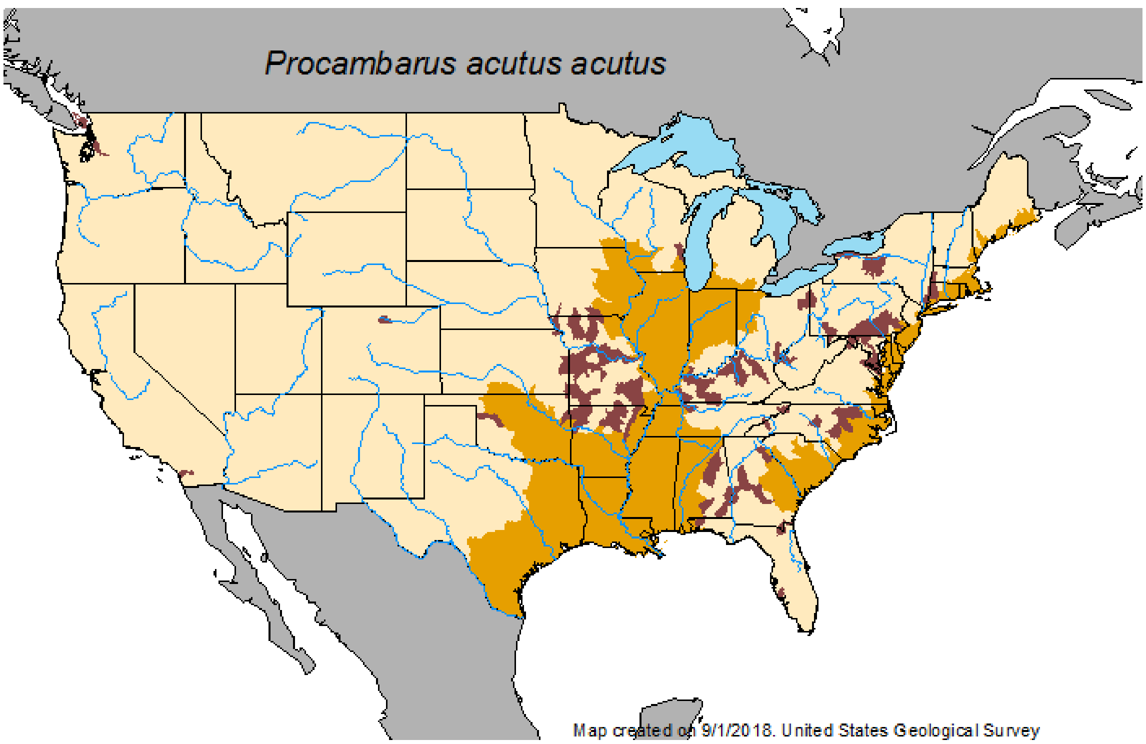
Pacifastacus leniusculus (Johnsen and Taugbøl 2010 NOBANIS – Invasive Alien Species Fact Sheet)

Distributions of some nonnative crayfishes are updated on the U.S. Geological Survey's Nonindigenous Aquatic Species webpage: https://nas.er.usgs.gov/.

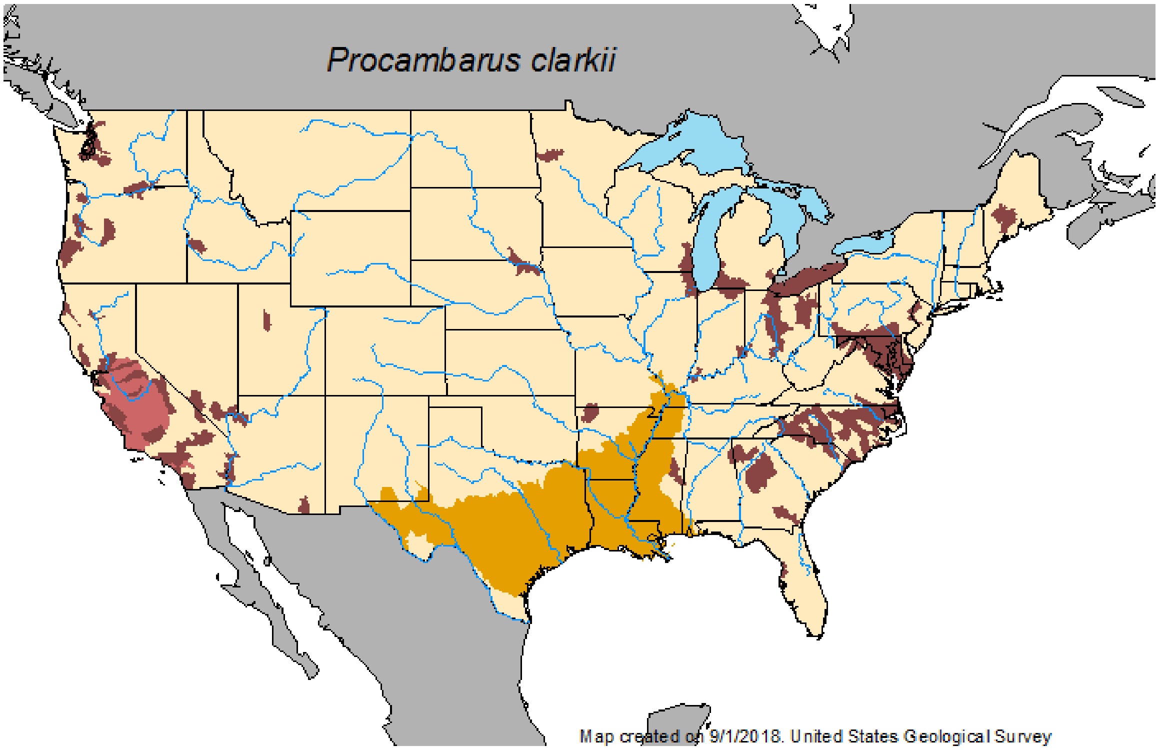
Below are examples of five species in the US, including *P. zonangulus, P. acutus, P. clarkii, F. virilis*, and *F. rusticus*. Maps on this web page are based on published information, with supporting literature citations. Range maps depict native and nonnative distributions, and can also be displayed as dot maps.



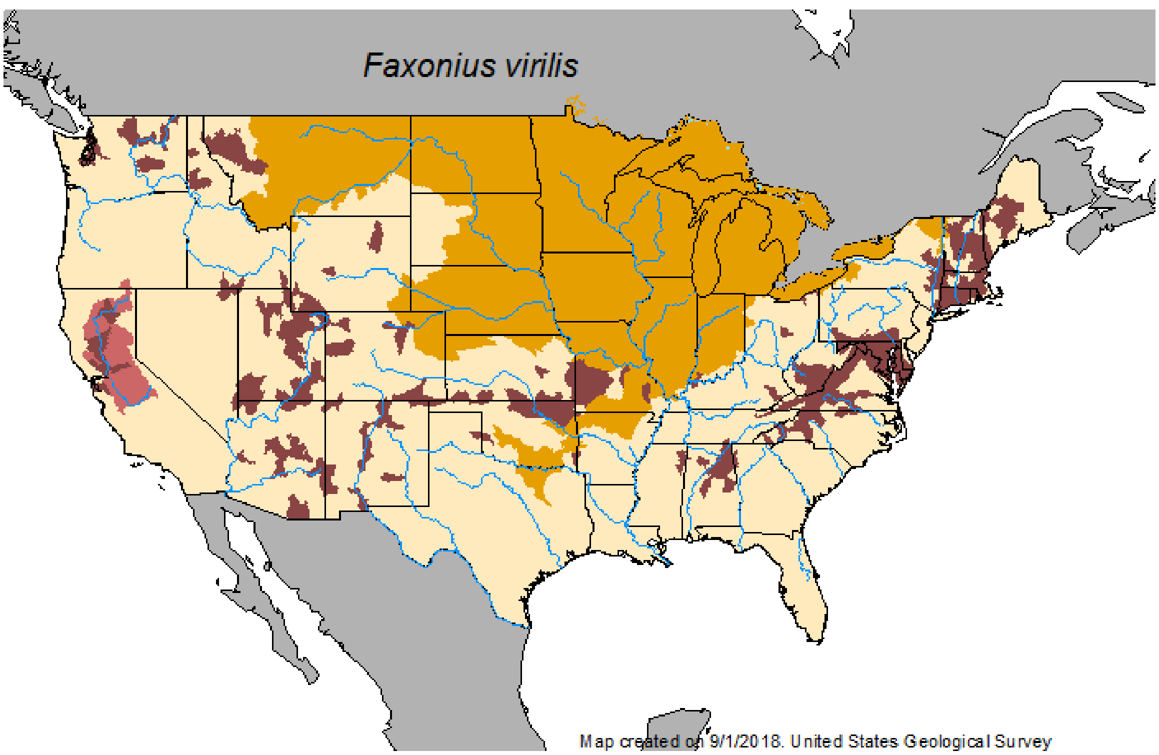
Native (orange) and nonnative (red) P. zonangulus distributions.



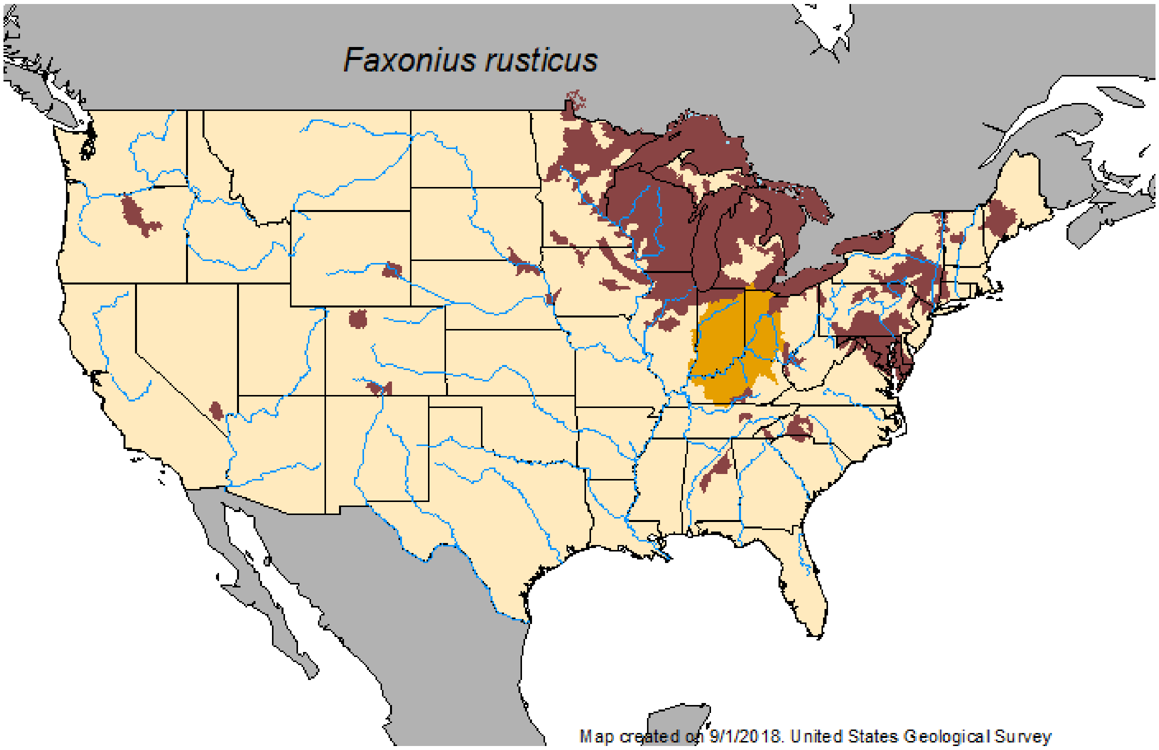
Native (orange) and nonnative (red) distribution of P. acutus.



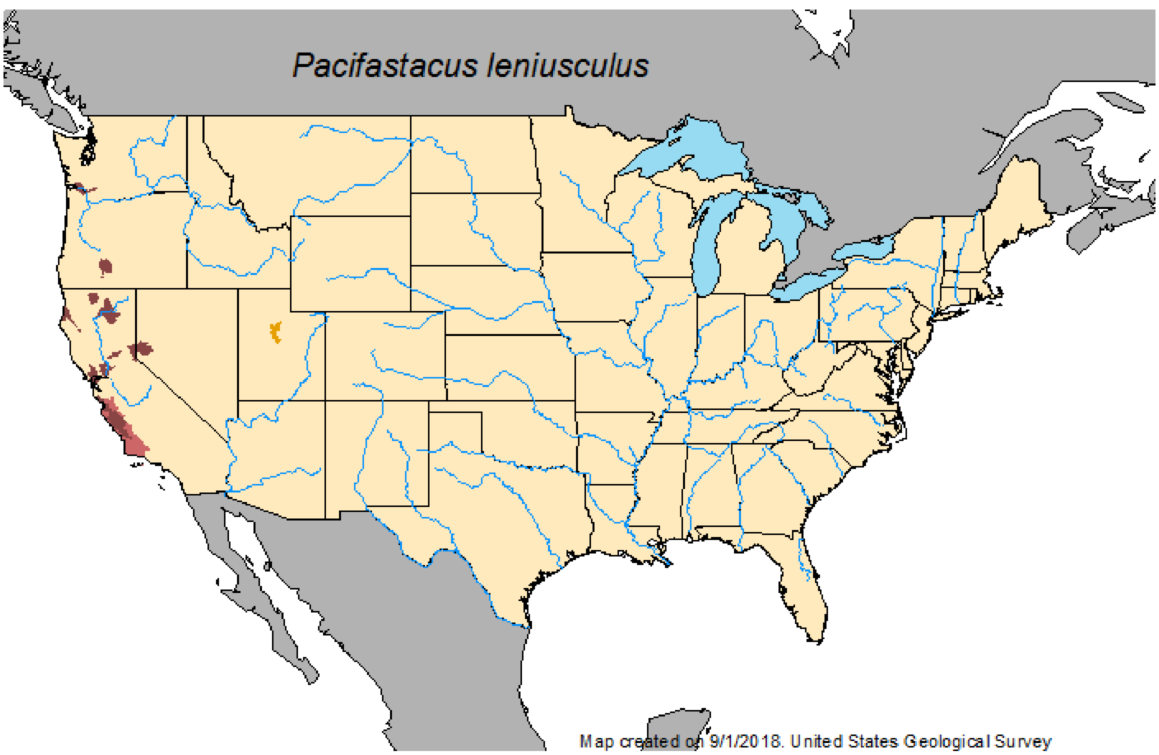
Native (orange) and nonnative (red) distributions of P. clarkii.



Native (orange) and nonnative (red) distributions of F. virilis.



Native (orange) and nonnative (red) distributions of F. rusticus.



Native (orange) and nonnative (red) distributions of P. leniusculus.

Overexploitation

Exploitation of crayfish populations include commercial harvest, collection for fish bait, and collection for aquarium trade. Few studies have examined these concerns, so little information is available to assess the impacts of overexploitation or overharvesting.

Chapter 6

Collection

Collecting Crayfishes

Important: *No collecting should be done until the proper agency has been consulted to determine whether or not collecting permits are required…*

Stream dwellers

Stream dwellers can be easily collected with seine or dip net. Set seines or nets a short distance downstream from rocks or gravel substrate. Turn rocks or kick gravel in direction of device. Crayfish are carried by current, or “tail flip” backwards, into net or seine. Baited minnow traps are an especially effective method of collecting. Traps left overnight will usually yield good results. Electrofishing also produces results, but should never be used as the primary means of epigean crayfish capture. Crayfish can autotomize their chelae voluntarily if they feel threatened. Electricity seems to initiate this response in crayfish rapidly and readily.

With the advent of both *Cambarus callainus* and *Cambarus veteranus* receiving federal protection, the need for a standardized collection method for crayfishes was paramount in order to compare apples to apples various individuals' results when competing surveys for these species. The following is a protocol developed by the Loughman laboratory, West Virginian Department of Natural Resources Wildlife Diversity program, and the West Virginia U.S.F.W.S. field office in Elkins. IT is presented “as is” and has served well as a straightforward way to approach crayfish collection as well as habitat evaluation. Habitat evaluation is completed through use of the Ohio E.P.A.'s Quality Habitat Evaluation Index (QHEI) form alongside crayfish collection.

Big Sandy and Guyandotte River Crayfish Survey Protocol

Project-specific survey plans shall be coordinated with and approved by the U.S. Fish and Wildlife Service (USFWS) at the address below prior to conducting any surveys within potential habitat for the Big Sandy crayfish (*Cambarus callainus*) or the Guyandotte River crayfish (*C. veteranus*). Survey plans should be submitted at least 30 days prior to the proposed start of surveys. When surveys are conducted to evaluate whether a proposed project may affect the species, surveys should be conducted early in project planning so that project modifications can be made to avoid and minimize project effects. Surveyors must have a valid Scientific Collecting Permit from the West Virginia Division of Natural Resources (WVDNR) prior to conducting the work.

Surveys are not permitted from July 20 through September 10 due to egg extrusion and rearing of juveniles by females. Surveys must be conducted when water conditions/temperatures are conducive to detecting *C. callainus/C. veteranus*. Water temperature must be above 50° F/ 10° C and surveys cannot be completed for 72 hours after a precipitation greater than 0.5in/1.3cm to ensure clear water and that suitable sampling conditions are present.

Surveys should be conducted throughout the entire reach of stream that may be affected by a potential project; total upstream and downstream distance to be sampled

from the point of direct impact will be determined for each project by the USFWS. Once the survey area has been delineated, the area should be divided into sampling reaches and each reach sampled following the approved protocol.
Each sampling reach should be approximately 125 meters (m) in length and include at least one riffle, run, or both riffle and run habitats. Crayfish sampling shall be performed using an 8'x4' seine, with double leads and double floats, and 1/8" netting. Sampling shall be performed by hauling a seine at a minimum of 10 locations within the 125m stream reach. Seine hauls will be completed by overturning every slab boulder (rocks approximately 1m wide x 1m long; 5cm high) present per 2m linear upstream/downstream distance in riffles and runs. One to two slab boulders can be sampled per seine haul.

Seine hauls should be completed with at minimum a two-person team using the seine. One crew member will hold both handles/brails, with the seine spread approximately 2m in width. Handles should be held at a 40°-50° angle from the stream surface. The other crew members should ensure that the seines lead line is making contact with the stream substrate and that the lead line is not resting on substrate items that are planned to be sampled in the ensuing haul. Once these conditions are met, surveyors charged with flipping substrate items should do so quickly and assertively. When each substrate item is overturned, the surveyor should kick in the direction of the seine over the area of stream substrate uncovered by moving rocks being sampled.

Slab boulders should always be given sampling priority given *C. callainus*/*C. veteranus*' association with them. If a sampling reach does not contain sufficient slab boulders, the following substrate features should be given sampling priority in the following order of importance: boulders, large cobble, course woody debris, and artificial cover. All substrate items should be placed back in their original position immediately following the seine hauls in which they were dislodged from the substrate.

At the end of each haul, surveyors must ensure that the lead line is removed from the water prior to the float line so all captured organisms remain in the net bellows and are not dumped back into the stream following sampling. At this time, crayfishes should be removed from the net and placed into trolling buckets. All substrate items should be placed back in their original position immediately following the seine hauls in which they were dislodged from the substrate.

All crayfishes collected shall be housed temporarily in trolling bait buckets that do not leave the stream proper until processing begins. No more than five adult *C. callainus/C. veteranus* are to be housed in one bucket at one time; multiple buckets are suggested. Buckets are to be anchored in the stream or attached to collectors during active sampling.

Data must be recorded on the standardized datasheets provided with your collecting permit. A minimum of ten seine hauls per sampling reach is required; the total number of seine hauls employed at a reach shall be recorded as well as the total number of crayfish collected of each species per seine haul. Electric fishing gear ***should never*** be used at potential *C. callainus* and *C. veteranus* sites. Electric fishing gear is not considered efficient gear for the collection of stream crayfishes.

When sampling is completed, collectors are required to identify all captured crayfish to species, sex all captured crayfish (Form I, Form II, Female, Female Glair, Female-Ovig, Female-Attached Juveniles), and record total carapace length (TCL) in millimeters for each *C. callainus*/*C. veteranus* encountered using calipers. Data shall be recorded on the standardized WVDNR Crayfish Morphometric Datasheet. A photographic voucher is required for all *C. callainus*/*C. veteranus* captured prior to release; representatives of other crayfish species should also be photographed. Every effort should be undertaken to ensure animals are outside of water for the briefest period of time possible (5 minute maximum, but a shorter period is preferred). Following data collection, animals are to be returned to the stream bottom upstream of their home rocks and guided back to their rock or other substrate debris.

Collection of water quality and physical habitat metrics are required at each collection locale. At each sampling site, pH, temperature, percent dissolved oxygen, turbidity, and conductivity are to be measured. In addition to water quality, physical habitat will be evaluated through completion of a Qualitative Habitat Evaluation Index (QHEI; OEPA 2006).

Lake and pond dwellers

Lake and pond dwelling crayfishes are most often found in the littoral zone, in or among vegetation and/or debris. Collected by running a seine or sturdy net through vegetation along shoreline. This technique also works for stream dwellers where vegetation is available. Another method involves taking a sturdy dip net, and “jabbing” vegetation with 5-10 times before checking the nets contents. Baited traps are also quite useful in lentic habitats, but generally have to be employed several nights in a row for best results.

Burrowers

Some crayfishes, known as “primary burrowers,” spend their entire lives in burrows that they construct. These may be located considerable distances from surface waters, and the openings to the burrows may be “capped” with clay or mud structures called “chimneys.” Burrowers can be collected by:

1. Burrow activity can and should be determined by the presence of chimneys or fresh mud pellets at burrow portals prior to an excavation attempt. Active burrows can excavated with trowels and shovels until enlarged ”resting chambers” are breached. Once the resting chamber is breached, burrows can be filled with water and plunged with the investigator’s hand and arm. This pumping action usually is enough to dislodge crayfish hiding within the confines of the burrow, drawing them into the resting chamber where they can be grasped. If initial plunging efforts are not successful in dislodging crayfish, burrows should be left undisturbed for several minutes. Crayfish, curious of this disturbance, often rise to the water/air interface where the waving of their antennae are

observed. In this situation crayfish should be quickly pinned to the sides of the burrow and extracted.

2. Trapping them by using **“pitfall” traps** consisting of gallon cans or wide-mouthed plastic containers. Bury the pitfalls in burrow areas so that the openings are flush with ground level. Keep in position overnight, but check early in the morning to prevent predators from finding the crays first. These traps can be baited, but that usually attracts lots of insects, and may attract predators. Traps are especially effective along drift fences.

3. Visiting burrows areas on warm, wet nights, and watching for crayfishes either sitting at the entrances of burrows or foraging in the area. This technique can also be used, of course, for stream and pond dwellers, which emerge from cover at night. Along these lines, utilization of baited lines can increase capture rates if conditions are conducive to their use.

Description of baited line method

Prior to deployment of baited line rigs, burrow colonies were located during daylight hours. Active burrows were flagged and returned to after dark. Baited line rigs were assembled using a 2 cm fishing hook (size 6) tied to a 30 cm section of fishing line (Fig. 2). Two 0.5 g split shot sinkers were placed 5 cm above the junction point of hooks to the lines. When the rig was completed, 2.5 cm sections of *Lumbricus terrestris* L. (Canadian night crawlers) were threaded onto the hooks. The use of a hook is crucial to the success of the rig. During initial trials with bait tied to the end of fishing line, crayfishes detached the bait from the rig by cutting the worms, retreated into their burrows, and rarely returned to the surface.

One hour after nightfall, researchers wearing headlamps searched burrow colonies for crayfish resting in burrow portals. Use of a headlamp was crucial during this process because it freed investigator hands to use the rig and grasp crayfishes. Crayfishes were initially spotted in burrows using the primary beam of headlamps. Following initial illumination, the periphery of the beam was used to illuminate animals during the capture process. Crayfishes rarely retreated into burrows when illuminated with the periphery of the beam, but often retreated quickly down the burrow when spotlighted at close range with the primary beam. A baited line was deployed when crayfish were within arm’s reach of the investigator. This entailed lowering the line into the burrow and gently tapping the crayfish’s antennae or chelae with the bait. Crayfishes typically responded to this stimulus by moving towards the bait, grasping at it with their chelae. If this response was initiated, crayfishes were led 5–10 cm from the burrow portal over a 5–20 second period and grasped, or pinned to the entrance of the burrow and extracted. It is important to note that crayfishes were not allowed to grasp the bait; once the bait was grasped most animals immediately began retreating into their burrows. If crayfishes retreated during any part of this process, burrows were revisited within 15 minutes and the process repeated.

Transportation

If specimens are not preserved in the field as collected, they are easily transported alive in deep, heavy-gauge plastic bags containing a moderate amount of water. The tops of such bags can readily be slipped under belts around the waist. Can also transport live specimens in buckets, plastic jars, etc.-- whatever's handy. It's important, though, to **not mix** large individuals with smaller ones; the smaller ones won't make it. It is also very important that specimens from different localities, or specimens collected on different dates (even from the same localities), not be mixed.

Preservation

***Cardinal Rule*: Unless absolutely necessary, crayfish should never be preserved in formalin.**

Preservation is simple; just drop specimens into (preferably) 70-75% ethanol, or into "drugstore" 60% isopropyl (rubbing) alcohol. Only specimens collected for DNA studies should be preserved in 95% ethanol. Denatured alcohol, especially with acetone, may damage permanent museum labels and should not be used as preservative.

Appendix

Field Data Sheets

Crayfish Collection Datasheet

Date: ______/______/__________

Stream name: ____ Collection #: _______________________

Trib. of: _______________________ Basin: ________________________Co: ________________________

Town: _________________ Geographic marker:____

_____miles (N S E W) of __________________ Elevation: _____________ Stream Order: ________

Road/bridge: ______________________________ LAT:_________________ LONG: ________________

Specific Location: ____

____

Stream Width: __________ Stream Depth: __________ Sediment :___________

Riparian Vegetation

Wooded _____% Grass: _____% Shrub-brush: _____% Marsh: _____% Other: _____%

Flow	Riffle	Run	Pool	Slack	Glide	Dry

Relative depth	Very Shallow	Shallow	Moderate	Deep	Very Deep

Dom. Substrate	S	Ss	C	Co	B	Bo	P	G	R	V	D	M
Sub. Substrate	S	Ss	C	Co	B	Bo	P	G	R	V	D	M

Compaction	Compact	Normal	Unconsolidated

Sand/Gravel Bars	None	Present	Rare	Common	Abundant

Woody Debris	None	Low	Average	High

Macrophytes	None	Low	Average	High

Bank Stability	Very Stable	Some Erosion	Unstable	Stabilization Present	n/a

Buffer Width	None	Narrow	Moderate	Wide

Land Use	Natural	Active Crop	Timber	Active Pasture	Urban	Rural	Road

Woodland Extent	Extensive	Intermediate	Not Extensive

Species Collected

Species	I♂	II♂	♀	B♀	Juv.	Total
1.)						
2.)						
3.)						
4.)						
5.)						

Photograph Numbers:______________________ Collectors:________________________________

Notes:____

Crayfish Field Morphometric Data Sheet

Date:	Stream:	Project:	Sheet #:

#	Species	Sex	TCL	PrL	PaL	AbL	AbW	Repro st.	Molt st.	Notes
1.										
2.										
3.										
4.										
5.										
6.										
7.										
8.										
9.										
10.										
11.										
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