ARTHROPOD LABORATORY

Phylum Arthropoda

Subphylum Cheliceriformes

Class Celicerata

Subclass Merostomata
1. *Limulus polyphemus* – horseshoe crab, identify external characteristics (see lecture notes)

Subclass Arachnida
1. *Latrodectus mactans* – Southern black widow spider
2. *Aphonopelma* sp. – Tarantula
3. *Lycosal tarantula* – Wolf spider
4. *Pruroctonus* sp. – American scorpion
5. Slides of ticks and mites (identify different body parts)

Subclass Pycnogonida
1. Sea spider demonstration specimens

Subphylum Crustacea

Class Brachiopoda
1. *Daphnia magna* – live specimens to study feeding activity and monitor heart rate using several stimulatory and inhibitory chemicals
2. *Daphnia magna* – body components (see lecture notes)
3. *Artemia salina* – brine shrimp, living specimens

Class Ostracoda

Class Copepoda
1. Live representative specimen to observe movement and body components (see lecture notes)

Class Branchiura
No representatives

Class Cirripedia
1. *Lepas* sp. – gooseneck barnacle
2. *Balanus balanoides* – acorn barnacle
3. Live barnacles to observe movement of cirri and feeding activity

Class Malacostraca
Order Stomatopoda
1. *Lysiosquilla* sp. – Mantis shrimp

Order Amphipoda
1. *Armadillium* sp. – pillbug or roly poly

Order Isopoda
Live isopods to observe movement

Order Decapoda
Suborder Natancia – swimming decapods
1. *Penaeus* – common shrimp
Suborder Reptancia
1. *Cambarus* sp. – freshwater crawfish for **dissection**
2. *Homarus americanus* – American lobster
3. *Pagurus* sp. – hermit crab
4. *Emerita* sp. – mole crab
5. *Callinectes sapidus* – blue crab
6. *Libinia* sp. – spider crab
7. Larval slide, megalops larva

Subphylum Uniramia

Class Chilopoda
Various specimens to observe characteristics

Class Diplopoda
Various specimens to observe characteristics and body

Class Insecta
Insect articulation mount to observe under dissecting microscope

Order Orthoptera
1. *Romalea* sp. – external and internal anatomy, **dissection** specimens
2. *Periplaneta americana* – external and internal anatomy, **dissection** specimens

Order Isoptera
No specimens

Order Odonata
Representative dragonflies

Order Hemiptera
Representative bugs

Order Homoptera
1. *Tibicen canicularis* – dog day cicada
Order Coleoptera
   Representative beetles

Order Diptera
1. Representative flies and mosquitoes
2. Mosquito and fly head slides to observe characteristics
3. Mosquito eye slides

Order Hymenoptera
Representative bees, wasps and ants
1. Apis mellifera – honey bee

Order Lepidoptera
Representative butterflies and moths
LABORATORY 8: PHYLUM ARTHROPODA

In numbers of species, the phylum Arthropoda outranks all other phyla, comprising over three fourths of all animal species. Arthropod body organization is obviously a successful one. Extreme structural flexibility and a high capacity to speciate are also implied by these numbers. The success of the phylum is obvious, whether measured by numbers of individuals, species, total biomass, structural variety, adaptability, or evolutionary plasticity.

The basic arthropod body type is characterized by: 1) bilateral symmetry; 2) segmentation; 3) a hardened exoskeleton, usually chitinoid; 4) jointed appendages; 5) a strong tendency toward the fusion of blocks of segments to form major body regions (head, thorax, and abdomen) referred to as tagmosis; 6) a strong tendency toward the formation of specialized segments and appendages; 7) no distinct trophophore-like larva; 8) discontinuous apparent growth, usually occurring immediately after shedding the exoskeleton (molting); 9) cephalization; 10) coelom reduction and the formation of a haemocoel; 11) retention of many annelid characters [dorsal heart with ostia, nerve ring around esophagus; ventral ganglia paired (primitively) in each segment].

The major groups of arthropods are classified according to their segmentation tagmosis, and appendage differentiation. One of the most important aspects of arthropod biology is the extraordinary impact that the chitinoid exoskeleton has had on the form, function, adaptability, and evolution of the group. Not only the name arthropod (jointed feet), but most other characters such as the manner of growth, circulatory and respiratory systems, size, musculature, and even habitat can be related to living within a tough, jointed, hollow skeleton. Keep this in mind in your examination of representative arthropods.

Any comparative study of arthropods is difficult, since its major elements become diversified before the earliest recognizable fossils were deposited in the cambrian, 500 million years ago. Arthropod diversity is so vast that innumerably examples are required to get any feeling at all for the patterns of change and groups that have evolved during this long period. We will have time today to study very few examples, but these will hopefully provide a brief introduction to some of the major patterns of arthropod adaptation.

Classically, two major groups of living arthropods are generally recognized: the MANDIBULATES, which are jawed or mandible-bearing; and the CHELICERATES whose first appendages bear claw-like pincers. This dichotomous division of arthropods probably does not have evolutionary significance and each of these groups is probably polyphyletic. In our work we will recognize four arthropod subphyla 1) Trilobitomorpha (extinct trilobed arthropods); 2) Chelicerata, which are arthropods whose first appendages are claw-like pincers and include the subclasses Arachnida (scorpions, spiders, mites, and ticks), Merostomata (horseshoe crabs) and Pycnogonida (sea spiders); 3) Crustacea which all have two pairs of antennae, biramous appendages and include crabs, lobsters, shrimp, barnacles, and a variety of small aquatic forms; and 4) Uniramia which have uniramous appendages and include the classes Insecta which all have bodies divided into head, thorax, and abdomen with three thoracic pairs of legs, Chilopoda which have body divided into head and trunk with each trunk segment having one pair of appendages (centipedes) and Diplopoda which have body divided into a head and trunk with two pairs of appendages on each trunk segment (millipedes). A simplified key to the classes of arthropods is given below. You should examine it carefully and use it to classify each of the specimens you look at in today's lab.
KEY TO CLASSES OF PHYLUM ARTHROPODA

1a. Arthropods with body divided by two longitudinal furrows into three distinct lobes; appendages (2 per segment) are numerous and similar; now extinct, known only as fossils. (Subphylum Trilobita)…………………………Trilobite fossil

1b. Arthropods without the above combination of characteristics………………………………… 2

2a. Without jaws and antennae; first pair of ventral appendages are chelicerae (Subphylum Chelicerata)……………………………………………………………………………………………………………… 3

2b. With jaws (mandibles) and antennae (Mandibulates)………………………………………………… 5

3a. Possessing abdominal appendages and a long spike-like telson……………………………………………………………………………………………………………………………………………………………………Subclass Merostomata

3b. Lacking the above characteristics……………………………………………………………………………… 4

4a. Body divided into cephalothorax and abdomen with a very large abdomen; four pairs of walking legs present on cephalothorax …………………………Subclass Arachnida

4b. Body divided into cephalothorax and abdomen with a very reduced abdomen; four to six pairs of walking legs present on cephalothorax …………………Subclass Pycnogonida

5a. Possessing gills, biramous appendages and 2 pairs of antennae… Subphylum Crustacea

5b. Possessing one pair of antennae and no gills in the adult form…………………………………….. 6

6a. Body divided into head, thorax and abdomen; 3 pairs of legs on thorax ……………………………………Class Insecta

6b. Body divided into head and trunk; numerous trunk appendages……………………………………7

7a. Possessing one pair of appendages on each trunk segment; first pair of trunk appendages (maxillipeds) are poison claws………………………………………Class Chilopoda

7b. Possessing 2 pair of appendages on each apparent trunk segment ………………………………………Class Diplopoda
GLOSSARY OF SOME TERMS USED IN THE IDENTIFICATION OF ARTHROPODS

Abdomen - The most posterior of the major body divisions.

Antennae - The most anterior head appendages of mandibulate arthropods. In crustaceans, which possess 2 pairs of antennae, the first pair are called antennules and the second pair are referred to as antennae.

Biramous - (adj.) Having two branches. Refers specifically to the two-branched condition of typical crustacean appendages.

Carapace - The part of the exoskeleton covering all or a portion of the cephalothorax. It is formed from the fusion of a number of individual skeletal plates.

Cephalothorax - A major body division formed by the fusion of the head and thorax.

Chelicerae - (sing. chelicera) The first pair of appendages on the cephalothorax of chelicerate arthropods; they are typically pincer-like.

Mandible - One of a pair of mouth appendages comprising a jaw in mandibulate arthropods.

Telson - Terminal segment-like part (not considered a segment proper) of an arthropod, which bears the anal opening. In horseshoe crabs (Class Merostomata) the terminal spike-like tail is called a telson, but it is not homologous to the telson of other arthropods.

Trunk - Portion of the body behind the head, consisting of a thorax and abdomen that cannot be easily differentiated.

In today's laboratory we will examine a variety of representative arthropods in order to get a feel for their form, function, and classification.
SUBPHYLUM CHELICERATA

*Limulus polyphemus* (horseshoe crab)

Obtain a preserved or dried specimen of the horseshoe crab *Limulus* and examine its external anatomy. On the dorsal surface note that the body is divided into an anterior prosoma and a posterior opisthosoma which terminates in a telson. The **prosoma** is composed of eight fused segments and carries the walking legs and chelicera. On its dorsal surface examine the simple and compound eyes. The **opisthosoma** houses the **reproductive organs** and **book gills**. The telson is used to right the body when overturned and in forward locomotion. On the ventral surface of the prosoma examine the appendages of *Limulus*. The clawlike chelicerae are located on the second prosomal segment, the third segment bears a pair of pedipalps and segments four through seven have ambulatory legs. On the base of the **walking legs** note the presence of masticating structures called **gnathobases**. The ventral surface of the opisthosoma bears six pairs of segments which are all fused at the midline to form flattened plate-like flaps, with each having an endopodite and exopodite division. The first flap is the genital operculum with paired genital opening on its posterior surface. On the posterior surface of the exopodites of the remaining five limbs are leaf-like gills termed a gillbook. The final two opisthalomal segments are fused and limbless.

Be sure you can key *Limulus* to class using the key at the beginning of the handout. *Limulus* lives on soft bottoms and is a carnivore/scavenger. Food is moved into the mouth region with the chelicera and walking legs and then ground by the gnathobases before entering the mouth. Like other arthropods, the coelom of *Limulus* is filled with perivisceral sinuses which constitute a hemocoel. The vascular system is open, consisting of arteries that discharge blood into the sinuses rather than into capillaries and veins. The blood surrounds and bathes the tissues and organs of the body. Blood eventually collects in the large pericardial sinus surrounding the heart. The heart is perforated with ostia, through which blood enters from the perivisceral sinus. The ostia have one-way valves so that when the heart contracts, blood is forced into the arteries with no backflow. The blood of *Limulus* as with most arthropods may function in transportation of food and gases, as well as being involved in phagocytic and clotting reactions. The copper containing pigment haemocyanin is the respiratory pigment found in *Limulus*, while haemoglobin is found in some insects and crustaceans.

Obtain preserved specimens of a representative terrestrial chelicere. We have spiders and scorpions. Using the key at the beginning of this exercise, key them to class. Examine these specimens.
On the ventral surface of your specimen, identify the piercing **chelicerae**, the **pedipalps** which are used for grasping prey, and the walking legs. Terrestrial chelicerates have respiratory organs (**book lungs**) on the opisthosoma which are considered by most biologists to have been derived from book gills. In some spiders silk glands and spinnerets will be seen behind the lungs.

**SUBPHYLUM CRUSTACEA**
Crustaceans are an extremely diverse group of primarily aquatic arthropods characterized by having biramous appendages and two pairs of antennae. We will systematically examine a variety of representative crustaceans.

**Class Branchiopoda** (gill feet). Possess four or more pairs of thoracic appendages which are leaf-like (flattened) and are provided with gills on their margins. Antennae are either adapted for swimming or reduced in size. Mostly freshwater organisms. Body long and slender with 11-19 pairs of thoracic appendages and stalked eyes. Fairy shrimp and brine shrimp belong to this order. They swim upside down, using their appendages for propulsion, respiration, and filter feeding.
Obtain a large *Artemia* (brine shrimp) specimen and examine it in a small watch glass. How does it move? Describe your observations. To get a closer look, mount a large *Artemia* ventral side up on a depression slide and examine under a dissecting scope. Can you see the gills? Describe the structure of the thoracic appendages. How many different types of appendages do you see? What is the function of each?

**Class Ostracoda** (see shrimp). Body laterally compressed with a disproportionately large head with well-developed antennae used for locomotion. The trunk appendages are generally reduced to two pairs.

**Class Copepoda**. Body small and cylindrical, ocelli fused to form a single median eye; no appendages on abdomen; thoracic appendages used in propulsion; first antennae are enlarged and function to prevent sinking when the thoracic or second antennae stop moving; freshwater and marine. Females are often seen carrying egg sacs between the thorax and abdomen. These are perhaps the most common organisms on earth being the primary herbivores that feed on marine phytoplankton. Some, however, are
carnivorous and feed raptorially on other copepods. Examine some copepods under the microscope on a depression slide. Use the diagrams in Walker and Taylor to identify body structures. Describe locomotion.

**Class Cirripedia** (barnacles). Sessile, either stalked or attached directly to the substratum; first antennae are vestigial except for the cement glands; second antennae present only in larva; six pairs of thoracic appendages (cirri) modified for feeding; body covered by calcareous plates; exclusively marine. Examine some live Balanus under the dissecting microscope in a watchglass. By introducing a small amount of a 3:1 milk : water suspension into the watchglass you should be able to observe and describe the feeding movements. Describe the structure of the shell in *Lepas*. How does a barnacle grow?

**Class Malacostracea** Trunk portion of the body composed of 14 segments, 8 thoraci and 6 abdominal, appendages on all segments, compound eyes usually stalked.

**Order Amphipoda** Carapace absent and body usually laterally compressed; gills and heart located in thorax; appendages show a high degree of differentiation; brood pouch. Commonly called "scud."

**Order Isopoda.** Carapace absent and body usually dorsoventrally flattened; gills and heart located in the abdomen; appendages show little differentiation. Examine the isopods available in the laboratory. Refer to Barnes for diagrams. How have isopods become adapted for a semiterrestrial existence? How is locomotion accomplished in isopods? How does this differ from amphipods?

**Order Decapoda** First three pairs of thoracic appendages are modified as maxillip' the remaining 5 pairs of thoracic appendages are legs (Decapoda = ten legs); carapace present and generally well-developed. This is the largest crustacean order and contains shrimp, lobsters, crabs etc. We will examine a number of decapods in the laboratory today.

**Procedure:**
Obtain preserved specimen of the crayfish, *Cambarus*. We will use *Cambarus* to illustrate basic crustacean anatomy. It represents a relatively primitive decapod crustacean. The crayfish is a common
inhabitant of freshwater streams and ponds. Crayfish feed chiefly on detritus, although they can use their claws to break open poorly armoured shells.

Closely examine the segmentation and appendages. The segments are grouped into three general regions: 1) head (segments 1-5 with antennae, mandibles, and 2 pairs of maxillae); 2) thorax (segments 6-13, with 3 pairs of maxillipeds, 1 pair of chelipeds, and 4 pairs of walking legs) and 3) abdomen (segments 14-19 with 5 pairs of pleopods and a uropod and telson). Are all the appendages jointed? How do the chelipeds operate? Remove one and examine the musculature. Examine the functional anatomy of the joints.

Starting from the anterior end of your specimen try to identify and assign a function to the 19 pairs of appendages. Remember that in the primitive condition (*Artemia*) little specialization is seen and the appendages are all similar. Each limb primitively consists of three portions: the protopod or stem, the inner endopod, and outer exopod. Examine and attempt to explain how and why modification of this basic plan have occurred.

Look at the posteriormost thoracic appendage. It is pereopod 5. Which thoracopod is it? The typical malacostracan thoracopod (including pereopods and maxillipeds alike) is composed of seven articles. The two proximal articles represent the subdivided protopod and the distal five are the five articles of the endopod.

Find the seven articles of pereopod 5. The proximal article is the coxa. It is wide and short and articulates with the sternite of the last pereomere. Distally it articulates with a short, narrow basis. The basis joins with the ischium along an oblique articulation.

Notice that the ischium appears to be composed of two articles in that it has an oblique groove encircling it near its articulation with the basis. This groove marks the location of the fracture plane where the lobster can deliberately autotomize (auto=self, tome=cut) its limb. This plane is specialized
for this function and the animal can lose its limb, at this plane only, with minimal trauma or blood loss.

The ischium articulates with a long narrow **merus**. Next there is a short **carpus** followed by a long **propodus**. The final article is a sharp, pointed **dactyl**, or nail.

A large, white, feathery **gill** is attached to the pleurite immediately dorsal to the coxa. Gills are associated with all thoracopods except maxilliped 1. Most appendages have more than one gill and they may be attached to the pleurite, coxa, or the articulating membrane between the pleurite and coxa.

Locate the major body openings including the mouth, anus, the excretory pore on each antennae, and the genital pores. The male genital ducts open at the base of the last leg. A trough for the transfer of spermatophores into the female seminal receptacle is formed from the fused first and second swimmerets. The oviducts open at the base of the second walking legs, while a pore at the base between the fourth walking legs serves to receive spermatophores. The first swimmeret in females is reduced or absent. The female telson and filamentous swimmerets hold the egg cluster to form an external brood pouch for the eggs and young.

Carefully cut away the laternal extension of the carapace covering the gills, the branchiostegite, from one side to expose the internal sheet of gills. Carefully remove the dorsal exoskeleton of the thoracic and abdominal regions. Once you have opened your specimen, examine its internal structures.
The statocysts of most crustaceans are located on the base of the antennules. Remove an antennule from your preserved *Cambarus* and try to locate and examine a statocyst. Statocysts function in response to gravity and enable the animal to orient itself. Removal of one or both statocysts from living crayfish demonstrate this well. Once you have thoroughly examined the crayfish you should take a look at the external anatomy of the other decapod crustaceans available in the laboratory. Examine the external anatomy of the Brachyuran (true-crabs) crab *Carcinus*. How does it differ from *Cambarus*? Try to identify the appendages. What has happened to the abdomen? Why? How can you quickly tell a male from a female brachyuran crab? Why this difference?

Examine the anatomy of one of the hermit crabs (all *Pagurus* sp.) available in the laboratory. How does the segmentation and appendages of *Pagurus* compare with *Cambarus* and *Carcinus*? How is *Pagurus* well-adapted morphologically to living in shells? How are they like the original gastropod owners of the shell morphologically? What is the advantage of inhabiting a shell and how could it have evolved?

**SUBPHYLUM UNIRAMIA**

The subphylum is made up of three arthropod classes which have a single antennae and uniramous segmental appendages. The classes are 1) *Chilopoda* which have a body divided into a head and trunk with the trunk bearing many segments, each with a single pair of appendages. (= centipedes). 2) *Diplopoda* which have a body divided into a head and trunk with the trunk bearing many segments that each have two pairs of appendages (millipedes). These first two classes are usually referred to as the Myriapoda, and 3) *Insecta* which have a body divided into a head, thorax, and abdomen with three pairs of legs on the thorax.

Examine preserved specimens of the two myriapod classes. Pay particular attention to the differentiation and basic form of the appendages.

To examine basic insect design, we will look at the grasshopper, *Romalea*. Obtain a preserved Romalea and place it in a wax-bottomed pan. *Romalea* is a hemimetabolous insect, which means that it goes through a gradual metamorphosis from larva to adult. This is in contrast to holometabolous insect which has complete metamorphosis divided into four distinct stages: egg, larva, pupa, and adult.

Identify the major body segments on your *Romalea*, the head, thorax, and abdomen. How do these body divisions compare to those of the crayfish? The insect exoskeleton is a complex, many-layered structure of chitin and other materials and is covered by a thin but critically important outer waxy layer that prevents desiccation. The head consists of six fused segments; the thorax consists of three segments, and the abdomen of eleven, including the terminal reproductive organ. The tendency toward segment fusion is marked in insects. Movement is restricted to soft joints between the segments. Apparent growth is limited to stretching during the brief period from molting of the old exoskeleton and hardening of the new one.
Closely examine the head. Locate the compound eyes and simple eyes between them. Identify the appendages, mouth parts and other details of *Romalea* anatomy. In doing this try to relate form to function.

To examine the internal structure of *Romalea*, pin your specimen to a dissecting pan through the base of the legs after trimming off the legs and hind wings. Carefully open the dorsal surface of the abdomen and examine the internal organs. This is extremely difficult in preserved specimens, but do your best.
The air-tube or tracheal system is an extremely important anatomical feature of insects which allows them to breath in a dry environment. It supplies oxygen from the outside air directly into the tissues and cells without a blood carrier, unlike most other animals. In this rapid gas exchange by diffusion air flows through major tubes and numerous smaller branches that penetrate tissues and reach each cell. If all but the trachea of the insect were taken away, you would see remaining a tight network of fine interlacing branches reaching around the cells, just as capillaries do in our own bodies. Air passes from the spiracles (openings) to the major tracheal ducts, then to air sacs and a multitude of smaller tubes. Remove a bit of muscle tissue from your Romalea, place it in a drop of water on a slide, tease it apart with needles, and then press a coverslip over it. Examine this preparation under to observe the tracheal branches among the muscle fiber.

Repeat dissection and identification of anatomical details using Periplaneta americana, the American cockroach. Species of Periplaneta are elongate, oval in outline and strongly dorso-ventrally depressed, or flattened. The body is divided into the three tagmata characteristic of insects, i.e. head, thorax, and abdomen. The head is inconspicuous in dorsal view but the pronotum of the thorax is very large. The remainder of the thorax and abdomen are hidden by the two pairs of wings. Six pairs of similar, strong, spiny legs are present. The dorsal anatomy of Periplaneta americana can be subdivided into the small non-distinct head, the pronotum, mesonotum, metanotum and the tergites (T1-10).
The large powerful legs are responsible for the cursorial competency for which cockroaches are renown. Three pairs are present, of course, one on each thoracic segment. The three pairs are similar but increase in size from anterior to posterior. Each consists of a large, flattened, proximal coxa, a small trochanter, a long femur, a tibia, and a long tarsus. The femur and tibia bear strong spines. The tarsus is a series of 5-articulate tarsomeres. Tarsomeres 1-4 each bear a posterior pad-like adhesive pulvillus. Tarsomere 5 ends with a pair of tarsal claws beside a pad-like arolium. This distal arrangement of claws and arolium is sometimes referred to as the pretarsus. The arolium is an adaptation for clinging to smooth surfaces and makes it possible for Periplaneta to climb smooth walls.

Begin the study of cockroach internal anatomy with a fresh, undamaged specimen if possible. The dissection will be made from the dorsal side so you must first remove the wings by cutting their attachments with a pair of fine scissors. The dissection is facilitated if the legs are removed by cutting across their trochanters. Organ systems will be considered in order of their appearance in dorsal dissection.

Place the specimen in a small dissecting pan of water or alcohol so it is completely immersed. Insert the blade of a pair of fine scissors under the posterior overhang of the right side of tergite 7 about 1 mm from the lateral margin of the tergite. Insert the blade only as deep as is necessary to penetrate the exoskeleton and be careful you do not damage internal organs with deep cuts. Cut anteriorly along the right side of the tergites all the way to the anterior end of the pronotum. Cut transversely across the anterior margin of the pronotum, just posterior to the head, and upon reaching the left side, change directions and cut posteriorly along the left side all the way back to tergite 7. Make a
transverse cut through the exoskeleton across the posterior border of tergite 7. You have now cut all of the way around the dorsum.

If you have a female cockroach you should be able to identify parts of the vitellarium by presence of mature oocytes at the posterior end of the abdomen (T2-7). Remove the gut, ventral diaphragm, and muscles from the thorax and abdomen. Remove the longitudinal body wall muscles (sternal muscles) and connective tissue as necessary from the floor of the abdominal cavity to reveal the ventral nerve cord (Fig 16-11). The cord consists to two side-by-side, parallel, longitudinal connectives which are united by transverse commissures passing between paired segmental ganglia. Notice the longitudinal ventral tracheal trunk lying beside the ventral nerve cord. Three thoracic ganglia are located in the thorax. From each extends several pairs of nerves to the abundant muscles of these segments. These are large ganglia. The abdominal ganglia will be hard to see, since they are merely slight protrusions of the ventral nerve cord.
One of the most interesting aspects of insect diversity is the variety of head structures and mouth parts that have evolved to tap different food sources. Examine prepared slides of insect heads and eyes available in the laboratory.