Myriapoda, Tardigrada, and Other Cryptozoics in Introductory Biology

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CRYPTOZOIC INVERTEBRATES can be used to develop interest in invertebrate ecology, behavior, physiology, morphology, and taxonomy in introductory-biology courses. These animals are readily available locally and may be collected and maintained with a minimum of equipment, space, and expense. Laboratory exercises using the invertebrates are easy to plan and can be adjusted to class size and ability.

Typical taxa of cryptozoic invertebrates of three groups—arthropods, near-arthropods, and nonarthropods—are, respectively, (i) the classes Insecta, Myriapoda, Arachnida, Crustacea, Symphyla, and Pauropoda; (ii) the class Tardigrada, tentatively placed in the phylum Arthropoda; and (iii) the phyla Annelida, Mollusca, Rotifera, and Nematoda. For details see the accompanying table. Two of the above taxa are infrequently used in introductory-biology courses but offer excellent possibilities for laboratory investigations: Myriapoda and Tardigrada. (I am following Brues and Melander [1932] in separating Symphala and Pauropoda from Myriapoda [centipedes and millipedes].)

A field trip to a nearby woods can be an occasion for collecting these organisms from decaying stumps and logs and from beneath leaf litter and small rocks—the microhabitats that give the cryptozoans ("hidden creatures") their name. Such a trip can be particularly fruitful in early fall, before cold weather drives these organisms deep into their microhabitats. Logs that are in the late stages of decay but are not dehydrated harbor the maximum number of cryptozoans (as well as a wide variety of other invertebrates). The near-arthropods—the tardigrades—are found primarily within the matrix of the microflora on the surface of woodland vegetation. Mats of moss and lichen on rocks and logs situated near a stream or in a shady place in a deciduous forest are excellent sources of tardigrades.

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Collection Methods

It is suggested that the instructor prepare a mimeographed page for the convenient summation of field data. The sheet should have blanks for the date, time, name of collector, weather conditions, temperature, number and kind of microhabitats observed, taxa collected per habitat, life stage of organism, and any unusual features in the sampling area.

Familiarize the students with the taxa to be collected. For example, the students need to know that the arthropod class Myriapoda includes the centipedes and millipedes, which are considered to represent a phylogenetic link between Annelida and the higher invertebrates; that centipedes have poison claws, and larger specimens must be handled with forceps; and so on. Similarly, it can be pointed out that Tardigrada is a taxon of zoological curiosities, of undetermined protostome ancestry and phylogenetic rank, that evolved at some point between arthropods and annelids (Barnes 1968).

Myriapods may be collected by rolling a rotten log over and carefully sorting through the debris beneath the log with forceps. In most instances, centipedes will dart for cover, in reaction against the light. Because they are orange or reddish-orange, they are easy to see. Also, they are surprisingly quick and elusive—a challenge to collectors. They must be captured with the forceps without hesitation, because they normally disappear within 3–4 seconds beneath any convenient cover, such as loose soil, leaves, or wood. Millipedes, on the other hand, frequently roll into a ball; for this reason, and because they have a much slower escape gait, they can be captured last.

If specimens are needed for studies in taxonomy, morphology, or ecology (relative abundance, activity per time of day, and the like), pitfall traps (Nelson 1970; H. Perkins, personal communication) can be established in the vicinity of a decomposing log. Pitfall traps that have been used with greatest success are 1-pint ice-cream cartons that are recessed until the top of the carton is flush with the ground. The cartons are filled with ethylene glycol to a depth of approximately 2.5 cm. Trap covers of plywood keep out debris and rain. (Covers are not needed on traps placed beneath logs.) Nails can be driven through the corners of the plywood so that the cover rests evenly over the carton and is anchored to the ground. The location of each trap can be marked, in places of dense vegetation, by a spot of orange paint on a nearby tree. To show nocturnal activity, the traps should be checked at 7–9 A.M. and 4–6 P.M.

Symphylans and pauropods are found on decaying roots, in plant debris beneath rotten logs, and in moldy leaf litter—material that can be carried to the laboratory in plastic bags (Von Wicklen 1963). The dead vegetation is then placed in Berlese funnels, and the animals are collected in 70% ethylene glycol for morphological and taxonomic studies. If living specimens of symphylans and pauropods are desired, the light intensity of the Berlese lamp should be decreased from 100 watts to 25 watts and, in the collecting vial, moist cotton substituted for the alcohol. Because symphylans and pauropods are time-consuming to collect, it is suggested that they be used in student research projects.

Centipedes and millipedes are brought back from the field in quart-sized jars filled with a small quantity of litter from beneath a log to reduce dehydration and light intensity. Centipedes are carnivorous and should not be kept together in containers. It is suggested that they be separated as soon as possible from each other and from millipedes. They can be isolated in finger bowls filled with wet sand and containing a small, moldy piece of wood (under which they can hide) and some of the microfauna from the original habitat. Bowls containing centipedes and millipedes should be placed in a refrigerator or in a cool place out of direct light. Millipedes are usually kept in Petri dishes containing moist, moldy wood or potato peels (Perkins 1973).

Tardigrades are collected from wet moss and lichens, particularly at the edges of streams. The vegetation can be gently detached from its substrate and brought to the laboratory in a grocery sack. The

Characteristic cryptic invertebrates and their microhabitats.

Group	*Typical representatives*	*Occurrence*	*Habitat* (for key letters see footnotes)	*Stage*	*Size (mm)*
Near-arthropod					
Tardigrada	Water bears	c	l, m	a, cy	0.5
Nonarthropod					
Annelida	Earthworm	c	lg, s	a	50–160
Nematoda	Roundworms	c	l, lg, s, m	a	0.1–1
Rotifera	Rotifers	c	l, m	a, cy	0.1–0.5
Mollusca	Snails	c	lg, s, ll, m	a	2–20
Arthropod					
Diplopoda	Millipedes	c	lg	a	2–100
Chilopoda	Centipedes	c	lg	a	4–125
Symphyla	Symphylans	o/r	s, ll	a, j	1–10
Pauropoda	Pauropods	c	s, ll	a, j	0.5–1
Crustacea	Pill bugs	vc	lg, s, ll	a, j	5–15
Arachnida					
Phalangida	Daddy longlegs	c	lg, ll	a, j	2–10
Pseudoscorpionida	Pseudoscorpions	o	b	a	1–5
Aranea	Spiders	vc	lg, s, ll, b	a	2–75
Acarina	Mites	c	l, lg, s, ll, b, m,	a, j	5–10
Insecta					
Coleoptera	Beetles	vc	lg, ll, b	a, lv, p	2–40
Zoraptera	Zorapterans	o	lg, s	a, j	1–3
Protura	Telsontails	r	s, ll	a, j	1–1.5
Collembola	Springtails	vc	lg, s, ll, b	a, j	5–6
Thysanura					
Japygidae	Japygids	o/r	s, ll	a, j	0.2–5
Campodeidae	Campodeids	c	s, ll	a, j	0.2–5
Machilidae	Jumping bristletails	c	lg, ll, b	a, j	0.8

lichens	*l*	very common	*vc*	adult	*a*
moss	*m*	common	c	juvenile	*j*
bark	*b*	occasional	*o*	larva	*lv*
leaf litter	*ll*	rare	*r*	pupa	*p*
log	*lg*			cyst	*cy*
soil	*s*				

vegetation is then soaked for 24–48 hours and washed. The tardigrades are transferred from the washed vegetation with micropipettes to deep-well slides; the wells are ringed with petroleum jelly, so that the animals will be asphyxiated after cover slips are applied. Gustafson (1963) recommends asphyxiation of tardigrades (to make them swell and become rigid) and the addition of phenol and lactic acid to polyvinyl alcohol, so that the tardigrades can be killed, fixed, cleared, and mounted in one process. (Gustafson's method is an improvement on the alcohol dehydration technique, followed by earlier workers; it was time-consuming, and the loss of specimens was high.)

Myriapod and Tardigrade Investigations

Studies on the external and internal morphology of myriapods are done with stereo-microscopes at X7–45. (See the table for relative sizes of the organisms.) Most observations on centipedes and millipedes for morphological detail can be accomplished at X7–30. Tardigrade anatomy is usually studied at X100–400.

Behavioral relationships and physiological relationships can be studied simultaneously. Some open-ended investigations are the following:

1. Relative running speed, in centimeters per second, of centipedes and millipedes. Innovative teachers will look for the reason why there is so much difference in running speed between these two groups of myriapods. In one of our investigations *Scolependra* (a centipede) averaged 4.1 cm/sec in 3 trials and *Julius* (a millipede) averaged 0.43 cm/sec in 3 trials.
2. Substrate influence on movement. How does each organism carry its body, and what velocities does it attain over various substrates (improvised by the teacher)?
3. Smell ability at set distances.
4. Light reactions. Because these animals are mainly nocturnal, investigations of movement away from light are readily performed. My students have made measurements in a photography darkroom, under red light.

Ecological topics that can be investigated include food sources, food chains, predator–prey relationships, microhabitat adaptation, size of organism in relation to the organism's relative abundance, and immediate reaction to an artificial environment.

Morphological investigations might be concerned with the comparison of centipedes and millipedes with each other, with other arthropods, and with near-arthropods as regards (i) number of legs, leg construction, and leg position; (ii) mouth parts and their modifications; (iii) placement of and number of spiracles and sensory appendages; and (iv) body architecture for a predatory, crawling, semisubterranean type existence.

Tardigrades can be investigated along lines suggested above for myriapods. Students will be interested to learn that tardigrades lack a circulatory system and a respiratory system and they are highly specialized for existence in a freshwater microhabitat (Pennak 1953). Inquiry into tradigrade behavior could begin with an investigation of (i) the movements of the animals in changing location and in obtaining food; (ii) the thrashing manner in which their legs move; and (iii) the possible use of claws and the sucking pharynx.

Some ecological relationships that can be studied are (i) the niche position of tardigrades in the freshwater microhabitat; (ii) the abundance of tardigrades in comparison with other freshwater invertebrates as seen per microscopic field; and (iii) the number of tardigrades seen per field in relation to the number of hours the substrate plants are soaked in water.

Physiological investigations can be based on temperature and dehydration phenomena. Tardigrades are particularly sensitive to water loss in their environment, and they become encysted during periods of drought (Marcus 1959). Experiments could be based on water and temperature as limiting factors of excystment; for example, how much time is required for tardigrades to emerge from the cyst stage (also known as the cryptobiotic stage) at room temperature? Mosses and lichens could be frozen for several months and recovery attempted from the cryptobiotic cysts by checking the melted water.

Conclusion

Teachers will, I hope, test and improve this outline of investigations—not only for Myriapoda and Tardigrada but also for other groups mentioned in the table. It is suggested that investigations be planned in the following order (time permitting): ecological (inclusive of field observations and data collection), behavioral, physiological, morphological and taxonomic.

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