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Albert J. Burky
University of Dayton, aburky1@udayton.edu

Daniel J. Hornbach

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COMPARISON OF CARBON AND NITROGEN CONTENT OF INFECTED AND UNINFECTED SNAILS, *SUCCINEA OVALIS*, AND THE TREMATODE *LEUCOCHLORIDIUM VARIAE*

Albert J. Burky and Daniel J. Hornbach*
Department of Biology, University of Dayton, Dayton, Ohio 45469

ABSTRACT: In June, 6.7% of adult *Succinea ovalis* collected near Urbana, Ohio, were infected with the trematode, *Leucochloridium variae*. The effects of parasitism were assessed as total organic carbon (equivalent to calorific values) and as total nitrogen. The parasite represents 23.8% of total (parasite + snail tissue) dry tissue weight, 21.4% of total carbon and 19.8% of total nitrogen of infected snails. The higher C:N ratio for parasite tissue indicates a higher proportion of nonproteinaceous compounds (e.g., fats and/or carbohydrates) as compared to host tissue. There is less snail tissue in parasitized *S. ovalis*. The C:N ratios for parasitized and nonparasitized snail tissue suggest identical percentage compositions of proteins, fats, and carbohydrates.

There is little information on the effects of *Leucochloridium variae* on their succineid hosts. However, the effects of other trematodes on their snail hosts have been reported (Rothschild, 1936, 1941a, b) and others. Cheng (1971) reviewed earlier studies and suggested that reports of gigantism in parasitized molluscs are subject to alternative interpretations, and that enhanced growth determined by shell dimensions or total weight can be shown to be a factor of shell deposition. He emphasized the need for more studies on the effects of parasitism on the whole organism in relation to the shell. Our study compares the carbon and nitrogen contents of *L. variae* and the snail, *Succinea ovalis* Say. An abstract of parts of this effort has been presented (Hornbach and Burky, 1976).

MATERIALS AND METHODS

Adult *S. ovalis* were collected from Cedar Bog Reserve near Urbana, Ohio, USA (USGS map quadrangle Urbana West, Champaign County, Ohio: 40°03.42'N, 83°47.98'W). Sporocysts of *L. variae* were identified by comparison to the banding pattern described by Lewis (1974). All nonparasitized snails were collected in June and fixed in the field with 12% neutral formalin. Parasitized snails (visible broodsacs) were collected May through August, relaxed in water with propylene phenoxetol and subsequently fixed in 12% neutral formalin. Shell length was measured to the nearest 0.1 mm with a vernier caliper. Nonparasitized snails were dissected to separate shell from body and to confirm the absence of worms. Parasite tissue is contained within the sporocyst, which facilitates separation from the host. Any host tissue remaining attached was small and, at most, could make a trivial contribution to the sporocyst data. Our use of the term sporocyst includes all parasitic tissue (sporocyst + broodsac + developmental stages). Each component (snail body, shell, sporocyst) was dried to constant weight at 95 C. The body and shell of nonparasitized snails, and the body, shell, and sporocyst of parasitized snails were analyzed for total organic carbon (wet-oxidation method of Russell-Hunter et al., 1968) and total nitrogen (Coleman model-29 micro-Dumas nitrogen analyzer; see Gustin, 1960). Organic carbon can be equated to calorific values where 10.94 Kcal/gC can be taken for average snail tissue (Russell-Hunter et al., 1968). For all measurements taken, means, standard deviations (SD) and/or 95% confidence limits (CL) were calculated. The C:N ratios were calculated from carbon and nitrogen data expressed per mg dry weight of component. Carbon and nitrogen data can not be obtained from the same specimen; therefore, C:N ratios were computed by pairing snails according to total dry weight (body + shell).

RESULTS

Measurements for snails and worms are summarized in Table I. There are no significant differences between the shell lengths of parasitized and nonparasitized snails. The total dry weight (combined from snails of C and N analyses) and body carbon of parasitized snails are lower ($P < 0.05$), but there is no significant difference in the nitrogen content. The differences in both weight and carbon reflect the removal of parasite tissue from these comparisons. There was a range of 1–3 broodsacs per sporocyst. Sporocysts represent 23.8% (SD = ±6.6) of the total

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* Present address: Department of Zoology, Miami University, Oxford, Ohio 45056.

TABLE I. *Total size, and carbon and nitrogen content of* Succinea ovalis *and sporocysts of* Leucochloridium variae.

	Nonparasitized		Parasitized	
	Analyzed for carbon	Analyzed for nitrogen	Analyzed for carbon	Analyzed for nitrogen
Number analyzed	6	6	5	6
Mean shell length (mm) ± SD	16.42 ± 2.02	14.75 ± 2.49	14.84 ± 2.26	15.13 ± 1.87
Mean dry body weight (mg) ± SD	46.95 ± 15.43	34.83 ± 21.36	29.44 ± 11.13	20.82 ± 6.52
Mean dry shell weight (mg) ± SD	64.28 ± 29.81	50.83 ± 29.81	49.32 ± 23.83	54.45 ± 28.88
Mean dry sporocyst weight (mg) ± SD	—	—	7.60 ± 3.85	7.38 ± 2.54
Mean body C or N (mg) ± SD	20.07 ± 6.31*	3.59 ± 1.75	12.43 ± 4.29*	2.38 ± 0.92
Mean shell C or N (mg) ± SD	0.30 ± 0.24	0.19 ± 0.09	0.27 ± 0.09	0.18 ± 0.07
Mean sporocyst C or N (mg) ± SD	—	—	3.28 ± 1.13	0.58 ± 0.24

* Statistically significant at the 0.05 level.

dry tissue weight [100 · sporocyst · (snail body + sporocyst)$^{-1}$], 21.4% (SD = ±6.3) of the total tissue carbon, and 19.8% (SD = ±5.5) of the total tissue nitrogen.

Component values are expressed per mg dry weight in Table II. For snails there were no significant differences in the carbon content of any component and between the nitrogen content of body or shell when considered separately. However, the nitrogen content of the combined body and shell from parasitized snails is lower ($P < 0.01$). There are no significant differences in the carbon content of sporocysts when compared to the body tissues of snails. However, the nitrogen content of the sporocyst is lower ($P < 0.01$) than that of the body tissue of parasitized or nonparasitized snails.

No significant differences are observed in the C:N ratios (Table III) for snails. However, the C:N ratio of sporocyst is higher than the body of nonparasitized ($P < 0.07$) and parasitized ($P < 0.10$) snails. Although these differences are not significant, they are strong indicators of differences because of the method necessary to calculate C:N ratios and because of the differences ($P < 0.01$) between sporocyst and snail body nitrogen (Table II). The higher C : N ratio for sporocyst suggests a higher proportion of nonproteinaceous compounds (e.g., fats and/or carbohydrates).

A collection of 60 *S. ovalis* (shell length range: 7.0–19.0 mm) without external evidence of parasitism (absence of broodsacs in tentacles) was made in June 1975 to assess the infection rate. Each snail was dissected under a microscope to determine the occurrence of early stages of parasite development. Four (6.7%) of these snails (shell lengths: 14.4, 15.2, 18.4, and 19.0 mm) showed early stages of infection with *L. variae*.

DISCUSSION

The absolute difference in tissue dry weight and carbon (Table I) reflects the lower

TABLE II. *Components of* Succinea ovalis *(body and shell) and sporocyst of* Leucochloridium variae *expressed as carbon or nitrogen per unit dry weight.*

	Nonparasitized		Parasitized	
	μgC/mg dry wt ±95% CL	μgN/mg dry wt ±95% CL	μgC/mg dry wt ±95% CL	μgN/mg dry wt ±95% CL
Number analyzed	6	6	5	6
Body	430.13 ± 15.54	107.64 ± 12.26*	430.26 ± 35.44	113.46 ± 28.44†
Shell	4.70 ± 4.29	4.14 ± 1.03	7.03 ± 5.19	3.58 ± 1.23
Sporocyst	—	—	458.35 ± 130.84	77.55 ± 10.98*†
Body + Shell	194.00 ± 34.75	47.51 ± 9.20‡	173.63 ± 44.99	34.86 ± 4.55‡
Body + Shell + Sporocyst	—	—	200.13 ± 58.08	38.76 ± 5.27

*,†,‡ Values designated by the symbol are significantly different at the 0.01 level.

TABLE III. *C:N ratio for* Succinea ovalis *(body and shell) and sporocyst of* Leucochloridium variae.

	Nonparasitized C:N ± 95% CL	Parasitized C:N ± 95% CL
Body	4.04 ± 0.48	3.95 ± 1.18
Shell	1.17 ± 1.06	2.01 ± 1.51
Sporocyst	—	6.27 ± 3.24
Body + Shell	4.12 ± 0.56	4.88 ± 1.47
Body + Shell + Sporocyst	—	5.08 ± 1.75

amount of parasitized snail tissue when compared to nonparasitized snails of comparable shell length. However, percentage composition of proteins, fats, and carbohydrates are probably the same (supported by C:N of Table III). The 23.8% dry weight tied up in the sporocyst of *L. variae* is equivalent to 32.0% calculated as $100 \cdot \text{sporocyst} \cdot \text{snail body}^{-1}$ and agrees with the 25% value reported for broodsacs alone (sporocyst tissue remained with snail) on another genus of Leucochloridiidae (Kagan, 1952). This suggests a lower potential of parasitized snails for their own reproduction, if in fact reproduction is possible. However, Kagan (1952) noted copulation and egg laying by parasitized snails.

The information on *S. ovalis* and *L. variae* (Tables I–III) do not suggest enhanced growth of host tissue or shell. However, energetic comparisons are possible. The Kcal/g ash-free dry weight can be estimated if there are 10.94 Kcal/gC (Russell-Hunter et al., 1968) and if all dry tissue considered is assumed to have between 5 and 10% ash. Such considerations give values of 4.97 to 5.24, 4.86 to 5.13, and 4.93 to 5.20 Kcal/g ash-free dry weight for sporocyst, parasitized snail and nonparasitized snail, respectively. Although there are no component analyses for fats, carbohydrates, and proteins (about 9.5, 4.1, and 5.7 Kcal/g ash-free dry weight, respectively, Brody, 1945), these values are satisfactory when compared to 5.234 and 5.415 Kcal/g ash-free dry weight for trematodes (Calow and Jennings, 1974) and *Succinea ovalis* (Slobodkin and Richman, 1961), respectively. Free-living animals often have greater than 6.0 Kcal/g ash-free dry weight (Slobodkin and Richman, 1961; Calow and Jennings, 1974). Low Kcal values for *S. ovalis* are associated with the storage of glycogen (low energy content per unit weight) rather than lipids and are claimed to be related to a sluggish life-style. Low energy content of trematodes also reflects glycogen storage and is claimed to support the hypothesis that high fecundity is linked to lower potential energy per unit weight in flatworms and is associated with a reduced need for long term energy reserves in parasites (Calow and Jennings, 1974).

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BOOK REVIEW . . .

Chemistry and Specifications of Pesticides, Second Report of the WHO Expert Committee on Vector Biology and Control, World Health Organization Technical Report Series, No. 620 (ISBN: 92-4-120620-9), Geneva, 1978. 36 p. Available through WHO Publications Centre USA, 49 Sheridan Avenue, Albany, N.Y. 12210, French and Spanish editions available. Paper $2.50.

With the chemical control of vectors of public health significance and concern for environmental contamination by such chemicals so timely, this short document serves as an interim accession to the WHO "Specifications for Pesticides used in Public Health" published in 1973. Spawned by the death of spraymen who worked with malathion during a malaria control program in Pakistan, this expert committee met to consider the former event and to reconsider, in light of advances in pesticide technology and environmental safety, specifications and analytical methods as they apply to pesticides.

Specifications for current insecticides, rodenticides, and molluscicides; new pesticides, bromophos, jodfenphos, chlorphoxim, and pirimiphos-methyl; new formulations of insecticides; and new insecticides including synthetic pyrethroids, insect growth regulators, and controlled release formulations are considered. Because mortality in the above mentioned spraymen was linked to isomalathion, an impurity in malathion, emphasis is placed on the need for the availability of high quality pesticide standards with clear definitions of their impurities. With this in mind, gas-liquid and high-pressure liquid chromatography (also termed high-performance liquid chromatography in this document) are indicated as methods that will be useful in achieving these goals. In addition, the development of methods for use in the assessment of environmental impact by pesticides are considered to be paramount in importance.

Two annexes and an appendix contain the bulk of the information provided by this report. The first annex presents recommended changes in the specifications for the insecticides DDT, methoxychlor, HCH, pyrethrum, diazinon, malathion, trichlorfon, fenthion, dichlorvos, fenitrothion, propoxur, and temephos; interim specifications recommended for the new insecticides; bromophos, jodfenphos, chlorpyrifos; and suggested interim specifications for chlorphoxim and pirimiphos-methyl. Interim specifications for trichlorfon, fenthion, dichlorvos, and chlorpyrifos-methyl were reviewed and left unchanged. It is worth noting that although dieldrin is still used in tsetse control, the committee recommended withdrawal of its specifications because of the danger of environmental contamination. Annex two presents recommended changes in methods used to determine specifications and indicates methods no longer needed. Specific methods considered are (1) visual suspensibility test for 75% DDT water-dispersible powders, (2) emulsion stability test, (3) revised Stepanow (total organic chlorine) method, and (4) standard water for suspensibility and emulsifiability tests. The analysis for the determination of isomalathion in malathion water-dispersible powder is presented in the appendix.

The report concludes with recommendations for the establishment of specifications for new insecticides and the revision of existing specifications and methods for pesticides. It also states "if resources are available" a fifth edition of "Specifications for pesticides used in public health" should be prepared and published. The theme of this document and the current role of pesticides throughout the world certainly supports the need for the latter.

Leslie S. Uhazy, Department of Biology, University of California, Los Angeles, California 90024.