

Wax esters in two species of freshwater zooplankton

Abstract—Lipid classes were determined in three Lake Michigan hypolimnetic calanoid copepods, *Limnocalanus macrurus*, *Diaptomus sicilis*, and *Senecella calanoides*. *Limnocalanus macrurus* and *S. calanoides* contained large stores of wax esters (57–80% of total lipid). Wax esters in this amount have not previously been reported for freshwater zooplankton. *Diaptomus sicilis* exhibits a typical freshwater lipid profile and contains triacylglycerols as its lipid reserves. Lipid storage sites are morphologically different in the copepods. *Limnocalanus macrurus* and *S. calanoides* store their wax esters in a large sac that surrounds the intestine, whereas *D. sicilis* maintains lipid droplet morphology typical of freshwater “triacylglycerol-storing” zooplankton. *Limnocalanus macrurus* and *S. calanoides* are “glacial relicts,” which may explain the origin of their typically marine wax ester lipid class.

Lipids are important biochemicals in freshwater and marine ecosystems (Lee et al. 1971; Morris 1972, 1984; Tessier et al. 1983). The different classes of lipids serve specific functions; for example, phospholipids and cholesterol are essential constituents of cell membranes (Geise 1966). Triacylglycerols and wax esters are important energy storage compounds that allow animals to save energy resources for reproduction (Goulden and Henry 1984) or for periods of irregular food supply (Griffiths 1977; Lee et al. 1970, 1971; Morris 1972). For example, animals living in the arctic and deep regions of the ocean may face intermittent food and be subject to long periods of starvation. As a result these species need to accumulate lipids when food is abundant (Lee et al. 1971; Lee 1975; Morris 1972).

Both triacylglycerols and wax esters occur in many marine fauna, but significant quantities of wax esters have never been ob-

served in freshwater animals (Morris 1984). We report here the presence of wax esters as a major lipid component (57–80% of total lipid) in the freshwater calanoid copepods, *Limnocalanus macrurus* and *Senecella calanoides*.

The exact functions of wax esters in marine environments are not fully understood, but likely include buoyancy, as well as energy storage (Lee et al. 1971; Morris 1972; Nevenzel et al. 1969). These same functions may also apply to triacylglycerols for freshwater organisms (Morris 1984). Organisms that store wax esters are more buoyant than those with similar levels of triacylglycerols because wax esters have a lower density than triacylglycerols (0.86 vs. 0.92 at 21°C) (Lewis 1970). As a result, wax esters provide 45% more lift than triacylglycerols (Sargent et al. 1976). Organisms living in very deep waters or cold surface waters have considerably more wax ester reserves than those living in tropical or subtropical regions, 70% of the dry weight vs. 10%, respectively (Lee and Hirota 1973; Lee 1975). Also, triacylglycerols are usually the major lipid reserve of epipelagic (upper 250 m) marine organisms whereas wax esters tend to be the dominant lipid reserve of the bathypelagic zone (below 1,000 m) organisms (Lee et al. 1971; Morris 1972).

Triacylglycerols are generally the major storage lipid of freshwater fauna (Goulden and Henry 1984; Arts and Sprules 1987). Although wax esters have not been reported in freshwater zooplankton, small quantities of wax esters have been reported for a few species of freshwater algae (Cranwell et al. 1988). In addition, chromatographic analysis suggested that they may be present in hypolimnetic sediment-trap materials from Lake Huron (Parrish 1986, 1987).

Upon observing a large elongate oil sac in *L. macrurus* from the Great Lakes that resembled the oil sac of many marine calanoid copepods, we suspected the occurrence of wax esters and determined the lipid class composition of *L. macrurus*. As a compar-

Acknowledgments

We thank C. Parrish for analysis of *D. sicilis* lipids and for helpful information on lipid class analysis, A. Place for data on marine *L. macrurus*, A. Beeton for useful references on the history of *L. macrurus* in the Great Lakes, and M. Quigley and G. Fahnenstiel for reviewing the manuscript.

GLERL Contribution 610.

ison, we examined the lipid composition of *Diaptomus sicilis*, another calanoid copepod that codominates the hypolimnion with *L. macrurus*. *Diaptomus sicilis* has scattered lipid droplets like other zooplankters that store triacylglycerols (Goulden and Henry 1984). Finally, we determined the lipid class composition of *S. calanoides*, another large calanoid copepod that occupies a niche very similar to that of *L. macrurus* (Dadswell 1974).

We collected *L. macrurus* with vertical tows of a 153- μ m net at an 80-m-deep Lake Michigan station off Grand Haven, Michigan, on 29 October 1987, 14 June, 14 July, and 29 August 1988 and in Lake Huron at a 60-m-deep site off Tawas Point, Michigan, on 2 August 1988. *Diaptomus sicilis* and *S. calanoides* were collected at the same Lake Michigan station on 20 October 1986 and 10 May 1988, respectively. Zooplankton samples were transported back to the laboratory in an insulated cooler and stored at 4°C. The next day, nongravid adult females or adult males were separated into five small test tubes (6-mm diam \times 50 mm long). Eleven to 16 *L. macrurus* or *S. calanoides* and 50 *D. sicilis* individuals were used per sample to provide sufficient biomass for lipid extraction. The five replicate samples were dried in a desiccator at 50°C for 2 d under a slow, steady flow of nitrogen. Dried samples were kept frozen under vacuum in a desiccator purged with nitrogen to prevent lipid oxidation.

Total lipids were extracted and quantified gravimetrically by a micro method (Gardner et al. 1985). A subsample of lipid extract was saved and stored below 0°C under nitrogen for later determination of lipid classes by thin-layer chromatography with flame ionization detection (TLC-FID) (Parrish 1986, 1987; Parrish et al. 1988). Degradation of lipids during drying, extraction, and storage was apparently minimal as <5% of the observed lipids occurred as free fatty acids. The lipid extract was spotted directly onto silica-coated Chromarods-SII (Ancal Inc.). Lipid classes were separated by sequentially developing the rods in increasingly polar solvent systems (Parrish 1986, 1987). Between solvent developments, rods were scanned with an Iatronscan Mark IV

(Iatron Labs., Tokyo) connected to a Hewlett-Packard 3392A integrator. A mixed lipid standard was used for TLC-FID calibration and quantification. It included one compound from each of the following lipid classes: hydrocarbon, sterol ester, triacylglycerol, free fatty acid, alcohol (aliphatic), sterol (alicyclic alcohol), and phospholipid.

On separate extracts, wax esters were separated from sterol esters by thin-layer chromatography (TLC) with molecule planarity as the means of separation (Stewart and Downing 1981; Nicolaidis 1970). Standards and the three calanoid-copepod lipid extracts were spotted directly onto TLC plates coated with magnesium hydroxide (Analtech). In addition to the TLC-FID standard, a wax ester (oleic acid stearyl ester) and a sterol ester (cholesteryl stearate) standard were used for TLC. Following the application of standards and extracts, the TLC plates were developed in a solvent system of hexane/acetone (99:1). The separated compounds were made visible by either spraying with a 0.2% solution of 2',7'-dichlorofluorescein in 96% ethanol in water or by charring the plate after spraying with 50% sulfuric acid.

The total lipid content of female *L. macrurus* ranged from $42.3 \pm 3.1\%$ (SE of the mean) for replicate extractions to $67.3 \pm 3.3\%$ of dry weight in spring and late summer, respectively. The total lipid content of male *L. macrurus* was $44.7 \pm 3.0\%$ in spring and $50.0 \pm 2.1\%$ in summer and resembled values for females sampled on the same dates (Fig. 1). *Senecella calanoides* had a total lipid content of $29.5 \pm 2.3\%$ in spring. The combination of wax esters and sterol esters composed up to $80.8 \pm 2.2\%$ of the total lipids measured by TLC-FID in *L. macrurus*, and $57.6 \pm 1.2\%$ in *S. calanoides* (Fig. 2).

Although the TLC-FID method does not separate wax esters from sterol esters, sterol esters usually constitute only a very small portion of zooplankton lipids (Lee and Hirota 1973). We verified this assumption by using magnesium hydroxide TLC to separate wax esters from sterol esters. Visual observation of the developed plates showed that most of the lipids in *L. macrurus* and *S. calanoides* had the same relative reten-

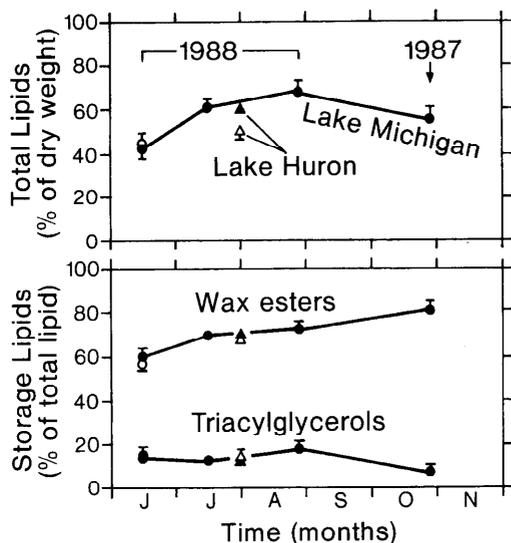


Fig. 1. Seasonal trend of total and storage lipids for *Limnocalanus macrurus* June through late October. Open symbols—males; closed symbols—females.

tion position as the wax ester standard; no lipids were observed at the relative retention position of sterol esters (Fig. 3). Other more polar lipids migrated more slowly and were less dramatically visualized in the lower region of the TLC plate for *L. macrurus* and *S. calanoides* spot developments. All *D. sicilis* lipids migrated more slowly than either the sterol ester or the wax ester standards (Fig. 3). Based on this verification we conclude that wax esters are the principal components of the TLC-FID wax ester-sterol ester peak observed in *L. macrurus* and *S. calanoides*; therefore we refer to these compounds as "wax esters."

Triacylglycerols accounted for up to $17.5 \pm 1.6\%$ of the total lipids in *L. macrurus*. The storage lipids, wax esters and triacylglycerols, together may constitute up to 90% of the total lipid content of adult *L. macrurus*, depending on time of year (Fig. 1). *Senecella calanoides* lipids contained 12.3% triacylglycerols and 70% total storage lipids, including wax esters on 10 May 1988, but more measurements are needed to establish seasonal trends. By comparison, *D. sicilis*, during October had a total lipid content of $36.3 \pm 5.3\%$ of dry weight, but only 1.1% of this amount consisted of wax esters-sterol esters. Triacylglycerols constituted $79.7 \pm 0.6\%$ of the total lipids in *D. sicilis*

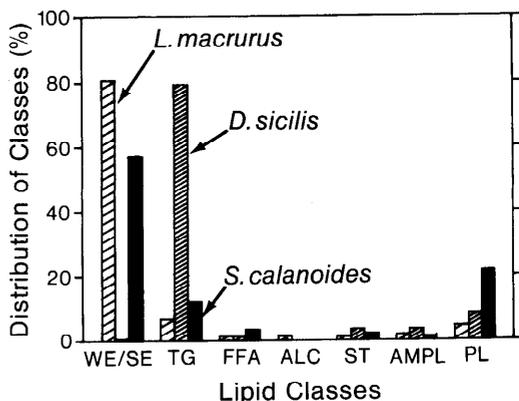


Fig. 2. Percent distribution of lipid classes measured by TLC-FID in *Limnocalanus macrurus*, *Diaptomus sicilis*, and *Senecella calanoides* collected in Lake Michigan on 29 October 1987, 20 October 1986, and 10 May 1988, respectively. Lipid class abbreviations are: WE/SE—wax ester/sterol ester; TG—triacylglycerol; FFA—free fatty acid; ALC—alcohol; ST—sterol; AMPL—acetone mobile polar lipid; PL—phospholipid.

(Fig. 2). Lipids in *D. sicilis* were typical of those observed in other freshwater species (Goulden and Henry 1984; Morris 1984). Minimal amounts of other lipid classes were

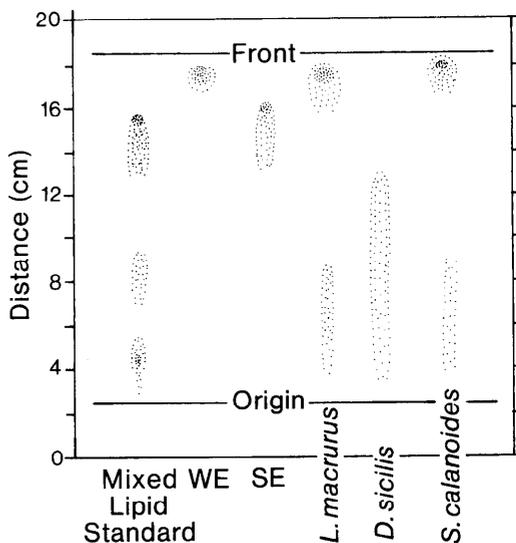


Fig. 3. Schematic representation of a developed magnesium-hydroxide TLC plate that demonstrates the separation of wax esters and sterol esters. The mixed lipid standard consisted of $45 \mu\text{g}$ of hydrocarbon, SE, TG, FFA, ALC, ST, and PL (abbreviations as Fig. 2). The amounts of lipid applied per single standard and for the copepods were as follows: WE, $100 \mu\text{g}$; SE, $60 \mu\text{g}$; *Limnocalanus macrurus*, $245 \mu\text{g}$; *Diaptomus sicilis*, $127 \mu\text{g}$; *Senecella calanoides*, $60 \mu\text{g}$.

present in the copepods (Fig. 2). Hydrocarbons were <1% of the total lipid and therefore are not presented in Fig. 2.

The occurrence of wax esters in *L. macrurus* and *S. calanoides* counters the paradigm that freshwater crustaceans do not contain wax esters. *Limnocalanus macrurus* is a "glacial relict." Glacial relicts were introduced mainly from marine sources into the Great Lakes and many other glacial lakes from proglacial pools following the glacial recession during the Pleistocene (Dadswell 1974; Segerstrale 1976; Gannon et al. 1978). *Limnocalanus macrurus* is tolerant of variations in salinity and has been known to travel through brackish water and the Arctic Ocean (Grainger 1965). Wax esters have recently been found in *L. macrurus* from the Beaufort Sea (A. Place pers. comm.). Thus the marine origin of *L. macrurus* seems to explain its wax ester content. Although it is also a glacial relict, the marine origin of *S. calanoides* is less obvious. It is believed to have existed in the Great Lakes region before the Pleistocene. *Senecella calanoides* is less tolerant of high salinity and high temperatures than *L. macrurus* and apparently exists only in freshwaters. Its relatives, members of the family Pseudocalanidae, however, are marine (Balcer et al. 1984; Dadswell 1974). The presence of wax esters in *L. macrurus* and *S. calanoides* suggests an aquatic source of animal-synthesized wax esters that have been observed in hypolimnetic sediment-trap materials from the Great Lakes.

Joann F. Cavaletto
Henry A. Vanderploeg
Wayne S. Gardner

U.S. Department of Commerce
Great Lakes Environmental
Research Laboratory
2205 Commonwealth Blvd.
Ann Arbor, Michigan 48105

References

- ARTS, M. T., AND G. W. SPRULES. 1987. Energy reserves of three zooplankton species from two lakes with different metal concentrations. *Can. J. Fish. Aquat. Sci.* **44**: 458-466.
- BALCER, M. D., N. L. KORDA, AND S. I. DODSON. 1984. *Senecella calanoides* Juday 1923, p. 73-74. *In* Zooplankton of the Great Lakes, a guide to the identification and ecology of the common crustacean species. Univ. Wisconsin.
- CRANWELL, P. A., M. E. CREIGHTON, AND G. H. M. JAWORSKI. 1988. Lipids of four species of freshwater Chrysophytes. *Phytochemistry* **27**: 1053-1059.
- DADSWELL, M. J. 1974. Distribution, ecology, and postglacial dispersal of certain crustaceans and fishes in eastern North America. *Nat. Mus. Can. Publ. Zool.* **11**: 110 p.
- GANNON, J. E., D. J. MAZUR, AND A. M. BEETON. 1978. Distribution of glacial relict crustacea in some Michigan inland lakes. *Mich. Acad.* **11**: 5-18.
- GARDNER, W. S., W. A. FREZ, E. A. CICHOCKI, AND C. C. PARRISH. 1985. Micromethod for lipids in aquatic invertebrates. *Limnol. Oceanogr.* **30**: 1100-1105.
- GEISE, A. C. 1966. Lipids in the economy of marine invertebrates. *Physiol. Rev.* **46**: 244-298.
- GOULDEN, C. E., AND L. L. HENRY. 1984. Lipid energy reserves and their role in Cladocera, p. 167-185. *In* Trophic interactions within aquatic ecosystems. AAAS Select. Symp. Ser. 85. Westview.
- GRAINGER, E. H. 1965. Zooplankton from the Arctic Ocean and adjacent waters. *J. Fish. Res. Bd. Can.* **22**: 543-561.
- GRIFFITHS, D. 1977. Caloric variation in crustacea and other animals. *J. Anim. Ecol.* **46**: 593-605.
- LEE, R. F. 1975. Lipids of arctic zooplankton. *Comp. Biochem. Physiol.* **51B**: 263-266.
- , AND J. HIROTA. 1973. Wax esters in tropical zooplankton and nekton and the geographical distribution of wax esters in marine copepods. *Limnol. Oceanogr.* **18**: 227-239.
- , AND A. M. BARNETT. 1971. Distribution and importance of wax esters on marine copepods and other zooplankton. *Deep-Sea Res.* **18**: 1147-1165.
- , J. C. NEVENZEL, AND G. A. PAFFENHÖFER. 1970. Wax esters in marine copepods. *Science* **167**: 1510-1511.
- LEWIS, R. W. 1970. The densities of three classes of marine lipids in relation to their possible role as hydrostatic agents. *Lipids* **5**: 151-153.
- MORRIS, R. J. 1972. The occurrence of wax esters in crustaceans from the northeast Atlantic Ocean. *Mar. Biol.* **16**: 102-107.
- . 1984. The endemic faunas of Lake Baikal: Their general biochemistry and detailed lipid composition. *Proc. R. Soc. Lond. Ser. B* **222**: 51-78.
- NEVENZEL, J. C., W. RODEGKER, J. S. ROBINSON, AND M. KAYAMA. 1969. The lipids of some lantern fishes (Family Myctophidae). *Comp. Biochem. Physiol.* **31**: 25-36.
- NICOLAIDES, N. 1970. Magnesium oxide as an adsorbent for the chromatographic separation of molecules according to their degree of flatness, e.g. the separation of wax esters from sterol esters. *J. Chromatogr. Sci.* **8**: 717-720.
- PARRISH, C. C. 1986. Dissolved and particulate lipid classes in the aquatic environment. Ph.D. thesis, Dalhousie Univ. 259 p.

- . 1987. Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. *Can. J. Fish. Aquat. Sci.* **44**: 722–731.
- , X. ZHOU, AND L. R. HERCHE. 1988. Flame ionization and flame thermionic detection of carbon and nitrogen in aquatic lipid and humic-type classes with an Iatroscan Mark IV. *J. Chromatogr.* **435**: 350–356.
- SARGENT, J. R., R. F. LEE, AND J. C. NEVENZEL. 1976. Marine waxes, p. 49–91. *In* P. E. Kolattukudy [ed.], *Chemistry and biochemistry of natural waxes*. Elsevier.
- SEGERSTRALE, S. G. 1976. Proglacial lakes and the dispersal of glacial relicts. *Commentat. Biol. Soc. Sci. Fenn.* **83**: 3–15.
- STEWART, M. E., AND D. T. DOWNING. 1981. Separation of wax esters from sterol esters by chromatography on magnesium hydroxide. *Lipids* **16**: 355–359.
- TESSIER, A. J., L. L. HENRY, AND C. E. GOULDEN. 1983. Starvation in *Daphnia*: Energy reserves and reproductive allocation. *Limnol. Oceanogr.* **28**: 667–676.

Submitted: 6 April 1988

Accepted: 2 December 1988

Revised: 20 March 1989

Announcement

No, there is no mistake on the front cover. *Limnology and Oceanography* will henceforth be published eight times per year. The two “new” issues will appear in June and December. This expansion is intended to have two effects. One is to lower the rejection frequency, which is currently >50%; the other is to accommodate special issues, such as those of July 1988, part 2, and November 1988, part 1.

While the manner in which special issues are born is known to regular attendees of ASLO Business Meetings, it would be advantageous for all of our readers to understand the essentials of the process. Any topic can be proposed at any Business Meeting or a suggestion can be sent for presentation at such meetings to any of the officers listed on the inside front cover of a recent issue of *L&O*. The topic may, but need not, fit within the scope of regular issues (cf. inside back cover).

Two ingredients are essential for approval of an idea. One is a guest editor who will solicit and handle the review of manuscripts for the special issue. The other is funding to defray part of the costs. For the two special issues of 1988 and the one in progress (on optical oceanography, guest edited by Rick Spinrad of the Office of Naval Research), diverse means have been used to obtain about half of the funds needed with the remainder supplied by ASLO. The per-issue cost of production at this time is close to \$110 per page. Thus, the proposer of a special issue of 200 pages would need to provide about \$11,000 of its cost. Such an amount could be raised by a page charge of \$55 per printed page, by a grant from one or more sponsoring agencies, or by some more creative means.

The advantages of publishing a symposium, workshop, or other edited volume in this format are several. A major one is the worldwide distribution and large circulation (>5,000) of *L&O*. Another is the technical editing and printing help provided by the *L&O* editorial staff—one is not likely to be disappointed by the look of the final product. Finally, the costs are relatively low compared to those of producing and distributing a like number of high-quality copies by most other means.