A comparative study of the lipids of water-striders from marine, estuarine, and freshwater environments: *Halobates*, *Rheumatobates*, *Gerris* (Heteroptera: Gerridae)[1]

Richard F. Lee

Skidaway Institute of Oceanography, P. O. Box 13687, Savannah, Georgia 31406

Lanna Cheng

Scripps Institution of Oceanography, P. O. Box 1529, La Jolla, California 92037

Abstract

The lipids of water-striders from three different aquatic environments (marine—*Halobates germanus* and *Halobates sericeus*; estuarine—*Rheumatobates aestuarius*; and freshwater—*Gerris remigis*) differ considerably in their fatty acid composition. The polyunsaturated 22:6 fatty acid, present only in *Halobates*, is presumably derived from its marine plankton food. The 20:5 acid is a major component of the lipids of *Gerris* and *Rheumatobates*, which feed on terrestrial insects known generally to lack long-chain (>C-18) polyunsaturated acids; it is presumably synthesized by chain elongation from C-18 precursors. Differences in food and habitat may account for some of the observed differences in fatty acid composition.

Neutral triglyceride lipids are stored in all three genera: in *Halobates* this fraction represents 74–92% of the total lipid, while in *Rheumatobates* and *Gerris* it is only 46–72%. The triglycerides are rapidly utilized during starvation by *Halobates* and *Rheumatobates*.

Pristane, the major hydrocarbon of many marine zooplankton, is apparently absent from the lipids of *Halobates*.

Aquatic insects occur predominantly in freshwater habitats, but representatives of a few orders, notably the Diptera and Heteroptera, are also found in brackish waters. Only the family Gerridae (Heteroptera) has successfully invaded fresh and brackish water as well as marine environments, a fact which prompted us to collect specimens from all three types of habitats for a comparative study of the insect lipids.

Members of the family Gerridae, commonly known as water-striders or pond-skaters, are often found on the surface of streams and lakes. The genus *Gerris*, which occurs exclusively in freshwater, has a cosmopolitan distribution (Cheng and Fernando 1969). The species chosen for this study, *Gerris remigis*, is probably the commonest gerrid in North American streams and creeks. The genus *Rheumatobates* has several brackish water species. The one chosen, *Rheumatobates aestuarius*, is common in mangrove swamps and streams in the La Paz area of Baja California (Cheng and Lewin 1971). The genus *Halobates*, with some 39 known species, is exclusively marine (Cheng 1973*b*). We have chosen two oceanic species, *Halobates germanus* and *Halobates sericeus*, for this study.

Halobates presumably originated from freshwater ancestors capable of invading saline habitats and must have had to alter its biology in many ways to live and breed in the open ocean. Some of the most striking adaptations for its oceanic existence are its propensity to lay eggs on floating objects instead of on submerged rocks, mud banks, or plant roots (as do most other gerrids) and to feed on marine organisms instead of terrestrial insects (Cheng 1974). This change in diet might be expected to influence the fatty acid composition of *Halobates* in contrast to that of its freshwater and brackish water relatives.

Gerrids occupy a specialized niche in the aquatic environment, the air–water in-

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terface, where they prey mainly on insects and other small invertebrates caught on the water surface. A water-repellent layer is consequently an essential feature of their outer surfaces: Stereoscan electron micrographs of its fine texture have been published elsewhere (Cheng 1973*a*). In this study, both surface and storage lipids of adults and juveniles of the four species chosen were analyzed, and the relative amounts of triglycerides and phospholipids were determined.

Insects store lipids in an organ called the fat body. In terrestrial insects, storage lipids are composed mainly of triglycerides of saturated or monounsaturated fatty acids. Freshwater and marine invertebrates generally also contain appreciable amounts of polyunsaturated fatty acids. Our interest was to determine whether water-skaters might, in this respect, show biochemical similarities to other aquatic organisms.

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Methods

Gerris remigis was collected from creeks in the vicinity of Englewood, Colorado (June 1972). Two species of *Halobates* were collected during two cruises of the RV *Thomas Washington* in subtropical regions of the Pacific Ocean: *H. sericeus* from cruise Southtow XIII in the North Central Pacific (February 1973) and *H. germanus* from cruise Aries III in the South Central Pacific (March 1971). *Rheumatobates aestuarius* was collected in marine littoral mangrove lagoons on two islands, Espíritu Santo and San José, in the Gulf of California during an expedition on the RV *Dolphin* (April 1973).

Table 1. Lipid data for Halobates sericeus, Rheumatobates aestuarius, *and* Gerris remigis. *A is lipid (% dry weight); B is lipid per individual (mg); C is triglycerides (% of lipid); D is phospholipid (% of lipid).*

Stage	A	B	C	D
Halobates sericeus				
nymphs (I + II)	38	0.03	74	24
nymphs (III)	32	0.14	30	69
♀ with eggs	62	1.2	92	7
♀ without eggs	41	0.7	82	16
♂	44	0.8	80	18
Rheumatobates aestuarius				
nymphs (III + IV)	34	0.06	77	21
nymphs (V)	32	0.11	79	20
adult ♀	26	0.20	72	26
adult ♂	22	0.12	61	36
Gerris remigis				
nymphs (I)	16	0.03	24	75
nymphs (II)	18	0.07	37	61
nymphs (III)	21	0.04	54	45
nymphs (IV)	25	1.0	57	41
nymphs (V)	24	2.0	59	40
adult ♀	12	2.7	53	45
adult ♂	12	2.2	46	52

The lipids were extracted with chloroform:methanol (2:1 v/v), and the dried carcasses, after lipid extraction, were then weighed to give the fat-free dry weight. Between 10 and 60 individuals of each stage were pooled for lipid analysis. Generally three to four such samples of each stage were analyzed and averaged. All experimental work on extracted lipids was carried out under nitrogen. The lipid was weighed and adsorbed on a silicic acid column, and four fractions were eluted with solvents of increasing polarity (Nevenzel et al. 1965). The individual fractions were weighed. The efficiency of the separation of lipid classes was monitored by thin-layer chromatography on silicic acid. The procedures for analyzing the four different fractions (hydrocarbons, fatty acids of triglycerides, sterols, and fatty acids of phospholipid) by gas-liquid chromatography are given elsewhere (Lee et al. 1971*b*). We used a gas chromatograph (Varian-Aerograph series 1800) equipped with an

Table 2. Fatty acids of triglycerides and phospholipids of Halobates. *TG is triglyceride; PL is phospholipid; T is trace (<0.5%).*

	H. sericeus								H. germanus	
	Female with eggs		Female without eggs		Male		Nymphs (stage 3)		Adults	
Fatty acid	TG	PL	TG	PL	TG	PL	TG	PL	TG	PL
	weight percent									
14:0	7	1	9	1	2	T	3	2	4	2
16:0	23	11	38	13	35	12	31	15	22	16
16:1	7	4	8	3	4	2	4	2	8	1
18:0	2	10	4	11	3	10	5	14	6	8
18:1	36	24	34	27	25	30	6	27	35	27
18:2	10	8	1	7	5	12	2	6	2	8
20:1	3	1	T	2	1	1	6	1	1	3
20:4	T	3	T	1	T	1	T	1	T	5
20:5	4	25	3	22	19	22	20	20	10	24
22:1	-	-	-	-	-	-	7	-	-	-
22:5	-	-	-	-	-	-	3	-	-	-
22:6	3	4	1	5	1	4	10	7	6	7

integrator for peak areas and two columns: 10% SP-2100 on 100/120 Supelcoport and 10% SP-22-PS on 100/200 Supelcoport (Supelco Inc., Bellefonte, Pennsylvania). Temperatures were programed: we ran hydrocarbons from 140° to 220°C at 2° min^{-1} on SP-2100, methyl esters of fatty acids from 160° to 220°C and from 180° to 190°C on SP-222, and sterol trifluoroacetates at 240°C on SP-2100. Fatty acids were hydrogenated to aid in identification. Authentic samples of fatty acids, sterols, and hydrocarbons were used for reference identifications. Each sample was run in duplicate or in triplicate. A shorthand notation is used throughout the text in referring to fatty acids; for example, 22:6 refers to a molecule 22 carbons long and containing 6 double bonds.

The procedures used to separate, identify, and quantitate the phospholipids involved thin-layer chromatography and phosphorus analysis (Parsons and Patton 1967). The identities of some phospholipids, deposited as thin films on KBr pellets, were confirmed by infrared spectrum analysis.

Surface lipids of *Halobates* and *Gerris* were removed by rinsing the insects for 30 min in hexane (Conrad and Jackson 1971); we assumed that this procedure would not extract internal lipids. Extracted lipids were weighed and portions were applied to thin-layer chromatographs for tentative identifications of their components. Other portions were examined in the SP-2100 column, temperature programed from 150° to 250°C at 2° min^{-1}.

Results

Halobates germanus and *sericeus*

The major neutral lipids extracted from all of the water-striders examined were triglycerides; in the adults and young nymphs of *Halobates* this was the major lipid class, accounting for 74–92% of the total lipid (Table 1). Living *Halobates* contained an orange oil, which could be withdrawn by a glass capillary after rupturing the exoskeleton: thin-layer chromatography of this oil likewise proved it to consist of triglyceride. Females without eggs had 0.7 mg of lipid while females with eggs had 1.2 mg of lipid per individual; the additional 0.5 mg was mainly triglyceride. The increased lipid content of gravid females suggests indirectly that lipids were stored in eggs, but these egg lipids were not directly examined. Starvation of adults for 1–9 days resulted in considerable depletion of lipid; after 9 days the lipid content per individual had decreased from 0.7 to 0.2 mg, mainly due to metabolism of triglyceride.

Table 3. Fatty acids of the triglycerides and phospholipids of Rheumatobates acstuarius. *TG is triglyceride; PL is phospholipid; T is trace (<0.5%).*

Fatty acid	Adult female		Adult male		Nymph (stages 3&4)	
	TG	PL	TG	PL	TG	PL
	weight percent					
14:0	2	T	3	T	1	T
16:0	24	14	31	12	27	15
16:1	8	5	1	6	8	6
18:0	4	14	2	14	3	13
18:1	41	28	45	26	44	28
18:2	11	21	9	25	8	26
18:3	3	4	3	3	4	3
20:4	T	2	T	1	T	1
20:5	4	8	2	9	3	8

The hydrocarbons, which accounted for about 1% of the lipid, comprised a series of paraffins from C_{19} to C_{36} with C_{27} and C_{29} the major components. The polyunsaturated hydrocarbon, 21:6, which is synthesized by marine phytoplankton (Lee et al. 1970) and found in some zooplankton, was only a minor component (2–4% of the hydrocarbon fraction) in *Halobates*. Pristane was absent. Since C_{29} hydrocarbon is a major component of the lipid of some insect cuticles (Conrad and Jackson 1971; Jackson 1972), we carried out a separate study of the surface lipids of *Halobates*. About 0.1 mg of lipids was collected from the surface of each adult *Halobates*; hydrocarbons accounted for only 5–7% of this cuticular lipid, the major components being tentatively identified, by their position on thin-layer chromatograms and by their retention times on gas-liquid chromatograms, as long-chain alcohols. No further work was done with these alcohols.

Other neutral lipid components, accounting for between 1 and 2% of the total lipids, were sterols (mainly cholesterol), diglycerides, and free fatty acids. Phospholipids, which accounted for over 90% of the polar lipid fraction, were identified as cardiolipin (a trace to 1% of the total phospholipids), lysophosphatidyl choline (a trace), phosphatidyl choline (50–67%), phosphatidyl ethanolamine (29–41%), phosphatidyl inositol (1–2%), phosphatidyl serine (1–2%), and sphingomyelin (1%).

The phospholipids of both species and all instars of *Halobates* have a similar fatty acid composition, whereas the triglyceride composition shows considerable variation, notably between nymphs and adults (Table 2). In the phospholipids the high content of 20:5 (20–25%) and 22:6 (4–7%) is of interest, since these fatty acids are associated with the lipids of other marine animals (Ackman et al. 1970; Culkin and Morris 1969; Lee et al. 1971*b*). There were no major differences in the amount of lipid, types of lipid, and fatty acid composition between *H. sericeus* from the North Central Pacific and *H. germanus* from the South Central Pacific.

Rheumatobates

In female *Rheumatobates* both the total dry weight per individual (0.8 mg) and the percent lipid content (22–26% of the dry weight) were less than in *Halobates* (1.7 mg and 41%: Table 1). Triglycerides accounted for most of the neutral lipid, which was 61–79%, with minor amounts of hydrocarbons, sterols, diglycerides, free fatty acids, and pigments (all less than 1% of the total lipid: Table 3).

In nonstarved adults, the phospholipids, accounting for between 26 and 36% of the lipid, were lysophosphatidyl choline (a trace), phosphatidyl choline (47–48%), phosphatidyl ethanolamine (44–48%), phosphatidyl inositol (a trace to 1%), phosphatidyl serine (a trace to 1%), and sphingomyelin (1–3%).

Table 4. Fatty acids of triglycerides and phospholipids of Gerris remigis. *TG is triglyceride; PL is phospholipid; T is trace (<0.5%).*

Fatty acid	Nymph (stage 4)		Nymph (stage 5)		Adult female		Adult male	
	TG	PL	TG	PL	TG	PL	TG	PL
	weight percent							
14:0	3	T	5	T	3	T	3	T
16:0	13	12	18	10	17	11	19	14
16:1	11	6	25	4	6	1	11	1
18:0	7	11	4	15	2	6	5	9
18:1	24	30	28	25	51	35	50	38
18:2	10	18	10	16	10	22	5	20
18:3	3	T	T	1	2	1	1	2
20:4	1	4	T	T	T	5	T	3
20:5	23	17	7	23	4	13	3	8

The triglycerides appear to be short term energy stores, since after a 3-day starvation period they decreased from 0.14 to 0.04 mg per individual.

Gerris remigis

The adults of this freshwater gerrid were larger (dry weight, 22.5 mg per individual) than either *Halobates* (1.7 mg) or *Rheumatobates* (0.8 mg); males were smaller than females (Table 1). The animals contained proportionately less lipid (12%) than their marine counterparts (*see Table 1*). The main neutral lipid was triglyceride (46–53% of the total lipid). Sterols and hydrocarbons accounted for 1% of the lipid. The surface lipids of *Gerris* were predominantly hydrocarbons (80–90% of the surface lipid), the major component being the straight-chain paraffin C_{29}, although small amounts of long-chain alcohols were also noted, as in *Halobates*. The phospholipids were quantitatively and qualitatively similar to those of *Rheumatobates* and *Halobates*: cardiolipin (a trace to 1%), lysophosphatidyl choline (a trace to 1%), phosphatidyl choline (48–51%), phosphatidyl ethanolamine (36–53%), phosphatidyl inositol (2–3%), phosphatidyl serine (1%), and sphingomyelin (1–2%). The phospholipid compositions of the different nymphal stages of *Gerris* were almost identical to those of the adults (Table 4).

The phospholipid fatty acids of the adults of *Gerris* were 30 and 40% polyunsaturated, comprising high levels of 18:2 and 20:5. The nymphs had a phospholipid fatty acid pattern similar to that of the adults, but the triglyceride fatty acids were markedly different, notably in the amounts of 18:1 (50% in adults, 25% in nymphs) and 20:5 (4% in adults, 23% in stage 4 nymphs).

Discussion

Although *Halobates* stores large amounts of lipid, the rapid utilization of this reserve observed during starvation suggests that this insect could survive only short periods without feeding. Because of the constancy of food supply all year in the tropical open ocean (Ryther 1963) it may rarely be subjected in nature to long periods of starvation. The normal food of *Halobates* in the open ocean has not been determined, but since it lives on the surface of the sea, zooplankton and small fish could possibly serve as prey. In the laboratory these insects take small amphipods, copepods, and fish larvae (Cheng 1974). Chemical analyses of Central Pacific zooplankton caught in a neuston net showed that most species had little or no storage lipid (Lee and Hirota 1973; Lee unpublished data). Another possible source of food would be fish eggs, which are high in lipid; in fact, eggs of certain pelagic fish were often collected in our neuston nets (Ahlstrom 1969; Zaitsev 1970). The water-striders probably are capable of converting proteins and carbohydrates of the prey to triglycerides.

The fatty acid composition of *Halobates* is quite unlike that of terrestrial insects in that it is characterized by a high content of 20:5 and 22:6 fatty acids. The main polyunsaturated fatty acids of terrestrial insects are 18:2 and 18:3, and they lack the long-chain polyunsaturated fatty acids. The lipids of *Halobates* also differ from those of *Rheumatobates* and *Gerris* by the presence of the 22:6 acid. Assuming that such polyunsaturated acids cannot be synthesized de novo (from two-carbon units) by water-striders, we must seek a source of these acids in their diet. In fact, most marine lipids have a relatively high content of 20:5 and 22:6 acids (Ackman et al. 1970; Brockerhoff et al. 1963; Lee et al. 1971*a*) which are often associated with the phospholipids of membranes. In marine zooplankton linoleic acid (18:2) accounts for less than 2% of the phospholipid fatty acids (e.g. Ackman and Hooper 1970; Culkin and Morris 1970; Sipos and Ackman 1968), whereas in insects it accounts for about 50% of the phospholipid fatty acids (Fast 1966; Henson et al. 1973).

The polyunsaturated acid content of phospholipids in fats of *Halobates* is similar to that of other insects, but in addition to 18:2 they also contain considerable amounts of 20:4, 20:5, and 22:6. The content of 18:2 phospholipid fatty acid, 8%, is low for an insect but high by comparison with zooplankton, whereas the level of 22:6 in the phospholipids, 4–7%, is low for a marine lipid but much higher than that recorded for any other insect. In the phospholipids of *Halobates* the high level of 20:5 (22–25%), which is apparently absent from terrestrial insects, resembles that of many zooplankton. Thus, in terms of fatty acid pattern, *Halobates* has both insect and marine zooplankton characteristics. The phospholipids of all three water-strider genera are similar to those reported for terrestrial insects, with phosphatidyl choline and phosphatidyl ethanolamine as the principal components (Fast 1966; Kok and Norris 1972).

The surface lipids of *Halobates*, which may contribute to the water repellency of the cuticle, have been tentatively identified as mainly alcohols; in *Gerris* the major surface lipids are long-chain hydrocarbons. It should be mentioned here that there are some significant differences among the surface fine structures of *Halobates*, *Ventidius*, a closely related freshwater gerrid (Cheng 1973*a*), and *Gerris* (Cheng unpublished data).

The absence of pristane from the hydrocarbon fraction should be especially noted, since this is a major hydrocarbon of marine zooplankton and fish (Blumer et al. 1964; Blumer 1967; Kayama et al. 1969; Lee et al. 1971*b*). Avigan and Blumer (1968) have suggested that in nature pristane generally results from the degradation of chlorophyll by zooplankton, and, since this hydrocarbon is found in many marine animals, Blumer (1967) has proposed that it is passed along the food chain without alteration. The absence of pristane from *Halobates* suggests either that *Halobates* feeds on animals that do not contain pristane or, more likely, that pristane is metabolized or excreted by *Halobates*. Freshwater copepods contain a small amount of pristane in the hydrocarbon fraction (Lee unpublished data) but some freshwater fish lack this hydrocarbon (Ackman 1971) so that pristane may not be passed up this freshwater food chain.

Although *Rheumatobates* lives in a marine estuarine environment, its phospholipid-fatty acid pattern is similar to that of the freshwater *Gerris*, with 18:2 as the major polyunsaturated fatty acid and the 22:6 acid lacking. The food of *Rheumatobates*, like that of most other gerrids (Cheng and Fernando 1970), is terrestrial insects, predominantly small dipterans that fall into the swamp water (Cheng unpublished data).

The major polyunsaturated fatty acids of terrestrial insects are linoleic (18:2) and linolenic (18:3), and, since insects cannot biosynthesize these acids, they are essential nutritional requirements (Gilbert 1967). From several studies Gilbert and O'Connor (1970) have concluded that insects generally cannot extend the molecu-

lar chains of unsaturated fatty acids, which means that the 20:4 and 20:5 fatty acids found in the fats of the water-striders would have to come from their diet. However, if the main prey of *Rheumatobates* and *Gerris* is terrestrial insects, which contain no long-chain polyunsaturated acids, these water-striders must be able to effect chain elongation of 18:2 and 18:3 fatty acids. Experiments involving the injection of radiolabeled linoleic and linolenic acids into water-striders might help to solve this problem.

The proportion of polyunsaturated fatty acids in the lipids of aquatic organisms from low-temperature environments is generally higher than in those of related species from warmer waters (Farkas and Herodek 1964; Morris 1971). Although the gerrids collected for this study came from habitats of different water temperatures (*Gerris*, 10–15°C; *Rheumatobates*, 17–22°C; *Halobates*, 15–20°C), the proportions of polyunsaturated phospholipids in all three genera were similar (about 40%). However, there were appreciable differences in the relative amounts of the three main polyunsaturated components, 18:2, 20:5, and 22:6, perhaps attributable to the differences in their diets. *Gerris* and *Rheumatobates* feed mainly on terrestrial insects, which have 18:2 as their major polyunsaturated acid, whereas *Halobates* feeds on marine organisms that have 20:5 and 22:6 as their major fatty acids and thus can be expected to have a higher content of 20:5 (22%, as compared to 8% and 15% in the other species) and a much lower content of 18:2 (8% as compared to 19% and 24%). The 22:6 acid, which is present in *Halobates* as we would expect, is absent in lipids of *Gerris* and *Rheumatobates*. Finally, the evolutionary history of the animals may be a factor in the development of different fatty acid patterns in different aquatic environments. A major change in *Halobates* during its development from a freshwater ancestor could be the formation of a membrane with 22:6 acid and a much lower content of 18:2 acid. The 20:5 and 22:6 acids are important in the phospholipids of both freshwater and marine zooplankton (Ackman et al. 1970; Farkas and Herodek 1964; Lee et al. 1971*b*), thus long-chain polyunsaturated chains are apparently needed for the membranes of aquatic insects.

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