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Effect of Long-term Fasting on the Use of Fatty Acids as Trophic Markers in the Opossum Shrimp *Mysis relicta*—A Laboratory Study

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ABSTRACT. *Fatty acids are potential trophic markers to trace feeding relationships in aquatic ecosystems primarily because lipid reserves of organisms broadly reflect dietary sources of lipids and can therefore provide information on the availability of key fatty acids in the food web. However, the use of fatty acids for such applications may be constrained by the degree to which the fatty acid composition of organisms is obscured by factors other than straightforward uptake from the diet. Thus, we studied the effect of long-term fasting, under controlled laboratory conditions, on the lipid content and fatty acid composition of field-caught* Mysis relicta*. Periods of 3 to 6 weeks are required to induce clear effects of fasting in* M. relicta*. Relative proportions of docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (ARA; 20:4n-6) increased with decreasing lipid contents during fasting. DHA proportions in the total fatty acid composition of fasting* M. relicta *were significantly higher in comparison to field-caught animals.* M. relicta *with high proportions of DHA (> 25%) and with low lipid contents (< 14% of dry weight) can be clearly identified as fasting animals. Such thresholds will help to improve the validity of fatty acids in trophic studies.*

INDEX WORDS*:* Mysis relicta*, fatty acids, trophic marker, fasting, calibration.*

INTRODUCTION

Fatty acids have been used as trophic markers to trace feeding relationships in marine and freshwater environments (Napolitano 1999, Dalsgaard *et al*. 2003). However, the fatty acid composition of aquatic organisms is not only useful to elucidate the dietary source of lipid reserves; it also provides information on the "availability" of key fatty acids in the food web. Zooplankton organisms, however, not only incorporate and transfer dietary fatty acids from lower to higher trophic levels, they have also the capacity for *de novo* synthesis and/or modification of fatty acids (Castell 1982, Weers *et al*. 1997, Bec *et al*. 2003). Such factors, as well as the mobilization and catabolism of fatty acids during starvation and reproduction, may further alter the fatty acid composition of organisms (Dalsgaard *et al*. 2003), thus obscuring our ability to make strong inferences about feeding relations and/or generalizations about dietary quality. These problems can be reduced by measuring the fatty acid composition of a given species of interest under controlled laboratory conditions in order to define the species-specific natural ranges and accompanying thresholds (minima and maxima) for lipid content and proportions of key fatty acids. Fasting experiments can provide information on expected changes in lipid content and fatty acid composition of animals facing a food deprivation stress. Here we describe how long-term fasting, under controlled laboratory conditions, affects the lipid content and fatty acid composition of field-caught *M. relicta*. The opossum

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shrimp *Mysis relicta* is an important diet component of benthivorous and planktivorous fish common in the Great Lakes (Rand *et al*. 1995). *M. relicta* feed on zooplankton, phytoplankton and detritus, and thus they are an important link between lower and higher trophic levels in the food web (Grossnickle 1982, Johannsson *et al.* 2003).

METHODS

Mysis Fasting Protocol

M. relicta were collected from Lake Ontario on two occasions: once during the fall (29 October 2003; station no. 41, 43°32′00″N; 76°49′16″E, "128 m depth") and once during the spring (06 April 2004; station no. 65, 43°35′48″N; 78°48′9″E, "160 m depth"). These sites are part of a permanent monitoring grid sampled by the Department of Fisheries and Oceans (DFO), Canada. *M. relicta* were sampled from the water column at night using 1 m^2 vertical net tows taken from 2 m above the substrate to the surface. The nets were fitted with windowless cod ends to keep the mysids in water and to prevent physical temperature shock. The animals were held in 4°C water during transport to the laboratory at the Canada Centre for Inland Waters (CCIW), Burlington, Ontario. The mysids were kept in full darkness in a temperate-controlled room (3°C ± 0.3°C) which approximated natural conditions as closely as possible. *M. relicta* sampled in the fall were kept first in 15-L aquaria for 12 d to ensure adaptation to laboratory conditions. During this time animals were fed live zooplankton collected from Lake Ontario. Mortality was very low during the acclimation period; therefore, in spring, experiments were started as soon as the animals arrived in the laboratory.

Centrifugation vials (polypropylene, 50 mL) with a window (1-cm diameter) at each end, covered with nylon screen (2-mm mesh size) served as the experimental containers. During the fasting experiment, single *M. relicta* were placed in separate vials to prevent cannibalism. All vials were held horizontally in submerged racks in 50-L aquaria (50–75 vials/aquarium), in a constant current of aerated water. Water in the tanks was changed weekly with municipal drinking water, which comes from Lake Ontario, and which was aerated for at least 1 week to remove chlorine and cooled to the experimental temperature (3°C).

The gender of field-caught *M. relicta* was determined prior to the fasting experiments. Male and female (without eggs) *M. relicta* (≥ 13 mm in body length) were sampled during the fall experiment. The fasting protocol consisted of sampling a set of animals at the beginning of the experiment (time zero) and then at regular time intervals thereafter. During the fall experiment fasting animals were sampled every 5 d. The sampling was changed to a once every 2 weeks schedule for the spring experiment, since the results of the first experiment suggested that we needed a longer time frame to reach a state of complete starvation. Each time, batches of eight male and eight female *M. relicta* were selected randomly. All samples were stored at –85°C. Dead *M. relicta* found during the experiments were counted and the cumulative mortality calculated. The fall experiment was terminated after 20 and 25 d for males and females, respectively. The spring fasting experiment lasted 42 d.

Lipid Extraction

All *M. relicta* samples were freeze-dried and weighed prior to fatty acid (FA) analysis. Fatty acid methyl esters (FAME) of individual *M. relicta* were obtained in a three-step process: extraction, derivatization, and quantification on a gas chromatograph (GC). Samples were extracted 3 times by grinding freeze-dried tissues in (2:1 v/v) chloroform: methanol (Bligh and Dyer 1959) followed by centrifugation at 3,300 r.p.m. to remove non-lipid material (e.g., exoskeletons). Extracted lipids were brought to a final volume of 2 mL. Duplicate 200-µL aliquots were dispensed into pre-weighed tin cups which were dried and reweighed on a Sartorius (Model ME5) microbalance with 1 µg precision to provide a quantitative measure of total lipid content. Lipid content (%) was reported on a dry weight basis. The remaining extract of 1.6 mL was then transferred into a Shimadzu vial (Sigma 27319U) and evaporated to dryness using nitrogen gas and stored at –85°C until derivatization.

Fatty Acid Analysis

The lipid extracts were resuspended in 2 mL hexane prior to derivatization. Two mL of BF_3-methanol (14% w/w) were added and the vials heated (70°C) for 2 h after which 1 mL of water was added to each vial. The hexane layer containing FAME was carefully removed and placed into a 2-mL Kuderma-Danish receiving vial (Sigma 6-4689U). One mL hexane was then added two times to the original Shimadzu vial to extract the remaining FAME. The FAME-hexane solution was evapo-

rated using nitrogen gas to 0.5 mL and transferred to a GC vial.

FAME concentrations were quantified on a Hewlett Packard 6890 GC with the following configuration: splitless injection; column = Supelco (SP-2560 column) 100 m × 0.25 mm ID × 0.20 μm thick film; oven = 140°C (hold for 5 min) to 240°C at 4°C min^{-1}, hold for 12 min; carrier gas = helium, 1.2 mL min^{-1}; detector = FID at 260°C; injector = 260°C; total run time = 42 min per sample. A 37-component FAME standard (Supelco 47885-U) was used to identify FAME in the samples by comparing their retention times to those of the FAME standard. Results are presented as percentage values of reported fatty acids.

Statistical Analysis

All results are presented as means ± standard error (SE). The difference between the mean values of two groups was analyzed by a t-test. A one-way analysis of variance (ANOVA) was used when three or more groups were compared and was followed by the Tukey test when a significant difference was detected. The application of the Bonferroni procedure did not affect these results. Differences were reported as statistically significant when $p < 0.05$. Percentage data were arcsine transformed (Sokal and Rohlf 1995). The relationship between the percentage values of individual polyunsaturated fatty acids and the lipid content of the animals was examined by linear regression analysis.

RESULTS

Male *M. relicta* collected in fall and spring had significantly different average dry weights, 6.4 mg and 4.8 mg, respectively (Table 1). However, the difference observed in the average dry weight of female *M. relicta* between fall and spring was not significant. Adult *M. relicta* (both sexes) sampled in fall had a significantly higher lipid content (~36%) compared to the animals sampled in spring (~21%).

Independent of seasonal effects and the sex of the animals, the fatty acid composition of field-caught *M. relicta* was generally characterized by a high proportion of polyunsaturated fatty acids (PUFA; 53.0%–58.2%), whereas saturated fatty acids (SAFA) and monounsaturated fatty acids (MUFA) showed lower proportions with 22.7% to 25.0% and 19.2% to 22.2%, respectively. PUFA were always characterized by a high proportion of n-3 fatty acids (43.3%–49.4%). The SAFA composition was generally dominated by palmitic acid (16:0; 17.3%–18.6%), whereas MUFA showed high proportions of oleic acid (18:1n-9; 12.7%–15.5%) (Table 1).

Female *M. relicta* collected in fall had a lower proportion of docosahexaenoic acid (DHA; 22:6n-3; 16.1%) than those sampled in spring (21.2%). Male *M. relicta* collected in fall and spring contained 21.4% and 20.4% DHA, respectively (Table 1).

Adults (both sexes) collected in spring showed lower levels of arachidonic acid (ARA; 20:4n-6) but higher levels of eicosapentaenoic acid (EPA; 20:5n-3) in comparison to adult mysids sampled in fall (Table 1). Independent of species and seasonal effects, all animals contained only minor proportions (< 3%) of the PUFA α-linolenic (ALA; 18:3n3) and linoleic acid (LIN; 18:2n6).

In the first fasting experiment (fall 2003), female *M. relicta* fasting for 25 d showed a significant decrease in dry weight from 8.9 mg to 5.8 mg, while the percent lipid content remained stable throughout the experiment (Table 2). However, the relative levels of individual PUFA showed different trajectories. The proportion of DHA increased significantly from 16.1% to 21.1% but the proportions of ARA and EPA remained stable. LIN and ALA decreased from around 3% to 2%. No effects were observed in male *M. relicta* after 20 d of fasting. During this experiment 12% of the animals died.

In the second experiment lasting 42 d (spring 2004), the average dry weight of female decreased significantly during fasting, whereas in male animals no change in weight was found (Table 3). The *M. relicta* collected for this experiment contained ~21% lipids. The lipid content of adult *M. relicta* declined during fasting to ~13% (Table 3). In both groups increasing ARA and decreasing EPA proportions were observed. However, all *M. relicta* sampled at the end of the fasting period contained higher proportions of DHA compared to animals collected at the beginning of the experiment. In contrast, proportions of LIN decreased during the fasting period. ALA proportions remained stable at ~1% (Table 3). The total mortality of female and male *M. relicta* during the experiment was 22.1% and 23.8%, respectively (Table 3).

In order to investigate the conservation of specific PUFA in fasting *M. relicta*, we examined the relationships between the proportion of individual PUFA and the total lipid content (% of DW) of the animals using linear regression. Regression relationships for ARA and DHA had significant nega-

TABLE 1. Fatty acid composition (% of identified FA) in total lipids, summary fatty acid indices, dry weight (DW), and total lipid (TL, % of dry weight) in* Mysis relicta *from Lake Ontario in two seasons. Mean values within a row with a different superscript letter are significantly different (n = 8; t-test; p = 0.05). SE = Standard Error; Σn-3 = sum of omega-3 fatty acids; Σn-6 = sum of omega-6 fatty acids; ΣSAFA = sum of saturated fatty acids; ΣMUFA = sum of monounsaturated fatty acids; ΣPUFA = sum of polyunsaturated fatty acids; nd = not detected.

	Mysis relicta							
	male				female			
fatty acid composition	fall	±SE	spring	±SE	fall	±SE	spring	±SE
12:0	0.1	0.0 b	0.5	0.1 a	0.2	0.0	0.3	0.1
14:0	2.3	0.2 b	3.1	0.1 a	3.2	0.1 a	2.5	0.2 b
14:1n-5	nd		nd		nd		nd	
15:0	0.4	0.0 b	0.5	0.0 a	0.5	0.0	0.5	0.0
16:0	17.3	0.4	17.8	0.2	18.6	0.7	17.9	0.2
16:1n-7	3.7	0.6 b	5.5	0.3 a	5.0	0.4	4.0	0.4
17:0	0.7	0.0 a	0.5	0.1 b	0.7	0.0 a	0.5	0.0 b
17:1	nd		nd		0.1	0.0 a	0.0	0.0 b
18:0	1.8	0.1	2.0	0.2	1.6	0.1 b	1.7	0.1 a
18:1n-9t	0.1	0.0 b	0.2	0.0 a	0.1	0.0 b	0.2	0.0 a
18:1n-9c	13.7	0.3 a	12.7	0.2 b	15.5	0.2	14.2	0.4
18:2n-6t	nd		nd		nd		nd	
18:2n-6c (LIN)	2.4	0.1	2.3	0.1	2.8	0.1 a	2.1	0.1 b
20:0	0.1	0.0 b	0.2	0.0 a	0.1	0.0	0.1	0.0
18:3n-6	0.3	0.0 a	0.2	0.0 b	0.3	0.0 a	0.2	0.0 b
20:1n-9	1.4	0.1	1.3	0.0	1.3	0.1	1.4	0.0
18:3n-3 (ALA)	2.1	0.2 a	1.0	0.1 b	3.0	0.2 a	1.1	0.1 b
21:0	0.0	0.0	0.1	0.1	nd		nd	
20:2	1.0	0.1	1.0	0.0	0.8	0.0 b	1.0	0.0 a
22:0	0.0	0.0 b	0.1	0.1 a	nd		0.1	0.0
20:3n-6	0.1	0.0 a	0.1	0.0 b	0.1	0.0 a	0.1	0.0 b
22:1n-9	0.1	0.0 a	0.1	0.0 b	0.1	0.0 a	0.1	0.0 b
20:3n-3	0.6	0.1	0.6	0.0	0.6	0.1	0.6	0.0
20:4n-6 (ARA)	7.5	0.5 a	3.4	0.2 b	5.6	0.3 a	3.7	0.2 b
23:0	nd		nd		nd		nd	
24:0	nd		nd		nd		nd	
20:5n-3 (EPA)	22.1	0.5 b	25.6	0.3 a	23.0	0.6 b	26.0	0.3 a
24:1n-9	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
22:5n-3	0.7	0.0	0.6	0.0	0.6	0.0	0.5	0.0
22:6n-3 (DHA)	21.4	1.0	20.4	0.5	16.1	0.8 b	21.2	0.6 a
Σn-3	46.9	0.8	48.2	0.5	43.3	1.4 b	49.4	0.6 a
Σn-6	10.3	0.5 a	5.9	0.2 b	8.9	0.4 a	6.0	0.2 b
ΣSAFA	22.7	0.4 b	25.0	0.3 a	24.8	0.8	23.6	0.2
ΣMUFA	19.2	0.8	19.9	0.4	22.2	0.5 a	19.9	0.4 b
ΣPUFA	58.2	1.0 a	55.2	0.5 b	53.0	1.1 b	56.5	0.5 a
DW (mg)	6.4	0.6 a	4.8	0.2 b	8.9	0.3	7.6	0.7
TL (%)	36.6	2.0 a	21.5	1.7 b	36.4	2.3 a	20.3	1.2 b

TABLE 2. Fatty acid composition (% of identified FA) in total lipids, summary fatty acid indices, dry weight (DW), total lipid (TL, % of dry weight), and cumulative mortality (see methods) in female and male* Mysis relicta *from Lake Ontario collected in fall and fasting for 5 to 25 d. Mean values within a row with a different superscript letter are significantly different (n = 8; Tukey test; p = 0.05). Abbreviations as described in Table 1.

females	days													
fatty acid composition	0	± SE		5	± SE		10	± SE		15	± SE		20	± SE		25	± SE	
18:2n-6 (LIN)	2.8	0.1	ab	3.0	0.1	a	2.6	0.1	bc	2.6	0.1	bc	2.5	0.1	bc	2.4	0.1	c
18:3n-3 (ALA)	3.0	0.2	a	3.1	0.3	a	2.7	0.1	ab	2.6	0.1	ab	2.4	0.2	ab	2.1	0.1	b
20:4n-6 (ARA)	5.6	0.3		5.5	0.3		5.8	0.3		5.9	0.3		5.8	0.2		5.7	0.1	
20:5n-3 (EPA)	23.0	0.6		22.1	0.5		23.4	0.4		22.4	0.5		22.4	0.5		22.6	0.3	
22:6n-3 (DHA)	16.1	0.8	b	16.5	1.2	b	18.9	1.0	ab	18.7	0.8	ab	18.7	0.9	ab	21.1	0.7	a
Σn-3	43.2	1.4		42.9	1.4		46.3	1.2		45.0	1.0		44.7	0.7		46.9	0.5	
Σn-6	8.9	0.4		9.0	0.3		8.8	0.3		9.0	0.2		8.8	0.2		8.4	0.1	
ΣSAFA	24.8	0.8	a	24.4	0.5	ab	23.3	0.7	ab	23.8	0.5	ab	24.0	0.4	ab	22.5	0.2	b
ΣMUFA	22.2	0.5		22.9	1.2		20.7	0.9		21.4	0.7		21.7	0.7		20.9	0.5	
ΣPUFA	53.0	1.1		52.7	1.6		56.0	1.4		54.8	1.1		54.4	0.8		56.6	0.6	
DW (mg)	8.9	0.3	a	6.1	0.5	b	7.1	0.5	ab	5.5	0.5	b	6.1	0.5	b	5.8	0.5	b
TL (%)	36.4	2.3		34.8	2.6		31.7	3.0		34.7	2.4		34.5	3.5		30.5	2.0	
cum. mort (%)																12.5		

males	days											
fatty acid composition	0	± SE		5	± SE		10	± SE		15	± SE		20	± SE	
18:2n-6 (LIN)	2.4	0.1		2.3	0.2		2.3	0.1		2.3	0.1		2.3	0.1	
18:3n-3 (ALA)	2.1	0.2		2.0	0.3		2.2	0.1		2.0	0.1		2.2	0.1	
20:4n-6 (ARA)	7.5	0.5		7.0	0.6		7.8	0.5		8.4	0.6		8.0	0.5	
20:5n-3 (EPA)	22.1	0.5		21.9	0.3		21.9	0.5		21.7	0.4		20.7	0.3	
22:6n-3 (DHA)	21.4	1.0		19.5	1.5		20.6	0.6		22.0	1.0		19.8	0.6	
Σn-3	46.9	0.8		44.7	1.3		46.1	0.8		47.1	1.1		44.3	0.7	
Σn-6	10.3	0.5		9.8	0.5		10.6	0.5		11.1	0.5		10.7	0.5	
ΣSAFA	22.7	0.4		23.9	0.6		22.6	0.4		21.9	0.6		23.5	0.6	
ΣMUFA	19.2	0.8		20.6	1.2		19.8	0.6		18.8	1.0		20.5	0.5	
ΣPUFA	58.2	1.0		55.5	1.7		57.6	0.9		59.3	1.4		56.0	1.0	
DW (mg)	6.4	0.6		6.7	0.5		6.1	0.4		5.2	0.3		5.0	0.2	
TL (%)	36.6	2.0		34.9	4.4		34.3	3.1		35.0	4.1		34.8	1.2	
cum. mort. (%)													12.2		

tive slopes indicating that the proportions of both fatty acids increased with decreasing lipid contents during fasting, with the exception of ARA in male *M. relicta* sampled in spring ($p>0.05$) (Table 4, Fig.1). The slopes for EPA, ALA, and LIN in mysids were all positive but not always significant.

DISCUSSION

Lipid content and composition of *Mysis relicta* differed in spring and fall, setting the baseline for two studies on the changes in lipid profiles in this species with fasting. The highest lipid content of *M. relicta* (~37% of dry mass) was found in adult animals sampled in fall which agrees with Adare and Lasenby (1994) who described maximum mysid lipid levels in the range of 30% to 40% of dry mass in smaller temperate Canadian lakes. Due to their size and developmental stage (≥ 1.5 to 2.5 years old), it can be expected that the adult *M. relicta* collected from Lake Ontario in the fall would have mated later that fall with young being released the following spring. Lipids are allocated to eggs to provide offspring with fuel for growth and develop-

TABLE 3. Fatty acid composition (% of identified FA) in total lipids, summary fatty acid indices, dry weight (DW), total lipid (TL, % of dry weight), and cumulative mortality (see methods) in female and male Mysis relicta from Lake Ontario collected in spring and fasting for 14, 28 or 42 d. Mean values within a row with a different superscript letter are significantly different (n = 8; Tukey test; p = 0.05). Abbreviations as described in Table 1.

females	days							
fatty acid composition	0	± SE	14	± SE	28	± SE	42	± SE
18:2n-6 (LIN)	2.1	0.1 a	1.8	0.1 ab	1.6	0.1 b	1.6	0.1 b
18:3n-3 (ALA)	1.1	0.1	1.0	0.2	0.7	0.1	0.7	0.1
20:4n-6 (ARA)	3.7	0.2 b	4.2	0.1 ab	4.1	0.2 ab	4.4	0.1 a
20:5n-3 (EPA)	26.0	0.3 a	24.8	0.3 b	24.4	0.2 b	24.0	0.2 b
22:6n-3 (DHA)	21.2	0.6 c	23.3	0.6 bc	24.3	1.0 ba	27.0	1.0 a
Σn-3	49.4	0.6 b	50.2	0.5 ab	50.5	0.9 ab	52.6	0.8 a
Σn-6	6.0	0.2	6.2	0.2	5.9	0.2	6.1	0.2
ΣSAFA	23.6	0.2 ab	24.2	0.5 a	23.6	0.7 ab	22.1	0.2 b
ΣMUFA	19.9	0.4	18.1	0.8	18.8	0.5	17.8	0.6
ΣPUFA	56.5	0.5 b	57.7	0.5 ab	57.5	1.0 ab	60.1	0.7 a
DW (mg)	7.6	0.7 a	7.4	0.8 ab	6.4	0.7 ab	4.6	0.3 b
TL (%)	20.3	1.2 a	16.5	1.4 ab	16.6	1.7 ab	12.4	1.1 b
cum. mort. (%)							22.1	

males	days							
fatty acid composition	0	± SE	14	± SE	28	± SE	42	± SE
18:2n-6 (LIN)	2.3	0.1 a	1.7	0.1 b	1.7	0.1 b	1.5	0.1 b
18:3n-3 (ALA)	1.0	0.1	0.9	0.1	0.9	0.1	0.7	0.1
20:4n-6 (ARA)	3.4	0.2 b	4.4	0.2 ab	5.2	0.4 a	5.3	0.2 a
20:5n-3 (EPA)	25.6	0.3 a	24.7	0.2 ab	23.6	0.3 bc	23.0	0.4 c
22:6n-3 (DHA)	20.4	0.5 c	22.1	0.4 bc	23.7	1.0 ab	25.8	1.1 a
Σn-3	48.2	0.5	48.9	0.6	49.4	1.1	50.7	0.9
Σn-6	5.9	0.2	6.3	0.2	7.2	0.5	7.0	0.4
ΣSAFA	25.0	0.3 a	26.5	0.6 a	23.3	0.3 b	23.0	0.4 b
ΣMUFA	19.9	0.4 a	17.2	0.4 b	18.9	0.8 ab	18.0	0.6 ab
ΣPUFA	55.2	0.5 b	56.3	0.5 ab	57.8	1.0 ab	59.1	0.8 a
DW (mg)	4.8	0.2	5.4	0.2	5.6	0.5	4.5	0.4
TL (%)	21.5	1.7 a	19.2	1.6 ab	17.7	2.3 ab	13.5	1.4 b
cum. mort. (%)							23.8	

ment and to buffer against periods of low food during early life stages. The accumulation of sufficient lipid energy reserves in fall is expected, therefore, to play a significant role in reproduction. Adult males die after reproducing while a very few adult females survive to reproduce again the next spring (Morgan and Beeton 1978). Adult *M. relicta* collected in the spring were smaller on average than the fall adults supporting the notion that these were younger animals of ~1 year of age. The lipid content of these animals was significantly lower compared to the relatively older adult mysids collected in fall (~21% of dry mass vs ~36%). Unfortunately, no animals from the same cohort of a similar or smaller size and developmental stage were sampled in fall. We can therefore only speculate how the lipid content of adult *M. relicta* sampled in spring was influenced by overwintering.

Our study showed several differences between the fatty acid profiles of field-caught *M. relicta*. The fatty acid composition of storage lipids is highly influenced by diet (Olsen 1999). While some

TABLE 4. Regression analysis for the relationships between individual polyunsaturated fatty acids (% of identified FA in total lipids) and lipid content (% of dry weight) in* Mysis relicta *sampled in fall or spring. ALA = 18:3n-3, α-linolenic acid; LIN = 18:2n-6, linoleic acid; ARA = 20:4n-6, arachidonic acid; EPA = 20:5n-3, eicosapentaenoic acid; DHA = 22:6n-3, docosahexaenoic acid. a = intercept and b = slope of linear regression. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001

	male *Mysis relicta*							
	fall (n = 33)				spring (n = 30)			
fatty acid	a	b	R^2	p	a	b	R^2	p
LIN	1.84	0.01	0.15	*	0.90	0.05	0.46	***
ALA	1.58	0.02	0.08	ns	0.12	0.04	0.51	***
ARA	11.52	–0.11	0.36	***	4.86	–0.02	0.01	ns
EPA	19.69	0.06	0.15	*	22.83	0.08	0.09	ns
DHA	24.75	–0.12	0.13	*	31.14	–0.46	0.66	***

	female *Mysis relicta*							
	fall (n = 45)				spring (n = 37)			
fatty acid	a	b	R^2	p	a	b	R^2	p
LIN	1.79	0.02	0.17	**	1.18	0.04	0.24	**
ALA	0.84	0.05	0.30	***	0.31	0.04	0.17	*
ARA	6.92	–0.04	0.14	**	4.73	–0.04	0.12	*
EPA	20.59	0.04	0.02	ns	23.47	0.09	0.13	*
DHA	24.66	–0.21	0.38	***	30.91	–0.43	0.46	***

algae (e.g., diatoms, cryptophytes) are rich in EPA, diet items consumed by carnivores (in this case zooplankton) contain relatively higher contents of ARA (Ballantyne *et al*. 2003, Kainz *et al*. 2004). Male *M. relicta* sampled in spring showed a high proportion of 16:1n-7 and EPA which have been suggested as potential fatty acid markers for diatom-based diets (Napolitano 1999). Our observations indicate that diatoms (spring bloom) might be an important food source for *M. relicta* not only during the diatom peak in May (Johannsson *et al*. 2001) but also during the winter and early spring.

Male and female *M. relicta* collected in fall showed higher ARA proportions compared to adult animals collected in spring. The difference suggests that the diet of adult *M. relicta* collected in fall was characterized by a higher proportion of zooplankton (e.g., cladocerans and copepods) which is in accordance with the seasonal diets described for *M. relicta* from Lake Ontario in the mid-1990s determined by stable isotope and gut content analyses (Johannsson *et al*. 2001). The feeding of live zooplankton during the brief acclimation period preceding the fasting experiment may also have strengthened this trend.

The statements about seasonal patterns of fatty acids made before are of a speculative nature since animals of two different cohorts were compared. However, the examples demonstrate how information about the fatty acid composition of field-caught animals can be used to make inferences about the dietary source of lipid reserves. Our laboratory experiments gave us the opportunity to investigate how long-term fasting might obscure the use of fatty acids as trophic markers.

During the first experiment on *M. relicta* sampled in fall, male and female animals were fasted for 20 and 25 d, respectively. Even though we observed a low cumulative mortality of ~12%, the period was obviously not long enough to produce clear effects on the high lipid content (> 30%) of adult *M. relicta*. Animals of both sexes showed a tendency to lower lipid levels, however these differences were not significant due to the high deviation observed in both groups which can be explained by the heterogeneous composition of the field caught animals. Nevertheless, the data indicate a highly efficient use of energy storage lipids in fasting *M. relicta* kept at 3°C which might be explained by the low metabolic rate at this temperature as well as the animals' restricted activity within our laboratory setting. In comparison to animals in the field which

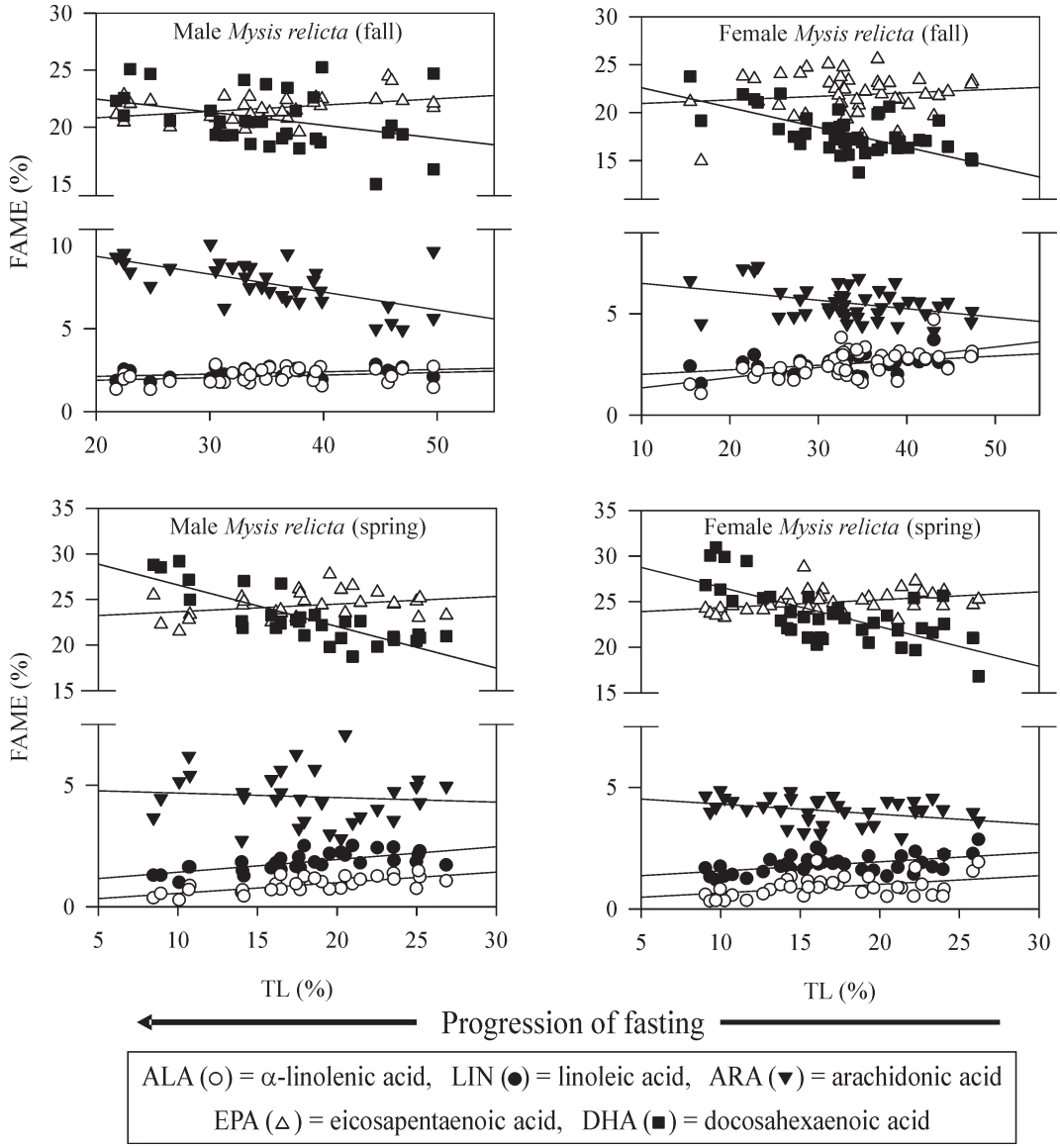

FIG. 1. Relationship among individual polyunsaturated fatty acids (% of identified FA in total lipids) and lipid content (% of DW) in* Mysis relicta *sampled in fall or spring. Regression statistics are described in Table 4. TL = total lipids; FAME = fatty acid methyl ester.

constantly lose energy when swimming (e.g., vertical migration), *M. relicta,* kept in the limited space of 50-mL centrifugation tubes, likely moved less.

No effects of fasting were observed on the fatty acid composition of male *M. relicta*. However, female *M. relicta* showed a significant increase of DHA from 16.1% to 21.1% during the 3 week fasting period providing the first indication of DHA retention during fasting.

Due to the limited effect of the 3 week fasting period on lipid content and fatty acid composition of adult *M. relicta*, the second experiment, carried out in spring, was extended to 6 weeks. The cumulative mortality of male and female *M. relicta* almost doubled in comparison to the first experiment which could be explained by the extended fasting period and the lower lipid level of the animals at the onset of the experiment.

Lipid content of the animals decreased significantly during fasting from > 20% to < 14% of dry mass. However, due to the relatively high survival rate of around 80%, it can be assumed that the fast-

ing period of 6 weeks was not long enough to reach the lower limit of storage lipids for *M. relicta*.

Several effects of fasting on the fatty acid composition of adult *M. relicta* could be observed. The relative contribution of individual PUFA to total FAME as a function of lipid content (% of DW) revealed that several fatty acids were selectively retained during fasting. For example, proportions of ARA and DHA (%) increased in all groups with decreasing lipid content. This can be explained by the following mechanism. Neutral (storage) lipids of *M. relicta* mainly consist of triglycerides (TAG) (Cavaletto and Gardner 1999). TAG serve primarily as a source of metabolic energy (adenosine triphosphate, ATP), and therefore decrease during fasting. Total phospholipid concentrations, however, have been shown to be relatively constant and independent of feeding conditions (Rainuzzo *et al*. 1994). When TAG decrease, the fatty acid composition of polar (membrane) lipids, which are relatively rich in ARA and DHA (Sargent *et al.* 2002), will therefore be increasingly reflected in the total fatty acid composition. The DHA and ARA-retentive metabolism observed in our fasting studies is similar, in this context, to fish (Bell *et al.* 1985, De Silva *et al.* 1997, Zenebe *et al.* 2003).

The observed effects of long-term fasting on the fatty acid composition of *M. relicta* confirm that fatty acids used as trophic markers must be interpreted cautiously. In nature, periods of food shortage occur that can, temporarily, result in food-limitation or starvation conditions for zooplankton. In the Great Lakes, mysids may suffer from food limitation or fasting conditions over the winter when the zooplankton and diatom densities are lowest. Also the recent invasion of the Great Lakes by exotic invertebrates (e.g., *Bythotrephes longimanus*, *Cercopagis pengoi*, *Dreissena polymorpha,* and *D. bugensis*), which has altered the base and intermediate levels of the food webs (Johannsson *et al.* 2000, Benoît *et al.* 2002, Laxson *et al*. 2003, Barbiero and Tuchman 2004) in these lakes, may temporarily result in food limitation for *M. relicta*.

Without knowing something about the synthetic and catabolic (and retentive) capabilities of an organism one cannot immediately reach the conclusion that high proportions of a fatty acid (e.g., DHA) necessarily reflect access to dietary resources rich in this fatty acid. We therefore suggest that an examination of fatty acid profiles in trophic studies should also be accompanied by measurements of total lipid content since total lipid content is generally useful as an index of overall feeding success, reproductive condition and physiological state.

We conclude that periods of 3 to 6 weeks are required to induce clear effects of fasting on lipid content and fatty acid composition of 'healthy' field-caught *M. relicta.* This species, therefore, seems to be highly adapted to periods of food shortage. Fatty acid compositions in fasting *M. relicta* change with decreasing lipid contents. DHA and ARA are highly conserved during fasting. Higher proportions of DHA associated with lower total lipid levels would indicate food shortages with proportions of DHA (> 25%) and lipid contents (< 14% of DW) clearly identifing fasting animals. Such thresholds will help to improve the validity of fatty acids in trophic studies.

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