Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence

Suzanne M. Budge, Sara J. Iverson, W. Don Bowen, and Robert G. Ackman

Abstract: The fat and fatty acid compositions of 28 species of fish and invertebrates (n = 954) from the Scotian Shelf, Georges Bank, and the Gulf of St. Lawrence were determined. Discriminant analysis of the 16 most numerous species ($n \geq 18$ each), using 17 major fatty acids, classified species with greater than 98% accuracy and grouped species into three general clusters (gadids, flatfish, and planktivores) with similar fatty acid compositions, and likely, similar diets. A number of species exhibited changes in fatty acid signatures with increasing size (multivariate analysis of variance), which corresponded with known dietary shifts reported from stomach contents analyses. Location effects were also observed among the three major geographical regions and were probably due to broad-scale variations in prey assemblages and phytoplankton composition in the northwestern Atlantic. Despite these effects, within-species variation was still substantially less than among-species variation. Thus, fatty acid signatures can be used to distinguish and characterize fish and invertebrate species in a given ecosystem, as well as to study finer-scale trophic interactions of these species. These data also have applications at higher trophic levels and will serve as a prey database for studying the diets of other fish and marine mammal predators using fatty acid signatures.

Résumé : On trouvera ici une analyse de la composition en graisses et en acides gras de 28 espèces de poissons et d'invertébrés (n = 954) de la plate-forme néo-écossaise, du banc George et du golfe du Saint-Laurent. Une analyse discriminante des 16 espèces les plus abondantes (nombre d'individus ≥ 18) basée sur 17 acides gras principaux classifie correctement les espèces avec une précision de 98 % et les rassemble en trois groupes généraux (les gadidés, les poissons plats et les planctonophages) qui possèdent des compositions en acides gras et probablement des régimes alimentaires similaires. Plusieurs espèces affichent un changement dans leurs signatures d'acides gras en fonction de la taille (analyse multidimensionnelle de variance), ce qui correspond à des variations du régime alimentaire déjà observées par l'analyse des contenus stomacaux. Il existe aussi des variations spatiales dans les trois principales régions géographiques, variations qui sont probablement dues à des différences à grande échelle dans les communautés de proies et la composition du phytoplancton dans le nord-ouest de l'Atlantique. Malgré ces effets, la variation intraspécifique est considérablement plus faible que la variation interspécifique. Les signatures d'acides gras peuvent donc être utilisées pour reconnaître et caractériser les espèces de poissons et d'invertébrés dans un écosystème donné, ainsi que pour étudier les interactions trophiques détaillées de ces espèces. Ces données peuvent s'appliquer aussi aux niveaux trophiques plus élevés et servir d'information de base sur les proies dans l'étude des régimes alimentaires d'autres poissons et mammifères marins prédateurs à l'aide des signatures d'acides gras.

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Introduction

The decline or collapse of many commercial fisheries around the world has underscored the need for alternatives to single-species assessments (Pauly et al. 1998). Among the alternative approaches is an attempt to understand stock dynamics within a broader ecosystem context (e.g., Christensen et al. 1996; Köster et al. 2001). These approaches recognize that the interrelationships among predators and prey, as well as changes in the physical environment, can influence the population dynamics of commercial stocks. Fish and marine mammals are significant consumers of commercially harvested species in a number of marine ecosystems (Bax 1991; Bowen 1997). In eastern Canada, attempts to understand bottom-up

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S.M. Budge[1] **and S.J. Iverson.** Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada.
W.D. Bowen. Marine Fish Division, Bedford Institute of Oceanography, Department of Fisheries and Oceans, Dartmouth, NS B2Y 4A2, Canada.
R.G. Ackman. Canadian Institute of Fisheries Technology, DalTech, Dalhousie University, Halifax, NS B3J 2X4, Canada.

[1]Corresponding author (e-mail: budges@is.dal.ca).

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or top-down trophic interactions of commercial fish stocks have been sporadic, with few long-term studies of the diets of the fish stocks of interest or of their fish or marine mammal predators (Lilly 1986; Albikovskaya et al. 1991). Determining the diet composition of predators is clearly fundamental to delineating food webs. The predominant methods that have been used to estimate the diets of marine predators are based on the recovery of prey structures resistant to digestion from stomachs and faeces (e.g., Popova 1978; Pierce and Boyle 1991). These methods have proved useful, and in fact, most of our understanding of fish and marine mammal diets has been derived from such studies. Nevertheless, these methods are subject to known biases, some of which can be corrected, whereas others continue to limit the value of estimated diets (e.g., Jobling and Breiby 1986). For example, prey without hard structures or with readily degradable structures may be underrepresented in diet reconstructions.

It has been known for some time that lipids, and especially fatty acids, can be used as biological markers and general diet indicators in marine ecosystems (e.g., Sargent et al. 1987). Fatty acids are components of the more general group of lipids and are ubiquitous in nature. They have a variety of structures but, in marine organisms, commonly contain from 14 to 24 carbon atoms and have varying degrees of unsaturation. Because of biochemical properties and limitations, many dietary fatty acids are incorporated into marine animals with little or no modification of the original structure, making these fatty acids useful as indicators or markers of the dietary source. Combinations of these markers, or the whole suite of fatty acids present, are referred to as the fatty acid signature of an organism (Iverson 1993).

Early studies recognized that the fatty acid composition of zooplankton lipids influenced the fatty acid composition of the blubber lipids of the baleen whales that fed on them (e.g., Ackman and Eaton 1966). However, Sargent et al. (1987) were among the first groups to suggest the use of marker fatty acids in the study of trophic relationships. Since that time, numerous studies have demonstrated that fatty acid signatures can be passed from prey to predator, both at the bottom (e.g., Fraser et al. 1989; Graeve et al. 1994) and near the top (e.g., Iverson 1993; Kirsch et al. 1998; Kirsch et al. 2000) of the food web. Once fatty acid patterns are characterized in prey, they can be used to trace food webs and diets of predators. For example, fatty acids have been used to study the diets of fish and copepods (e.g., Sargent et al. 1989; Fraser et al. 1989; St. John and Lund 1996). Fatty acids have also been used to indicate the presence of fish and other prey in the diets of terrestrial and aquatic carnivores (e.g., Rouvinen et al. 1992; Colby et al. 1993), the degree to which plants have been consumed by terrestrial carnivores (Iverson et al. 2001), and spatial or temporal differences in diets both within and between marine mammal species (Iverson et al. 1997*a*, 1997*b*; Smith et al. 1997).

Thus, the fatty acid signatures of marine fish and invertebrates can be used to study their ecology and trophic interactions (i.e., bottom-up) but can also be characterized to study the diets of their fish or marine mammal predators. To use fatty acids to estimate the diets of apex marine carnivores, we need to understand the degree to which prey species can be distinguished on the basis of their fatty acid signatures and how stable these signatures are with respect to ecological variability. We expect marine fish and invertebrate fatty acid signatures to vary predominantly with the diets characteristic of the species, but also with changes in primary and secondary production related, in turn, to temporal and spatial variability in the physical environment. Changes in the diet of a fish can result in a significant change in its fatty acid signature in as little as 3 weeks (Kirsch et al. 1998); thus we can expect to rapidly detect shifts in diet. Fatty acid composition is also influenced to varying degrees by the biochemistry of the organism and its reproductive status (i.e., the presence of lipid-rich eggs prior to spawning) (Sargent et al. 1989; Sargent 1995). Lastly, for some fish species, including gadids, diets change with increasing body size such that fatty acid signatures may vary among age groups within a species (e.g., Iverson et al. 1997*b*).

The objectives of this study were (*i*) to determine the fatty acid composition of selected commercial and noncommercial marine fishes and invertebrates from the Scotian Shelf, Georges Bank, and Gulf of St. Lawrence that are known or potential prey of marine mammals; (*ii*) to determine whether species can be reliably differentiated on the basis of their fatty acids; and (*iii*) to examine the effects of geographic location and body size on fatty acid signatures within prey species.

Materials and methods

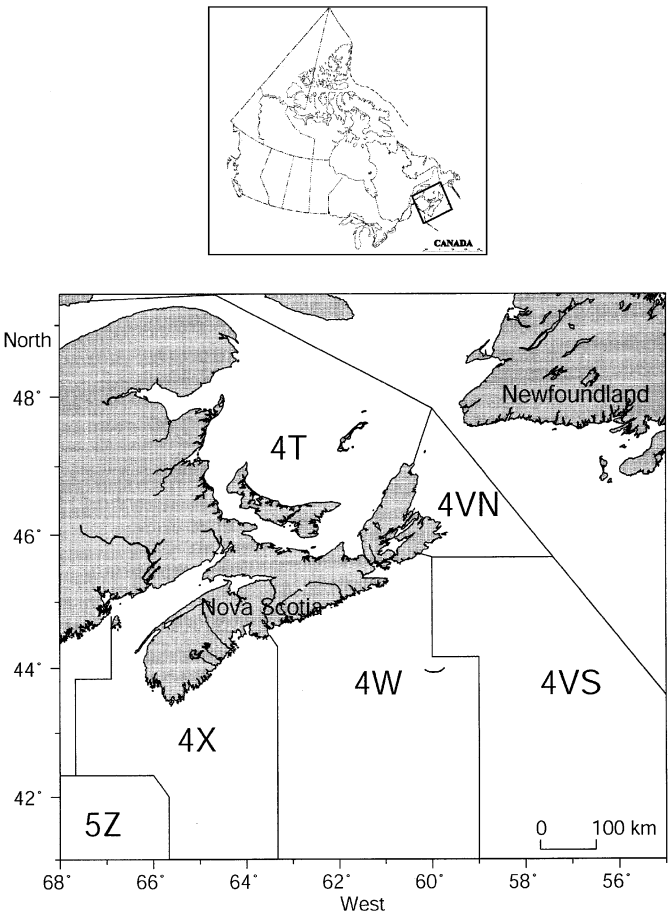
Sample collection

Fish and invertebrate samples were collected during stratified-random bottom-trawl surveys conducted in the spring, summer, or fall on the Scotian Shelf (Northwest Atlantic Fisheries Organization (NAFO) subareas 4V, 4W, and 4X), and on Georges Bank (5Z) in 1993, 1994, 1995, and 1996, and in the southern Gulf of St. Lawrence (4T) in 1999 (Fig. 1; Table 1). Additional invertebrate samples were obtained from research cruises and commercial fisheries on the Scotian Shelf. As our initial interest was to determine the fatty acid signatures of potential prey of marine mammals, we restricted the length of fish collected to between 10 and 40 cm (Bowen et al. 1993). No restriction was placed on the size of invertebrates collected. Although we would have preferred to collect species in all seasons, locations, and years, this was not possible because of constraints associated with collection and sampling. At collection, individuals or groups of individuals of each species were stored frozen at –20°C in airtight plastic bags until analysis (<6 months).

Lipid and fatty acid analysis

Individual fishes and invertebrates were thawed and fork length or carapace width of each individual was measured to the nearest 0.1 cm; body mass was measured to the nearest 0.1 g. Each individual was then homogenized in a blender or food processor. Although it may have increased within-species variability, we made no attempt to remove stomach contents of individuals before homogenization because our objective was to characterize them as prey, which are generally eaten whole by fish and marine mammal predators. Lipids were quantitatively recovered in duplicate from samples of the homogenized tissue using a modified Folch et al. (1957) method. Briefly, 1.5-g tissue aliquots were extracted with 30 mL 2:1 chloroform–methanol and the entire lower

Fig. 1. Northwest Atlantic Fisheries Organization (NAFO) fishing zones surrounding Nova Scotia, Canada, referred to in the present study.



phase was collected. This fraction was then washed, filtered through anhydrous sodium sulphate, evaporated under nitrogen, and vacuum sonicated to obtain total lipid weight. Fatty acid methyl esters (FAME) were prepared and analyzed in duplicate according to Iverson et al. (1997*b*) using a Perkin-Elmer Autosystem II capillary gas chromatograph (GC) (Norwalk, Conn.) with a flame ionization detector (ID) using a flexible fused silica column (30 × 0.25 mm ID) coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness; J&W DB-23; Folsom, Calif.). Helium was used as the carrier gas and the gas line was equipped with an oxygen scrubber. The following temperature program was used: 153°C for 2 min, hold at 174°C for 0.2 min after ramping at 2.3°C·min^{-1}, and hold at 220°C for 3 min after ramping at 2.5°C·min^{-1}. Up to 66 FAME were identified according to Iverson et al. (1997*b*, 2001) and reported as weight percent of total fatty acids. Each fatty acid was described using the shorthand nomenclature of *A*:*B*n–*X*, where A represents the number of carbon atoms, *B* the number of double bonds, and *X* the position of the double bond closest to the terminal methyl group.

Data analysis and interpretation

Fatty acid data were analyzed using a combination of classification and regression trees (CART), discriminant function analysis, and multivariate analysis of variance (MANOVA). Most multivariate methods require that the data meet several requirements. With discriminant analysis and MANOVA, the number of samples must exceed the number of variables to offer some assurance that the covariance matrices are homogeneous (Stevens 1986). In this study, we have set the number of fatty acid variables to 17 allowing all species with sample numbers of 18 or greater to be included in the analysis. We used the 17 fatty acids that were either most abundant and (or) exhibited the greatest average variance across all species, accounting for approximately 86% of total fatty acids identified. In addition, for both discriminant analysis and MANOVA, the data were normalized using a log transformation according to the following equation: $x_{trans} = \ln(x_i/c_r)$, where x_i is a given fatty acid expressed as percent of total fatty acids, x_{trans} is the transformed fatty acid data, and c_r is the percentage of a reference fatty acid, in this case, 18:0 (Aitchison 1986).

We also investigated differences in the fatty acid compositions of samples with CART, which has been used previously to classify fatty acid signatures (Iverson et al. 1997*a*, 1997*b*; Smith et al. 1997). CART has the advantage of making fewer assumptions than most other multivariate methods. Homogeneous covariance matrices are not required, the number of variables is not limited by sample size, and variables need not be normally distributed, so that untransformed percentage data may be used. This permitted us to use all fatty acids and a greater number of species in the analysis. CART analyses were conducted with the S-PLUS 4.0 software (Statsci Division, Mathsoft Inc., Seattle, Wash.). All other analyses were carried out with SPSS 9.0 (SPSS Inc., Chicago, Ill.). The standard deviations (SD) are given as a measure of variability about the mean.

Results

A total of 954 samples representing 28 species were analyzed for total fat content and fatty acid composition (Tables 1 and 2). Scientific names of species referred to in the text are given in Table 1. We also present average lipid concentration, length, body mass, and fatty acid composition of all species (Table 2). (Note that individual data from all 954 samples are available from the authors.) Data from all 28 species (i.e., $n \geq 5$) were used in classifications with CART (classification success given in Table 2). Of these, 16 species had sample sizes ≥18 and were examined in more detail using discriminant analysis and MANOVA.

Among-species variation in fatty acid composition

Major fatty acid characteristics

Of the 66 fatty acids identified, 14:0, 16:0, 16:1*n*–7, 18:1*n*–9, 20:1*n*–9, 22:1*n*–11, 20:5*n*–3, and 22:6*n*–3 made up 60–80% of total fatty acids in the 28 species (Table 2). Other fatty acids, including 18:0, 18:1*n*–7, 20:1*n*–11, 22:1*n*–9, 18:2*n*–6, 18:4*n*–3, 20:4*n*–6, and 22:5*n*–3 were often present in amounts >1%. A preliminary examination of the average fatty acid compositions among different species (Table 2) revealed some obvious similarities and differences. For example, in the Gadidae species (cod, haddock, pollock, red hake, silver hake, and white hake), levels of 22:6*n*–3 tended to be

Table 1. Distribution of species collected with respect to location (NAFO subarea; see Fig. 1) and season.

Species	4VsW	4Vs	4Vn	4W	4X	5Z	4T	Unknown	Total
American plaice (*Hippoglossoides platessoides*)	—	25sp	—	3sp	29s	24sp	18f	—	99
Argentine (*Argentina silus*)	—	—	—	—	10s	—	—	—	10
Butterfish (*Peprilus triacanthus*)	—	—	—	10sp	—	—	—	—	10
Capelin (*Mallotus villosus*)	—	6s	—	29sp	—	—	21f	—	56
Cod (*Gadus morhua*)	23u	—	—	9sp, 16s	—	15sp	21f	—	84
Gaspereau (*Alosa pseudoharengus*)	—	—	—	19sp	—	—	22f	—	41
Haddock (*Melanogrammus aeglefinus*)	—	3sp	—	10sp, 6s	28s	7sp	—	—	54
Halibut (*Hippoglossus hippoglossus*)	—	—	—	8sp	—	—	—	—	8
Herring (*Clupea harengus*)	—	—	—	38s	—	—	21f	15f	74
Lobster (*Homarus americanus*)	—	—	9u	—	—	—	—	—	9
Longhorn sculpin (*Myoxocephalus octodecemspinosus*)	—	10sp	—	10sp	—	—	—	—	20
Mackerel (*Scomber scombrus*)	—	—	—	10sp	—	—	—	—	10
Northern sand lance (*Ammodytes dubius*)	—	27sp	—	44s	—	—	—	—	71
Ocean pout (*Macrozoarces americanus*)	—	—	—	11sp	7s	—	—	—	18
Pollock (*Pollachius virens*)	—	—	10sp	4s	7s	4sp	—	—	25
Red crab (*Geryon quinquedens*)	—	—	—	—	14u	—	—	—	14
Red hake (*Urophycis chuss*)	—	—	—	7sp	—	—	—	—	7
Redfish (*Sebastes* sp.)	—	—	8s	3s	8s	—	30f	—	49
Rock crab (*Cancer irroratus*)	—	—	10u	—	—	—	—	—	10
Sea raven (*Hemitripterus americanus*)	—	—	—	4sp	2s	—	—	—	6
Shrimp (*Pandalus borealis*)	—	—	25sp	21f	—	—	—	—	46
Silver hake (*Merluccius bilinearis*)	—	—	—	18sp	15s	5sp	—	—	38
Smooth skate (*Raja senta*)	—	5sp	—	—	—	—	—	—	5
Thorny skate (*Raja radiata*)	—	12sp	—	—	—	—	—	—	12
White hake (*Urophycis tenuis*)	—	11sp	—	5sp	—	—	30f	—	46
Winter flounder (*Pseudopleuronectes americanus*)	—	—	—	4sp, 10s	11s	—	—	—	25
Winter skate (*Raja ocellata*)	—	—	—	15sp	—	—	—	—	15
Yellowtail flounder (*Limanda furruginea*)	5sp, 26s	—	—	15sp, 10s	4s	12sp	20f	—	92
Total	54	99	62	339	135	67	183	15	954

Note: NAFO, Northwest Atlantic Fisheries Organization; sp, spring; s, summer; f, fall; u, unknown.

higher than in most other species, whereas 20:1*n*–9 and 22:1*n*–11 were more abundant in planktivores such as capelin, herring, and sandlance.

CART analysis

We initially investigated variation in fatty acid signatures of the 28 species using all 66 fatty acids. A tree using 20:5*n*–3 at the root node generated 65 terminal nodes and an overall classification success rate of 89% (Table 2). Although the tree was complex, the root node generally separated species according to feeding patterns and life histories with, for instance, all capelin and sand lance classified into the left branch and all invertebrates and most of the flatfish into the right branch of the tree. Tenfold cross-validation with forcing of 20:5*n*–3 at the root node produced a reduced classification success of 73%, as would be expected (see below). Selection of other fatty acids for the root node generated trees with similar separation of species along each branch but with poorer classification success.

Despite the overall high classification success of nearly 90%, there was considerable variation in the proportion correctly classified among species. In 18 of the 28 species, ≥80% of individuals were correctly classified (12 species >90% correct; Table 2). However, in the other 10 species, classification percentage generally varied from 40 to 73%, with one case of 0% in the sea ravens (n = 6). We expected that one source of this variability in success might be sample size, which ranged from 5 to 99 among species. Species represented with data from <20 individuals were significantly more likely to have a classification rate <80% than species represented by a larger number of samples (χ^2 = 11.9, df = 1, P = 0.001).

Discriminant analysis

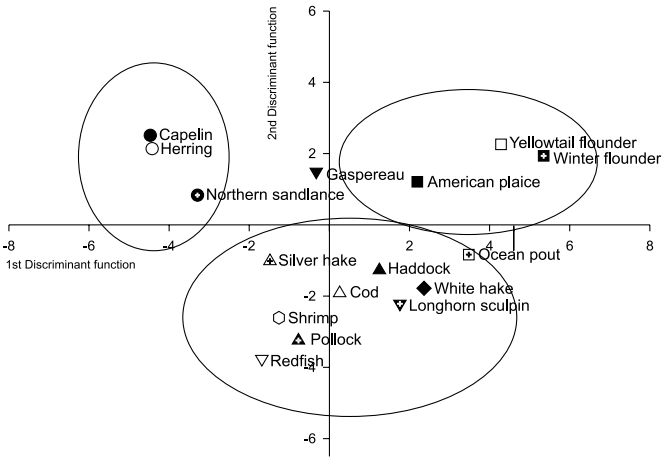
To investigate among-species variation in greater detail, we conducted a discriminant function analysis of the 16 most numerous species. Fifteen discriminant functions, or linear combinations of variables, were generated with the first five functions accounting for 88% of the variance. A plot of the average scores of the first two discriminant functions, representing 57% of the variance, showed three major groups of species that tended to have similar fatty acid compositions (Fig. 2). The first discriminant function separated species into two groups, roughly representing flatfish and gadids in the upper and lower right quadrants and the planktivores (including sand lance, herring, and capelin) in the upper left quadrant. The second discriminant function separated Gadidae species and shrimp from the flatfish. Al-

Table 2. Fat and fatty acid composition of fish and invertebrate species from the Scotian Shelf, Georges Bank, and the Gulf of

	American plaice (n = 99)	Argentine (n = 10)	Butterfish (n = 10)	Capelin (n = 56)	Cod (n = 84)	Gaspereau (n = 41)	Haddock (n = 54)
Length (cm)	27.4 ± 8.5	26.2 ± 1.2	15.5 ± 1.9	13.3 ± 2.2	36.5 ± 6.7	22.8 ± 3.4	27.0 ± 5.8
Mass (g)	217.8 ± 201.9	180.1 ± 27.0	73.1 ± 29.8	12.4 ± 5.9	481.1 ± 264.4	126.3 ± 48.2	202.2 ± 133.2
Lipid content (%)	2.2 ± 1.3	6.6 ± 2.7	7.2 ± 3.1	8.3 ± 4.4	2.1 ± 1.0	12.6 ± 6.7	1.4 ± 0.6
Saturated fatty acids (%)							
14:0	3.52 ± 1.46	6.56 ± 0.44	5.00 ± 0.96	6.26 ± 1.17	2.06 ± 0.84	5.17 ± 1.04	1.97 ± 0.94
16:0	14.35 ± 2.03	11.90 ± 0.89	16.48 ± 1.00	12.48 ± 3.10	14.32 ± 1.79	16.83 ± 1.02	14.39 ± 1.13
18:0	3.73 ± 1.15	2.13 ± 0.19	4.84 ± 0.77	1.14 ± 0.42	3.63 ± 0.95	2.87 ± 0.72	4.08 ± 0.73
Subtotal	21.61 ± 2.31	20.59 ± 0.82	26.32 ± 1.26	19.88 ± 3.98	20.00 ± 2.12	24.87 ± 1.05	20.44 ± 0.93
Monounsaturated fatty acids (%)							
16:1n–7	6.29 ± 3.88	4.77 ± 0.56	3.11 ± 0.74	9.96 ± 2.67	5.19 ± 3.10	3.85 ± 0.56	3.07 ± 1.20
18:1n–9	8.42 ± 1.62	7.40 ± 1.65	22.69 ± 5.37	7.26 ± 3.63	10.14 ± 1.67	15.46 ± 3.47	8.82 ± 1.91
18:1n–7	4.18 ± 1.09	2.04 ± 0.33	2.10 ± 0.32	2.87 ± 1.54	4.71 ± 1.33	3.28 ± 0.66	4.13 ± 0.86
20:1n–11	1.02 ± 0.79	0.65 ± 0.11	0.23 ± 0.22	0.46 ± 0.16	0.71 ± 0.30	0.83 ± 0.20	0.72 ± 0.24
20:1n–9	3.26 ± 2.02	13.52 ± 1.60	4.50 ± 1.27	12.42 ± 4.72	3.96 ± 2.49	5.91 ± 1.28	3.10 ± 1.94
20:1n–7	1.44 ± 0.86	0.56 ± 0.13	1.25 ± 0.31	0.74 ± 0.29	0.74 ± 0.45	0.56 ± 0.17	0.83 ± 0.30
22:1n–11	2.71 ± 2.61	17.67 ± 3.09	2.70 ± 2.65	15.34 ± 6.59	2.64 ± 2.30	5.80 ± 2.93	1.65 ± 1.58
22:1n–9	0.56 ± 0.42	1.69 ± 0.20	3.07 ± 0.84	1.40 ± 0.57	0.48 ± 0.28	0.75 ± 0.43	0.49 ± 0.24
24:1	1.09 ± 0.44	0.43 ± 0.12	0.72 ± 0.28	0.91 ± 0.31	1.06 ± 0.68	0.53 ± 0.22	1.17 ± 0.41
Subtotal	28.96 ± 8.30	48.73 ± 2.92	40.37 ± 3.42	51.36 ± 9.24	29.62 ± 7.99	36.97 ± 3.52	23.98 ± 3.62
Polyunsaturated fatty acids (%)							
18:2n–6	0.93 ± 0.32	0.96 ± 0.12	0.75 ± 0.23	1.19 ± 0.32	0.78 ± 0.20	1.36 ± 0.37	0.76 ± 0.16
18:4n–3	0.84 ± 0.63	1.77 ± 0.42	0.76 ± 0.42	1.34 ± 0.45	0.83 ± 0.47	1.63 ± 0.72	0.75 ± 0.51
20:4n–6	2.51 ± 1.18	0.70 ± 0.07	1.63 ± 0.39	0.34 ± 0.19	1.83 ± 0.81	0.86 ± 0.29	2.54 ± 0.67
20:5n–3	13.90 ± 2.76	7.61 ± 1.13	5.06 ± 0.84	7.39 ± 2.56	13.81 ± 2.23	7.48 ± 0.87	14.77 ± 2.34
22:5n–3	2.65 ± 0.76	1.40 ± 0.24	2.35 ± 0.20	0.74 ± 0.13	1.44 ± 0.30	1.64 ± 0.32	1.90 ± 0.37
22:6n–3	17.03 ± 5.76	9.60 ± 1.72	10.76 ± 2.26	9.65 ± 4.53	22.77 ± 7.50	15.04 ± 3.13	24.77 ± 4.69
Subtotal	37.86 ± 7.17	22.04 ± 2.58	21.31 ± 2.87	20.66 ± 6.10	41.47 ± 6.66	28.00 ± 3.16	45.49 ± 3.92
Total	88.43 ± 1.65	91.36 ± 0.39	88.00 ± 0.79	91.91 ± 0.69	91.09 ± 0.99	89.84 ± 1.00	89.91 ± 1.15
CART Classification	92.9%	100.0%	80.0%	96.4%	90.5%	92.7%	83.3%

Note: Values are mean ± standard deviation of the 17 fatty acids (and 18:0 reference) used in discriminant analysis and MANOVA. Complete data, including individuals correctly classified to species (89.3% overall).

Fig. 2. Discriminant analysis of the 16 species represented by ≥18 individuals and using the 17 fatty acids that exhibited the greatest average variance (see Table 2). Plot shows the average scores of the first 2 of 15 discriminant functions that classified individuals to species with a 98% success rate. Ellipses surrounding the three major clusters of groups are based on the data point clouds of individuals, rather than just the average scores.



though shrimp were grouped with the Gadidae species using only the first two discriminant functions, they separated from those species when the eighth and ninth functions were considered. Similarly, capelin and herring, while grouped closely together on the first two functions, separated well on the fourth and fifth discriminant functions (Fig. 3). Based on both the function coefficients and function-variable correlations, discriminant function 1 was primarily defined by 22:1n–11 and 20:1n–9, whereas function 2 mainly represented the influence of 16:0. Together, the 15 discriminant functions classified species with a 98% success rate using a quadratic classification rule (Fig. 2). Tenfold cross-validation using discriminant analysis, like CART, also resulted in a reduced classification success of 88%. These lower percentages from cross-validation of results of both CART and discriminant analyses are expected simply because the data being classified are not used to create the classification function. Cross-validation gives a more realistic estimate of the classification success that can be expected when the function is applied to a new data set (Stevens 1986).

Within-species variation in fatty acid composition

Size

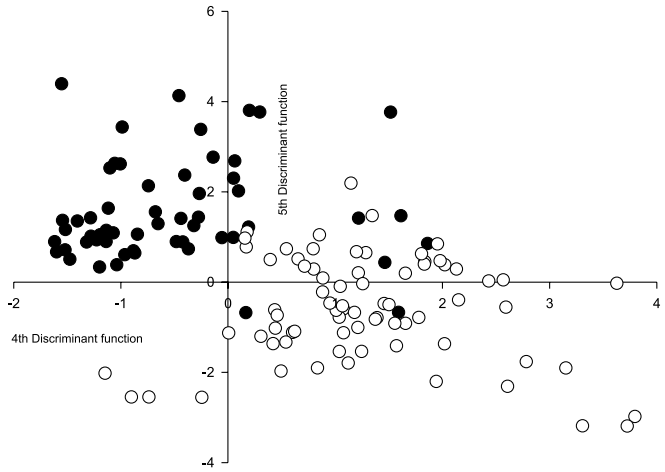
As fish grow, especially in species with a large body size range, they often change their diet. To investigate within-species variation in fatty acid composition as a function of fish length, data for each species were divided into two groups based on either median length in our sample or a length from the literature at which significant changes in diet might be ex-

St. Lawrence (n = 954). Table 2 continues on following pages.

Halibut (n = 8)	Herring (n = 74)	Lobster (n = 9)	Longhorn sculpin (n = 20)	Mackerel (n = 10)	Northern sand lance (n = 71)	Ocean pout (n = 18)	Pollock (n = 25)	Red crab (n = 14)
30.2 ± 4.3	26.0 ± 4.2	19.7 ± 0.8	25.0 ± 3.1	32.5 ± 2.2	18.5 ± 5.0	26.6 ± 6.1	24.8 ± 9.8	10.2 ± 0.9
245.0 ± 139.2	196.0 ± 91.1	243.7 ± 23.5	166.4 ± 72.0	280.5 ± 73.8	14.5 ± 10.1	81.0 ± 57.7	221.1 ± 256.5	354.8 ± 102.4
1.1 ± 0.3	7.7 ± 3.9	2.0 ± 0.6	1.4 ± 1.0	3.4 ± 2.0	5.6 ± 4.3	2.0 ± 1.0	3.0 ± 1.9	1.8 ± 0.4
0.99 ± 0.28	5.33 ± 1.35	2.65 ± 0.51	2.63 ± 0.89	3.59 ± 1.19	5.43 ± 1.49	2.46 ± 1.06	2.89 ± 1.37	1.95 ± 0.32
17.58 ± 0.56	13.65 ± 2.18	11.41 ± 0.67	12.46 ± 1.27	16.41 ± 0.95	13.44 ± 1.69	12.95 ± 1.20	14.53 ± 2.32	10.15 ± 0.71
5.65 ± 0.61	1.39 ± 0.54	3.16 ± 0.28	3.78 ± 0.84	4.58 ± 1.03	2.04 ± 0.81	4.69 ± 1.08	4.26 ± 0.44	2.40 ± 0.50
24.21 ± 0.87	20.37 ± 2.47	17.22 ± 0.78	18.88 ± 1.54	24.59 ± 0.90	20.92 ± 2.20	20.10 ± 0.85	21.68 ± 3.46	14.51 ± 0.69
3.32 ± 1.45	6.24 ± 2.60	6.52 ± 0.43	6.71 ± 2.38	2.96 ± 0.79	6.23 ± 2.12	6.14 ± 2.73	3.53 ± 0.97	6.17 ± 1.00
7.34 ± 1.18	7.25 ± 3.48	10.40 ± 1.50	11.26 ± 1.19	10.99 ± 2.57	5.88 ± 1.59	10.61 ± 3.16	10.77 ± 1.98	15.28 ± 2.11
4.37 ± 0.77	2.34 ± 0.50	6.53 ± 0.73	4.77 ± 0.91	3.46 ± 0.58	2.47 ± 0.64	5.79 ± 1.15	3.51 ± 0.58	4.84 ± 1.01
0.30 ± 0.25	0.96 ± 0.36	1.68 ± 0.37	0.63 ± 0.17	0.66 ± 0.23	0.49 ± 0.16	1.32 ± 0.66	0.71 ± 0.32	1.78 ± 0.37
0.82 ± 0.28	11.09 ± 3.41	4.53 ± 1.55	3.87 ± 1.48	4.92 ± 1.94	7.50 ± 3.02	1.42 ± 0.26	4.40 ± 1.76	6.65 ± 1.22
0.60 ± 0.32	0.50 ± 0.28	1.69 ± 0.20	0.46 ± 0.18	0.52 ± 0.12	0.45 ± 0.23	1.65 ± 0.82	0.40 ± 0.16	0.92 ± 0.38
0.19 ± 0.22	17.27 ± 5.68	3.51 ± 2.26	1.88 ± 1.36	6.07 ± 3.44	9.39 ± 4.20	0.53 ± 0.24	2.68 ± 1.45	6.69 ± 2.04
0.16 ± 0.06	1.23 ± 0.65	0.71 ± 0.21	0.41 ± 0.11	0.99 ± 0.36	1.11 ± 1.06	0.29 ± 0.09	0.49 ± 0.23	1.00 ± 0.21
1.18 ± 0.34	0.75 ± 0.39	0.18 ± 0.05	1.20 ± 0.51	1.25 ± 0.21	1.34 ± 0.33	1.10 ± 0.60	1.01 ± 0.38	0.44 ± 0.11
18.27 ± 3.76	47.61 ± 8.90	35.76 ± 2.91	31.19 ± 3.92	31.81 ± 6.72	34.84 ± 7.70	28.84 ± 4.54	27.51 ± 5.84	43.78 ± 2.92
0.61 ± 0.15	1.15 ± 0.34	0.84 ± 0.08	1.27 ± 0.40	1.49 ± 0.17	1.49 ± 0.50	0.86 ± 0.11	1.00 ± 0.18	0.83 ± 0.06
0.17 ± 0.08	1.51 ± 0.80	0.83 ± 0.18	0.67 ± 0.36	1.44 ± 0.70	1.98 ± 1.17	0.43 ± 0.26	1.35 ± 0.39	0.25 ± 0.05
5.43 ± 0.84	0.42 ± 0.28	6.33 ± 1.17	3.00 ± 1.20	1.50 ± 0.68	0.59 ± 0.31	4.06 ± 1.29	1.11 ± 0.44	3.04 ± 0.74
9.59 ± 1.04	7.77 ± 1.57	17.04 ± 1.10	13.78 ± 1.84	8.03 ± 1.21	12.96 ± 1.87	15.07 ± 2.76	11.03 ± 2.19	12.13 ± 1.32
2.56 ± 0.27	0.83 ± 0.13	1.29 ± 0.20	1.66 ± 0.32	1.58 ± 0.27	1.03 ± 0.32	1.98 ± 0.51	1.00 ± 0.36	2.25 ± 0.99
30.60 ± 4.38	12.46 ± 6.89	7.69 ± 1.06	19.86 ± 3.14	19.34 ± 5.89	16.17 ± 4.90	14.32 ± 4.58	25.58 ± 8.39	11.93 ± 2.46
48.95 ± 3.97	24.15 ± 7.71	34.01 ± 2.27	40.24 ± 4.01	33.37 ± 6.98	34.23 ± 6.09	36.71 ± 6.87	41.08 ± 8.91	30.42 ± 2.18
91.44 ± 0.83	92.13 ± 1.05	86.99 ± 0.85	86.21 ± 1.57	89.76 ± 0.84	89.99 ± 1.70	85.64 ± 3.57	90.26 ± 0.70	88.70 ± 2.19
50.0%	98.6%	100.0%	70.0%	70.0%	94.4%	50.0%	80.0%	42.9%

all 66 fatty acids quantified among individuals, are available on request from the corresponding author. CART classification is the percentage of

Fig. 3. Plot of scores for capelin and herring fatty acids based on the fourth and fifth discriminant functions (see Fig. 2) (solid circles, capelin; open circles, herring).



pected (i.e., for American plaice, cod, redfish, winter flounder, and yellowtail flounder). Confounding of length with geographic location of sampling was a concern with most species. Thus, we conducted a MANOVA on the main effects of both length and location, as well as their interaction, for each species (Table 3). For some species, the number of fatty acids (i.e., 17) exceeded the number of samples in the subsets; thus, fatty acids accounting for the greatest variance were retained with the constraint that the number of fatty acids was less than the number of samples within each length or area class.

Nine species exhibited a significant effect of length on fatty acid signature ($P < 0.05$) and two others were close to significance ($P < 0.06$; Table 3). Of all of the fish species considered, only longhorn sculpin displayed a significant length effect without a concurrent significant interaction with location. For this species, Tukey's test identified 22:1n–11 as the only univariately significant fatty acid (Table 3). To examine some of the variation in fatty acid composition with length in other species, we selected only a single area, each with the largest sample size, for illustration (Fig. 4). Redfish and sand lance had the greatest number of significantly different fatty acids among the two size classes, including 16:1n–7, 22:1n–11, and 20:5n–3, as well as several other fatty acids not shown. Fewer differences were apparent among size classes of capelin and herring with only 16:1n–7, 22:1n–11, and 22:6n–3 being significantly different in capelin and 14:0 and 18:4n–3 being significantly different in herring. To further illustrate the variation with size, significant correlations of selected fatty acid levels with fish length may be shown (Fig. 5). In the examples shown, there were clear decreases in levels of the indicated fatty acid with increasing size. However, among all four species, there were no consistent trends in variation of fatty acid levels with size. For example, in capelin, redfish, and sandlance, levels

Table 2 (*concluded*).

	Red hake (n = 7)	Redfish (n = 49)	Rock crab (n = 10)	Sea raven (n = 6)	Shrimp (n = 46)	Silver hake (n = 38)	Smooth skate (n = 5)	Thorny skate (n = 12)
Length (cm)	29.3 ± 5.1	27.6 ± 9.2	8.1 ± 0.6	27.2 ± 5.5	11.5 ± 1.0	22.8 ± 6.9	29.7 ± 8.2	33.0 ± 3.0
Mass (g)	183.7 ± 102.8	405.6 ± 339.3	184.4 ± 44.5	389.9 ± 238.8	10.5 ± 4.1	88.9 ± 74.4	120.7 ± 91.1	297.1 ± 64.4
Lipid content (%)	1.7 ± 0.8	6.3 ± 2.9	0.8 ± 0.2	0.8 ± 0.3	2.6 ± 0.7	2.2 ± 1.4	1.4 ± 0.4	1.1 ± 0.2
Saturated fatty acids (%)								
14:0	1.32 ± 0.40	3.74 ± 0.74	1.69 ± 0.55	1.20 ± 0.74	2.89 ± 0.43	2.34 ± 0.96	1.66 ± 0.60	1.77 ± 0.56
16:0	15.08 ± 1.14	9.39 ± 1.84	10.44 ± 0.72	13.88 ± 1.85	11.42 ± 0.94	16.80 ± 1.25	16.65 ± 2.16	16.82 ± 0.65
18:0	5.87 ± 0.45	2.43 ± 0.51	3.18 ± 0.27	5.31 ± 1.55	1.93 ± 0.33	3.72 ± 1.06	4.23 ± 1.16	4.04 ± 0.59
Subtotal	22.27 ± 1.10	15.56 ± 2.67	15.31 ± 1.15	20.40 ± 2.63	16.24 ± 1.10	22.86 ± 1.32	22.54 ± 2.69	22.63 ± 0.74
Monounsaturated fatty acids (%)								
16:1*n*–7	2.87 ± 1.37	7.63 ± 2.23	5.93 ± 0.78	6.71 ± 3.77	8.74 ± 1.59	3.20 ± 1.54	7.60 ± 2.52	6.57 ± 1.74
18:1*n*–9	12.13 ± 2.63	8.66 ± 3.34	8.16 ± 1.39	13.29 ± 1.20	11.76 ± 2.63	11.29 ± 1.81	10.68 ± 0.48	12.63 ± 1.63
18:1*n*–7	5.59 ± 1.34	3.33 ± 0.63	7.45 ± 1.83	7.28 ± 0.69	6.83 ± 1.93	3.23 ± 0.76	6.99 ± 0.26	6.40 ± 0.54
20:1*n*–11	0.52 ± 0.15	1.07 ± 0.43	1.37 ± 0.35	0.48 ± 0.27	1.40 ± 1.03	1.02 ± 0.32	0.49 ± 0.18	0.73 ± 0.19
20:1*n*–9	1.94 ± 0.58	14.91 ± 3.05	3.85 ± 1.49	1.77 ± 0.87	4.85 ± 1.45	5.16 ± 2.36	1.08 ± 0.39	1.92 ± 0.49
20:1*n*–7	0.46 ± 0.19	1.37 ± 0.63	1.84 ± 0.62	0.74 ± 0.32	1.53 ± 0.32	0.33 ± 0.12	0.86 ± 0.41	1.03 ± 0.15
22:1*n*–11	0.81 ± 0.33	15.91 ± 3.70	3.63 ± 2.01	0.50 ± 0.49	6.74 ± 2.35	4.16 ± 3.07	0.24 ± 0.19	0.66 ± 0.59
22:1*n*–9	0.26 ± 0.06	3.02 ± 1.74	0.61 ± 0.23	0.24 ± 0.05	1.56 ± 0.71	0.54 ± 0.36	0.30 ± 0.07	0.45 ± 0.11
24:1	1.29 ± 0.46	0.87 ± 0.41	0.30 ± 0.08	0.72 ± 0.16	0.30 ± 0.21	1.48 ± 0.73	0.27 ± 0.09	0.31 ± 0.09
Subtotal	25.86 ± 4.94	56.76 ± 7.66	33.15 ± 2.81	31.73 ± 5.57	43.71 ± 2.30	30.40 ± 6.52	28.50 ± 3.62	30.69 ± 2.23
Polyunsaturated fatty acids (%)								
18:2*n*–6	0.79 ± 0.20	0.90 ± 0.22	1.00 ± 0.13	0.91 ± 0.31	1.00 ± 0.18	1.05 ± 0.22	1.50 ± 0.29	1.41 ± 0.10
18:4*n*–3	0.44 ± 0.23	1.07 ± 0.41	0.42 ± 0.13	0.48 ± 0.42	0.71 ± 0.29	0.70 ± 0.38	0.90 ± 0.50	0.65 ± 0.31
20:4*n*–6	2.59 ± 0.96	0.57 ± 0.39	4.05 ± 1.11	4.40 ± 1.63	1.66 ± 0.72	1.45 ± 0.51	3.09 ± 0.48	3.46 ± 0.74
20:5*n*–3	9.90 ± 3.16	7.39 ± 1.81	20.74 ± 2.19	12.44 ± 1.53	15.26 ± 1.30	9.66 ± 2.56	10.02 ± 2.24	8.30 ± 1.45
22:5*n*–3	2.25 ± 0.44	0.79 ± 0.14	2.06 ± 0.40	2.32 ± 1.00	0.74 ± 0.21	1.11 ± 0.23	1.75 ± 0.25	2.35 ± 0.25
22:6*n*–3	25.16 ± 7.13	8.23 ± 3.10	10.35 ± 1.74	18.73 ± 3.57	11.37 ± 1.99	23.64 ± 6.28	20.87 ± 3.20	21.89 ± 2.14
Subtotal	41.13 ± 4.79	18.95 ± 4.60	38.62 ± 3.12	39.27 ± 4.14	30.75 ± 2.27	37.61 ± 6.28	38.14 ± 1.37	38.06 ± 1.71
Total	89.26 ± 1.14	91.27 ± 0.96	87.08 ± 0.87	91.40 ± 1.14	90.70 ± 1.86	90.87 ± 0.69	89.18 ± 0.51	91.39 ± 0.54
CART Classification	57.1%	100.0%	70.0%	0.0%	100.0%	89.5%	40.0%	91.7%

of 22:1*n*–11 were greater in the larger size class, but in herring, the opposite effect was observed, likely reflecting different types of diet shifts in the species considered.

Geographic location

Given that there are broad-scale geographic differences in prey assemblages, and therefore, in the diets of fishes, we also investigated the effect of sampling location on fatty acid composition within species. Species with <4 samples from one area were dropped from the analysis and the number of fatty acids included was chosen as described above, based on maximizing retained variance. All species had a significant Wilks' Lambda, indicating differences among geographic locations, but only six of these did not exhibit a significant interaction between fish length and area (Table 3). To illustrate some of these differences, discriminant analysis was carried out on three of the better-represented species (i.e., American plaice >25 cm in length, cod, and yellowtail flounder), again adjusting the number of fatty acids used (Fig. 6). In each species, there was evidence that different stocks differed in fatty acid composition. In American plaice, there was some overlap of samples from subareas 5Z and 4Vs, indicating similarity in the composition of the fatty acids that are most closely related to those two discriminant functions (20:5*n*–3 and 16:1*n*–7 on the first function and 20:1*n*–9 and 22:1*n*–11 on the second function). Nevertheless, a plot of individual scores for these and other selected species according to subarea and placed in the original discriminant analysis plot for all 16 species (Fig. 7) illustrates that despite the clear separation among stocks on a fine scale, members of each species were more similar to each other in fatty acid signature than to the other species.

Discussion

The results of this study demonstrate that fatty acid signatures of fish and invertebrate species can be used not only to distinguish and characterize species in a given ecosystem, but also to study finer-scale within-species variation with size, and possibly, within regional food webs. In general, individuals were accurately classified to their species based on fatty acid signature. As stated earlier, we made no attempt to remove stomach contents of the individuals we analyzed because we wished to characterize them as prey that would be eaten whole by a predator. However, this may have increased variability within species, resulting in a more conservative classification success.

When all 66 fatty acids were considered, CART successfully classified 89% of the samples, demonstrating the information contained within fatty acid signatures. These signatures were distinctive for most species, with 18 of the 28 species having a classification success rate greater than 80% (12 of which were >90%). In the 10 more poorly classified species with sample sizes of <20, the within-species variance in the fatty

White hake (n = 46)	Winter flounder (n = 25)	Winter skate (n = 15)	Yellowtail flounder (n = 92)
32.9 ± 5.3	27.0 ± 6.6	35.6 ± 4.0	26.8 ± 6.1
281.1 ± 142.8	274.8 ± 189.8	262.2 ± 112.3	189.8 ± 138.6
1.3 ± 0.8	1.9 ± 0.8	1.5 ± 0.6	2.7 ± 1.3
1.49 ± 0.61	1.96 ± 0.69	1.58 ± 1.09	2.72 ± 0.87
14.39 ± 1.05	15.04 ± 0.86	16.94 ± 2.20	14.01 ± 1.53
5.37 ± 0.92	4.51 ± 0.85	3.98 ± 0.90	3.56 ± 0.96
21.26 ± 1.40	21.51 ± 0.91	22.50 ± 2.02	20.30 ± 1.78
3.59 ± 1.52	5.21 ± 2.92	4.30 ± 1.20	6.47 ± 2.90
12.08 ± 2.82	7.31 ± 1.61	9.30 ± 0.73	9.75 ± 2.57
5.69 ± 1.08	3.61 ± 0.86	5.24 ± 0.96	4.25 ± 0.75
0.72 ± 0.35	0.55 ± 0.27	0.59 ± 0.35	0.76 ± 0.32
3.18 ± 2.37	1.27 ± 0.30	2.10 ± 2.10	1.34 ± 0.38
0.66 ± 0.24	2.52 ± 1.57	0.81 ± 0.36	1.65 ± 0.74
1.75 ± 1.80	0.23 ± 0.09	1.19 ± 2.18	0.49 ± 0.28
0.36 ± 0.22	0.35 ± 0.11	0.37 ± 0.15	0.25 ± 0.11
0.90 ± 0.46	0.61 ± 0.49	0.54 ± 0.27	0.69 ± 0.41
28.92 ± 7.25	21.67 ± 5.72	24.44 ± 4.47	25.65 ± 4.83
0.80 ± 0.17	0.60 ± 0.17	1.34 ± 0.24	1.03 ± 0.24
0.43 ± 0.27	0.48 ± 0.28	0.64 ± 0.41	0.95 ± 0.57
2.29 ± 0.94	3.58 ± 0.99	3.24 ± 1.22	2.55 ± 1.25
9.61 ± 1.54	14.43 ± 2.14	7.78 ± 1.91	15.02 ± 3.32
2.69 ± 0.61	3.82 ± 0.97	2.93 ± 0.75	3.31 ± 0.97
24.83 ± 5.90	20.10 ± 4.26	26.06 ± 4.61	18.73 ± 4.84
40.66 ± 6.67	43.01 ± 5.65	41.99 ± 3.71	41.59 ± 4.19
90.84 ± 0.89	86.19 ± 2.32	88.93 ± 2.24	87.53 ± 2.07
91.3%	84.0%	73.3%	89.1%

acid signature was too large to allow accurate identification. There were several exceptions where species with only 9–12 samples (e.g., argentine, lobster, and thorny skate) were nevertheless accurately classified (92–100%), perhaps because these species had fatty acid signatures that were particularly unusual or relatively invariant. One limitation of CART is that it is based on the calculation of greatest deviation among samples remaining at each node, and such dichotomous splits provide no indication of the overall relationships of fatty acid composition among species. In this study, with the exception of the crabs, the 10 poorly classified species tended to be randomly misidentified rather than misidentified as a related species. For example, the three misclassified smooth skates (n = 5) were classified as cod and longhorn sculpin rather than thorny or winter skates.

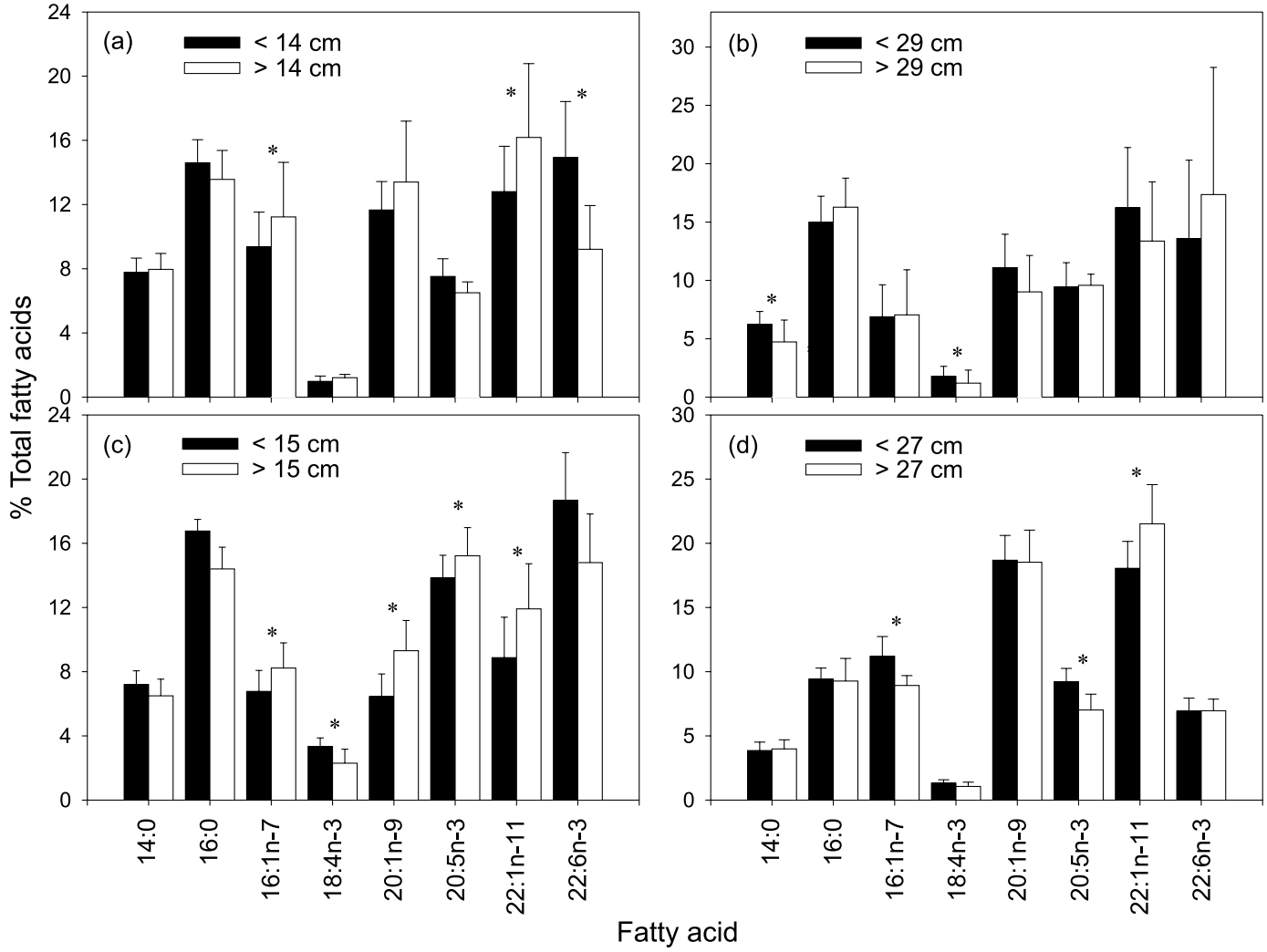
Discriminant analysis of the 16 most numerous species resulted in high (98%) classification success. Plots of these analyses also serve to illustrate the relationships between species' fatty acid signatures. For instance, all of the Gadidae species (cod, haddock, pollock, silver hake, and white hake) grouped together, whereas the Pleuronectidae species (American plaice, winter flounder, and yellowtail flounder) formed a separate group. Certainly, some similarity in fatty acid composition among such closely related species may be expected because of shared patterns in life histories and feeding strategies. However, ocean pout, redfish, and sculpin were also included in the Gadidae cluster,

Table 3. Results of MANOVA of fish species ($n \geq 18$ individuals) showing main effects of body length and location (NAFO subarea; see Fig. 1), as well as their interaction, on fatty acid composition.

			Variation with length			Variation with location	
	$P_{interaction}$	Median length (cm)	P_{length}	Univariately significant fatty acids	Locations	$P_{location}$	Univariately significant fatty acids
American plaice	<0.001	25[b]	<0.001	20:1*n*–11, 20:1*n*–7, 22:6*n*–3	4Vs, 4X, 5Z, 4T	<0.001	All
Capelin	ND	13	0.008	22:6*n*–3	4Vs, 4W, 4T	<0.001	16:0, 20:1*n*–9, 22:6*n*–3
Cod	0.052	35[a]	0.056	—	4W, 5Z, 4T	<0.001	All
Gaspereau	<0.001	23	0.003	18:1*n*–9, 20:1*n*–7, 22:6*n*–3	4W, 4T	<0.001	20:4*n*–3, 22:5*n*–3, 22:6*n*–3
Haddock	<0.001	27	<0.001	14:0, 16:1*n*–7, 20:5*n*–3	4X, 4W	<0.001	14:0, 20:1*n*–9, 22:1*n*–11
Herring	ND	27	<0.001	16:0, 20:5*n*–3, 24:1	4W, 4T	<0.001	16:0, 22:1*n*–7, 22:5*n*–3
Longhorn sculpin	0.48	25	0.035	22:1*n*–11	4Vs, 4W	<0.001	18:1*n*–9, 20:4*n*–6
Northern sand lance	0.013	17	<0.001	18:2*n*–6, 20:4*n*–6, 22:6*n*–3	4Vs, 4W	<0.001	18:1*n*–7, 18:2*n*–6, 20:5*n*–3
Ocean pout	0.48	28	0.33	——	4X, 4W	0.001	18:1*n*–9, 18:1*n*–7, 22:6*n*–3
Pollock	ND	17	0.75	——	4Vn, 4X	0.006	16:0, 20:1*n*–9, 22:6*n*–3
Redfish	<0.001	27[c]	<0.001	18:1*n*–9, 22:1*n*–11	4Vn, 4X, 4T	<0.001	16:1*n*–7, 22:1*n*–11, 22:1*n*–9
Silver hake	<0.001	23	<0.001	16:0, 18:1*n*–9, 18:4*n*–3	4W, 4X	<0.001	18:1*n*–7, 20:5*n*–3, 24:1
White hake	0.62	33	0.13	——	4Vs, 4T	0.044	None
Winter flounder	0.26	25[b]	0.088	——	4W, 4X	0.002	16:1*n*–7, 18:1*n*–9, 20:1*n*–7
Yellowtail flounder	0.097	25[b]	0.055	——	4W, 5Z, 4T	0.003	18:1*n*–9, 18:4*n*–3, 20:5*n*–3

Note: Significant diet changes were expected in species at the length denoted by a superscript based on [a]Bundy et al. (2000), [b]Martell and McClelland (1994), and [c]Cannalejo et al. (1989). Univariate significance was determined using Tukey's test. ND, not determined.

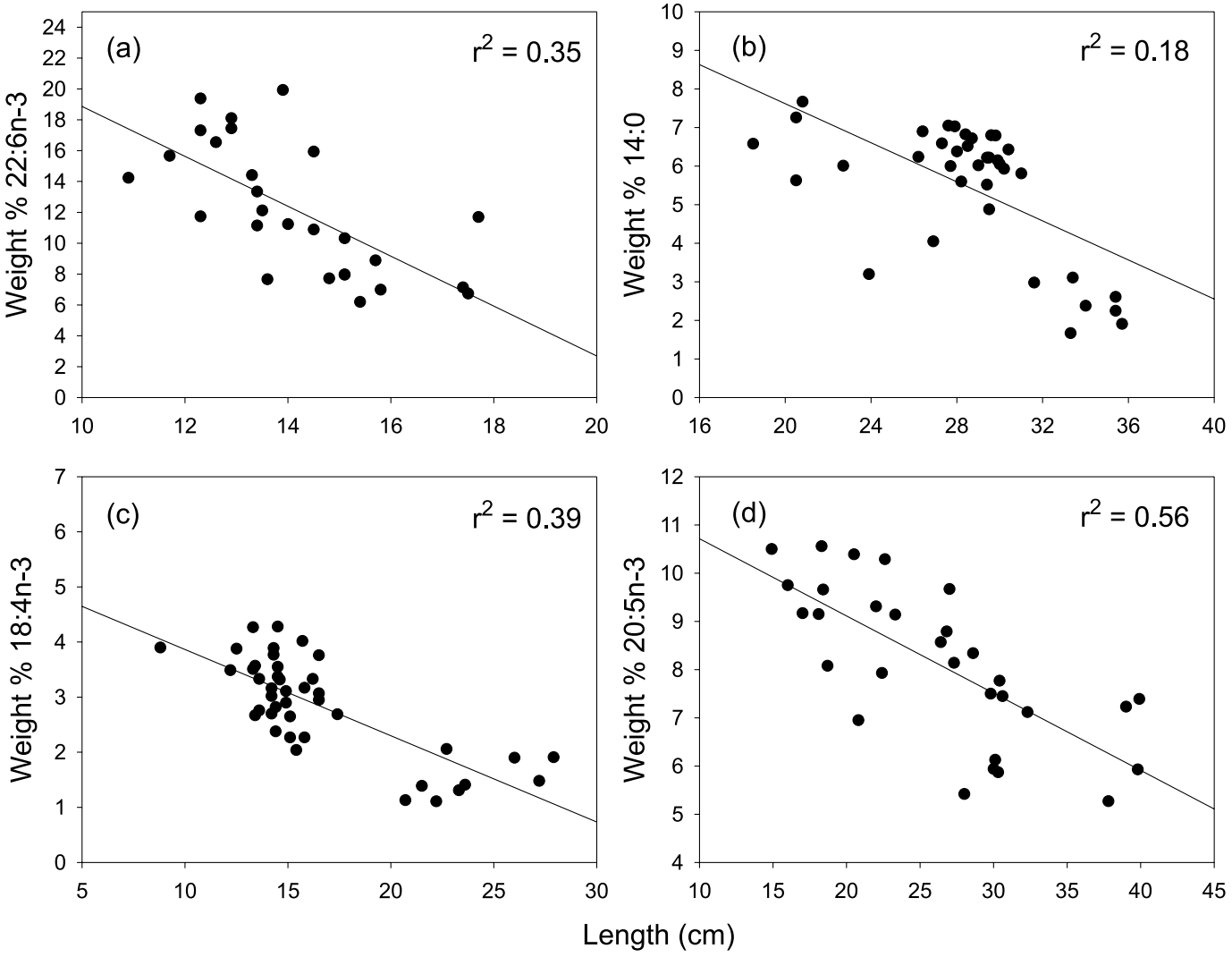
Fig. 4. Variation in selected fatty acids with body length within one major location for (*a*) capelin (4W), (*b*) herring (4W), (*c*) northern sand lance (4W), and (*d*) redfish (4T). For each species, the median length for that specific location was used to classify species as small and large. The fatty acids illustrated included those that were the most univariately significant (e.g., Table 3) across the four species. Asterisks indicate statistically significant differences. A greater number of fatty acids are univariately significant in this plot than indicated in Table 3 because only data from a single area were considered, removing constraints placed on the number of fatty acids that could be used in the analyses. Use of a single location also eliminates any potential interaction of length and area.



indicating that factors other than phylogeny, i.e., diet, play an important role in fatty acid composition (e.g., Kirsch et al. 1998). That is, unrelated species with similar diets would be expected to share similar patterns in fatty acid signatures. For instance, capelin, herring, and sand lance, members of three different families that share a similar and predominantly planktivorous diet (Scott and Scott 1988), were located in a third separate group, further illustrating the influence of diet on fatty acid composition. The classification of shrimp with the Gadidae cluster was somewhat unexpected, particularly because shrimp and gadids have very different diets. However, gadids eat shrimp. In fact, shrimp may compose up to 20% by weight of the diet of Gadidae species (Bundy et al. 2000). Thus, if the gadids that we analyzed were consuming diets high in shrimp, this could explain the similarity in some of the signatures displayed in the first two discriminant functions. However, without analyses of stomach contents, we can only speculate as to the diets of the gadids analyzed. Gaspereau was the only species that was not included in any group, suggesting that its fatty acid signature was unusual, possibly because of its anadromous nature. For instance, lipids from freshwater fish typically contain higher proportions of saturated fatty acids, C18 polyunsaturated fatty acids (PUFA) and *n*–6 PUFA than marine fish owing to consumption of different diets (Henderson and Tocher 1987). In our data set, Gaspereau did contain elevated levels of both saturated fatty acids and C18 PUFA.

The 17 fatty acids with greatest variance across all species were selected for the multivariate analyses based on the assumption that the fatty acids with the largest fluctuations in levels would provide the most useful information in differentiating among species. It is possible that a different subset of fatty acids may generate a better classification scheme. For example, the use of only dietary fatty acids excludes such major fatty acids as 16:0 and 18:1*n*–9 (which can be readily biosynthesized) and produces a less accurate classification

Fig. 5. Correlation of the indicated fatty acid with body length within one major location for (*a*) capelin (4W), (*b*) herring (4W), (*c*) northern sand lance (4W), and (*d*) redfish (4T). The fatty acids illustrated were both univariately significant (see Fig. 4) and significantly correlated with length.

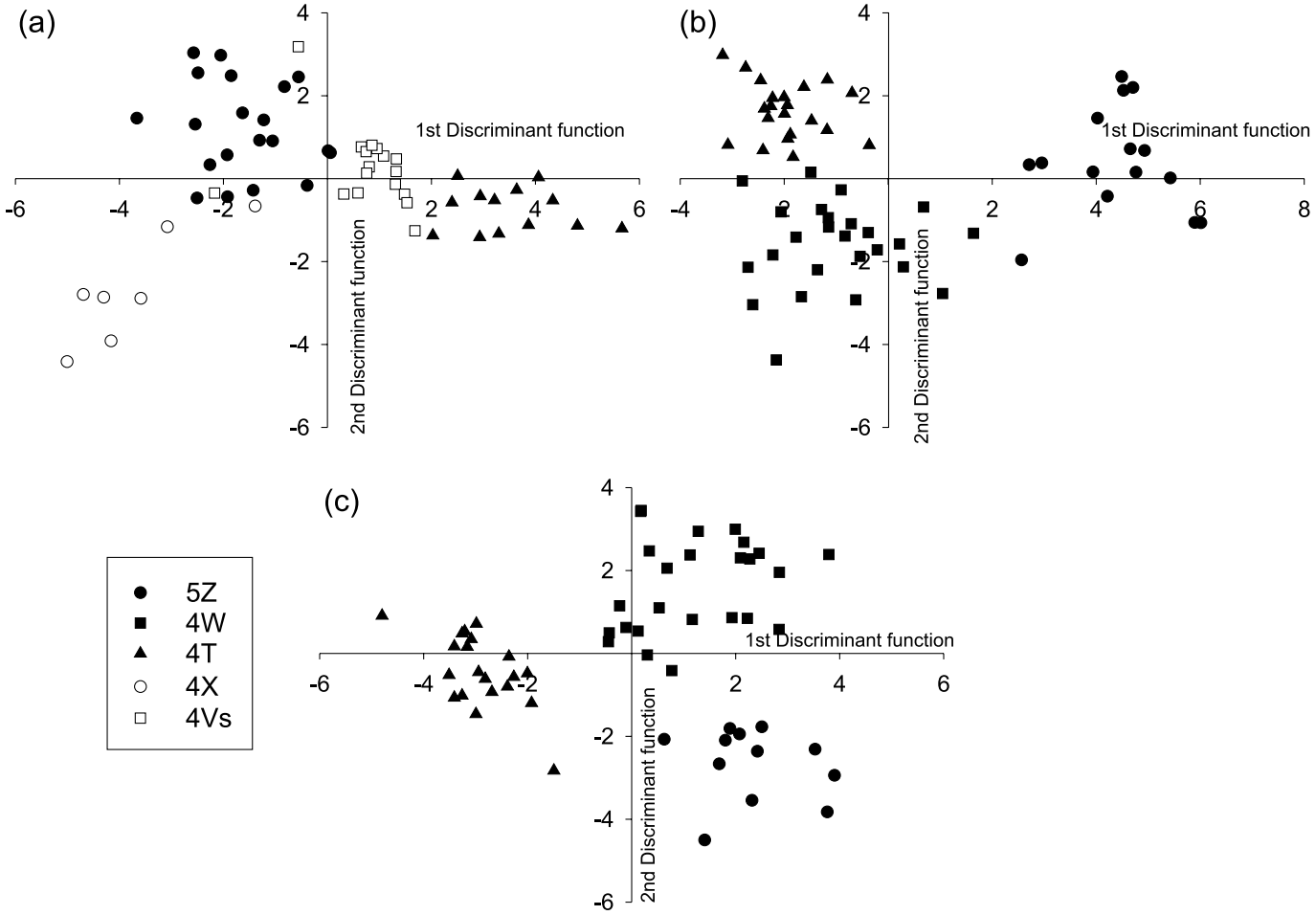


scheme, but one that more clearly represents fish with similar diets. Thus, it may still be useful in supplementing stomach content analyses when determining fish diets. It is also possible that using a larger number of fatty acids would result in better resolution, for instance, in the separation of shrimp from the Gadidae species. This would be possible with a greater number of samples for each species collected and would avoid subjective variable selection.

A number of investigations have reported variation in diet with fish size (Lilly 1986; Canalejo et al. 1989; Martell and McClelland 1994); thus, it is not surprising that we found differences in fatty acid composition as a function of fish length. Both capelin and herring are planktonic feeders, but at larger body sizes, they are known to consume other small fish (Gerasimova 1994). Similarly, changes in the diet of redfish with body size are well documented in stomach content analyses (Canalejo et al. 1989). Thus, dietary fatty acid intake and deposition should differ in fish consuming these differing diets. For instance, the fatty acids 20:1*n*–9 and 22:1*n*–11 are associated with zooplankton, specifically copepod lipids (Graeve et al. 1994), and variation in levels of these two fatty acids likely reflects varying amounts of zooplankton in their diets. However, the variation in fatty acid composition in northern sand lance (*Ammodytes dubius*) was unexpected. The diet of sand lance is reported to be exclusively planktonic (Scott 1973), so a shift in diet to small fish species with increasing body size, as seen in capelin and herring, seems unlikely. Nonetheless, the significant differences observed in the fatty acid composition of the larger sand lance (with lengths up to 28 cm) indicate consumption of diets that are different than those of the smaller fish.

More detailed information is available on diets from stomach content analyses in American plaice, winter flounder, and yellowtail flounder. In both flounders, Martell and McClelland (1994) found shifts in the diet of small fish to large fish. Although small flounder consumed mainly small cumaceans, amphipods, and tunicates, large flounder consumed larger infauna such as polychaetes and tubaceous amphipods. The diets of American plaice have also been found to vary markedly with length (Martell and McClelland 1994),

Fig. 6. Discriminant analyses displaying geographic effects on fatty acid composition within single species for (*a*) American plaice (>25 cm), (*b*) cod, and (*c*) yellowtail flounder. Discriminant scores on the first and second functions for three separate analyses are plotted. The species selected were sampled in at least three areas and did not exhibit a significant interaction between area and length, or in the case of American plaice, only samples from one size class were used.

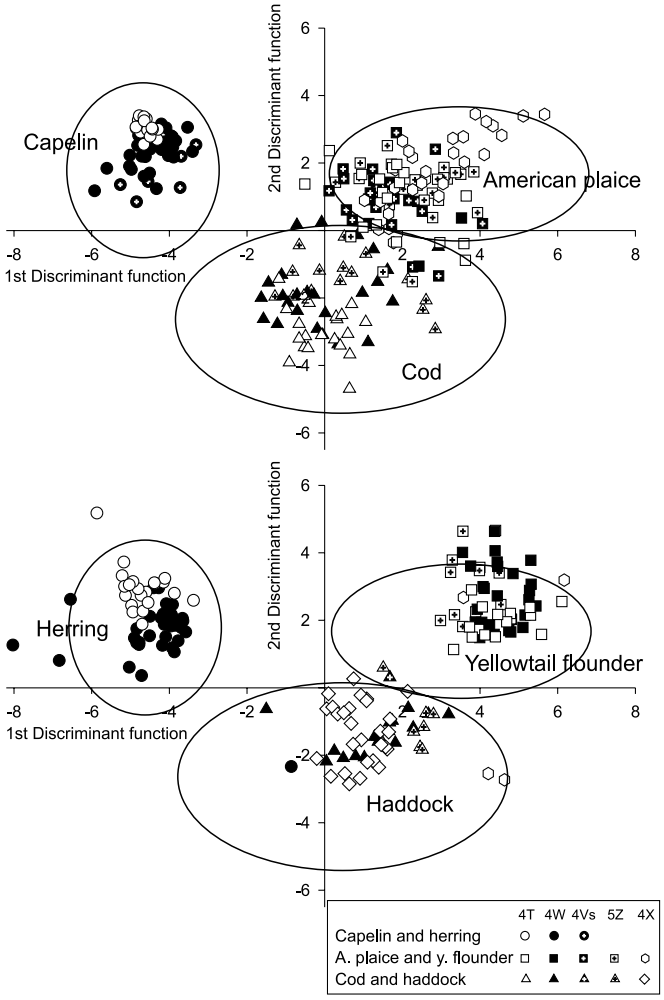


with small fish ingesting small invertebrates, such as amphipods and mysids, and larger fish consuming decapods, echinoderms, and fish. In American plaice and yellowtail flounder, the previously described diet shifts occurred primarily at 20–30 cm (Martell and McClelland 1994), which agreed well with our results from fatty acid analyses.

The diets of Atlantic cod and redfish also vary with fish length (Canalejo et al. 1989). In cod, at lengths greater than 50 cm, a dramatic increase in the amount of teleosts and a decrease in the amount of invertebrates were reported in the diet. At 30 cm, a more subtle change was apparent with more shrimp and molluscs consumed and fewer polychaetes, euphausiids, and mysids. Similarly, it was reported that in redfish at lengths greater than 27 cm, more chaetognaths and finfish and fewer invertebrates were consumed. The fatty acid data are consistent with this information, with differences in composition being observed at 35 and 27 cm for cod and redfish, respectively. The average length of cod in our data set was 37 cm, with only one sample exceeding 50 cm. Had we sampled larger cod (>50 cm), perhaps a significant change in fatty acid composition would also have been apparent. The agreement of changes in fatty acid signatures with changes in diets as reported from stomach content analysis in flatfish, cod, and redfish suggests that the differences in fatty acid composition for the other species considered in this study likely indicate general dietary differences with size. Larger fish are capable of consuming larger prey, and it has been suggested that changes in mouth morphology are responsible for diet variation (Hacunda 1981).

Variability in fatty acid composition among geographic locations may also reflect changes in fish diet. There are known to be broad-scale differences in prey assemblages in the Northwest Atlantic, and some variation may exist among the regions we sampled. Additionally, phytoplankton composition at the base of the food web fluctuates seasonally (Parrish et al. 1995), and the fatty acid composition varies among classes of phytoplankton (Volkman et al. 1989; Viso and Marty 1993) and with characteristic water temperature, salinity, incident light, and available nutrients, all of which may differ geographically. This can result in differences in the fatty acid signatures of phytoplankton in a study area, and thus, the fatty acid composition of food available to higher trophic levels. For instance, Fraser et al. (1989) documented changes in zooplankton fatty acids corresponding to changes in phytoplankton fatty acids. Such changes in fatty

Fig. 7. Plot of individual scores for six selected species according to sampling location from discriminant analyses of all 16 species (see Fig. 2). Ellipses represent point clouds as drawn in Fig. 2.



acid composition in zooplankton are, in turn, expected to influence the fatty acid signature of fish consuming that zooplankton (St. John and Lund 1996). Although we might not expect differences in prey assemblages and primary production to be particularly great across the Scotian Shelf, some differences in fatty acid signatures within species were evident. In the species most broadly sampled, it appeared that the largest differences within species occurred between the three major regions of the Scotian Shelf (4V, 4W, 4X), Georges Bank (5Z), and the Gulf of St. Lawrence (4T), where we would expect different water temperatures, salinities, and nutrient-supplying currents. However, despite these broad-scale and more subtle geographical differences, within-species variation in fatty acid composition was still less than between-species variation.

Although our main objective was to demonstrate the value of using fatty acid signatures to differentiate among fish species, there exist many potential uses of fatty acids in understanding trophic dynamics and predator–prey interactions. In addition to identifying species with similar diets, it may be possible to determine interannual changes in the diets of separate stocks of fish. In migratory fish, like mackerel, fatty acid signatures may provide information on spawning and feeding locations. Furthermore, the fatty acid data reported here will have applications at higher trophic levels. If fish and other prey species from a given geographic region can be distinguished by their fatty acid signatures, then they can be used to estimate the prey composition in the diet of predators like marine mammals (S.J. Iverson, C. Field, W.D. Bowen, and W. Blanchard, unpublished data). This paper represents the first step toward documenting differences in fish and invertebrate fatty acid compositions on the Scotian Shelf, Georges Bank, and Gulf of St. Lawrence. These data, combined with other techniques, will be valuable in investigating ecological relationships at a number of trophic levels.

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