Dietary effects on the fatty acid signature of whole Atlantic cod (*Gadus morhua*)

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Abstract: Understanding the influence of dietary fatty acids on whole fish is necessary to evaluate the degree to which fatty acids may be used for understanding foraging patterns in fish, as well as in marine mammals that consume their prey whole. Adult Atlantic cod (*Gadus morhua*) were fed two prey items that differed significantly in fat content and fatty acid compositions. Cod were first fed squid *(Illex illecebrosus*, 2.0% fat) for 6 weeks, followed by Atlantic mackerel (*Scomber scombrus*, 15.7% fat) for 8 weeks. Twenty whole cod were individually analyzed at each of 0, 3, 6, 11, and 14 weeks. Despite being on a low-fat squid diet, in only 3 weeks, cod fatty acid patterns changed significantly to reflect the patterns found in squid and did not further change at 6 weeks. When switched to a high-fat mackerel diet, total body fat of cod increased and the fatty acid composition of cod changed significantly in the direction of patterns found in mackerel. Despite changes in cod fatty acid signatures, cod were readily distinguished from the fatty acid signatures of their diets. Our results provide support for the use of fatty acids as indicators of diet at upper trophic levels.

Résumé : On doit connaître l'influence des acides gras alimentaires sur le poisson entier pour établir dans quelle mesure les acides gras peuvent être utilisés pour comprendre les comportements de recherche de nourriture des poissons et des mammifères marins qui consomment leurs proies entières. On a nourri des morues (*Gadus morhua*) adultes avec deux types de proie dont les teneurs en gras et les compositions en acides gras différaient significativement. Les morues ont d'abord consommé du calmar (*Illex illecebrosus*, 2,0 % de matières grasses) durant 6 semaines, puis du maquereau (*Scomber scombrus*, 15,7 % de matières grasses) durant 8 semaines. Vingt morues entières ont été individuellement analysées à chacune des étapes suivantes : 0, 3, 6, 11 et 14 semaines. Bien que les morues aient été exposées à un régime de calmar faible en matières grasses, en seulement 3 semaines, les profils d'acides gras des morues ont changé significativement pour refléter ceux du calmar, et ils étaient les mêmes à 6 semaines qu'à 3 semaines. Quand les morues sont passées au régime de maquereau riche en matières grasses, leur teneur corporelle totale en matières grasses s'est accrue et leur composition en acides gras a changé significativement pour s'approcher de celle du maquereau. Malgré les changements dans les profils d'acides gras de la morue, ces derniers demeuraient nettement distincts des profils d'acides gras de ses proies. Nos résultats indiquent qu'il est possible d'utiliser les acides gras comme indicateurs du régime alimentaire dans les niveaux trophiques supérieurs.
[Traduit par la Rédaction]

Introduction

The concept of using lipids as biomarkers in marine ecosystems has received considerable attention in the past few decades (e.g., Sargent et al. 1988). Some of the first evidence for the conservative transfer of these fatty acids in neutral lipids came from experiments on phytoplankton and copepods (Lee et al. 1971), and tracer lipids in plankton continue to be the most studied. For example, diatoms contain high levels of 20:5*n*–3 whereas dinoflagellates are rich in 22:6*n*–3, green algae in 18:4*n*–3, and red algae in 20:4*n*–6 (Sargent 1989). Recently, there has been growing interest in the use of fatty acids to examine the foraging ecology and diet of a wider variety of marine species, including those of higher trophic levels. Kharlamenko et al. (1995) used long-chain fatty acids, unique to bacteria and algae, to determine food sources of macrozoobenthic species in a shallow-water hydrothermal ecosystem. Similarly, diet information was gained from studying the transfer of the fatty acid 18:2*n*–6 (Klungsøyr et al. 1989), or the ratio of 16:1*n*–7 to 16:0 (St. John and Lund 1996), from diatoms to copepods and on to larval Atlantic cod (*Gadus morhua*). Sargent (1989) reported that zooplanktivorous fish, caught during the spring and autumn algal blooms, had large amounts of the fatty acids 20:1*n*–9 and 22:1*n*–11, which corresponded to the fatty alcohol of the wax esters laid down by the copepods feeding on the algae. These zooplanktivorous fish are consumed in turn by other commercially important fish species such as cod, haddock (*Melanogrammus aeglefinus*), and pollock (*Pollachius virens*). Hence, the livers of these piscivorous fishes were found to be rich in 20:1*n*–9 and 22:1*n*–11 and also contained high levels of the *n*–3 polyunsaturated fatty acids (Sargent 1989), which were characteristic of the phytoplankton.

Several studies have examined the fatty acid patterns at yet higher trophic levels, such as in the milks and blubber from various marine mammals, in relation to dietary intake

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(reviewed in Iverson 1993). For instance, Antarctic fin whales (*Balaenoptera physalus*), whose diet consists primarily of herbivorous euphausiids, and North Atlantic fin whales, who feed on zooplankton, were easily differentiated by levels of 20:1 and 22:1 which were characteristic of their prey (Ackman and Eaton 1966). Similarly, populations of freshwater and saltwater harbour seals (*Phoca vitulina*) in northern Quebec could be distinguished using stable-isotope ratios and dietary fatty acids (Smith et al. 1996). More recently, fatty acids have been used to study the spatial scales of foraging and temporal variations in diets of other pinnipeds (Iverson et al. 1997*a*, 1997*b*).

It is well established in various fish species that the fatty acid composition of diet influences the lipid composition of tissues such as liver and muscle (Cowey and Sargent 1972; Yu et al. 1977; Lie et al. 1986; Nelson 1992; Dos Santos et al. 1993; Xu et al. 1993). Indeed, because the liver is the primary site of lipid deposition and storage in fish, it has been the tissue most frequently studied. However, higher trophic level predators including seals and other marine mammals generally consume their prey whole. Thus, to use fatty acids as an ecological tool, we must understand the influence of diet on the fatty acid signature of the whole body, rather than on individual fish tissues. Additionally, it may be the entire array of fatty acids in a species (i.e., the "signature," Iverson 1993; Iverson et al. 1997*b*), rather than the few selected components, that are the focus in biomarker studies, which will eventually allow quantitative assessments of diets.

Our study had two objectives within a larger program on the development of fatty acid signature analysis. Our first aim was to determine whether the fatty acid signature of whole cod changes with a change in diet, whether these changes reflect the fatty acid signatures of the prey being consumed, and finally what kind of time course would be required for such changes to occur. This information would provide the opportunity to use fatty acids to study the foraging ecology of fish predators as well as marine mammal predators. Fatty acid signatures of whole Atlantic cod are of particular interest, as this species is both commercially important and believed to be a significant prey of seals in Atlantic Canada (e.g., Bowen and Harrison 1994). Our second objective was to determine the stability of the cod fatty acid signatures by statistically comparing cod on dietary treatments with signatures of their experimental prey and with that of wild cod, i.e., can a cod still be identified as a cod by its fatty acid signature despite changes in and influences by diet. If fatty acids are to be used to study the foraging ecology of seals, it is important to establish the ability to distinguish all prey based on their fatty acid compositions despite the influence of diet on those compositions.

Materials and methods

Maintenance and experimental design

In December 1993, about 100 large adult Atlantic cod (47.4 ± 0.63 cm, 1.0 ± 0.04 kg) were captured by bottom trawl near Devil's Island, Eastern Passage, Dartmouth, N.S. The cod were housed in four flow-through tanks (6 m^3) to maintain them at a density that favours increased survival and appetite. Lighting was controlled to simulate the seasonal photoperiod. Temperature of the filtered seawater (31‰) delivered to the tanks varied seasonally (December to mid-April, 0.3–4.0°C). During the experiment, cod were fed every second day ad libitum, as Dos Santos et al. (1993) reported that cod fed at a reduced frequency showed greater growth rates and food utilization than cod fed daily. We had no a priori reason to expect large changes in fatty acids in these nonreproducing fish over the duration of the experiment if on a constant diet. Thus, cod were randomly divided into groups of equal size for diet treatments and sampling. Because the time of the study coincided with greatly reduced availability of wild cod for use in our study, the experiment was set up as a basic intervention design to maximize the number of cod that were analyzed for changes in fatty acid composition at each time period. There were a total of five sampling periods, and at each sampling, five cod were randomly selected from each tank for analysis.

To establish a baseline signature of the wild cod during acclimation to our facilities, they were fed other cod from the same net haul for the first 4 weeks, after which the first set of cod samples (CodC, week 0, $N = 20$) were obtained for proximate and fatty acid analysis. Cod were then fed squid (*Illex illecebrosus*, 2% lipid), cut into 5- to 8-cm strips, for 6 weeks. Cod were sampled at 3 weeks (CodS1, $N = 20$) and 6 weeks (CodS2, $N = 20$) after the start of the squid diet. During these two 3-week periods, tank water temperatures averaged 4.0 and 2.8°C, respectively. The cod were then switched to a diet of Atlantic mackerel (*Scomber scombrus*, 16% lipid), cut into 5- to 8-cm pieces with the heads, tails, and viscera removed, for 8 weeks. This second diet was fed slightly longer due to a significant reduction in food consumption that coincided with lower water tempertaures during the final two periods (1.7 and 2.3°C, respectively). The cod on the mackerel diet were sampled at 5 weeks (CodM1, $N = 20$) and 8 weeks (CodM2, $N = 24$, all remaining cod in the tanks) after the start of the mackerel diet. All squid and mackerel fed to the cod were purchased from the same vendor at the same time.

At each sampling the cod were killed by placing them in a bath containing lethal concentrations of tricainemethanesulfonate (MS 222) and stored for several weeks in sealed plastic bags at –20°C until processing. Samples of the diet (18 squid and 25 mackerel in total) were taken at each feeding and frozen for subsequent proximate and fatty acid composition analysis. The squid and mackerel taken for analysis were treated the same way as those fed to the cod.

Laboratory and data analysis

Cod and prey samples were thawed and individually ground and analyzed for dry matter (by forced convection at 100°C for about 5 h), protein (by the Kjeldahl method, Bradstreet 1965), fat (Bligh and Dyer 1959), and fatty acid composition. Fatty acid methyl esters were prepared from the lipid extract (filtered and dried over anhydrous sodium sulfate), using 8% boron trifluoride in methanol (v/v) and hexane, capped under nitrogen gas, and heated at 100°C for 1 h. Using fresh reagents, this method produces results identical to those using 0.5 N sulfuric acid in methanol (Iverson 1988; Iverson et al. 1997*b*). Fatty acid methyl esters were analyzed in duplicate using temperature-programmed capillary gas–liquid chromatography (GLC) according to Iverson (1988) and Iverson et al. (1992), but on a Perkin Elmer (PE) Autosystem II Capillary FID gas chromatograph fitted with a 30 m × 0.25 mm inside diameter column coated with 50% cyanopropyl polysiloxane (0.25 film thickness, J&W DB-23, Folsom, Calif.) and linked to a computerized integration system (PE Nelson). Fatty acids were identified and quantified according to Iverson et al. (1997*b*) using a combination of standard mixtures (Nu-Chek-Prep, Inc., Elysian, Minn.) and silver nitrate chromatography, as well as an ion-trap mass spectrometer (R.G. Ackman, personal communication). The fatty acids are designated in IUPAC nomenclature by carbon chain length:number of double bonds and the position (*n*–*x*) of the first double bond with respect to the methyl end. Seventy-two fatty acids and isomers were regularly identified in all cod and prey samples. Additionally, isomers (*n*–11 and *n*–9) of two long-chain monounsaturated fatty acids, 20:1 and 22:1, and the ratio of these isomers have been found to be good indicators of diet (Iverson 1993; Iverson et al.1997*b*). Thus, ratios of 20:1*n*–11/20:1*n*–9 (ratio 20:1)

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and 22:1*n*–11/22:1*n*–9 (ratio 22:1) were also included in the fatty acid data sets for analysis. Data are presented as mean ± SEM.

Cod treatment comparisons, as well as any between-tank differences, were tested using two-way multivariate analysis of variance (MANOVA) on arcsin-transformed fatty acid data. To perform MANOVA, four cod were randomly removed from the final sampling period to create equal sample sizes for all sampling periods. Because of the group sample size ($N = 20$), up to 19 fatty acids could be used for this statistical analysis. Therefore, this data set consisted of the 18 fatty acids and one isomer ratio, which were either present at the most abundant levels and (or) were relevant to dietary differences (Table 1). One-way ANOVA was used to compare fat content and selected individual fatty acids of prey and cod.

To examine the overall differences in cod signatures with diet, all fatty acids were analyzed using classification and regression tree (CART) analysis in S-plus (Statistical Sciences Inc., Seattle, Wash.) according to Smith et al. (1997) and Iverson et al. (1997*a*). CART is a nonparametric multivariate classification technique that enables the statistical interpretation of fatty acid patterns containing a high number of variables (fatty acids) per observation. CART sequentially selects the "best" variable, and the best splitting point of that variable, to separate the data into two groups (nodes) that are as different as possible. This splitting continues until one of two stopping criteria (based on deviance and number of observations) is met and a classification is made along with the associated misclassification or error rate. We also used deviances of other fatty acids calculated in the CART analysis to determine other fatty acids that could be used for the initial split at the root node (Clark and Pregibon 1992; Venables and Ripley 1994). Although CART compares quite favourably with more traditional multivariate techniques, such as discriminant function analysis, CART may be advantageous because subjective variable selection is not required when the number of fatty acids exceeds the number of samples (Smith et al. 1997).

Results

Prey composition

The total fat and fatty acid compositions of the prey species fed to the cod differed considerably. The fat content of squid averaged 2.0% (CV = 45.6%) compared with 15.8% fat (CV = 19.4%) in mackerel ($P < 0.0001$) (Table 1). The percentage of many of the major fatty acids also differed significantly. Squid and mackerel fatty acid signatures differed based on the 18 fatty acids and one isomer ratio (MANOVA, $P < 0.0001$) (Table 1). For example, squid contained high levels of 20:4*n*–6, 20:5*n*–3, and 22:6*n*–3 (1.52, 12.93, and 33.77%, respectively) compared with much lower levels of these components in mackerel (0.44, 7.54, and 13.35%, respectively) (Table 1; Fig. 1). In contrast, squid contained relatively low levels of 14:0, 16:1*n*–7, 18:2*n*–6, 18:4*n*–3, 20:1*n*–9, 20:4*n*–3, and 22:1*n*–11 and a low 22:1 isomer ratio in comparison with much higher levels of these components and ratios in mackerel (Table 1; Fig. 1).

Although average values of fatty acids differed between prey species, there was considerable intraspecific variability. For example, the levels of 18:2*n*–6 and 20:4*n*–3 in squid ranged between 0.19 and 1.00% (CV = 52.6%) and between 0.08 and 0.58% (CV = 66.2%), respectively, and in mackerel ranged between 0.81 and 1.87% (CV = 18.4%) and between 0.58 and 1.10% (CV = 15.7%), respectively. Similar variation was found in major fatty acids, such as 20:1*n*–9 (3.91–14.42% in mackerel and 3.04–8.46% in squid) and 22:6*n*–3 (8.35–17.96% in mackerel and 23.82–39.80% in squid).

Cod composition

During the first 6 weeks on the squid diet, the fat content of cod remained at 2.3%. However, during the following 8 weeks on the mackerel diet, the fat content of the whole cod increased to 3.7% ($P < 0.02$) (Table 1). The fatty acid composition of cod also changed significantly over the course of the experiment (MANOVA on cod treatments, $P < 0.0001$) (Table 1). Between-tank differences were found only when two minor fatty acids (iso16:0 and 16:3*n*–1) were included in the two-way analysis ($P < 0.0025$); with their removal, no tank differences were detected ($P > 0.1909$). Hence, data from all tanks were pooled for further analyses. After 3 weeks on the squid diet (CodS1), levels of 22:6*n*–3 significantly increased from those observed in baseline CodC, while 14:0, 16:1*n*–7, 18:2*n*–6, 18:4*n*–3, 20:1*n*–9, 20:1*n*–9, and 22:1*n*–11 significantly decreased ($P < 0.05$) (Fig. 1; Table 1). Fatty acid 20:5*n*–3 also increased and 20:4*n*–3 and the 22:1 ratio decreased; however, these changes were less pronounced, reflecting the relative similarity in levels of these components in the two diets (CodC and squid). After 6 weeks on the squid diet (CodS2), levels of fatty acids still differed from the baseline cod but there were no significant differences between CodS1 and CodS2 ($P > 0.1$).

After the first 5 weeks on the mackerel diet (CodM1), the levels of 14:0, 16:1*n*–7, 18:2*n*–6, 18:4*n*–3, 20:1*n*–9, 20:4*n*–3, and 22:1*n*–11 and the 22:1 ratio increased significantly ($P < 0.005$), while the levels of 20:4*n*–6, 20:5*n*–3, and 22:6*n*–3 decreased significantly ($P < 0.002$) from levels found at the end of the squid diet. These changes corresponded to the respective differences in these components found between mackerel and squid (Fig. 1; Table 1). The fatty acid signature of cod fed mackerel for 8 weeks (CodM2) again differed from the cod fed squid (CodS1 and CodS2) but did not differ further from CodM1 ($P > 0.1$). The percentage of one major fatty acid, 18:1*n*–9, did not decrease from CodC to CodS1 and CodS2 as might have been expected by low levels found in squid (Table 1). However, after the switch from squid (3.40% 18:1*n*–9) to mackerel (9.15% 18:1*n*–9), this component significantly increased as expected in CodM1 and CodM2 ($P < 0.01$). In general, the levels of most fatty acids changed between dietary treatments, but did not differ further within treatments, i.e., between CodM1 and CodM2 or between CodS1 and CodS2.

CART analysis was performed using all 74 fatty acids to determine which fatty acids best identified the differences among cod dietary treatments and to determine how well the dietary treatments could be correctly classified (Fig. 2). The CART algorithm selected a minor component, 16:3*n*–1, as the fatty acid that maximized the change in deviance (91.8) between groups at the root node. All 20 of the baseline cod (CodC) were correctly classified on the left-hand side of the tree and all cod in the dietary treatment groups, with the exception of five CodS1, were grouped on the right-hand side of the tree. Subsequent splits resulted in most of the cod fed squid (CodS1 and CodS2) being grouped closer to the baseline cod (CodC) and classified in center branches, while cod fed mackerel (CodM1 and CodM2) were grouped in the right-hand branches and separated from both baseline cod and cod fed squid (Fig. 2). This tree correctly classified 93% (97 of 104) of the cod based upon their fatty acid signatures; five of the seven individuals misclassified were the result of difficulty separating CodM1 from CodM2 and CodS1 from CodS2.

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Table 1. Fatty acid composition (mass %) of prey species (squid and Atlantic mackerel) and Atlantic cod after dietary treatments.

	Squid ($N = 18$)	Mackerel ($N = 25$)[a]	CodC ($N = 20$)	CodS1 ($N = 20$)	CodS2 ($N = 20$)	CodM1 ($N = 20$)	CodM2 ($N = 24$)
% lipid	2.0±0.21	15.8±0.61	2.3±0.23	1.8±0.21	2.3±0.22	3.7±0.26	3.6±0.31
Fatty acid							
12:0	0.02±0.00	0.05±0.00	0.02±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.02±0.00
13:0	0.00±0.00	0.26±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.27±0.09	0.00±0.00
iso14	0.06±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
14:0[b]	2.04±0.20	4.19±0.12	2.32±0.15	1.56±0.11	1.77±0.09	2.48±0.07	2.40±0.08
14:1*n*–9	0.06±0.01	0.29±0.02	0.13±0.01	0.10±0.00	0.11±0.01	0.22±0.00	0.21±0.00
14:1*n*–7	0.05±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
iso15	0.08±0.01	0.21±0.00	0.14±0.00	0.10±0.00	0.10±0.00	0.15±0.00	0.15±0.00
anti15	0.05±0.01	0.10±0.00	0.03±0.00	0.01±0.00	0.01±0.00	0.06±0.00	0.05±0.00
15:0	0.78±0.12	0.47±0.01	0.37±0.01	0.32±0.00	0.32±0.00	0.37±0.00	0.36±0.00
iso16[b]	0.29±0.04	0.17±0.00	0.09±0.00	0.21±0.02	0.22±0.00	0.16±0.00	0.15±0.01
16:0[b]	19.57±0.50	13.45±0.35	14.47±0.37	14.70±0.27	14.41±0.23	12.77±0.24	12.29±0.27
16:1*n*–11[b]	0.26±0.02	0.59±0.04	1.05±0.68	0.39±0.01	0.41±0.00	0.49±0.02	0.55±0.03
16:1*n*–9	0.09±0.01	0.24±0.01	0.34±0.00	0.40±0.01	0.38±0.00	0.33±0.00	0.37±0.00
16:1*n*–7[b]	1.30±0.27	5.48±0.33	4.47±0.28	3.36±0.28	3.64±0.21	4.94±0.18	5.10±0.22
7Me16:0	0.16±0.02	0.37±0.01	0.26±0.00	0.24±0.00	0.24±0.00	0.29±0.00	0.30±0.00
16:1*n*–5	0.27±0.02	0.38±0.01	0.31±0.01	0.34±0.02	0.38±0.00	0.32±0.01	0.29±0.02
16:2*n*–6	0.10±0.02	0.01±0.00	0.00±0.00	0.03±0.02	0.00±0.00	0.03±0.00	0.03±0.00
iso17	0.12±0.01	0.20±0.01	0.17±0.01	0.17±0.00	0.18±0.00	0.18±0.00	0.18±0.00
16:2*n*–4	0.29±0.03	0.46±0.03	0.47±0.06	0.53±0.03	0.58±0.02	0.56±0.02	0.56±0.02
16:3*n*–6	0.04±0.02	0.26±0.03	0.17±0.07	0.15±0.04	0.17±0.02	0.25±0.01	0.25±0.02
17:0	0.68±0.03	0.38±0.02	0.23±0.01	0.33±0.02	0.33±0.01	0.32±0.00	0.31±0.00
16:3*n*–4	0.12±0.02	0.15±0.03	0.24±0.01	0.13±0.02	0.11±0.02	0.16±0.00	0.17±0.01
17:1	0.16±0.02	0.44±0.03	0.39±0.01	0.39±0.02	0.34±0.02	0.38±0.00	0.40±0.01
16:3*n*–1[b]	0.34±0.08	0.31±0.02	0.05±0.00	0.22±0.03	0.26±0.01	0.22±0.01	0.24±0.01
16:4*n*–3	0.13±0.03	0.05±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.00	0.00±0.00
16:4*n*–1	0.35±0.03	0.33±0.03	0.38±0.02	0.51±0.03	0.46±0.04	0.15±0.01	0.18±0.03
iso18	0.17±0.02	0.02±0.00	0.38±0.04	0.46±0.05	0.36±0.03	0.21±0.02	0.21±0.03
anti18	0.11±0.02	0.00±0.00	0.28±0.04	0.12±0.05	0.05±0.02	0.20±0.02	0.18±0.02
18:0[b]	3.95±0.21	2.48±0.12	3.03±0.11	3.37±0.12	3.11±0.07	2.63±0.06	2.71±0.05
18:1*n*–13	0.11±0.02	0.11±0.02	0.00±0.00	0.08±0.02	0.03±0.01	0.03±0.01	0.00±0.00
18:1*n*–11	0.20±0.03	0.48±0.03	0.87±0.09	1.10±0.09	1.22±0.09	1.14±0.04	1.20±0.09
18:1*n*–9[b]	3.40±0.33	9.15±0.73	10.30±0.31	10.97±0.32	10.47±0.36	11.61±0.28	12.14±0.35
18:1*n*–7	1.42±0.12	3.13±0.12	4.15±0.65	4.03±0.47	3.87±0.59	4.10±0.62	4.20±0.40
18:1*n*–5	0.39±0.03	0.63±0.02	0.39±0.08	0.38±0.07	0.38±0.09	0.54±0.09	0.54±0.10
18:2d5,7	0.03±0.00	0.04±0.00	0.03±0.03	0.06±0.03	0.05±0.04	0.10±0.05	0.09±0.06
18:2*n*–7	0.06±0.01	0.01±0.00	0.08±0.04	0.02±0.03	0.00±0.00	0.00±0.00	0.00±0.00
18:2*n*–6[b]	0.44±0.06	1.47±0.05	0.95±0.16	0.85±0.18	0.84±0.14	1.20±0.12	1.24±0.17
18:2*n*–4	0.18±0.02	0.12±0.01	0.18±0.05	0.12±0.04	0.12±0.04	0.13±0.03	0.14±0.03
18:3*n*–6	0.08±0.00	0.12±0.00	0.05±0.03	0.08±0.03	0.08±0.01	0.10±0.01	0.10±0.01
18:3*n*–4	0.09±0.00	0.11±0.00	0.05±0.02	0.12±0.04	0.09±0.06	0.08±0.02	0.07±0.03
18:3*n*–3[b]	0.30±0.05	1.43±0.09	0.66±0.22	0.44±0.20	0.48±0.19	0.93±0.13	1.00±0.20
18:3*n*–1	0.05±0.00	0.02±0.00	0.06±0.03	0.10±0.02	0.11±0.02	0.12±0.04	0.12±0.05
18:4*n*–3[b]	0.36±0.09	2.73±0.12	1.04±0.49	0.75±0.43	0.82±0.41	1.72±0.30	1.82±0.53
18:4*n*–1	0.06±0.01	0.08±0.01	0.10±0.04	0.07±0.03	0.08±0.04	0.10±0.03	0.10±0.03
20:0	0.11±0.00	0.12±0.00	0.07±0.02	0.05±0.03	0.05±0.02	0.05±0.00	0.05±0.01
20:1*n*–11	0.60±0.04	0.73±0.03	0.58±0.23	0.56±0.20	0.61±0.19	0.72±0.11	0.69±0.12
20:1*n*–9[b]	4.81±0.41	8.01±0.53	4.64±1.83	3.49±1.15	4.05±1.35	5.35±1.05	5.51±1.01
Ratio 20:1	0.13±0.00	0.10±0.00	0.13±0.03	0.16±0.02	0.15±0.02	0.14±0.02	0.13±0.02
20:1*n*–7[b]	0.21±0.03	0.84±0.07	0.40±0.12	0.31±0.07	0.30±0.06	0.46±0.13	0.45±0.11
20:1*n*–5	0.05±0.00	0.11±0.00	0.09±0.05	0.09±0.03	0.08±0.02	0.10±0.02	0.09±0.01
20:2*n*–6	0.33±0.00	0.31±0.01	0.25±0.05	0.26±0.04	0.25±0.04	0.30±0.02	0.31±0.04
20:3*n*–6	0.02±0.00	0.05±0.00	0.05±0.01	0.06±0.02	0.05±0.01	0.06±0.00	0.07±0.01
20:4*n*–6	1.52±0.09	0.44±0.05	2.45±1.45	2.98±1.55	2.17±0.83	1.32±0.43	1.31±0.57
20:3*n*–3	0.75±0.09	0.19±0.01	0.11±0.03	0.12±0.03	0.13±0.04	0.18±0.04	0.19±0.04

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Table 1 (*concluded*).

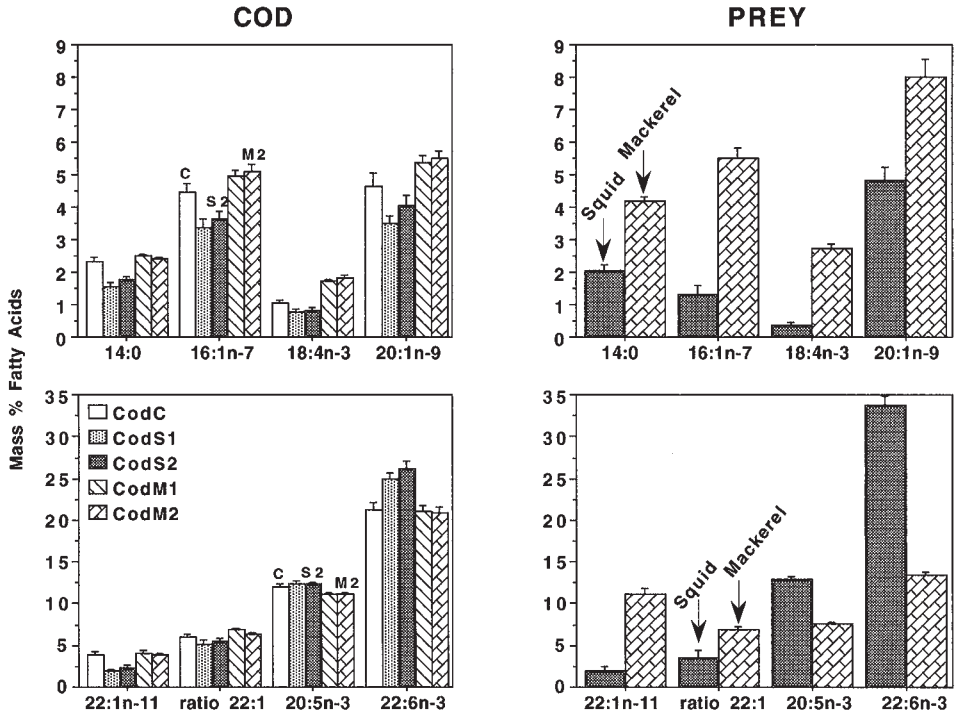
	Squid ($N = 18$)	Mackerel ($N = 25$)[a]	CodC ($N = 20$)	CodS1 ($N = 20$)	CodS2 ($N = 20$)	CodM1 ($N = 20$)	CodM2 ($N = 24$)
20:4*n*–3[b]	0.23±0.04	0.84±0.03	0.47±0.14	0.45±0.16	0.48±0.13	0.78±0.10	0.82±0.13
20:5*n*–3[b]	12.93±0.32	7.54±0.16	11.98±1.50	12.43±1.01	12.32±1.06	11.06±0.94	11.08±1.28
22:0	0.03±0.00	0.00±0.00	0.07±0.04	0.03±0.04	0.00±0.00	0.00±0.00	0.00±0.00
22:1*n*–11[b]	1.96±0.60	11.09±0.82	3.84±1.82	1.94±1.04	2.36±1.20	4.10±1.10	3.80±1.08
22:1*n*–9[b]	0.43±0.04	1.69±0.11	0.62±0.30	0.34±0.12	0.41±0.12	0.61±0.22	0.61±0.16
Ratio 22:1[b]	3.57±0.81	6.81±0.40	5.94±1.94	5.12±2.11	5.44±1.70	6.89±1.12	6.31±1.23
22:1*n*–7	0.09±0.00	0.28±0.03	0.10±0.03	0.11±0.03	0.08±0.02	0.09±0.03	0.07±0.04
22:1*n*–5	0.12±0.00	0.00±0.00	0.05±0.04	0.04±0.03	0.02±0.03	0.02±0.03	0.00±0.03
22:2*n*–6	0.02±0.00	0.03±0.00	0.00±0.01	0.03±0.03	0.01±0.02	0.03±0.01	0.02±0.02
21:5*n*–3	0.18±0.01	0.30±0.00	0.22±0.06	0.20±0.07	0.22±0.06	0.31±0.04	0.32±0.06
22:4*n*–6	0.11±0.00	0.06±0.00	0.19±0.12	0.18±0.11	0.15±0.07	0.11±0.04	0.12±0.05
22:5*n*–6	0.49±0.03	0.29±0.02	0.38±0.08	0.38±0.05	0.34±0.05	0.35±0.07	0.32±0.06
22:4*n*–3	0.04±0.00	0.08±0.00	0.04±0.03	0.04±0.02	0.05±0.02	0.06±0.01	0.09±0.08
22:5*n*–3	0.59±0.04	1.39±0.04	1.77±0.22	1.78±0.22	1.69±0.16	1.61±0.08	1.68±0.16
22:6*n*–3[b]	33.77±1.14	13.35±0.43	21.15±4.20	24.99±3.14	26.10±3.93	21.02±2.83	20.84±3.53
24:1	0.58±0.04	1.09±0.04	1.06±0.24	1.35±0.50	1.20±0.30	0.81±0.20	0.78±0.22

Note: Values are means ± SEM. CodC, baseline cod on cod diet, week 0; CodS1 and CodS2, cod on squid diet for 3 and 6 weeks, respectively; CodM1 and CodM2, cod on mackerel diet for 5 and 8 weeks, respectively.

[a]Mackerel as fed to cod, i.e., with heads, tails, and viscera removed.

[b]Fatty acids chosen ($N = 19$) to test treatment and tank differences using MANOVA, based upon abundance and (or) relevance to dietary differences.

Fig. 1. Relative amounts (% by mass) of selected abundant fatty acids in Atlantic cod dietary treatment groups and prey. Bars are means and vertical lines are 1 SEM for CodC (baseline cod fed cod), CodS1 and CodS2 (cod fed squid diet for 3 and 6 weeks, respectively), and CodM1 and CodM2 (cod then changed to a mackerel diet for 5 and 8 weeks, respectively).



As stated previously, the minor fatty acid 16:3*n*–1 was originally chosen at the first node of the tree (Fig. 2), as it produced the greatest deviance between groups. Choosing one of the more abundant fatty acids (e.g., 18:2*n*–6, 18:3*n*–3, 18:4*n*–3, 20:4*n*–3) with relatively high deviances (61.5–89.0) for the root node resulted in similar classifications of cod diet groups, but with higher misclassification rates (16–18 of 104).

Composition of cod dietary treatments in relation to prey composition

Although MANOVA showed that there were signifcant differences in the fatty acid composition of squid, mackerel, and wild cod, we used CART analysis to identify which fatty acids best identified these fish species and to determine how well the groups were classified. CART analysis accurately separated

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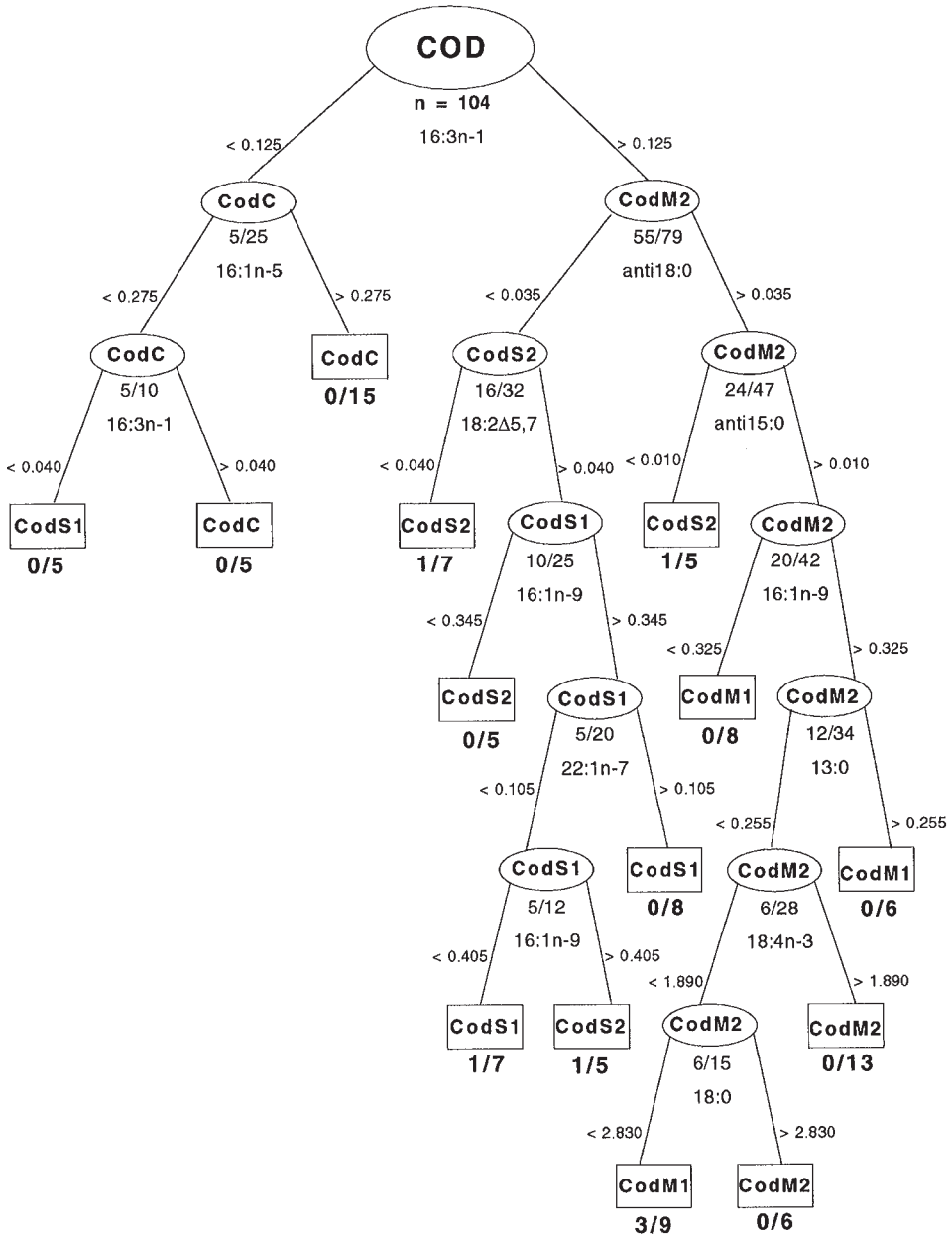
Fig. 2. Classification tree of all Atlantic cod dietary treatment groups. Elipses indicate intermediate splitting nodes and rectangles indicate terminal nodes where a classification is made. The labels within the ovals and rectangles indicate the classification of the group with the largest number of observations at that node. The fractions located beneath each split indicate the number of misclassifications over the total number of observations for that node. The fatty acids listed at each split, as well as their optimal splitting levels, were chosen by the algorithm to create that split (values < *X* down left extension, values > *X* down right extension). Total misclassification rate was 7/104. Rate of cod groups correctly classified was 93%: CodC (20/20), CodS1 (19/20), CodS2 (19/20), CodM1 (20/20), and CodM2 (19/24).



baseline cod, squid, and mackerel into their respective groups with no misclassifications (0 of 63) using only the two fatty acids 20:5*n*–3 and 16:1*n*–9.

Clearly, fatty acid patterns of cod changed in the direction of that found in their prey (Fig. 1). To examine whether cod could still be differentiated from squid and mackerel after having been fed pure squid and mackerel diets, CART analysis using all 74 fatty acids was performed on cod treatment groups and their diets (Fig. 3). To avoid an overly large and cumbersome tree, CodS1 and CodS2 were combined into one group and CodM1 and CodM2 were combined into another. Squid were clearly distinguished from the cod fed squid (CodS), while mackerel were readily distinguished from the cod fed mackerel (CodM). In this analysis, the cod dietary treatment groups continued to be distinguished from one another, although baseline cod and cod fed squid tended to group on the left-hand side of the tree with squid, while cod fed mackerel grouped on the right-hand side with mackerel (Fig. 3).

Discussion

To examine the influence of diet on the fatty acid signature of

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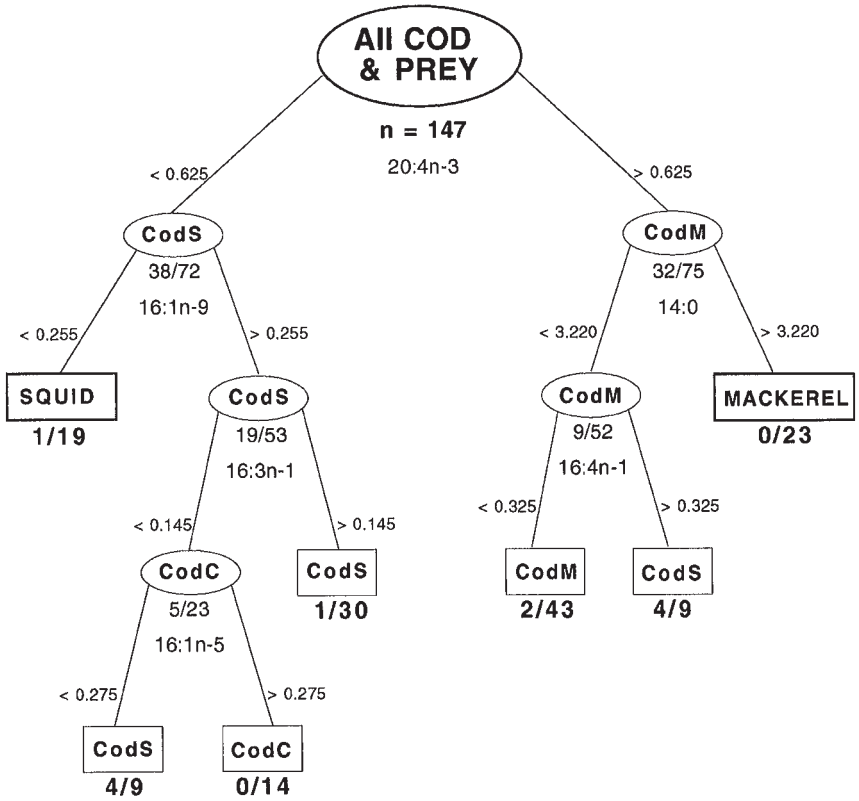
Fig. 3. Classification tree of prey and all Atlantic cod dietary treatment goups. See Fig. 2 for an explanation of the tree. For analysis, all cod on the squid diet (CodS1 and CodS2) were combined as CodS and all cod on the mackerel diet (CodM1 and CodM2) were combined as CodM. Total misclassification rate was 12/147. Rate of prey and cod groups correctly classified was 92%: squid (18/18), Atlantic mackerel (23/25), CodC (14/20), CodS (39/40), and CodM (41/44).



Atlantic cod, two natural prey items, which differ significantly in lipid content and fatty acid composition, were used in our study. Although adult wild cod feed on various prey items, including a wide variety of fish species as well as various invertebrates (Lilly 1991), we used these single-species diets to maximize the dietary effects on the cod fatty acid signature. Squid and mackerel were indeed quite different in their patterns of fatty acids (Fig. 1) and were easily differentiated from one another, even when only major fatty acids were considered.

Adult cod used in this study initially contained about 2.3% total body fat and did not change in fat content during the 6-week squid diet (containing 2.0% lipid) (Table 1). However, despite maintenance of total body fat, the composition of the fat changed significantly. After only 3 weeks of being fed a pure squid diet, the fatty acid patterns in cod changed in the direction of those as found in squid (Fig. 1). The magnitude of the changes in cod fatty acids also tended to reflect the relative magnitude in squid; for instance, levels of 20:5*n*–3 increased only slightly from baseline cod (CodC) to CodS1 and CodS2, while levels of 22:6*n*–3 increased more dramatically, reflecting the level of differences in these components between CodC and squid (Fig. 1). Additionally, despite being on a low-fat diet, the time course required for diet to influence total body lipids in cod appeared to be short. In only 3 weeks, cod fatty acid patterns were significantly altered in the direction expected by patterns in squid and did not differ further by 6 weeks.

After a second change in diet, cod again responded rapidly to changes in lipid intake. As expected on a high-fat diet (mackerel, 15.7%), adult cod deposited more total body fat than on a lower fat diet of either cod or squid (Table 1). As in the previous case with a switch to the squid diet, cod fatty acid patterns changed significantly to reflect the mackerel diet (Fig. 1). Most of the changes that had occurred on a diet of squid were reversed and further changed with a change to a diet of mackerel, and the changes reflected the direction of differences in the fatty acid compositions of the squid and mackerel diets. The finding that 18:1*n*–9 remained high in cod fed squid, despite a reduction in diet, suggests its synthesis in cod; however, it did increase as expected with its increase in the mackerel diet (Table 1). Initially, we did not expect that cod fatty acid signatures would change as rapidly as found in our study; thus, when cod reduced their food intake during the mackerel diet, they were not sampled until 5 and 8 weeks to ensure that any dietary effects could be measured. But again, the time course required for diet to influence body lipids was relatively short. Cod fatty acid patterns changed significantly by 5 weeks and did not differ further at 8 weeks. It is not not known whether cod fatty acid patterns would have continued to change further with a longer time on each of the test diets. However, it would appear from our results that most changes had taken place within the first 3–5 weeks and subsequent changes over the next few weeks were not detectable.

Clearly, when switched first to a squid diet and then to a mackerel diet, the fatty acid signature of cod changed

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significantly toward the fatty acid signatures of these respective prey, thus demonstrating the value of fatty acid signatures in predators as indicators of diet. However, when applied to higher trophic level predators such as seals (Iverson 1993; Iverson et al. 1997*a*, 1997*b*), then the cod, as well as squid and mackerel, become potential prey. In the present study, fatty acid patterns in squid differed greatly from those of mackerel, and both were also readily distinguished from the original wild or baseline cod (CodC) using either MANOVA or CART. These findings suggest that fatty acid patterns in higher trophic level predators might be used to distinguish species compositions of diets. However, an additional question that arises is, if a predatory fish (i.e., cod) feeds upon another prey item and begins in part to resemble its prey item in fatty acid patterns, and both cod and prey are consumed by a larger predator, can the predatory fish and its prey items still be distinguished from one another. Our results indicate that the answer to this question is yes. The cod treatment groups were readily differentiated from one another using both univariate and multivariate analyses (e.g., Fig. 2). However, despite the significant changes in cod fatty acid signatures, cod were still readily separated from their dietary items using CART analysis (Fig. 3). In Alaska, Iverson et al. (1997*b*) also found that despite large within-species variation in fatty acid signatures with geographical region and likely diet, fish species were still readily differentiated from one another. Additionally, even though walleye pollock (*Theragra chalcogramma*) and Pacific herring (*Clupea pallasi*) of similar size-classes in similar locations were known to have similar diets, they were still distinguished by species based upon their fatty acid signatures.

The species-specific differences in the fatty acid signatures of cod, squid, and mackerel provide support for the use of fatty acid signatures to investigate the diet of higher trophic level predators such as marine mammals (e.g., Iverson et al. 1997*b*). However, the changes within cod also point to the possibility of using fatty acids to study the foraging ecology of cod. We conclude that fatty acid signatures should allow useful analyses of trophic interactions and predator–prey relationships in both space and time. Our study shows that the deposition of dietary fatty acids accounts for a large part of the variation in Atlantic cod signatures and, as a result, suggests their use as an indicator of dietary intake. A feature, not to be overlooked, is that the characteristic fatty acid signatures appear to be integrated over a time course of weeks, offering the opportunity to extend our knowledge of fish diets obtained from research trawl surveys beyond that possible from stomach content data alone.

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