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RÉGIME ALIMENTAIRE DU BÉLUGA, *DELPHINAPTERUS LEUCAS,*

de l'estuaire du Saint-Laurent, Canada, tel que révélé par l'analyse des acides gras du lard

Mémoire présenté
à la Faculté des études supérieures de l'Université Laval
dans le cadre du programme de maîtrise en biologie
pour l'obtention du grade de maître ès sciences (M. Sc.)

DÉPARTEMENT DE BIOLOGIE
FACULTÉ DES SCIENCES ET DE GÉNIE
UNIVERSITÉ LAVAL
QUÉBEC

JUILLET 2006

Résumé

Une connaissance du régime alimentaire des espèces constitue un élément clé pour la compréhension de l'écologie des espèces. Or, peu de techniques existent afin d'examiner le régime alimentaire contemporain du béluga du Saint-Laurent étant donné le statut précaire de cette population et la rareté de contenus stomacaux chez les individus retrouvés morts. L'analyse des profils d'acides gras dans le lard et dans leurs proies potentielles constitue un outil susceptible de fournir certains indices concernant leur régime alimentaire. C'est donc dans ce but qu'une étude comparative des profils d'acides gras a été menée. Dans une première étape, le profil d'acides gras a été examiné chez environ 60 espèces de proies potentielles du béluga provenant de l'estuaire et du golfe du Saint-Laurent. Cette analyse a révélé la nécessité de considérer les effets des lipides totaux, de la taille, des saisons et des lieux d'échantillonnage lors de l'examen des profils d'acides gras des espèces afin d'améliorer le couplage des profils des proies potentielles avec ceux d'un prédateur tel que le béluga. Les profils d'acides gras du lard ont ensuite été comparés entre les bélugas et quatre espèces de pinnipèdes qui se trouvent dans l'estuaire et le golfe du Saint-Laurent. Les espèces de même que les sexes et classes d'âge ont généralement pu être distingués sur la base des profils d'acides gras. Une gamme d'analyses multivariées ont révélé des différences qui semblaient liées à des acides gras d'origine alimentaire, confirmant ainsi le potentiel de ces techniques dans l'analyse comparative de la diète des mammifères marins. La caractérisation des signatures d'acide gras des proies potentielles et des prédateurs constitue une étape essentielle dans le cheminement menant à une meilleure compréhension de l'écologie alimentaire de ces animaux.

Abstract

Knowledge of diet is an important element for understanding the ecology of species. Currently, there are few techniques to examine the contemporary diet of St. Lawrence belugas considering the precarious status of the population and the rarity of prey remains in the stomach of stranded individuals. Fatty acid analyses of prey and blubber may provide the tools to indicate diet. It was with this purpose that a comparative study of fatty acid profiles was undertaken. As a first step, about 60 potential prey species for belugas were collected in the Estuary and Gulf of St. Lawrence and were examined for their fatty acid profiles. The analysis revealed the need to consider the effects of lipid content, size, season, and sampling location when evaluating species fatty acid profiles in order to improve evaluations of prey profiles of a predator such as the beluga. Blubber fatty acid analyses were subsequently compared between belugas and four species of pinnipeds that are encountered in the Estuary and the Gulf. Species along with other classes such as sex and age could be distinguished on the basis of their fatty acid signatures. A suite of multivariate analyses confirmed the role of diet-linked fatty acids in differentiating the groups, thereby confirming the potential for these techniques to conduct a comparative analysis of diets among these marine mammals. The characterization of fatty acid signatures of potential prey and predators represents essential steps on the path towards a better understanding of trophic ecology in these animals.

Avant-propos

À chacune des étapes, ce projet ne se serait pas réalisé sans les contributions de beaucoup de monde, mais je dois donner des remerciements particulièrement profonds à mes superviseures.

Avant tout, à ma co-directrice, Dr Véronique Lesage, de l'Institut Maurice-Lamontagne (IML), de Pêches et Océans Canada, Région du Québec, qui est la raison d'être, autant que le ciment qui permettait à tous ces liens et ces opérations d'exister. C'est grâce à sa grandeur d'esprit que mon projet a fonctionné et dépassé tout obstacle, grand et petit. Je suis vraiment chanceux comme étudiant de la connaître comme amie en même temps que 'boss'! Si ma réussite se fait remarquer, il faudra considérer ses talents de chercheuse autant que son amitié afin de comprendre la source.

À ma directrice, Dr Helga Guderley de l'Université Laval, pour son soutien et sa patience. Elle est une chercheuse exemplaire dont la sagesse en même temps que la bonne humeur sont des sources de plaisir pour tous ceux qui ont eu la chance de la rencontrer.

To Dr. Sara Iverson (Dalhousie University), for accepting to collaborate in the project, thus making it all possible in the first place! Thanks also for providing timely advice and inspiration, and for setting an example of scientific scholarship, through your seminal publications and presentations in the intriguing field of lipid physiology and ecology.

Thank you to Shelley Lang, and all the technicians of the 'Fat lab' at Dalhousie, for the thousand-plus chemical extractions analyses they performed over the course of my project.

Merci aux échantillonneurs du MPO et MAPAQ pour leurs conseils et contributions, incluant tous les membres des équipages du NGCC *Calanus II* et du NGCC *Alfred Needler* (2000 - 2002).

Merci aux Drs Ladd Johnson et Jacques Larochelle pour leur soutien au comité et les conversations fulgurantes dans les cours d'écologie et physiologie. Merci aussi au Dr Larochelle d'inciter les étudiants à réfléchir entre les domaines de l'écologie et de la physiologie, ce qui a en partie inspiré ma conclusion ici.

Merci à tous mes amis de l'IML, surtout à Diane Archambault, Denis Chabot, Louise Savard, Lyne Morissette et Yves Morin pour leurs conseils bien appréciés. Et surtout à trois amis spéciaux, qui, comme Véro, m'ont supporté (et même toléré) avant et durant ce projet: Pierre Carter, Jean-François Gosselin et Mike Hammill.

Dès mon arrivée à Québec, tout le monde me tendait une main de bienvenue qui ne lâchait pas, ce qui ne cessait pas de me surprendre. Si jamais je suis regardé comme un type correct, c'est en espérant suivre un peu leurs exemples de professionnalisme autant que d'amitié. Mais mon chemin a commencé sur une plage à Vancouver, et pour tous ces souvenirs et les voyages, je tiens fort à remercier mes parents, Claude et Marguerite.

Aux enfants

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Chapitre 1

Introduction générale

1.1. Contexte

Un objectif continuel de l'écologie est de progresser de l'observation et de la description à des explications causales et prédictives (May 1999, Underwood *et al.* 2000, Power 2001, Bowen 2005). En ce sens, la maturation des sciences biologiques cherche à suivre le chemin parcouru par les sciences physiques. Par exemple, les disciples de l'astronomie ont dû développer les concepts de Copernic (c'est-à-dire le soleil, et pas la terre, est le centre de notre système planétaire) dans un premier temps et ceux de Kepler (les planètes ont des orbites elliptiques, pas circulaires) dans un deuxième temps pour arriver à un ensemble de théories qui constituait un véritable progrès. Antérieurement, les observations d'orbites excentriques de Ptolémée étaient plus correctes en prévisions que les héliocentriques de Copernic avant l'appui des lois de Kepler. De la même manière, il se peut qu'une période de transition soit nécessaire afin de relier les observations accumulées avec les idées contemporaines en écologie et en biologie des organismes. C'est dans ce contexte qu'est lancé le défi d'étudier les interactions trophiques entre les prédateurs et leurs proies à l'aide des biomarqueurs. Ce défi consiste à examiner d'abord la variabilité spatio-temporelle écologique, puis les raisons pour lesquelles les lipides constitueraient de bons indicateurs écologiques et enfin la manière dont les méthodes statistiques multivariées peuvent nous aider à présenter ces informations, de façon à mettre de l'avant des hypothèses rigoureuses.

L'écologie trophique régionale

L'analyse des écosystèmes est une démarche complexe, ambitieuse et coûteuse à cause du grand nombre de variables d'intérêt potentiel liant les organismes et leur

environnement. C'est pourquoi elle nécessite souvent des études prolongées et des échelles d'étude variées afin de documenter les patrons et les processus qui les affectent (Werner *et al.* 2004, Witman *et al.* 2004, Anderson *et al.* 2005). Ces analyses visent non seulement à dégager des principes généraux liés au fonctionnement des écosystèmes, mais aussi à discerner les tendances majeures et mineures en faisant des inférences solides, non-biaisées par des cas spéciaux ou du bruit dans les données (Underwood *et al.* 2000, Smayda *et al.* 2004, Bowen 2005, McIntyre et Mckitrick 2005). Une façon d'examiner ces patrons est de se référer à la variabilité spatio-temporelle pour documenter les différences et les ressemblances entre les sites et les périodes. Dans ce contexte, il est essentiel de comprendre le choix des acteurs autant que les variables qui vont servir à retracer les liens écologiques.

La documentation des interactions trophiques des populations, comme la consommation ou la compétition pour un niveau trophique ou des espèces-clés, qu'il s'agisse d'espèces fouragères ou de prédateurs majeurs, est souvent la cible principale des analyses écosystémiques (Bruno *et al.* 2003, Fanshawe *et al.* 2003, Roberge et Angelstam 2004). La révélation des associations avec la géographie, telle que l'hydrologie locale et le climat régional, est aussi d'intérêt puisqu'elle peut parfois identifier les facteurs régissant ces liens (Thacker et Lewandowicz 1997, Zamon 2001, Abookire et Piatt 2005). Par exemple, on peut tenter de déceler si les prédateurs varient leur régime alimentaire en fonction de l'abondance des proies (l'opportunisme) ou s'ils affichent des préférences alimentaires (sélection dirigée), comme démontrent les études directes des oiseaux alimentant leurs petits ainsi que les études indirectes des statistiques de pêche (Wilson *et al.* 2002, Santos et Pierce 2003, Litzow 2004). Les tendances ainsi dévoilées pourront être

utiles pour formuler des hypothèses lors de la reconstruction des interactions trophiques historiques ou encore pour prédire les interactions trophiques futures (Pitcher 2001, Pauly et Maclean 2003, Choi *et al.* 2004, Frank *et al.* 2005).

L'un des risques associés à l'étude des processus et des espèces-clés est la possibilité de sous-estimer l'importance de certaines composantes moins remarquées et de les exclure *a priori* de l'analyse, réduisant ou biaisant de ce fait notre compréhension du fonctionnement des écosystèmes (Underwood 2000). Deux exemples viennent à l'esprit. Dans le premier, on étudie l'écosystème dans le contexte des transferts de biomasse tel que celui du carbone entre certains groupes taxonomiques, ignorant le rôle des composantes ou des éléments mineurs, ou encore celui des classes de taille ou d'âge qui sont égalements importantes dans le contexte des interactions trophiques (Jennings *et al.* 2002, Birkeland et Dayton 2005, Woodward *et al.* 2005). Malgré les critiques potentielles associées a une telle simplification, cette approche visant à modéliser selon un processus clé (le transfert de carbone) a connu beaucoup de succès dans le cadre d'études régionales, réalisée généralement à l'aide du logiciel 'ECOPATH' (Christensen et Pauly 1992). Un deuxième cas de biais possible des études écosystémiques est un intérêt démesuré pour certains vertébrés de grande taille tels que les oiseaux, les poissons commerciaux et les mammifères marins, non pour leur importance écologique mais pour leur caractère charismatique ou leur intérêt pour les humains (Schmelzer 2001, Sergio *et al.* 2005). Plusieurs espèces d'invertébrés et de petits poissons sont méconnues, n'ont pas de valeur commerciale et sont souvent peu remarquées ou échantillonnées par les engins de pêche, malgré leur présence et leur importante contribution au régime alimentaire de prédateurs tels que les oiseaux et les mammifères marins (Bryant *et al.* 1999, Hjelset *et al.* 1999, Payne *et al.* 1999, Santos et

Haimovici 2001). Les amphipodes (*Themisto* spp.) et le lançon (*Ammodytes* spp.) sont des exemples de ce phénomène, en tant que source importante de nourriture pour les prédateurs, mais leur biologie et leur abondance demeurent encore peu connues (Nilssen *et al.* 1995, Bowen *et al.* 2002, Dunham et Duffus 2002).

L'écologie alimentaire des mammières marins

L'estimation de la disponibilité des proies est d'intérêt pour plusieurs questions fondamentales liées à la gestion des populations de mammifères marins (Shelton *et al.* 1997, Flaaten 1998, Bundy 2001, DeMaster *et al.* 2001, MacKenzie *et al.* 2002, Read and Brownstein 2003, Pitcher 2004).

Les populations les plus abondantes, surtout celles de pinnipèdes, attirent l'attention de par leur potentiel de nuire à la pêche et ce, particulièrement dans les régions où les stocks de poissons commerciaux sont en déclin ou en mauvaise condition (Trites *et al.* 1997, Swain et Sinclair 2000, McLaren *et al.* 2001, Yodzis 2001, Pauly et Maclean 2003). Par contre, plusieurs espèces de cétacés ont été surexploitées historiquement, au point où la taille de leur population a subi des baisses importantes (Mitchell 1974, Reeves *et al.* 1999, Nichol *et al.* 2002, Roman et Palumbi 2003, Baker et Clapham 2004). Malgré les mesures de conservation des récentes décennies, plusieurs populations de cétacés semblent croître à des taux sub-optimaux (Caswell *et al.* 1999, Anderson 2001, Stevick *et al.* 2003, Baker et Clapham 2004). La perception d'un manque de reprise des populations soulève la question des facteurs pouvant limiter leur rétablissement, incluant la disponibilité et la qualité des ressources alimentaires.

Bien que la nourriture semble l'une des contraintes les plus logiques à la croissance des populations, sa contribution au statut des espèces demeure l'une des contraintes les plus difficiles à établir de façon non équivoque (Fritz et Hinckley 2005). Pourtant, l'identification de changements dans l'assemblage d'espèces de la chaîne alimentaire pourrait constituer un bon indicateur des problèmes courants ou éventuels (Wanless *et al.* 2005). À cet égard, l'état des populations de mammifères marins pourrait servir d'indicateur de l'état des écosystèmes. Des exemples de monitorage de communautés utilisant comme indice les populations d'espèces en péril incluent les études des otaries de Steller en Alaska (Merrick *et al.* 1997, Trites et Donnelly 2003, Fritz et Hinckley 2005), des baleines franches de l'Atlantique Nord (Greene et Pershing 2004) et des baleines grises du Pacifique Nord (Perryman *et al.* 2002).

Qu'ils soient en croissance ou menacés d'extinction, il s'avère prudent d'intégrer ces prédateurs du sommet de la pyramide trophique dans tout discours visant les interactions trophiques, compte tenu des biomasses importantes de proies qu'ils ont le potentiel de consommer (Kenney *et al.* 1997, Schweder *et al.* 1998, Pauly et Maclean 2003, Trites *et al.* 2004). Toutefois, la difficulté de mener des études dans le milieu aquatique fait en sorte que notre connaissance de l'écologie alimentaire de ces animaux reste souvent incomplète. Une exception importante à cette règle de méconnaissance est le cas des loutres de mer (*Enhydra lutris*). Le déplacement relativement restreint de ces mammifères marins et de leurs proies benthiques qu'ils consomment lorsqu'ils sont allongés à la surface facilite leur étude. Ainsi, l'impact de ces prédateurs sur certaines communautés marines spécifiques a pu être évalué (Estes et Duggins 1995). Par contre, la plupart des mammifères marins ont une tendance à parcourir de longues distances et à s'alimenter sous

l'eau où ils sont difficiles à repérer et à observer (Gaskin 1982, Bowen et Harrison 1996, Bowen 1997, Pauly *et al.* 1998b, Trites 2003). Le développement de techniques alternatives pour complémenter l'information obtenue par les méthodes plus traditionnelles, telles que les observations en surface ou l'examen des traces non-digérées dans les fèces s'est avéré nécessaire pour approfondir nos connaissances du rôle des mammifères marins au sein des écosystèmes (Tollit *et al.* 1998).

Les biomarqueurs du régime alimentaire

Les techniques traditionnelles utilisées en écologie alimentaire comportent certains biais et peuvent être difficilement applicables à l'étude de certaines espèces (*e.g.*, Pierce et Boyle 1991). Les techniques d'analyse du régime alimentaire vont de l'analyse des derniers repas, par l'identification des restes des proies à travers l'examen du tractus digestif ou des fèces et la reconstruction de la diète, à des méthodes indirectes intégrant le régime alimentaire sur des périodes plus longues et qui sont fondées sur la proportion détectées de certains molécules ou composés. Un exemple de ce genre de traceur indirect du régime alimentaire, ou biomarqueur, et qui est maintenant très répandu est l'analyse des rapports de certains isotopes stables tels que le carbone et l'azote dans les tissus des proies et des prédateurs (DeNiro et Epstein 1981, Tieszen *et al.* 1983, Lesage *et al.* 2001). Une autre classe de biomarqueurs est celle des acides gras, une composante des lipides remarquée depuis des décennies comme une source potentielle d'identification et de quantification de certains processus incluant le régime alimentaire des organismes (Ackman *et al.* 1971, Ackman 1989, Dalsgaard *et al.* 2003). Comme pour les études impliquant les isotopes stables, l'intérêt pour ce type de biomarqueurs s'est accrû avec la disponibilité

d'équipement analytique et leur potentiel pour l'emploi de méthodes moins invasives (Connor *et al.* 2000, Dalsgaard *et al.* 2003).

Les écosystèmes marins sont remarquables par leur diversité en lipides biologiques et en acides gras (Ackman 1989, Zhukova et Aizdaicher 1995, Volkman *et al.* 1998). Les acides gras sont composés de chaînes de carbones, dont la longueur et le degré de saturation (nombre des liens simples et doubles entre les carbones) leur donnent des propriétés distinctes (Fig. 1.1). Bien que plusieurs acides gras puissent être synthétisés et modifiés par une variété d'organismes, les acides gras polyinsaturés proviennent typiquement des végétaux incluant le phytoplancton. De nombreux acides gras (souvent plus d'une soixantaine) qui composent les lipides peuvent être identifiés lors d'une analyse typique de chromatographie en phase gazeuse (Table 1.1). Plusieurs de ces acides gras proviennent exclusivement de la diète alors que d'autres peuvent être produits par l'organisme, mais à un taux insuffisant pour répondre aux besoins ou pour expliquer les niveaux observés (Sprecher 2000, Leonard *et al.* 2004; Fig. 1.2). Ainsi, la composition en acides gras peut discerner certaines tendances générales, comme l'alimentation herbivore vs carnivore (Auel *et al.* 2002), pélagique vs benthique (Graeve *et al.* 1997), poisson vs invertébré (Phillips *et al.* 2003), ou eau douce vs eau de mer (Smith *et al.* 1996). Il existe également un potentiel d'élucidation des patrons plus spécifiques allant du groupe d'espèces, surtout pour les prédateurs sténophages, à l'identité taxonomique des proies ingérées.

Il est possible d'atteindre une plus grande spécificité dans l'identification des proies lorsque l'ensemble du profil d'acides gras, communément appelé la signature d'acides gras, est utilisé plutôt que seulement un ou quelques acides gras (Smith *et al.* 1997). C'est aussi le cas avec d'autres biomarqueurs tels que les isotopes stables individuels.

L'enrichissement ou la déplétion des tissus des prédateurs en regard de certains isotopes représente une des méthodes répandues pour adresser les questions relatives aux interactions trophiques (Hobson 1999, Lesage *et al.* 2001, Jennings *et al.* 2002, Das *et al.* 2003). Bien que les isotopes stables de carbone et d'azote soient les plus populaires, d'autres isotopes tels que le baryum, le mercure, le plomb, le césium, et l'arsenic peuvent également servir à l'identification de sources environnementales pouvant révéler l'origine des ressources alimentaires. La combinaison des traceurs atomiques (isotopes) avec les structures moléculaires (chaînes d'acides gras) constitue un exemple de technique permettant d'augmenter la spécificité du traçage des sources alimentaires (Stott *et al.* 1997, Mottram *et al.* 2003, Chamberlain *et al.* 2004). Outre la combinaison de traceurs déjà communément employés, l'utilisation de nouvelles versions d'analyses chromatographiques et spectroscopiques promettent d'offrir un rehaussement de la précision analytique (Christie 2003, Takaichi *et al.* 2003, Wetzel et Reynolds 2004). Cependant, comme toute nouvelle initiative technologique, le potentiel de ces outils doit se réaliser au coût de l'inexpérience et d'essais répétés lors de leur développement pour ultimement être utiles dans de nombreuses études futures. Néanmoins, poussé par les intérêts commerciaux en santé, les innovations en technologie lipidique ont connu un développement pratique et rapide (Ackman 2002).

Bien que la continuation du développement des techniques analytiques ajoutera à la précision des analyses, parallèlement, l'un des besoins actuellement les plus criants est de gérer les données issues de ces activités. Une meilleure compréhension du rôle de la physiologie sur le métabolisme des acides gras servira à nous assurer de ne pas tomber dans le piège de 'la collection de timbres' comme ironisé par la fameuse boutade du physicien

Rutherford, suggérant que les biologistes pourraient simplement amasser des observations sans formuler des métapatrons causals (Gallagher et Appenzeller 1999, Underwood *et al.* 2000, Bowen 2005). Jusqu'à présent, plusieurs études portant sur les acides gras, dans le but de comprendre le rôle dans la nutrition et la santé des organismes, ont été menées sur des humains ou des animaux domestiques (Dos Santos *et al.* 1993, Arts *et al.* 2001, Christie 2003, Dalsgaard *et al.* 2003, Speake 2004). Toutefois, les voies d'assimilation et de circulation des acides gras des proies au prédateur sont encore méconnues, particulièrement chez les grandes espèces sauvages telles que les cétacés. Cet état de fait est lié principalement à la difficulté d'accéder à ces animaux, de les manipuler et donc de les comparer aux espèces terrestres ou plus petites. Ainsi, contrairement à nos attentes dérivées des expériences avec des organismes modèles, les quelques études effectuées jusqu'à présent indiquent que plusieurs espèces sauvages possèdent des stratégies physiologiques particulières pour exploiter les propriétés des différents acides gras. Les espèces pourraient ainsi différer dans l'assimilation et la modification des lipides selon leurs besoins métaboliques, de reproduction ou de thermorégulation (Hill *et al.* 1992, Käkelä et Hyvärinen 1996, Best *et al.* 2003b, Pierce *et al.* 2004, Speake 2004, Munro *et al.* 2005).

L'analyses des données ou « data mining »

En plus de l'intérêt de mieux comprendre l'écologie alimentaire à travers la chimie analytique, il existe un besoin d'élaborer des méthodes robustes d'analyse exploratoire des données (Aitchison 1986, Andersson 2000, De'ath et Fabricius 2000, Hastie *et al.* 2001). Le grand nombre de variables issu des analyses de composés peut facilement dépasser le nombre d'échantillons. Leur utilisation comme traceurs trophiques requiert souvent

l'application de méthodes statistiques multivariées et non-paramétriques (Smith *et al.* 1997, Dalsgaard *et al.* 2003, Thiemann *et al.* 2004a). Ces techniques exploratoires permettent d'évaluer les patrons et les associations potentielles qui pourraient ne pas être évidents, ou même possibles, par l'application de méthodes statistiques traditionnelles ou de modèles linéaires (De Veaux 1995). Ainsi, les techniques d'analyse exploratoire des données sont devenues populaires dans plusieurs domaines scientifiques, allant de la chimie environnementale à l'écologie et à la génétique (Breiman *et al.* 1984, Brereton 1990, Yunker *et al.* 1995, Dudoit *et al.* 2000).

La popularité des méthodes multivariées est en grande partie liée à l'augmentation fulgurante de puissance et de convivialité des micro-ordinateurs et des progiciels (Afifi et Clark 1999). Par contre, l'évolution d'outils puissants est suivie par le potentiel d'un usage peu approprié ou incomplet pour l'analyse des données (James et McCulloch 1990, Smith *et al.* 1999, Legendre *et al.* 2002, Beaugrand 2003, Thiemann *et al.* 2004a, Hobbs et Hilborn 2006). En outre, il est souvent préférable de percevoir les méthodes multivariées comme un moyen d'indiquer les associations potentielles, et non comme des tests rigoureux de modèles basés sur des théories probabilistiques (Field 2000). Des mesures de puissance d'association et la réplication des résultats peuvent être appliquées à certaines procédures. Toutefois, la plus grande force des méthodes statistiques multivariées réside dans l'utilisation concomitante de plusieurs types d'analyse et dans la comparaison des résultats issus de chacune plutôt que dans l'exploitation d'une seule méthode statistique appliquée à toutes les situations et à tous les types de données (Fig. 1.3, Table 1.2).

Jusqu'à présent, on peut déceler un biais vers certaines méthodes ou progiciels dans la littérature scientifique. Par exemple, les analyses de fonctions discriminantes et les

transformations de données par 'log ratios' ou la mesure de distance Kullback-Leibler sont des concepts connus chez les écologistes traitant d'analyses spatiales (McClean *et al.* 1998, Burnham et Anderson 1999, Iverson *et al.* 2004, Silverman *et al.* 2004). D'autre part, la validation statistique des composantes principales, dérivée des pratiques de contrôle de qualité de la fabrication des produits chimiques en usine, est mieux connue par les chimistes marins (Frank et Lanteri 1989, Eriksson *et al.* 2002, Grahl-Nielsen *et al.* 2004). L'application d'analyses exploratoires des données ne devrait pas être vue comme une solution pour pallier aux problèmes liés à des biais de collecte ou à des données insuffisantes (James et McCulloch 1990, Underwood 2000). Néanmoins, ces techniques ont le potentiel de mettre en évidence des patrons utiles à tester et à modéliser dans l'avenir, en même temps que d'indiquer leurs limitations (Fowler-Walker et Connell 2002, Beaugrand 2003, Iverson *et al.* 2004).

1.2. Objectifs généraux

À la lumière des considérations sur l'écologie, la chimie des lipides et les méthodes d'analyse de données présentées précédemment, les objectifs principaux de cette étude étaient d'examiner les patrons inter- et intra-spécifiques des signatures en acides gras dans l'écosystème marin du Saint-Laurent tel que révélés par :

1) l'analyse du gras du lard des mammifères marins, et particulièrement le béluga;

2) l'analyse de spécimens entiers d'une gamme d'espèces de poissons et d'invertébrés qui représentent des proies potentielles pour les mammifères marins tels que le béluga.

Au cours de cette étude, ces objectifs ont été explorés de façon plus spécifique par l'examen de l'écologie régionale, les différences d'acides gras comme marqueurs trophiques et l'interprétation plus élargie des différences en acides gras telles que dévoilées par une variété de méthodes statistiques multivariées. Le corps de cette thèse est écrit sous forme de deux chapitres indépendants pour publication ultérieure dans la littérature scientifique primaire. Les objectifs seront présentés ainsi dans les chapitres qui suivent :

Le **Chapitre 2** vise à définir les profils d'acides gras pour une banque de proies potentielles de mammifères marins séjournant dans le secteur à l'étude pour la période 1999–2002. Les variations des profils d'acides gras des proies documentées dans ce chapitre, tant entre les espèces qu'entre les saisons ou endroits serviront de base pour l'évaluation quantitative ultérieure du régime alimentaire des principaux mammifères marins prédateurs de l'estuaire et du golfe du Saint-Laurent. En ce faisant, cette banque de données permettra également une mise à jour des informations sur les variations dans la qualité des espèces de proie (à partir de leur teneur en lipides), ce qui permettra de mieux comprendre la bioénergétique et le rôle trophique des prédateurs.

Le **Chapitre 3** vise à comparer les profils d'acides gras de certaines espèces de mammifères marins fréquentant l'estuaire et le golfe du Saint-Laurent: quatre espèces de pinnipèdes et une d'odontocète (le béluga) qui sont soit des résidents ou des visiteurs de l'aire d'étude pour la période de 1996-1998. Le béluga est d'un intérêt particulier compte tenu de son statut d'espèce menacée dans cette région et du manque d'information sur son régime alimentaire contemporain. À partir d'une série d'analyses multivariées, la classification des individus basée sur leurs profils d'acides gras permettra de distinguer ou d'associer les différents groupes. Ces comparaisons représentent un pas essentiel vers

l'évaluation future d'hypothèses concernant la compétition et le rôle trophique de ces espèces et des classes de sexe et d'âge.

Enfin, le **Chapitre 4** résume les résultats des analyses de profils d'acides gras des proies et des prédateurs. Il fournit aussi des recommandations quant à l'orientation d'études futures afin d'approfondir les résultats présentés dans cette étude.

Table 1.1. Liste des acides gras fréquemment détectés, organisés par le nom chimique, la longueur de chaîne, et le degré d'insaturation.

Nom spécfique	Nom court	Nom commun
	ACIDES GRAS SATURÉS	
tétradécanoïque	14:0	myristique
pentadécanoïque	15:0	pentadécanoïque
hexadécanoïque	16:0	palmitique
octadécanoïque	18:0	stéarique
éicosanoïque	20:0	arachidique
docosanoïque	22:0	béhénique
tétracosanoïque	24:0	lignocérique
	ACIDES GRAS MONOINSATURÉS	
9-tétradecenoïque	14:1n-5	myristoléique
9-hexadécenoïque	16:1n-7	palmitoléique
11-octadécenoïque	18:1n-7	vaccénique
9-octadénoïque	18:1n-9	oléique
11-éicosénoïque	20:1n-9	eicosénoïque
5,8,11-éicosatriénoïque	20:3n-9	hydromel
13-docosénoïque	22:1n-9	érucique
15-tétracosanoïque	24:1n-9	nervonique
	ACIDES GRAS POLYINSATURÉS (n-3)	
9,12,15-octadécatrienoïque	18:3n-3	*a*-linolénique
6,9,12,15-octadécatétraénoïque	18:4n-3	stearidonique
11,14,17-éicosatriénoïque	20:3n-3	éicosatriénoïque
8,11,14,17-éicosatétraénoïque	20:4n-3	éicosatétraénoïque
5,8,11,14,17-éicosapentaénoïque	20:5n-3	éicosapentaénoïque
7,10,13,16,19-docosapentaénoïque	22:5n-3	docosapentaénoïque
4,7,10,13,16,19-docosahexaénoïque	22:6n-3	docosahexaénoïque
6,9,12,15,18,21-tétracosahexaénoïque	24:6n-3	tétracosahexaénoïque
	ACIDES GRAS POLYINSATURÉS (n-6)	
9,12-octadécadiénoïque	18:2n-6	linoléique
6,9,12-octadécatriénoïque	18:3n-6	*g*-linolénique
11,14-éicosadiénoïque	20:2n-6	éicosadiénoïque
8,11,14-éicosatriénoïque	20:3n-6	homo-*g*-linolénique
5,8,11,14-éicosatétraénoïque	20:4n-6	arachidonique
13,16-docosadiénoïque	22:2n-6	docosadiénoïque
7,10,13,16-docosatétraénoïque	22:4n-6	docosatétraénoïque

Table 1.2. Sommaire des techniques statistiques multivariées fréquemment employées lors d'analyses de données biochimiques.

Méthode Multivariée (acroynme)	Type de classification	Exemple tiré de la littérature
Analyse en composantes principales (PCA)	Apprentissage non-supervisé, ordination	(Joensen *et al.* 2000)
Modélisation de classes (SIMCA)	Apprentissage supervisé, ordination	(Marengo *et al.* 2001)
Régressions multiples	Apprentissage non-supervisé, régression	(Dalsgaard *et al.* 2003)
Analyses discriminantes (DFA)	Apprentissage supervisé, régression	(Iverson *et al.* 2002)
Analyse de groupements (HCA)	Apprentissage non-supervisé, ordination	(Howell *et al.* 2003)
Arbres de classification (CART)	Partitionnement récursif binaire	(De'ath et Fabricius 2000)

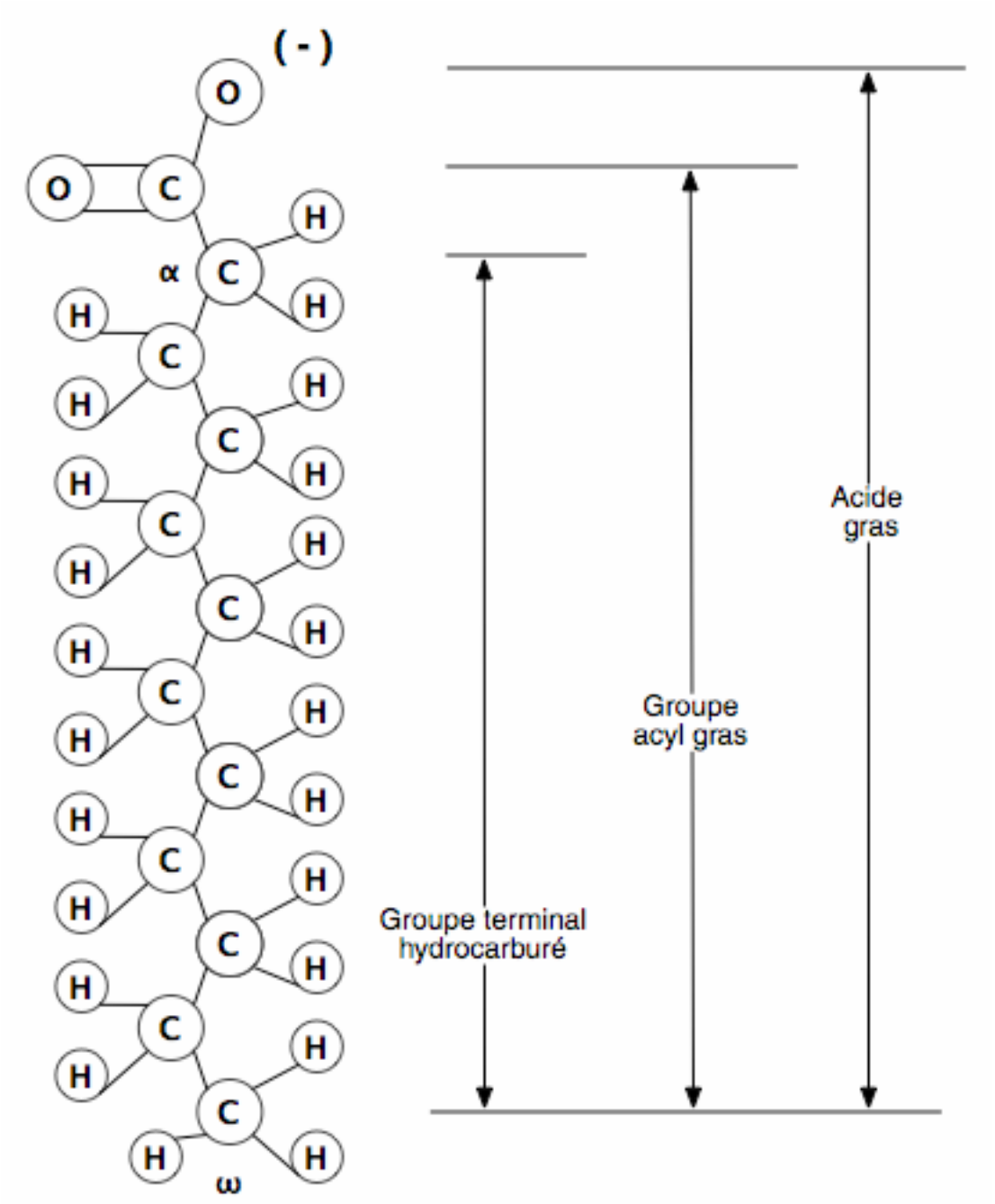


Figure 1.1. Schéma d'un acide gras typique, avec un carbone estérifié en tête (COO^-) et un groupe méthyle (CH_3) terminal. L'exemple présenté ici concerne une chaîne saturée (*i.e.,* pas de lien double entre les carbones) avec le 12^e carbone en position terminale (ω).

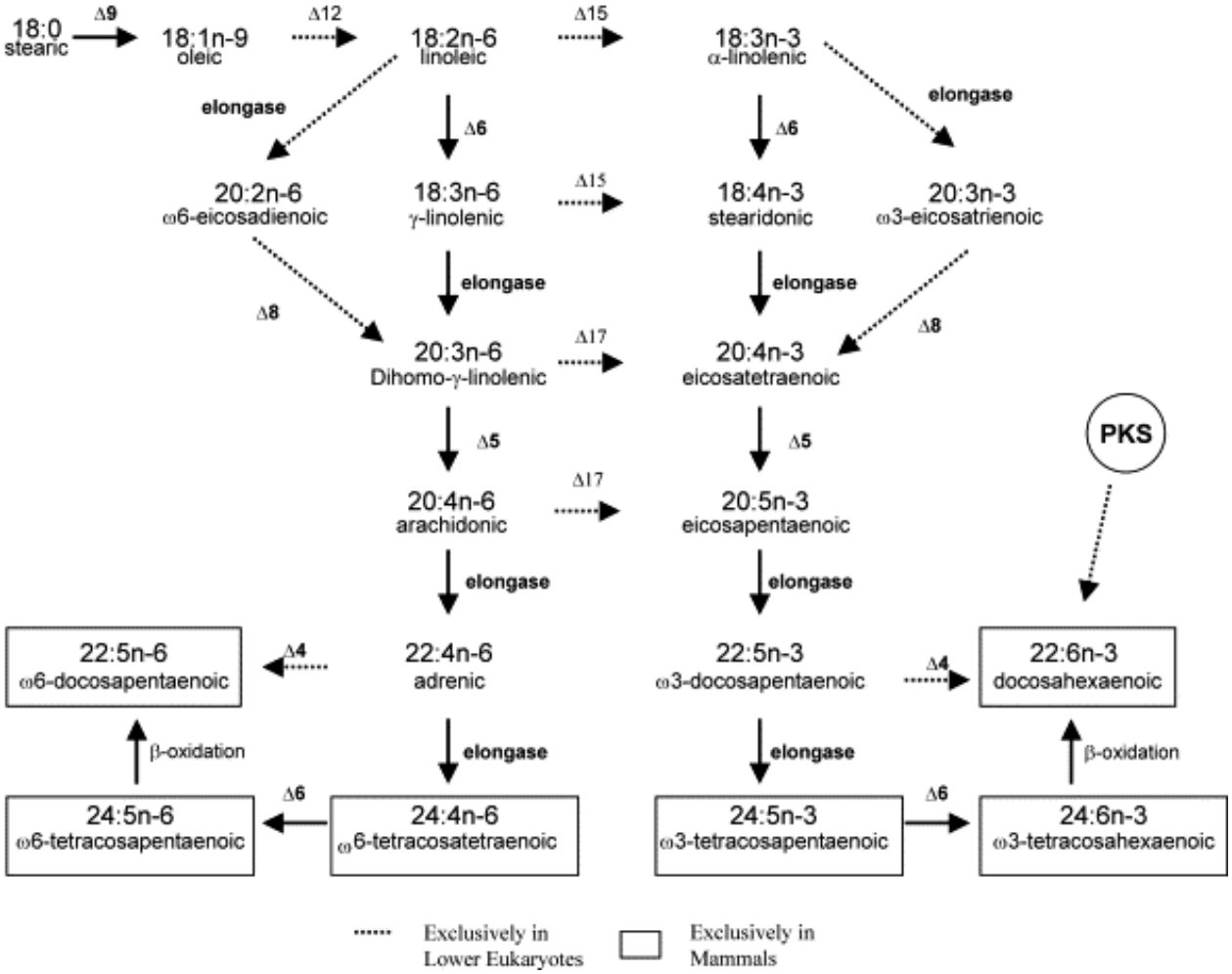


Figure 1.2. Exemple de spécificité des voies de synthèse pour les acides gras à longue chaîne polyinsaturée. Les flèches solides indiquent les voies retrouvées chez les mammifères tandis que les flèches pointillées représentent les voies exclusives aux autres types d'organismes. Tiré de la Fig. 1 de Leonard *et al.* (2004).

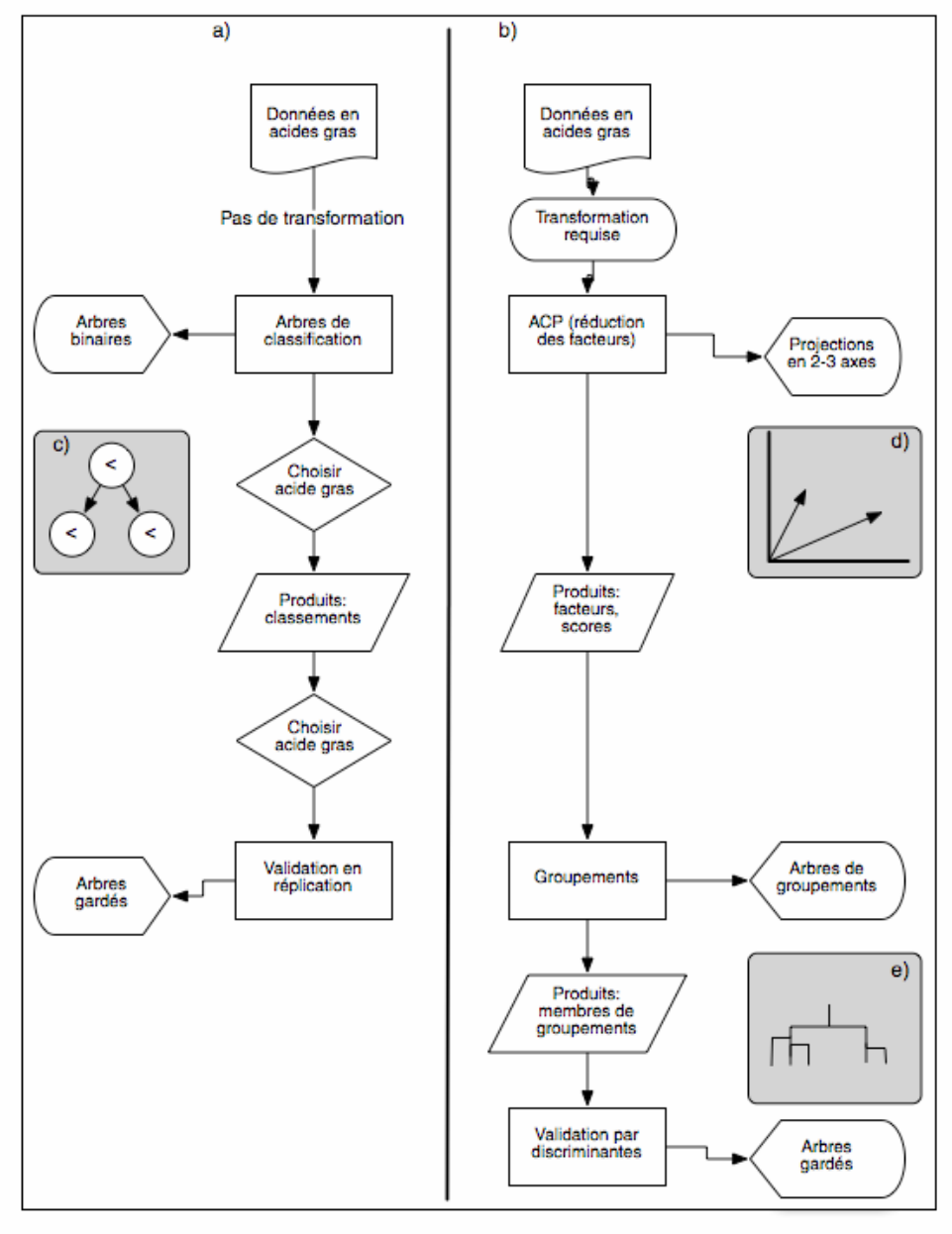


Figure 1.3. Schémas typiques de l'analyse des données d'acides gras à l'aide des techniques multivarieés semblables à celles utilisées au chapitre 3 : a) démarche lors d'une analyse suivant un arbre de classification. b) démarche lors d'une analyse impliquant une suite d'analyses en composantes principales, de groupements, et des fonctions discriminantes. Les encadrés gris illustrent des exemples de sorties graphiques associées avec ces techniques de classification, soit; c) séparation en arbre de décisions, d) projections linéaires, et e) association de cas lors d'analyses de groupements.

Chapitre 2

Among- and within-species variability in fatty acid signatures of fish and invertebrate prey of the St. Lawrence beluga whale

Résumé

Les profils d'acides gras de 60 espèces de proies potentielles du béluga (*Delphinapterus leucas*) de l'estuaire et du golfe du Saint-Laurent ont été examinés pour leur variabilité par espèces et autres classes. Les similaritiés et les différences révélées par les statistiques multivariées ont suggéré que plusieurs espèces sont relativement homogènes en composition, tandis que d'autres pouvaient varier selon la saison et l'endroit. Les tendances générales présentées ici pourraient être utiles pour le classement des groupes de proies tels que pélagiques ou benthiques, estuariens ou marins, et pour la modélisation future de la diète de prédateurs. Néanmoins, la variabilité potentielle en contenu lipidique et en composition en acides gras invite à la prudence dans l'emploi des valeurs moyennes pour répondre à des scénarios spécifiques.

Abstract

Fatty acid signatures of 60 potential prey species of the beluga whale (*Delphinapterus leucas*) of the Estuary and Gulf of St. Lawrence were examined for their variability by species and other classes. The similarities and differences revealed through multivariate statistics suggested that a number of species were relatively homogenous in composition, while others could vary by season and location. The general trends shown here might be useful for prey group classifications, such as pelagic or benthic, estuarine or marine, et will be useful in future modelling exercises of predator diet. Nonetheless, the potential variability in lipid content and fatty acid composition suggests caution in the application of mean values for answers to more specific situations.

2.1 Introduction

The use of fatty acids to examine the diet of marine predators has interested researchers ever since the development of tools for detailed lipid analysis made comparisons readily possible, and it was observed that fatty acid profiles of consumers would generally reflect those seen in their diet (Folch *et al.* 1957, Ackman and Eaton 1966, Dalsgaard *et al.* 2003). Aquatic and marine systems, in particular, are notable for their abundance of lipids containing longer-chained (*i.e.*, 14 – 22 carbons) unsaturated fatty acids that arise from plants and plankton (Beach *et al.* 1974, Ackman *et al.* 1980, Dunstan *et al.* 1994). By comparison, vertebrates are greatly limited in their capacities to synthesise polyunsaturated fatty acids and must obtain these nutrients in their diet (Kanazawa *et al.* 1979, Tinoco 1982, Leonard *et al.* 2004). This limitation represents the starting point for using fatty acid profiles as indicators of assimilated prey items in predators (Tjonneland *et al.* 1993, Iverson *et al.* 1997a, Brown *et al.* 1999).

As a trophic tracer, fatty acids have already been used in a variety of ways, depending on the subject and hypothesis being investigated (Dalsgaard *et al.* 2003). In some situations, the presence of certain fatty acids in a dietary item has been used to identify its uptake. This has been particularly the case with consumers that specialise on classes of plants or invertebrates that are often characterised by these specific fatty acids which otherwise may be uncommon in the local environment (Ackman and Hooper 1973, Holland *et al.* 1990, Mansour *et al.* 2005). In order to gain use as a general tool, studies comparing among or within species of marine vertebrates have also been conducted using the relative abundance of a range of fatty acids, also called the fatty acid signature (Iverson

et al. 1997b, Budge *et al.* 2002, Iverson *et al.* 2002). Results of the signature approach, both from captive feeding trials and from samplings of free-ranging organisms, have shown promise for investigating trophic interactions (Kirsch *et al.* 1998, Phillips *et al.* 2003, Walton and Pomeroy 2003, Jeffs *et al.* 2004). This potential for tracing diets has led to studies attempting to relate the fatty acid signatures of predators to those of potential prey, both qualitatively and quantitatively (Dahl *et al.* 2000, Kirsch *et al.* 2000, Olsen and Grahl-Nielsen 2003, Andersen *et al.* 2004). These studies reveal the challenges in making inferences between prey and predators using fatty acids (Smith *et al.* 1997, Budge and Iverson 2003, Budge *et al.* 2004, Iverson *et al.* 2004). A number of hurdles remain before this approach can be used with confidence on a variety of marine mammal species in the wild (Grahl-Nielsen *et al.* 2004, Staniland and Pond 2004, Theimann *et al.* 2004). Central to this debate is the manner in which fatty acid differences are interpreted, in particular, how to evalutate the similarities/divergences between the signatures of assimilated prey and those of predators.

Documenting the dynamics of fatty acid composition of potential prey is an important step towards their use in trophic linkages (Budge *et al.* 2002, Iverson *et al.* 2002, Phillips *et al.* 2002, Speake 2004). Along with species differences, fatty acid compositions may vary with size, season or location, potentially reflecting changes in diet, but also in response to environmental conditions or life history including population genetics (Henderson *et al.* 1984, Joensen *et al.* 2000, Morais *et al.* 2003, Krahn *et al.* 2004). At the same time, predators may forage over large areas, getting access to a range of potential prey species that may vary in quantity and quality. Ostensibly, the ideal prey base would encompass all prey types for all areas covered by a predator, although in practice, most

spatial diet studies should take life history into account to improve sampling design (Iverson *et al.* 2004, Fritz and Hinckley 2005). The impracticalities arise because the logistics of field sampling, along with the time and expense of chemical analyses make it prohibitive to collect all possible variations of a prey species with replicates of 20 or more, as might be expected for statistical evaluations using several fatty acids as variables. Furthermore, it is not usually possible for the prey sampling to fully coincide with the locations, seasons or years of predator sampling (Dahl *et al.* 2000, Hooker *et al.* 2001). Therefore, examining the relative stability of the fatty acid signatures of prey, between species and for other classes including size, season and location, is of particular interest to validate the use of available prey data in long-term studies of marine mammal predators.

Such a need to understand prey variability is evident in the context of our long-term study of beluga whales, *Delphinapterus leucas*, of the St. Lawrence Estuary, Canada (48°N, 70°W). Belugas in the Estuary form a small and relict population of approximately 1200 individuals (Gosselin *et al.* 2001). Although an unknown portion of the population migrates to the northwestern Gulf of St. Lawrence during winter, many of the individuals appear to remain in the Estuary throughout the year (reviewed in Lesage and Kingsley 1998). The population was estimated at several thousand individuals and was broader in distribution at the end of the 19th century, but it was decimated by intensive hunting in the first half of the 20th century (Sergeant and Hoek 1988, Kingsley 2002). Census surveys carried out over the past two decades suggest that the population has only been increasing at a slow rate, if at all, despite the absence of mortality from predation or hunting (Gosselin *et al.* 2001).

The enigma of their slow recovery, and the vulnerability associated with being a small, endemic population are matters of continuing concern for the long-term viability of belugas in the St. Lawrence Estuary (Sergeant and Hoek 1988, Smith *et al.* 1990, COSEWIC 2004). Pollution, habitat loss or degradation associated with coastal development and marine traffic (noise, ship strikes, harassment), along with shifts in prey abundance, availability or quality (energy density) are among the factors proposed to explain the limited growth of this population (Sergeant and Hoek 1988, Muir *et al.* 1990, Martineau *et al.* 1994, De Guise *et al.* 1995, Kingsley 2002). The lack of recent information on beluga diet in the St. Lawrence limits our capacity to evaluate the impact of prey sources on population health, for example when considering pathways for contaminant accumulation or when proposing food limitation.

The only detailed information on the diet of St. Lawrence beluga comes from an extensive study carried out in the 1930s (Vladykov 1946). This study remains the reference for beluga feeding even though its value for understanding contemporary diets is unclear. For example, in his study, Vladykov examined stomach contents of mostly adult males that were hunted principally from river mouths that are no longer being used (Laurin 1982, Reeves and Mitchell 1984, Kingsley and Reeves 1998). Secondly, changes in prey species assemblages are believed to have occurred in the St. Lawrence marine ecosystems and elsewhere in the Northwest Atlantic following the collapse of the commercial Atlantic cod fishery in the 1990s (Myers *et al.* 1997, Sinclair and Murawski 1997, Frank *et al.* 2005).

The contemporary diet of beluga whales in the Estuary cannot be documented using traditional methods such as sampling of digestive tract contents because of the precarious status of the present population. The only beluga samples available are from beach-cast

strandings, of which approximately fifteen are reported annually in the St. Lawrence. Those carcasses recovered in sufficiently good condition for a necropsy examination often show signs of chronic poor health leading up to their death, and thus it is hardly surprising that prey remains are very rarely reported in their stomachs. Only a few cases have been diagnosed as trauma, *i.e.*, individuals experiencing sudden death from ship strikes. While these case may be good opportunities to obtain prey remains and thus information on recent feeding events, it remains difficult to evaluate diet at the population level over long periods.

Longer-term dietary information might be obtained by examining the fatty acid composition of blubber collected since 1988 from beluga carcasses that have stranded. Information on diet has previously been obtained in fatty acid studies of fishes and pinnipeds (Kirsch *et al.* 1998, Kirsch *et al.* 2000). A great deal of interest has since been generated by the potential to identify and quantify prey contributions in predator signatures using maximum likelihood-based approaches to modeling, such as QFASA or Quantitative Fatty Acid Signature Analysis model developed in the laboratory of Dr. Sara J. Iverson (Iverson *et al.* 2004). In order to be accurate and predictable, and therefore useful, a number of conditions are required prior to undertaking predator diet modelling exercises (reviewed in Iverson *et al.* 2004). Inferring potentially diet-linked variations in the signatures of beluga carcasses first requires information concerning the relative stability of prey fatty acid signatures and lipid contents, across species and over time and space. This is necessary because belugas may be feeding in a number of different areas, from the Estuary downstream to the northern Gulf, and the collection of carcasses span over several seasons and years.

In this study, we propose to screen fatty acid compositions and lipid contents of potential prey of beluga for the variables that are of greatest relevance for models regarding their diet. We first consider the local ecology (prey availability) and the absolute amounts of fatty acids (prey lipid content), before examining fatty acid compositions (prey signatures) of potential prey of St. Lawrence beluga whales. Given the range of species and environmental conditions that may be encountered, the characterisation of inter- and intra-specific variability of fatty acid composition of potential prey constitutes an essential step towards a better understanding of the contemporary feeding ecology and diet in this endemic population of whales.

2.2. Materials and methods

Species selection

Species for the fatty acid prey base were selected using two principal sources of information. First, we consulted local records of beluga prey, as determined from observations and stomach contents (Préfontaine 1932, Vladykov 1935, 1946, Pippard and Malcolm 1978). Second, we examined current prey species distributions and relative abundance, examples of which are recorded during fisheries and scientific surveys in the Estuary and adjacent Gulf of St. Lawrence (Therriault 1991, Bérubé and Lambert 1999, Caron *et al.* 2001, Benoît *et al.* 2003b). As a result, approximately 60 species of fish and invertebrates were targeted as potential prey of the beluga.

While most samples were 10–40 cm in length, smaller and larger specimens were also sampled. Copepods (*Calanus hyperboreus*), euphausiids or krill (*Meganyctiphanes norvegica, Thysanoessa* spp.), and hyperiid amphiphods (*Themisto libellula*), were included

to better understand the variability of fatty acid composition in the planktivorous fishes and invertebrates that may be consumed by belugas (Dahl *et al.* 2000, Auel *et al.* 2002). Concerning maximum prey size, there have been reports of adult male belugas consuming specimens such as eels and sturgeon up to 80 cm in length (Vladykov 1946). However, belugas are constrained by their relatively small oesophagus and inability to handle prey, making them more suited to swallowing whole, relatively small-bodied prey. The other consideration regarding maximum prey size was partially historical. Observations from local fisheries and surveys indicate that belugas are presently less likely to encounter larger specimens, especially of eels, salmon and groundfishes (*e.g.*, Atlantic cod, Atlantic halibut, Atlantic sturgeon), in the St. Lawrence compared to past decades or other areas such as Cook Inlet, Alaska (Castonguay *et al.* 1994, Bérubé and Lambert 1999, Huntington 2000, Christensen *et al.* 2003). Consequently, only larger specimens of salmon and eel were available and thus included for species and size-class comparisons relative to other potential prey.

Sample collection

The primary source for open-water samples were bottom-trawl surveys for groundfish and shrimp conducted during the ice-free period (late April to late October) in the Estuary and northern Gulf of St. Lawrence during 2000–2002 (Fig. 2.1). Additional specimens of spawning and littoral species were obtained during near-shore dives and from hand-collection by local partners at tidal fishing weirs along both the north and south shores of the estuary. The bulk of sampling took place in 2001, with additional specimens collected in 1999, 2000, and 2002. A number of the targeted species were encountered on repeated occasions, either as residents or during their seasonal migrations through the

Estuary and Gulf. Repeated sampling for species that were at once abundant and presumed significant prey for beluga was carried out when possible to investigate their variability across the study region, by size-class and season. This sampling sought to compare specimens from the Upper Estuary, the Lower Estuary, as well as the northern Gulf of St. Lawrence, for spring, summer and autumn, between 2000-2002 (Table 2.1, Annexe II). All samples were stored frozen at -20°C in plastic bags until processed for lipid extraction and fatty acid analysis. Storage time before processing ranged from 6 mo. to 2 yrs.

Lipid content and fatty acid analysis

Individual specimens were thawed and measured (standard length) to the nearest 0.1 cm and weighed to the nearest 0.1 g. Samples of smaller invertebrates (zooplankton) were comprised of pooled sets (usually 2–5) of individuals to obtain sufficient wet material for lipid extractions. After being thawed and measured, samples were homogenised individually in a blender or food processor. No attempt was made to remove stomach contents prior to homogenisation, so that the specimen represented the prey item as consumed by a predator. In some cases, additional specimens of fish that were not processed for analysis had their stomach contents qualitatively examined for general information regarding recent feeding events (Annexe I).

Using a modified Folch method, lipids were quantitatively recovered in duplicate from samples of the homogenate (Budge *et al.* 2002). In brief, 1.5 g aliquots were extracted with 30 ml 2:1 chloroform-methanol, and the entire lower phase was collected. This fraction was then washed, filtered through anhydrous sodium sulphate, evaporated under nitrogen, and vacuum sonicated to obtain total lipid mass. Fatty acid methyl esters (FAME)

were prepared in duplicate according Iverson *et al.* (1997b). The analysis of FAMEs was conducted using a Perkin-Elmer Autosystem II capillary gas chromatograph (GC) (Norwalk, CT) with a flame ionisation detector (FID) using a flexible fused silica column (30 x 0.25 mm ID) coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness; J&W DB-23; Folsom, CA). 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, 2001) and reported here as weight percent of total fatty acids. Each fatty acid was described using the shorthand nomenclature of *X*:*Yn–Z*, where *X* represents the number of carbon atoms, *Y* the number of double bonds, and *Z* the position of the double bond closest to the terminal methyl group. During the analyses, several samples were identified as containing large amounts of wax esters. These samples were subsequently reprocessed for their fatty alcohols and then methylated to be combined with the FAMEs as derived from the acyl lipids as per Budge and Iverson (2003), thus ensuring the quantification of all assimilated prey lipid components.

Statistical analysis

A common requirement in statistical analyses is that the variables used be fewer in number than the smallest sample size (Hair *et al.* 1998, Field 2000). This constraint poses difficulties because of the very large number (60 or more) of fatty acids that might be selected to serve as explanatory variables in a signature analysis (Smith *et al.* 1997). Statistical analyses are therefore usually performed on a subset (n = sample size - 1) of fatty acids that are of greatest interest (Iverson *et al.* 2004). In this study, several species

were represented by 18 or more samples. As a result, statistical evaluation was conducted using 17 fatty acids: 16 of the generally most abundant and diet-linked fatty acids, along with the reference fatty acid 18:0 (often present in relatively small and stable amounts). Prior to all tests, the data were renormalised over 100% and logratio transformed over the fatty acid 18:0, which is the recommended data treatment for this type of compositional data (Aitchison 1986, Budge *et al.* 2002).

To examine fatty acid compositions while including those species only available in small sample sizes, one solution is to conduct a Principal Component Analysis (PCA). This approach summarises the original set of variables into a smaller set of independent factors. Although PCA is not strictly limited in the number of variables to be used, measures of adequacy and the interpretation of results are usually improved with a restraint on the number of input variables and the use of appropriate data transformations (Bonn 1998, Osborne and Costello 2004). The PC analyses were therefore restricted to using the set of 16 logratio fatty acids. From the analysis, only those PC factors with eigenvalues >1 were retained as significant. Following a Varimax rotation of axes (Kaiser 1958), the greatest absolute loadings of the fatty acid variables contributing to each retained factor were evaluated. The results were also summarised as two-way plots by factor for the individual scores.

Establishing predictive rules with which to distinguish samples into known classes while using several variables is a typical application of Discriminant Function Analysis, or DFA (Bradshaw *et al.* 2003). For this investigation, DFA was used in a more exploratory fashion, to reveal the extent to which defined groups may be readily distinguished using the set of fatty acids (Field 2000). To achieve this goal, the classification results were visually

summarised by the first two significant discriminant functions, or canonical variates, and presented in biplots of the fatty acid variables along with individual scores and their 95% confidence intervals over group means.

Variability in lipid contents between samples was examined using one-way ANOVAs followed by a pairwise comparison of means using post hoc Tukey's test. Significance was established throughout at $a = 0.05$ All statistical analyses were performed using the JMP 5.1© (SAS Institute Inc, Cary, NC) software package.

2.3. Results

Representation in prey base

A total of 1028 samples were analysed for their lipid content and fatty acid composition, with major taxa including five species of zooplankton, 14 species of crustaceans (shrimps and crabs) and molluscs (cephalopods and gastropods), and 40 species of demersal and pelagic fishes (Table 2.1). Among the repeated samples were three pelagic (herring, capelin, and smelt) and four demersal (plaice, flounder, tomcod and shrimps) species. Other potentially important prey of beluga, at least historically, failed to be obtained for all desired periods or location, as in the case for Atlantic cod and sand lance in autumn, or Arctic cod and shortfin squid that were encountered only in the Gulf.

Lipid content

Mean amounts of total lipids in percent wet mass ranged from < 1% to over 20% among species groups (Table 2.2). In general, lipid contents were highest (over 12%) in copepods and anguilliform fish (eels, lampreys, and hagfish), followed by the large pelagic fish species (salmon, shad, herring, barracudina, and Greenland halibut) with mean amounts

of 6–12% lipids, the smaller pelagic species (sand lance, capelin, shortfin squid, smelt, daubed shannies, euphausiids and amphipods) with 3–6.5% lipids, and the decapod crustaceans (shrimps and crabs) and most of the demersal fish species (sculpins, flatfishes, and codfishes) with mean lipid contents of 2% or less. Exceptions were longfin hake and pollock that occasionally had lipid contents several times those of other codfishes.

As expected, season significantly affected lipid contents in a number of groups, but these variations differed with size class and sampling location. For example, lipid contents were generally lower in the spring compared to summer or autumn in large herring (>18 cm) and capelin whereas lipid contents of smaller herring (<18 cm) did not change significantly between spring and autumn. The lipid content of smelt also varied significantly with season, but patterns varied regionally. Smelt from the Upper Estuary differed significantly in their lipid contents between May (1.6%) and October (3%), while smelt from the Lower Estuary showed high lipid contents in July (6%) and October (6.6%) and lower levels in intervening periods. In contrast to the pelagic species, lipid contents of shrimps and most demersal fishes were less affected by seasonal changes, but these latter groups often had lower absolute amounts of lipids. For example, lipid levels in similarly sized tomcod rose from 2% in spring to 3.5% in late summer. For plaice, individuals were only marginally richer in lipid in spring compared to summer or autumn (means = 2.2% vs. 1.4% and 1.5%, respectively, one-way ANOVA $F_{2,39} = 3.3$, $P = 0.049$).

General fatty acid compositions

The analysis identified up to 67 fatty acids and their isomers, 25 of which had global mean amounts >0.4% each (Table 2.2). The subset of 17 fatty acids retained for statistical comparisons were 14:0, 16:0, 16:1n-7, 16:4n-1, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 18:4n-

3, 20:1n-11, 20:1n-9, 20:4n-6, 20:5n-3, 22:1n-11, 22:1n-9, 22:6n-3, and the reference fatty acid 18:0, which together accounted for an average 88% of the total fatty acids by mass.

The selection of abundant fatty acids or those known to be associated with diet is one approach towards facilitating the identification of dietary components. However, the use of a restricted set (17 vs. 25 or 67) of fatty acids can result in the loss of useful information for this task. Several fatty acids were detected in significant amounts in certain species, but were in very low levels when averaged across all species. This was the case for the fatty acid 22:1n-7 (global mean = 0.27%), which averaged >0.4% in daubed shannies, plaice, herring, and several species of crustaceans (Fig. 2.2). Similarly, the fatty acid 14:1n-9, was found in amounts > 0.5% in gastropods including whelks, and in flatfishes such as witch flounder, Greenland halibut, and plaice. However, as neither of these monounsaturated fatty acids is well understood with regards to dietary origins, they were not considered further in comparisons. Several other fatty acids were detected at similarly low levels (*i.e.*, ≤ 0.5%), but were retained because of their better-known association with dietary sources. This was the case for fatty acid 18:2n-6, 18:3n-3 and 18:4n-3 that were found in substantial levels only in estuarine species such as salmon smolts, eels, nereid worms, snailfishes, and smelt (Fig. 2.3). Similarly, the fatty acid 16:4n-1 was generally detected in trace amounts with the notable exception of herbivorous zooplankton including *Thysanoessa* spp. (krill) and *Calanus hyperboreus* (copepod), but also certain fishes, notably herring sardines, sand lance, and smelt.

Other polyunsaturated and monounsaturated fatty acids were detected globally in substantial amounts, and exhibited wide inter-specific variability (Table 2.2). The fatty acid 22:6n-3 was especially abundant in marine demersal fishes (hake, cod, rockling,

haddock) and in squid, octopus, lumpfish, and skates, although each of these also had a low fat content and therefore relatively low absolute amounts of fatty acids. Similarly, the polyunsaturated fatty acid 20:5n-3 was often abundant in several species that had low total lipid contents, such as crabs, shrimps, whelks, and octopus. Another marine-linked fatty acid, the monosaturate 20:1n-9, was seen in greatest relative abundance in copepods, followed by hyperiid amphipods, the larger specimens of sand lance, herring, and shad (each averaging >15% for this fatty acid). In contrast, eels, salmon smolts, and shad sardines had very low amounts of this fatty acid, averaging 1% or less.

Multivariate comparisons among and within species

An exploratory PC analysis examined all specimens, both the species in low numbers alongside the more-abundant groups. This analysis reduced the set of 16 fatty acid logratios into four significant factors that together accounted for 82% of the original variance (Table 2.3). Following Varimax rotation, Factors 1, 2, 3, and 4 explained 18, 25, 18, and 21% of the variance, respectively. Correlations of fatty acids 20:5n-3 and 22:6n-3 were negative with Factor 1. Fatty acids 18:2n-6 and 18:3n-3 were strongly positively (>0.8) on Factor 2, along with high loadings for 16:0, 16:1n-7, 18:1-9 and 18:1n-7. Factor 3 was strongly negative for 20:4n-6 (>0.9), but positive for 14:0, 16:5n-3, 18:4n-3. Factor 4 was characterised by positive loadings for the 20-series mononunsaturated fatty acids of 20:1n-9, 20:1n-11, 22:1n-9, and 22:1n-11. A two-way matrix plot of the rotated PC factors revealed that most samples had relatively similar scores, although some species were segregated (Fig. 2.3). Species that were projected farthest from the origin of axes included estuarine fishes (salmon smolts, shad sardines, and eels) and smaller crustaceans (eualid shrimps, amphipods, and copepods).

We next carried out a series of pairwise comparisons of the retained factors, aimed at elucidating patterns among selected sets of samples. Thus, in a new PCA based only on eight prey species commonly found in the Estuary in autumn, two PC factors were sufficient to summarise 80% of the total variance. Examining the rotated factor loadings, Factor 1 (51% of variance) was characterised by a negative correlation with 20:4n-6 and a strongly positive correlation with the marine-linked fatty acids 16:4n-1, 18:4n-3, 20:1-9, 20:5n-3, 22:1n-9, 22:1n-11, and 22:6n-3. Factor 2 (29% of variance) was most strongly correlated with the polyunsaturated fatty acids 18:2n-6 and 18:3n-3, but also with the common monounsaturated fatty acids 16:1n-7, 18:1n-7, and 18:1n-9 (Table 2.4). Plotting sample scores on these two factors had eels segregated from pelagic species on the basis of low values on Factor 1 and from demersal species on high values on Factor 2, with smelts interspersed among the latter two groupings (Fig. 2.5a). While seeking to enhance the segregation of species in the two-way plots, it was observed that groups with positive scores on Factor 2 also demonstrated relatively high lipid contents (Fig. 2.5b). A reevalution of of the two-way plot in the PCA using all specimens also indicated that the additional character of lipid content could serve along with the PC Factors to highlight certain species group, such as eels that scored positive on Factor 1, and copepods on Factor 3, while hagfish had positive scores on both of these factors (not shown; Annexe III b).

As a separate PCA for autumn samples was similar in projection relative to the the analysis when using all specimens (not shown), an examination of selected species scores was continued based on the results of the original PCA. In one example, the factor scores of four species of the cod family were compared because they represented a diverse and ecologically-important group. Partial segregation between Atlantic cod and several tomcod

was suggested on Factor 1. While there was considerable overlap in scores between the two species, the majority of tomcod were positive on Factor 1 and revealed smaller contributions of 20-series polyunsaturated fatty acids compared to Atlantic cod (Fig 2.6). Also seen with Factor 1 was a possible separation between pollock, with the three specimens from the Gulf scoring closer to Atlantic cod than were three specimens from the Estuary. Positive scores on Factor 3 appeared to segregrate pollock and Arctic cod from Atlantic cod. Another potential distinction was seen with Factor 4, due to greater amounts of the 20-series mononunsaturated fatty acids in many of the larger Atlantic cod that scored positive as compared to Arctic cod and most tomcod. When combined, Factor 1 and 4 appeared to be the best segregators of Atlantic cod from the other gadid specimens in this total PCA series.

The factor score plots for the pelagic group of capelin, smelt, and herring were next explored in order to evaluate the limits of the information that may be obtained from these similar species when using PCA. A plot restricted to the first two PC Factors for the three species confirmed their broad overlap in scores, principally on the basis of all polyunsaturated fatty acids, with the exception of 20:4n-6 (Fig. 2.7). A full matrix plot suggested that Factor 3 could be used to segregate most capelin and herring from smelt, largely on the basis of the greater amounts in fatty acid 20:4n-6, while Factor 4 generally distinguished capelin from herring, due to greater amounts of 20-series monounsaturated fatty acids in the latter species. Among these repeated samples, capelin varied the least on the first two Factors, as sugggested by their smaller distribution of scores (grey-filled convex area) relative to herring or smelt (Fig. 2.7). Detailed examination of the species revealed that capelin, on the whole, were least variable on Factor 1 (grouping near -1), but

some separation between samples, according to location, season and year, was apparent along Factor 2 (Fig. 2.8; see full matrix, Annexe III c). In contrast, when examining all three species for one subset of samples (autumn in the Upper Estuary in 2001), once again no single PC Factor clearly separated the groups, except that sardines (small herring) loaded negative relative to large herring (18 cm or more) on Factor 1 (Fig. 2.9).

As seen earlier, when presented with similarity in factor scores, or species overlap, an examination of lipid content occasionally served to enhance group segregation, though not necessarily by species. For example, some of the fattier smelt were noticeable as scoring higher (>1) on Factor 2 than other smelt and the capelin or herring, while the fattest herring (>10% lipid) more closely resembled capelin on both Factors than they did to either the leaner herring or the smaller (and also leaner) herring sardines (Fig. 2.10).

Following these general explorations using PCA and total lipids, differences in fatty acid signatures were next compared using a series of discriminant function analyses. An initial analysis was restricted to seven of the most-abundantly sampled species groups. In this scenario, plotting on the first discriminant function had capelin scoring closest to herring, while tomcod, crangon shrimp, and flatfish appeared closer to the origin (less negative scores). Smelt were distinguished from all other species by their positive scores on the second discriminant function. Despite some overlap in individual scores (not shown), each of the species group means were significantly segregated, as suggested both by the separation of their 95% confidence interval ellipses of group means and by the very low number of individual misclassifications 4%, or 20 of 499), half of which were between capelan and herring (Fig 2.11).

A subsequent discriminant analysis was conducted on all groups with sample sizes greater than 17, thereby making full use of the logratio set of fatty acids. All smaller groups were also plotted on the resulting discriminant functions in order to suggest potential associations for future exploration with larger sample sizes. For the groups used in the analysis, the misclassification rate was 10% (70 of 699). In plotting individual scores on Discriminant Functions 1 and 2, the whelks were strongly segregated from all other species, principally on the basis of their lower amounts of fatty acids 16:0 and 16:1n-7. Eels were the next most distinctive species from the remaining groups, essentially on the basis of very low amounts of several monounsaturated fatty acids.

A subsequent analysis that excluded whelks and eels showed more clearly-discernible groupings of demersal fishes, pelagic planktivores (fishes and macrozooplankton), and decapod crustaceans (Fig. 2.12). The first discriminant function appeared to separate marine and pelagic from estuarine and demersal species, while the second discriminant function mostly segregated crustaceans from fishes. Those samples grouped in the scores plot as ‘marine’ or ‘pelagic’ were generally associated with an abundance in ‘plankton-linked’ fatty acids, 16:0 and 22:1n-11, and lower amounts of ‘plant detritus-linked’ 20:4n-6 as compared with those samples that scored towards the ‘estuarine’ or ‘benthic’ labels. The decapod shrimps were associated with high levels of fatty acids 16:1n-7 and 18:1n-7. Smelt continued to be intermediate between the other two putative fish groupings on the first discriminant function, and oriented towards eels when examined on the second discriminant functions. Of interest among the individual classifications, four Greenland halibut, obtained near the Upper Estuary, scored closer to the estuarine demersals compared to other specimens that appeared in the group of pelagics.

In light of these results, further discriminant analyses tested the efficacy of group classifications as suggested from the species analysis. For example, spring of 2001 was a period when a number of of both pelagic and demersal species were obtained at a time when the spring spawning of capelin is the focus of activity in the local marine ecosystem. A discriminant analysis for this period was highly successful (91%, or 95 of 102 correctly classified) at placing specimens into one ot two functional groups as either pelagic or demersal primarily on the basis of fatty acids 14:0, 18:4n-3 and 22:6n-3. Of note, five of the nine misclassified specimens were of larger specimens of tomcod (18–32 cm). These individuals, along with two flounder and one crangon shrimp, were predicted as belonging to the pelagic group that included capelin, principally on the basis of their higher amounts of 22:6n-3 (Annexe III d).

Autumn was another period chosen to examine group patterns in signatures, to evaluate potential differences after a summer of feeding. A discriminant analysis classifying species from autumn samples (2000 and 2001) highlighted the segregation of eels on the basis of their low amounts of 20:1n-9, 22:1n-11 and 22:6n-3 (not shown). Once again, excluding the distinctive species (eel) and forming composite species groups revealed four broad, but still-significant, groupings. In this successful analysis (0 of 156 misclassified), the pelagic (smelt, herring, and capelin), demersal (flatfishes) and marine shrimp (eualid and striped shrimps) groups scored apart from the crangon shrimp group, principally on the basis of the major polyunsaturated and 18-series mononunsaturated fatty acids that contributed to the first discriminant function. On the second discriminant function, with major contributions from 20-series monounsaturated fatty acids, pelagic

fishes were segregated from flatfishes, marine shrimp in one direction and crangon shrimp in the other (Annexe III e).

Digestive tract contents

Most fish species in this exploratory study had small sample sizes and consequently all available specimens were processed for the chemical analyses. However, some sample groups, especially of smelt and tomcod, were obtained in greater numbers than was needed. The dissection of digestive tracts in these extra specimens was an opportunity for potential insights into regional and seasonal feeding activities of a limited number of groups in parallel to the lipid/fatty acid analyses (Annexe I). The dissections were subjective and selective, and the small sample sizes were not intended to quantify stomach contents, as general diets may be found in the literature for these common species. The purpose was to examine members within a group (by species, location, or period) for 'significant' lipid-rich prey remains that could be assumed to contribute to the fatty acid signature of a similar specimen when homogenized whole, and in turn, to the variability of the signature for that group. This bias in using whole specimens is usually assumed rather than being examined, and therefore even fragmentary observations may be helpful in suggesting recent trophic ecology, especially for a particular region and period of a species group.

For the Upper Estuary in May, several of the larger (>17 cm) tomcod had stomachs with capelan (4 of 16 specimens) and crangon shrimps (7 of 17). Also seen for spring samples were copepods in 9 of 23 tomcod (14–22 cm), 3 of 4 smelt, and 2 of 2 sand lance. Krill filled the stomachs and intestines of examined barracudinas from the Lower Estuary, with mostly *Thysanoessa* sp. for 5 individuals in May, while *Meganyctiphanes norvegica*

were seen in 3 individuals for July. Krill (non-specific) were also seen in 1 of 2 specimens each of snailfish and Atlantic poacher from the Lower Estuary in July, and in 1 of 9 smelt from the Upper Estuary in August. While 12 of 27 smelt examined were empty, all tomcod specimens contained some material in their stomachs, even if the prey items were not always recognizable. Both smelt and tomcod were similar in containing traces of polychaete worms and amphipods in a number of specimens from spring through autumn. In contrast, traces of insects were only seen in 3 of 60 specimens of tomcod and smelt. Shad sardines were the only other group examined that had insect remains, with flies and larvae in 2 of 4 specimens that contained prey items.

2.4. Discussion

The comparative analysis of the lipid biochemical fraction of potential prey of St. Lawrence beluga revealed both intra- and inter-specific patterns in lipid contents and fatty acid compositions. While this study illustrated the possibility to distinguish prey species on the basis of fatty acid composition, it also suggests that success at classification will be enhanced, both at the individual level and for broader classes, by making use of complementary information and methods. Examples of complements demonstrated here include accounting for different species origins or trophic niches, total lipid quantity, successive multivariate analyses on subsets of data, and digestive tract contents. Several of these elements may be routinely applied in diet-based and fatty acid studies, but less common is the attempt to make simultaneous use of all this information to understand species and spatio-temporal patterns in fatty acid signatures. It is with this frame in mined that the study results and the choice of examples in each section are discussed in detail below.

Species sampling

The first step towards optimising fatty acid analysis for diet modeling is to establish the availability of potential prey, as rare or less-preferred species are unlikely to be detected in the predator signature (Iverson *et al.* 2004). Failure to detect infrequent dietary items is also a concern for techniques with specific identification including stomach contents and fecal DNA (Deagle *et al.* 2005, Svenning *et al.* 2005). This constraint makes it difficult at times to establish evidence of significant predation on threatened stocks of commercial species including cod, salmon and eels, which is a frequent objective of diet studies involving marine mammals and seabirds (Vladykov 1981, Shelton *et al.* 1997, Orr *et al.* 2004, Svenning *et al.* 2005). Another example where large prey species information might be desired but unavailable is the identification of pathways of environmental contamination in predators, such as linking eels with belugas (Hickie *et al.* 2000). At the same time, this limitation when using fatty acid signatures can be helpful, by focusing attention on known-significant prey, *i.e.*, because of abundance or preference, as being most likely responsible for the patterns detected (Andersen *et al.* 2004, Iverson *et al.* 2004). In the case of eels, the consumption of this potential prey by belugas might fall into doubt, given the marked decline in recent years of eel populations (Castonguay *et al.* 1994, Savaria *et al.* 2003).

Along with relative abundances, environmental changes are suspected to have led the St. Lawrence to harbour different species assemblages than was observed in past decades (Hanson and Chouinard 2002, Benoît *et al.* 2003a, deYoung *et al.* 2004). In this study, two pelagic species, shortfin squid and Arctic cod, have been reported as prey of beluga either historically in the St. Lawrence or elsewhere in the Arctic. During sampling

from 2000-2002, both of these schooling species were only encountered in the the Gulf. Although it is possible that these species were present in the Estuary, it is suggested that only the occasional migration of individuals would have likely have experienced significant access to these and other common Gulf species, such as larger members of the cod family.

This regional difference in potential prey distribution may be an example of species association with alternating environmental conditions (Dawe *et al.* 2000, Colbourne *et al.* 2002). For example, squid embark on feeding migrations, coming into the Gulf from the South (Nova Scotia) in late summer, occasionally being reported as far as the Estuary, presumably in years with warmer water temperatures (Préfontaine 1930, Mercer 1970). Conversely, colder-water species such as Arctic cod and its principal prey the hyperiid amphipod, *Themisto libellula*, descend into the Gulf from the North (Labrador) during cold events, as attested to by marine survey reports and predator stomach contents in the years leading up to the the sampling period of 2000-2002 (Dempson *et al.* 2001, Harvey *et al.* 2004). The Northwest Atlantic on the whole was associated with a negative condition index during this period, even though water temperatures in the Gulf increased relative to the 1990s (deYoung *et al.* 2004, Drinkwater and Gilbert 2004, Gilbert *et al.* 2004, Harrison *et al.* 2004).

This appeal in correlating observations of a species with an environmental condition index is thus often tempered by conflicting information when examined from several different indices, in both scale and type, making it a challenge to draw conclusions regarding impacts and causes of changes in prey species, and in turn, to predators (Hjermann *et al.* 2004). Notable other examples of linkages that have been attempted include the role of water temperature with capelin and seabirds (Carscadden 2005, Davoren and Montevecchi 2005), atmospheric depressions (*i.e.*, NAO) with copepods and right

whales (Greene and Pershing 2004), and Alaskan water circulation with walleye pollock and Steller sea lions as reviewed in Fritz and Hinckley (2005)

Environmental changes aside, sampling success was not necessarily a reliable indication of the availability of prey in the study region. For example, autumn-winter is an important feeding period for several marine mammal species, possibly including the beluga (Lydersen *et al.* 1989, Martin and Smith 1999, Winship *et al.* 2002, Laidre and Heide-Jørgensen 2005). Unfortunately, limited sampling opportunities in autumn made it difficult to evaluate several prey species for spatiotemporal differences, in particular Atlantic cod, capelin, and sand lance. Thus, cod may have been unavailable in autumn because of low abundance or insufficient fishing effort in the estuary. Eels were available from the autumn fishery, but this brief appearance (usually a few weeks in October) arises because of their reproductive migration to the sea, and it represents the only opportunity for predation during the year. Capelin were not commonly encountered in summer and rarely captured in autumn, although harp seals are known to consume large amounts in winter (Beck *et al.* 1993, Stenson *et al.* 1997) and this fish is ubiquitous during the spring spawning period.

The seasonal differences in availability is probably an indication of the unreliability of sampling pelagic species when using groundfish and shrimp nets, rather than the absence of these species in the ecosystem. Such a difficulty was most apparent by the virtual absence of sand lance during fish surveys except when using smaller dredges and sampling gear (pers. obs.). Sand lance may represent a significant prey species of belugas in autumn (Vladykov 1981), as also revealed by digestive contents in a sturgeon, seals, and a minke whale from the Lower Estuary during this period (pers. obs.). Other species, both resident (tomcod, shrimps and flatfishes) and migrant (herring) were both available and abundant in

all seasons from inshore fishing activities, which may be valid indications of their potential as important prey sources and for evaluation by standard fishing practices,

Lipid contents

Along with regional and seasonal availability, the lipid content of prey species may play a significant role in the diet of a marine predator, which may explain the attention given to this trait among prey species when examining declines of predator populations, notably of seabirds and pinnipeds (Kitts *et al.* 2004, Abookire and Piatt 2005, Rosen and Trites 2005, Wanless *et al.* 2005). Prey preferences are often assumed to be based on energetic values as indicated by the lipid content (Lawson *et al.* 1998b, Lea *et al.* 2002b). If all else is equal in foraging effort, a predator may be expected to seek higher-lipid prey items. Furthermore, while changes in fatty acid signatures can be detected even when feeding on a low-fat diet, *e.g.*, Kirsch *et al.* (2000), the prey signature is usually assumed to be more readily apparent (*i.e.*, faster detection of significant changes to predator composition) when consuming fatty specimens relative to leaner prey (Santos and Pierce 2003, Iverson *et al.* 2004).

From the samples obtained in this study, the lipid contents of flatfishes and decapod crustaceans varied little with season, location, or size-class, thereby suggesting that the mean values presented for these groups will not likely be improved by additional sampling. Other species demonstrated significant variability, thereby highlighting the need to confirm patterns such as the generally increased lipid contents seen in larger fish specimens (>18 cm) and in later seasons (spring vs. summer and autumn). Within-species differences in lipid contents were the most pronounced for herring, capelin and smelt. Herring were

relatively systematic, with predictable differences being seen between seasons and size-classes, as compared to smelt and capelan which could reveal greater variability between months and locations. These forage species were obtained in repeated samplings, but under varying conditions (on shore or at sea) while they were spawning, feeding, or migrating during the sampling period. The range of somatic and environmental conditions encountered by these forage species makes it pertinent to examine their variance in addition to their group (*i.e.*, by size, season, or location) means in order to accurately capture the lipid dynamics of interest. For example, documenting temporal variability in lipids of capelin may point to the impact on the fitness of seabirds that depend on this prey species (Davoren and Montevecchi 2003).

Unlike most pelagic fishes, codfishes are typically characterised as lean, with about 2% lipid by wet mass. Of interest was the relatively high amounts of lipid seen in pollock which is very similar morphologically and overlaps in distribution with the Atlantic cod. The pollock from autumn revealed the greatest amounts, averaging >5% lipid, while other specimens of pollock and other codfishes were all sampled earlier in the year. In the Northeast Pacific, walleye pollock (*Theragra chalcogramma*) increased in lipid content from spring to autumn with levels comparable to the pollock sampled here (Kitts *et al.* 2004). However, pollock are not caught often in the Estuary and the Gulf (pers. obs.), and are more commonly associated with banks in the Northwest Atlantic (Scott and Scott 1988, Benoît *et al.* 2003b). Thus, in parallel with the previous example given with eels, the potential prey value from the higher lipid content in pollock is likely to be discounted because of their less-favorable distribution for predation by St. Lawrence belugas.

General fatty acid comparisons

This study indicated that the greatest source of variability in a selection of fatty acids from the collected prey species were polyunsaturated fatty acids, and to a lesser extent, mononunsaturated fatty acids. While several of the fatty acids may originate through diet, the levels of specific fatty acids were not always easily attributed to differences in diet. Thus, a number of the fatty acids may be used as diet-linked tracers at certain trophic levels, *e.g.*, 16:0, 16:1n-7, and 16:4n-1 in plankton, but were less likely to be attributed to their direct prey origins, for example, the consumption of diatoms, when assimilated into top predators. Mammals commonly modify a number of saturated and mononunsaturated fatty acids (*i.e.*, 14:0, 16:0, 16:1n-7, 18:1n-7, and 18:1n-9) or contain only trace-level amounts in their adipose tissues of polyunsaturated fatty acids, as seen with fatty acid 16:4n-1 in this and other studies (Dalsgaard *et al.* 2003, Iverson *et al.* 2004).

When associated with origins in certain prey species, fatty acids of dietary interest may sometimes be characteristic of their environment. In a common example, 18:2n-6 is abundant in terrestrial plants and freshwater microorganisms (Desvilettes *et al.* 1997), which lead to food sources for estuarine fishes that includes eels, smelt, shad sardines and salmon smolts. By comparison, most species examined in this study revealed an abundance of marine-linked fatty acids, suggesting the common base of phytoplankton with fatty acids 20:5n-3 and 22:6n-3 (Sargent *et al.* 1985, Fraser *et al.* 1989) and copepods with fatty acids 20:1-9 and 22:1n-11 (Kattner and Krause 1987) in supporting a variety of species at higher trophic levels. Examples seen in this study were the relative amounts detected for cod and crabs (20:5n-3, 22:6n-3), and sand lance and herring (20:1n-9, 22:1n-11). Conversely, certain polyunsaturated fatty acids, such as 20:4n-6 and 22:5n-3, may be present in

vertebrate tissues in major quantities, but are thought to reflect metabolic rather than trophic activities (Imfeld 2000, Ferdinandusse *et al.* 2001). Occasionally abundant in invertebrates such as crabs and whelks, the fatty acid 20:4n-6 is found in relatively-low amounts in storage fats of mammals, perhaps due to its essential role in membrane function rather than energy storage (Cooper *et al.* 2005). Conversely, the fatty acid 22:5n-3 may be detected in relative abundance in higher vertebrates, but it is viewed as a metabolic intermediate between 20:5n-3 and 22:6n-3, thereby lessening its value as a dietary tracer if compensation is not performed (Pawlosky *et al.* 2003, Iverson *et al.* 2004).

Inter- and intra-specific fatty acid variability

Comparisons using PCA and discriminant analysis on a diet-linked subset of fatty acids provided insights into general and specific patterns, both when examining the entire prey base, and when evaluating selected groups between and among species. A PCA using all prey samples had their variance in fatty acid composition summarised into four significant factors, confirming the segregation of estuarine fishes such as eels, along with salmon smolts and shad sardines from most other species which scored as being relatively indistinct. The similarities among most of the remaining species may be a broad reflection of mixed, but mainly marine-linked (*i.e.*, marine plankton-based) diets, especially for abundant forage species like herring, capelin, and smelt, followed by piscivores such as larger codfishes and flatfishes.

Broad similarities in diets among the groups shown here might be expected to result in approximately similar fatty acid profiles, particularily given the emphasis on evaluating the generally abundant and diet-related fatty acids rather than potentially-discriminating traces. The suggestion of shared diets was also indicated partially by the stomach contents

of extra specimens that were occasionally available. For example, a number of specimens contained krill and copepods in several species for all locations, especially for the period of May through July. Lipid analyses that seek to relate prey contributions to a predator's diet-linked fatty acid signatures are best conducted using whole, homogenized prey items in order to represent actual diets, as opposed to removing digestive tracts or analysing selected tissues such as muscle (as is often done with squid and fish). In the case of belugas, it may of be particular interest to examine further the intermediate prey remains in their direct prey. An example is the copepod- and krill-related signatures that may be supplemented in fishes such as sand lance and barracudina that ingest these items, especially in spring and summer. A fatty acid food web study in Svalbard suggested that the copepod-like signature in sampled belugas in Svalbard was an indication of consumption of arctic cod, which in turn preyed on copepods, rather than to the direct ingestion of copepods by belugas themselves (Dahl *et al.* 2000). Thus knowledge of local food webs as revealed by examining intermediate prey may help in interpreting the path of lipids revealed in analyses at higher trophic levels.

The examination of specimens belonging to the same set as those analysed for fatty acids thus represents an opportunity for partial information on fatty acid signature variability as arising from digestive tract contents. For example, a large fish such as cod or turbot may contain sufficient shrimp biomass in their stomachs such that a whole-specimen analysis is affected by recent feeding on shrimps, in addition to the past dietary history of the predator as stored in its body lipids.

Along with identifying idividual constituents in terms of fatty acid, the analyses also provided information on total lipid content of samples. Segregation of potential prey groups for predator diet models might be assisted by considering lipid content as a

supplementary variable when statistical analyses produce broad or indistinct groupings. Such a scenario might be seen with the PCA results. In this case, the characteristic of lipid content could enhance the differences among groups from that seen when plotting individual factor scores derived from the set of fatty acids. An example was in the analysis for autumn samples, where several species groups that had similar factor scores on the retained PCs were further distinguishable when considering their lipid contents, specifically between individual smelt, between smelt and herring, and between large and small herring.

Overall, PCA was most useful at characterising the fatty acid variables (as compared to cases, or species) by summarising the selected set of fatty acids into functional groups. Thus, PC Factors 1 and 4 were most strongly-associated with the marine plankton-linked fatty acids, 20:5n-3 (diatoms) and 20:1n-9 (copepods), respectively. PC Factor 2 was defined more by the minor polyunsaturated fatty acids 18:2n-6 and 18:3n-3, likely to be associated with freshwater species (Desvilettes *et al.* 1997, Budge 1999). Contributions to Factor 3, while not as strong, could still be linked to diet when considering the plankton-linked minor fatty acids of 16:4n-1 and 18:4n-3 (Budge 1999). Factor 3 also exhibited a strong negative correlation with 20:4n-6, making it likely an indicator of benthic detrivory, as may have been suggested by the scores of species such as gammarid amphipods and eualid shrimps.

While the factors produced through the PCA could be associated with functional groups of species, understanding the variability within species was less straightforward. Hence, even when subsequent PC analyses were restricted to selected groups, such as samples from a particular season (autumn) or a family (codfishes), few groups could be strongly differentiated in the two-way factor score plots. This is a concern because inferences will be less persuasive when based on overlapping groups. At the same time, the

lack of differences suggests a large deal of commonality, as might be expected in food webs with sympatric groups of species (Dahl *et al.* 2000, Lesage *et al.* 2001, Andersen *et al.* 2004). In summary, PCA was useful because it enabled the inclusion of information from smaller sample sizes, which was the case for the majority of the 60 species analysed here. However, it also appeared to lose desired information for tracing diets, as groupings among and within species were largely not differentiated when using this data-reduction technique.

Discriminant analysis had greater success at segregating samples, as was shown by the significant separation of group means projected in score plots and relatively low misclassifications rates (usually <10%). The relative success, however, was accompanied by the requirement for sufficient samples in order to make use of a large number of classification variables. With this in mind, of additional interest was the graphical use of the classification results to reveal novel patterns, both in logical classes such as species and season, but also for trophic-linked, functional groups, *i.e.*, pelagic vs. demersal species. Individuals of species or other categories can be closely associated, as indicated by the apparent clustering of group means along the discriminant functions. Thus, even when known classes can be significantly-discriminated when using large numbers of samples and variables, it may be useful for subsequent tests if categories could be established that were readily distinct as well as interpretable for diet sources. In the series of discriminant analyses performed here, groups were readily characterised as pelagic vs. demersal, marine vs. estuarine, and fish vs. invertebrate, based on the proximity of specimen scores on the first two discriminant functions, along with the proportional contributions of the fatty acid variables and presumed life-history of species (Scott and Scott 1988, Squires 1996, Bourget 1997, Nozères *et al.* 2003)

Obtaining knowledge of potential enclosing groups with associated variables is useful for two reasons. First, it may allow diet determinations when specific identification of prey sources is less feasible due to similarities in fatty acid composition or insufficient sample sizes for full testing. New data for other prey species, or even for predators, could then be projected on the derived functions, enabling their comparison with the specified groups, to test the identity of proposed relationships. Such tests have commonly sought to establish differences between predators' diets based on fishes vs invertebrates (molluscs or crustaceans) (Bradshaw *et al.* 2003, Phillips *et al.* 2003). In one example seen in this study, whelks and eels were projected into distinct groups of benthic invertebrates and estuarine fishes, respectively, based on discriminant functions calculated on the more abundant samples (Fig. 2.12). Two other groups, hyperiid amphipods and shortfin squid (plotted, but not labelled in the figure because of small sample sizes) scored nearer to smelt than they did to pelagic invertebrates when projected on the same discriminant analysis. This pattern may be indicative of characters for these two important prey species not covered by the proposed group labels such as 'invertebrate' or 'estuarine', and not necessarily apparent in the PCA, thereby demonstrating the risk of generalisations based on limited samples and reduction techniques on fatty acid data. Thus, the discriminant analyses revealed that the signature of whelks is not wholly representative for cephalopods, and that that signatures for euphausiids (krill) and gammarids might not be adequate for hyperiids, as these would have failed in predicting the placement of squid and hyperiids. The result of these patterns demonstrates the value of a very broad sampling, even if only low numbers of a variety of species are available initially and therefore cannot be used for rigorous analysis or predictions using discriminant functions.

The second reason for examining potential groupings is to propose subsequent tests that will isolate those factors leading to the spread of scores, in particular, the intra-specific variablity. Our results, suggest that ecological factors such as recent feeding, size, season, and location affected the fatty acid compositions of individual specimens leading to their separation from the expected species groupings. Both the observed group means and the individual misclassifications provided examples of such inferences. Thus, even when available in large sample sizes (>20) or for selected periods (*i.e.*, autumn), pelagic and demersal species can present wide variability, overlapping in individual scores by species, but readily interpretable when presented as larger functional groups, such as pelagic, demersal, fish, or invertebrate.

From the results with the repeated species, tests with more samples to control for the size, season and location would have likely further improved class distinctions for some species. However, from the perspective of a predator signature, further sampling may not produce greatly different identifications beyond the significant group means already presented here. For example, continued sampling of the pelagic species of herring, capelin, and smelt, may be of limited value for diet-modelling beyond that which may be inferred here when using a set of 17 fatty acids. Their fatty acid composition and the large variability in lipid content of these species can likely be attributed to changes in their zooplankton prey, in species, numbers, and composition, which is difficult to predict (Henderson *et al.* 1984, Fraser *et al.* 1989, Iverson *et al.* 2002, Wanless *et al.* 2005).

Documenting patterns of similarities and differences among samples can also reveal fine-scale trophic relationships (Iverson *et al.* 1997b, Iverson *et al.* 2002). In two separate discriminant analyses, four turbot from the Upper Estuary and four large tomcod in spring, specimens were classified away from their respective species and trophic groups. It is

possible that stomach contents of these individuals contained secondary prey items affecting their average fatty acid signatures. Support for this hypothesis was seen when similar-sized specimens of tomcod from the same period had stomach contents filled with capelin, comprising a substantial fraction of the specimens' total mass. Capelin in these stomachs could account for the classification of larger tomcod in the pelagic rather than the demersal species group. These misclassifications might be useful for indicating potential trophic differences associated with a particular location or period. Hence, capelin-influenced signatures can be anticipated during the spring spawning period, when capelin are especially abundant and preyed upon by many larger piscivores, including marine mammals.

Similarly-targeted tests might reveal other abundant and intermediate prey groups as being responsible for overlapping species classifications by taking into account feeding preferences and prey life history. Possible examples might include shrimps, krill, herring, and sand lance rather than capelin as being consumed by halibut, plaice, and cod in summer and autumn months. A related study of a fatty acids prey base was unable to examine stomach contents, but also suggested that intermediate prey such as shrimps might be a related factor in the variance and misclassification errors for piscivores such as codfishes (Budge and Iverson 2003). In a discriminant analysis of four trophic groups in autumn, marine shrimps overlapped considerably with flatfishes, with pelagic fishes and estuarine sandshrimps (crangons) scoring further apart, which is suggestive of trophic similarity in the former two groups (Annexe III e).

Having discussed the diet-related factors that could account for the observed patterns in fatty acids and specimens, a final discussion may be warranted to show how a study might be conducted to examine other kinds of questions. Given the differences in

patterns inferred by the two multivariate methods shown in this study, it may be insightful to consider why other techniques were not considered, namely, classification trees and clustering analysess.

Classification trees, or CART, are most useful at revealing which variables are the most useful for segregating similar cases into known groups. In our broadly-based study, the variables of interest were already defined as the selection of 17 diet-linked fatty acids in relative abundance. Thus, the most important feature of CART (*i.e.*, objective selection of a few variables) was no longer of interest, while other useful information was not possible when using this approach. For example, discriminant analysis, using as many variables as permitted for given class sizes, provided statistically-robust results along with the identification of individuals that are classified into defined categories. Moreover, we showed that the relative position of the classified groups in discrimiant analysis can reveal patterns that can then be subsequently tested, such as trophic groups (pelagics vs. demersals).

Demonstrating unknown associations among cases is usually the purview of cluster analyses. Hierarchical cluster analysis, or HCA, seeks to iteratively join cases according to an index of similarity. Similar to PCA, HCA can reveal hidden patterns based on a number of variables. Unlike CART, HCA is sensitive to parameters including the number of variables, correlations among variables, data transformations, and the distance measures used in the analysis. Furthermore, as in the case of PCA, when potential group classes such as species overlap, the resulting associations can take many forms, with the result that it becomes difficult to assess their significance.

For these reasons, neither CART nor HCA were used to examine overall species and spatio-temporal variability in fatty acid signatures in this study. Nonetheless, these

complementary methods would be valuable in other exploratory tests. For example, CART can be used to establish the most important fatty acids that separate intraspecific groups by year, season, and location—even when only small sample sizes are available (*i.e.*, <6), the cases are similar (within-species differences), and the important variables are unknown. This type of analysis would be more appropriate for a detailed study restricted towards understanding the biology and diet of a species, such as capelin, because such small-scale differences would not be considered in predator diet-modeling. Similarly, cluster analysis can reveal associations if samples were to differ on a smaller group of variables of interest, as when using PCA with a larger number of fatty acids to produce a reduced set of independent factors. An example here might be to examine in greater detail the effect of size-classes of a species on compositions, as between sardines and herring.

2.4. Conclusion

We have documented the lipid contents and fatty acid compositions of a wide range of species, with an additional sampling effort aimed at assessing spatial and temporal variability of potential prey of beluga. By highlighting the similarities and differences between specimens when using a selection of fatty acids, both of the multivariate methods we used revealed their capacities to distinguish among the signatures of prey species. Perhaps of greater interest from this exploratory study (*i.e.*, when restricted in the number of variables and samples) was the suggestion of apparent functional groups among species that were both expected, as in the case of herring and capelin as pelagic fishes in all

analyses, but also novel, such as the intermediate scores of smelt between pelagic and demersal species in discriminant analysis but not in PCA.

The discrimination of prey species achieved using the diet-linked fatty acids suggests these signatures have the potential to trace trophic linkages. However, of some concern was the similarity (relative proximity of scores) among specimens of pelagic species such as herring and capelin, and the wider variability (dispersion of scores) seen among demersal species like tomcod and flatfishes, but also among smelt and invertebrates. The detailed examination of fatty acid compositions reiterated the need for additional considerations such as an awareness of prey species distributions, weighting by lipid contents, and the differences in output of multivariate analyses when evaluating fatty acid signatures for trophic-level studies.

Overall, our analyses improved the understanding of the fatty acid compositions of prey items available in the local food web for the St. Lawrence beluga whale. If the use of sampled beluga carcasses can be validated (to compensate for quality issues and calibrate for predator biology), the information available in this prey base will lead to better knowledge of diet linkages through the use of models such as QFASA, thereby helping to shed new light on persistent questions, including the source of environmental contaminants in predators like belugas. Finally, information derived from the prey base and future diet models would confirm the importance of presumed prey species of belugas, and therefore help identify the habitats upon which they depend, that are thus deserving of protection to ensure the viability of this unique population of whales.

Table 2.1. Summary of samples collected in 2000-2002 by species, season and sub-region: Upper Estuary (A), Lower Estuary (B), Sept-Iles (C), and northern Gulf (D). Seasons are spring (April–June), summer (July–Aug.), autumn (Sept.–Oct.) and winter (Feb.). Samples were analysed either as whole specimens or as a pooled set of specimens in the case of zooplankton, i.e, amphipods, copepods, and euphausiids (krill).

				n by sub-region			
Species name	Common name	Season	**Total**	A	B	C	D
Alosa sapidissima	American shad	summer	**13**	13	-	-	-
Amblyraja radiata	Thorny skate	summer	**6**	5	1	-	-
		autumn	**6**	6	-	-	-
Ammodytes sp.	Sand lance	spring	**18**	-	-	6	-
		summer	**12**	12	6	6	-
		winter	**12**	-	12	-	-
Anarhichas lupus	Atlantic wolffish	summer	**8**	-	-	-	8
Anarhichas minor	Striped wolffish	summer	**1**	-	-	-	1
Anguilla rostrata	American eel	autumn	**14**	14	-	-	-
Arctozenus risso	White barracudina	spring	**6**	-	6	-	-
		summer	**12**	-	12	-	-
Argis dentata	Argid shrimp	autumn	**12**	-	12	-	-
Bathypolypus arcticus	Boreal octopus	spring	**5**	-	-	5	-
Boreogadus saida	Arctic cod	summer	**4**	-	4	-	-
Buccinum undatum	Waved whelk	spring	**18**	6	6	6	-
		summer	**6**	-	-	-	6
Calanus hyperboreus	Copepod	spring	**1**	-	1	-	-
		summer	**11**	-	11	-	-
Chionoecetes opilio	Snow crab	spring	**6**	-	6	-	-
Colus sp.	Whelk	autumn	**2**	-	2	-	-
Clupea harengus	Atlantic herring	spring	**48**	24	24	-	-
		summer	**42**	30	-	12	-
		autumn	**36**	36	-	-	-
Coregonus sp.	Whitefish	spring	**2**	2	-	-	-
		autumn	**3**	3	-	-	-
Crangon septemspinosa	Sand shrimp	spring	**6**	6	-	-	-
		summer	**6**	6	-	-	-
		autumn	**17**	17	-	-	-
Cyclopterus lumpus	Lumpfish	spring	**7**	-	7	-	-
		summer	**5**	-	2	3	-

Table 2.1 (cont'd)

				n by sub-region			
Species name	Common name	Season	**Total**	A	B	C	D
Enchelyopus cimbrius	Fourbeard rockling	summer	**3**	-	3	-	-
		autumn	**4**	-	4	-	-
Eualus macilentus	Eualid shrimp	spring	**9**	-	9	-	-
		summer	**3**	-	3	-	-
		autumn	**6**	-	6	-	-
Gadus morhua	Atlantic cod	spring	**5**	-	5	-	-
		summer	**9**	-	9	-	-
Gammarus sp.	Gammarid amphipod	spring	**4**	-	-	4	-
Glyptocephalus cynoglossus	Witch flounder	summer	**5**	5	-	-	-
Gymnacanthus tricuspis	Arctic staghorn sculpin	summer	**7**	-	1	-	6
Hippoglossoides platessoides	American plaice	spring	**6**	-	4	2	-
		summer	**22**	5	9	8	-
		autumn	**12**	-	10	2	-
Hyas araneus	Toad crab	summer	**1**	-	1	-	-
Illex illecebrosus	Shortfin squid	summer	**6**	-	-	-	6
Leptagonus decagonus	Atlantic poacher	summer	**8**	-	8	-	-
		autumn	**4**	-	4	-	-
Limanda ferruginea	Yellowtail flounder	summer	**1**	-	1	-	-
Liparis gibbus	Snailfish	spring	**6**	6	-	-	-
		summer	**7**	-	7	-	-
		autumn	**5**	-	5	-	-
Lophius americanus	Monkfish	summer	**4**	-	-	-	4
Leptoclinus maculatus	Daubed shanny	summer	**6**	-	6	-	-
Lycodes vahlii	Checker eelpout	summer	**1**	-	1	-	-
		autumn	**7**	-	7	-	-
Mallotus villosus	Capelin	spring	**71**	23	18	30	-
		summer	**17**	11	6	-	-
		autumn	**7**	7	-	-	-
Meganyctiphanes norvegica	Northern krill	spring	**27**	-	21	6	-
		summer	**6**	-	6	-	-
Melanogrammus aeglefinus	Haddock	summer	**2**	-	2	-	-
Merluccius biinearis	Silver hake	summer	**6**	-	6	-	-
Microgadus tomcod	Atlantic tomcod	spring	**18**	18	-	-	-
		summer	**12**	12	-	-	-
		autumn	**9**	9	-	-	-
Myoxocephalus scorpius	Shorthorn sculpin	spring	**1**	-	1	-	-
		summer	**7**	-	7	-	-
Myxine glutinosa	Hagfish	summer	**12**	-	12	-	-

Table 2.1 (cont'd)

Species name	Common name	Season	**Total**	*n* by sub-region A	B	C	D
Nereis virens	Polychaete worm	spring	**6**	6	-	-	-
		autumn	**6**	6	-	-	-
Nezumia bairdii	Marlin-spike	summer	**6**	6	-	-	-
Osmerus mordax	Rainbow smelt	spring	**42**	36	6	-	-
		summer	**28**	12	16	-	-
		autumn	**57**	45	12	-	-
Pandalus borealis	Northern shrimp	summer	**6**	-	6	-	-
		autumn	**6**	-	6	-	-
Pandalus montagui	Striped shrimp	spring	**9**	-	9	-	-
		summer	**3**	-	3	-	-
		autumn	**6**	-	6	-	-
Petromyzontidae	Lampreys	autumn	**3**	2	1	-	-
Pseudopleuronectes americanus	Winter flounder	spring	**12**	12	-	-	-
		summer	**18**	16	2	-	-
		autumn	**13**	12	1	-	-
Pollachius virens	Pollock	summer	**6**	3	1	-	2
Reinhardtius hippoglossoides	Greenland halibut (turbot)	spring	**6**	-	6	-	-
		summer	**19**	4	15	-	-
		autumn	**1**	-	1	-	-
Salmo salar	Atlantic salmon	spring	**15**	-	15	-	-
Sclerocrangon boreas	Scampi shrimp	summer	**6**	-	-	-	6
Sebastes sp.	Deepwater redfish	spring	**12**	-	12	-	-
Semirossia tenera	Bobtail squid	summer	**7**	-	-	-	7
Themisto libellula	Hyperiid amphipod	spring	**8**	-	3	5	-
		summer	**3**	-	3	-	-
		autumn	**5**	-	5	-	-
Thysanoessa raschii	Krill	autumn	**5**	-	5	-	-
Triglops murrayi	Moustache sculpin	summer	**9**	-	9	-	-
Urophycis chesteri	Longfin hake	summer	**6**	-	6	-	-
Urophycis tenuis	White hake	summer	**7**	-	7	-	-
Zoarces americanus	Ocean pout	autumn	**1**	1	-	-	-

Table 2.2. (FOLLOWING PAGES)

Composition (% of total by weight) for 25 fatty acids, each averaging >0.4%, among all species groups analysed (= fatty acid not retained in statistical analyses). Shad, herring, and salmon were each segregated into small (<18 cm) and large (>18 cm) groups. Specimen standard length, wet weight, and lipid content are also presented. Values are means ± SEM.

Table 2.2.

Common name Species group	Shad-sardines *Alosa sapidissima*	Shad (large) *Alosa sapidissima*-L	Sand lance *Ammodytes* sp.	Atlantic wolffish *Anarhichas lupus*	Spotted wolffish *Anarhichas minor*
n	12	1	42	8	1
c14:0	2.57 ± 0.38	4.93	3.97 ± 0.19	2.72 ± 0.35	1.87
c16:0	20.54 ± 0.26	14.21	14.66 ± 0.59	13.51 ± 0.75	11.04
c16:1n-7	4.89 ± 0.92	3.05	6.77 ± 0.31	8.89 ± 0.98	5.64
c16:3n-6	0.65 ± 0.18	0.33	0.57 ± 0.04	0.15 ± 0.02	0.23
c16:4n-1	0.22 ± 0.01	0.23	0.85 ± 0.08	0.19 ± 0.04	0.24
c17:0	0.74 ± 0.02	0.40	1.17 ± 0.18	0.36 ± 0.06	0.50
c18:0	6.10 ± 0.21	2.52	2.74 ± 0.18	3.43 ± 0.31	4.79
c18:1n-11	0.05 ± 0.01	0.89	0.39 ± 0.03	0.66 ± 0.07	0.67
c18:1n-9	8.03 ± 0.32	9.08	6.91 ± 0.37	17.18 ± 2.25	10.86
c18:1n-7	3.94 ± 0.07	3.13	3.61 ± 0.23	6.37 ± 0.42	9.23
c18:1n-5	0.11 ± 0.01	0.45	0.52 ± 0.02	0.66 ± 0.05	0.98
c18:2n-6	2.02 ± 0.24	0.99	0.86 ± 0.04	0.66 ± 0.02	0.59
c18:3n-3	1.07 ± 0.19	0.75	0.34 ± 0.02	0.20 ± 0.02	0.16
c18:4n-3	0.69 ± 0.12	1.52	1.03 ± 0.07	0.33 ± 0.06	0.45
c20:1n-11	0.12 ± 0.02	1.62	0.44 ± 0.03	2.54 ± 0.84	2.65
c20:1n-9	0.36 ± 0.06	15.58	8.37 ± 0.76	2.99 ± 0.37	3.38
c20:1n-7	0.22 ± 0.01	0.26	0.61 ± 0.04	0.96 ± 0.17	1.39
c20:2n-6	0.19 ± 0.02	0.24	0.15 ± 0.01	0.31 ± 0.04	0.36
c20:4n-6	3.57 ± 0.26	0.60	0.57 ± 0.06	4.28 ± 1.29	8.96
c20:5n-3	8.45 ± 0.23	6.08	12.71 ± 0.33	11.21 ± 0.84	12.49
c22:1n-11	0.20 ± 0.14	13.50	9.46 ± 0.88	1.87 ± 0.33	1.24
c22:1n-9	0.05 ± 0.01	0.87	1.13 ± 0.10	0.43 ± 0.04	0.38
c22:5n-3	4.08 ± 0.12	2.08	0.88 ± 0.04	1.11 ± 0.15	1.04
c22:6n-3	22.29 ± 1.72	10.31	14.65 ± 0.92	10.13 ± 0.42	10.05
c24:1n-9	0.48 ± 0.04	0.79	0.94 ± 0.04	0.98 ± 0.10	0.83
∑	91.63	94.36	94.28	92.10	89.95
length (cm)	10.26 ± 0.12	46.40	11.48 ± 0.38	34.61 ± 3.23	38.60
weight (g)	8.90 ± 0.31	1543.90	4.04 ± 0.40	619.26 ± 147.07	698.50
lipid (%)	1.47 ± 0.14	7.55	3.37 ± 0.28	1.90 ± 0.29	1.26

Table 2.2. (cont'd)

Common name Species group	Thorny skate *Amblyraja radiata*	American eel *Anguilla rostrata*	White barracudina *Arctozenus risso*	Argid shrimp *Argis dentata*	Boreal octopus *Bathypolypus arcticus*
n	12	14	18	12	5
c14:0	0.96 ± 0.14	3.56 ± 0.14	5.74 ± 0.32	1.47 ± 0.09	1.22 ± 0.06
c16:0	16.08 ± 0.56	17.75 ± 0.38	16.03 ± 0.45	13.34 ± 0.16	15.97 ± 0.97
c16:1n-7	4.63 ± 0.36	10.36 ± 0.21	9.76 ± 0.18	9.07 ± 0.47	1.48 ± 0.66
c16:3n-6	0.12 ± 0.02	0.26 ± 0.03	0.60 ± 0.02	0.32 ± 0.02	0.04 ± 0.01
c16:4n-1	0.17 ± 0.03	0.12 ± 0.01	0.30 ± 0.01	0.23 ± 0.03	0.17 ± 0.02
c17:0	0.58 ± 0.08	0.51 ± 0.02	0.30 ± 0.08	0.72 ± 0.04	0.85 ± 0.03
c18:0	4.15 ± 0.40	2.85 ± 0.08	2.42 ± 0.09	2.44 ± 0.07	3.93 ± 0.17
c18:1n-11	0.94 ± 0.15	0.02 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.34 ± 0.06
c18:1n-9	12.24 ± 0.30	28.78 ± 0.79	18.68 ± 0.75	10.13 ± 0.24	3.32 ± 0.74
c18:1n-7	7.09 ± 0.24	5.25 ± 0.31	4.82 ± 0.15	9.80 ± 0.14	3.85 ± 0.57
c18:1n-5	0.53 ± 0.04	0.23 ± 0.02	0.42 ± 0.02	0.77 ± 0.02	0.64 ± 0.08
c18:2n-6	1.31 ± 0.07	3.25 ± 0.32	0.65 ± 0.02	0.73 ± 0.02	0.57 ± 0.07
c18:3n-3	0.24 ± 0.02	2.25 ± 0.23	0.32 ± 0.01	0.16 ± 0.01	0.11 ± 0.03
c18:4n-3	0.18 ± 0.04	0.18 ± 0.06	0.70 ± 0.04	0.45 ± 0.06	0.08 ± 0.03
c20:1n-11	0.94 ± 0.08	0.51 ± 0.07	0.47 ± 0.03	1.24 ± 0.13	0.99 ± 0.22
c20:1n-9	4.42 ± 1.13	1.10 ± 0.05	8.00 ± 0.50	0.72 ± 0.03	6.85 ± 0.59
c20:1n-7	1.05 ± 0.10	0.15 ± 0.01	0.55 ± 0.02	1.70 ± 0.09	1.36 ± 0.33
c20:2n-6	0.54 ± 0.05	0.60 ± 0.04	0.14 ± 0.00	0.42 ± 0.01	0.74 ± 0.09
c20:4n-6	4.27 ± 0.53	3.20 ± 0.18	0.25 ± 0.02	1.87 ± 0.06	4.30 ± 0.31
c20:5n-3	8.08 ± 0.55	2.77 ± 0.17	7.54 ± 0.31	20.86 ± 0.45	19.86 ± 0.74
c22:1n-11	3.20 ± 1.13	0.00 ± 0.00	9.82 ± 0.70	0.14 ± 0.02	1.43 ± 0.41
c22:1n-9	0.64 ± 0.10	0.05 ± 0.00	1.31 ± 0.07	0.22 ± 0.02	1.62 ± 0.24
c22:5n-3	2.48 ± 0.31	2.52 ± 0.14	0.72 ± 0.04	2.29 ± 0.12	1.22 ± 0.18
c22:6n-3	18.86 ± 0.81	3.69 ± 0.37	5.05 ± 0.22	10.49 ± 0.32	21.25 ± 1.13
c24:1n-9	0.36 ± 0.03	0.07 ± <0.01	0.55 ± 0.03	0.13 ± 0.01	0.32 ± 0.05
∑	94.05	90.03	94.78	89.72	92.23
length (cm)	21.07 ± 1.09	79.49 ± 1.58	24.11 ± 0.35	8.60 ± 0.22	15.38 ± 3.23
weight (g)	76.31 ± 15.03	1094.90 ± 60.52	24.61 ± 1.65	6.01 ± 0.50	51.68 ± 24.79
lipid (%)	1.57 ± 0.20	13.29 ± 0.34	12.53 ± 0.70	1.99 ± 0.13	1.13 ± 0.12

Table 2.2. (cont'd)

Common name Species group	Arctic cod *Boreogadus saida*	Waved whelk *Buccinum undatum*	Copepod *Calanus hyperboreus*	Snow crab *Chionoecetes opilio*	Herring-sardines *Clupea harengus*
n	4	24	12	6	42
c14:0	2.83 ± 0.13	2.08 ± 0.12	3.37 ± 0.54	1.33 ± 0.14	5.17 ± 0.15
c16:0	18.89 ± 0.32	10.97 ± 0.26	4.87 ± 0.21	11.39 ± 0.09	13.74 ± 0.44
c16:1n-7	8.22 ± 0.77	2.51 ± 0.25	8.82 ± 0.11	5.32 ± 0.44	6.64 ± 0.24
c16:3n-6	0.87 ± 0.12	0.12 ± 0.02	0.93 ± 0.04	0.17 ± 0.02	0.73 ± 0.03
c16:4n-1	0.30 ± 0.03	0.19 ± 0.02	1.96 ± 0.16	0.29 ± 0.03	0.83 ± 0.05
c17:0	0.10 ± 0.00	0.60 ± 0.03	0.04 ± 0.00	0.45 ± 0.05	0.49 ± 0.08
c18:0	4.10 ± 0.31	5.34 ± 0.29	0.32 ± 0.02	2.49 ± 0.15	1.53 ± 0.08
c18:1n-11	0.60 ± 0.16	0.48 ± 0.10	0.06 ± 0.03	0.53 ± 0.05	0.41 ± 0.03
c18:1n-9	9.59 ± 0.36	4.64 ± 0.28	1.94 ± 0.36	10.79 ± 0.75	6.74 ± 0.51
c18:1n-7	7.79 ± 0.62	4.74 ± 0.29	1.37 ± 0.04	6.80 ± 0.20	3.18 ± 0.19
c18:1n-5	0.27 ± 0.02	0.43 ± 0.05	0.52 ± 0.01	0.73 ± 0.05	0.45 ± 0.02
c18:2n-6	0.57 ± 0.02	1.38 ± 0.07	0.23 ± 0.02	0.76 ± 0.04	0.56 ± 0.02
c18:3n-3	0.24 ± 0.01	0.45 ± 0.06	0.10 ± 0.01	0.20 ± 0.01	0.25 ± 0.01
c18:4n-3	0.83 ± 0.05	0.44 ± 0.06	1.49 ± 0.12	0.28 ± 0.05	0.85 ± 0.07
c20:1n-11	0.43 ± 0.08	5.67 ± 0.23	0.57 ± 0.11	1.71 ± 0.08	0.70 ± 0.03
c20:1n-9	3.03 ± 0.46	3.97 ± 0.41	21.65 ± 0.52	4.92 ± 0.43	12.08 ± 0.72
c20:1n-7	0.41 ± 0.02	3.46 ± 0.28	2.56 ± 0.15	1.68 ± 0.19	0.58 ± 0.03
c20:2n-6	0.23 ± 0.00	3.86 ± 0.30	0.07 ± 0.00	0.63 ± 0.05	0.13 ± 0.01
c20:4n-6	0.60 ± 0.05	4.60 ± 0.63	0.22 ± 0.01	3.27 ± 0.25	0.41 ± 0.03
c20:5n-3	17.19 ± 0.32	20.91 ± 0.68	7.29 ± 0.28	19.18 ± 1.11	9.05 ± 0.41
c22:1n-11	3.06 ± 0.54	2.57 ± 0.64	29.14 ± 0.41	5.26 ± 0.73	15.41 ± 0.77
c22:1n-9	0.50 ± 0.06	0.41 ± 0.06	3.43 ± 0.20	0.65 ± 0.06	1.15 ± 0.07
c22:5n-3	0.69 ± 0.06	5.09 ± 0.37	0.56 ± 0.03	1.11 ± 0.07	0.81 ± 0.03
c22:6n-3	14.22 ± 1.17	7.60 ± 0.65	2.76 ± 0.17	12.83 ± 0.63	12.32 ± 0.74
c24:1n-9	0.61 ± 0.12	0.08 ± 0.02	0.41 ± 0.05	0.31 ± 0.02	0.73 ± 0.03
∑	95.66	92.50	94.31	92.79	94.26
length (cm)	10.63 ± 0.17	7.87 ± 0.14	- - -	6.82 ± 0.24	13.46 ± 0.26
weight (g)	8.77 ± 0.39	61.42 ± 3.73	- - -	104.90 ± 8.05	20.59 ± 0.98
lipid (%)	2.84 ± 0.37	1.30 ± 0.12	14.81 ± 1.49	1.06 ± 0.16	3.94 ± 0.30

Table 2.2. (cont'd)

Common name Species group	Herring (large) *Clupea harengus*-L	Whelk *Colus* sp.	Whitefish *Coregonus* sp.	Sand shrimp *Crangon septemspinosa*	Lumpfish *Cyclopterus lumpus*
n	84	2	5	29	12
c14:0	4.58 ± 0.10	1.65 ± 0.06	1.64 ± 0.26	1.37 ± 0.10	3.23 ± 0.36
c16:0	11.41 ± 0.24	8.59 ± 0.32	16.80 ± 0.68	15.39 ± 0.32	11.33 ± 0.31
c16:1n-7	6.53 ± 0.19	1.64 ± 0.01	8.76 ± 1.88	5.01 ± 0.37	4.25 ± 0.63
c16:3n-6	0.48 ± 0.02	0.06 ± 0.00	0.34 ± 0.08	0.18 ± 0.04	0.24 ± 0.03
c16:4n-1	0.62 ± 0.03	0.07 ± 0.01	0.25 ± 0.12	0.19 ± 0.03	0.11 ± 0.02
c17:0	0.26 ± 0.04	0.71 ± 0.08	0.53 ± 0.09	0.95 ± 0.08	0.29 ± 0.02
c18:0	1.17 ± 0.04	5.24 ± 0.40	3.83 ± 0.30	3.40 ± 0.20	3.00 ± 0.22
c18:1n-11	0.69 ± 0.03	1.19 ± 0.75	0.76 ± 0.35	0.32 ± 0.05	3.69 ± 0.32
c18:1n-9	7.45 ± 0.33	5.96 ± 0.51	17.17 ± 1.77	10.30 ± 0.28	13.19 ± 0.49
c18:1n-7	2.75 ± 0.10	5.26 ± 0.25	5.06 ± 0.56	4.98 ± 0.16	4.29 ± 0.12
c18:1n-5	0.45 ± 0.01	0.81 ± 0.11	0.38 ± 0.06	0.47 ± 0.04	0.60 ± 0.03
c18:2n-6	0.58 ± 0.02	1.61 ± 0.24	2.16 ± 0.41	1.47 ± 0.08	1.02 ± 0.04
c18:3n-3	0.23 ± 0.01	0.15 ± 0.02	1.47 ± 0.35	0.51 ± 0.05	0.41 ± 0.03
c18:4n-3	0.66 ± 0.04	0.07 ± 0.02	0.39 ± 0.11	0.19 ± 0.04	0.71 ± 0.14
c20:1n-11	1.28 ± 0.08	7.91 ± 0.02	1.02 ± 0.38	1.33 ± 0.08	1.25 ± 0.17
c20:1n-9	15.03 ± 0.35	4.36 ± 1.70	1.63 ± 0.68	3.07 ± 0.62	7.28 ± 0.72
c20:1n-7	0.99 ± 0.03	3.40 ± 0.62	0.65 ± 0.13	1.45 ± 0.10	1.07 ± 0.10
c20:2n-6	0.10 ± 0.00	3.32 ± 0.25	0.77 ± 0.13	0.55 ± 0.03	0.25 ± 0.01
c20:4n-6	0.30 ± 0.01	6.41 ± 1.00	3.53 ± 1.05	3.32 ± 0.25	0.70 ± 0.11
c20:5n-3	6.01 ± 0.16	15.04 ± 5.63	7.76 ± 0.85	19.81 ± 0.63	13.45 ± 0.48
c22:1n-11	22.80 ± 0.47	2.01 ± 1.25	0.88 ± 0.65	3.06 ± 0.75	3.67 ± 0.47
c22:1n-9	2.06 ± 0.06	0.39 ± 0.15	0.23 ± 0.08	0.47 ± 0.10	1.70 ± 0.25
c22:5n-3	0.68 ± 0.02	6.31 ± 0.73	2.53 ± 0.22	1.88 ± 0.13	1.56 ± 0.16
c22:6n-3	8.08 ± 0.30	8.53 ± 0.44	11.92 ± 3.84	11.92 ± 0.54	16.63 ± 1.40
c24:1n-9	0.64 ± 0.02	0.22 ± 0.05	0.50 ± 0.23	0.33 ± 0.03	0.51 ± 0.06
∑	95.21	90.73	90.67	91.62	94.00
length (cm)	24.83 ± 0.27	10.80 ± 2.00	37.84 ± 2.58	5.76 ± 0.13	26.65 ± 1.66
weight (g)	159.92 ± 5.11	65.55 ± 17.88	612.67 ± 97.93	1.68 ± 0.14	887.33 ± 106.27
lipid (%)	7.35 ± 0.40	0.81 ± 0.03	3.93 ± 1.22	1.42 ± 0.10	2.41 ± 0.52

Table 2.2. (cont'd)

Common name Species group	Fourbeard rockling *Enchelyopus cimbrius*	Eualid shrimp *Eualus macilentus*	Atlantic cod *Gadus morhua*	Gammarid amphipod *Gammarus* sp.	Witch flounder *Glyptocephalus cynoglossus*
n	7	18	14	4	5
c14:0	1.28 ± 0.13	1.71 ± 0.16	1.99 ± 0.19	2.14 ± 0.28	3.40 ± 0.21
c16:0	16.47 ± 0.59	15.60 ± 0.34	15.07 ± 0.37	13.68 ± 0.79	15.48 ± 0.23
c16:1n-7	3.66 ± 0.55	7.53 ± 0.80	4.79 ± 0.37	10.20 ± 1.27	8.88 ± 1.03
c16:3n-6	0.14 ± 0.02	0.42 ± 0.08	0.25 ± 0.03	0.21 ± 0.03	0.26 ± 0.01
c16:4n-1	0.39 ± 0.12	0.31 ± 0.08	0.08 ± 0.02	0.05 ± 0.01	0.32 ± 0.09
c17:0	0.50 ± 0.08	0.34 ± 0.03	0.18 ± 0.01	0.19 ± 0.06	0.70 ± 0.09
c18:0	4.38 ± 0.28	2.10 ± 0.10	3.17 ± 0.10	0.84 ± 0.19	3.48 ± 0.17
c18:1n-11	1.21 ± 0.12	0.39 ± 0.11	1.83 ± 0.14	0.53 ± 0.06	0.62 ± 0.12
c18:1n-9	11.83 ± 0.89	10.95 ± 0.48	10.37 ± 0.47	22.98 ± 3.03	6.68 ± 0.91
c18:1n-7	5.11 ± 0.32	10.00 ± 0.37	4.91 ± 0.25	5.76 ± 0.68	5.32 ± 0.45
c18:1n-5	0.43 ± 0.03	0.62 ± 0.02	0.37 ± 0.02	0.69 ± 0.14	0.55 ± 0.08
c18:2n-6	0.56 ± 0.04	0.91 ± 0.05	0.56 ± 0.02	1.29 ± 0.10	1.00 ± 0.03
c18:3n-3	0.10 ± 0.01	0.28 ± 0.01	0.17 ± 0.02	0.38 ± 0.06	0.23 ± 0.03
c18:4n-3	0.20 ± 0.03	0.48 ± 0.09	0.29 ± 0.04	0.51 ± 0.15	0.24 ± 0.05
c20:1n-11	0.76 ± 0.06	0.54 ± 0.05	1.15 ± 0.08	1.84 ± 0.13	2.11 ± 0.14
c20:1n-9	2.90 ± 0.23	2.15 ± 0.48	5.53 ± 0.48	4.84 ± 0.24	3.52 ± 0.59
c20:1n-7	0.91 ± 0.10	1.06 ± 0.09	0.51 ± 0.03	1.36 ± 0.36	2.38 ± 0.24
c20:2n-6	0.25 ± 0.01	0.36 ± 0.01	0.21 ± 0.01	0.47 ± 0.12	0.57 ± 0.06
c20:4n-6	3.17 ± 0.23	3.59 ± 0.44	1.92 ± 0.17	1.59 ± 0.32	2.85 ± 0.31
c20:5n-3	11.55 ± 0.38	17.69 ± 0.76	13.18 ± 0.47	11.83 ± 0.47	9.63 ± 0.28
c22:1n-11	0.93 ± 0.17	2.43 ± 0.80	3.98 ± 0.47	2.23 ± 0.64	3.22 ± 0.95
c22:1n-9	0.36 ± 0.03	0.38 ± 0.06	0.55 ± 0.04	0.42 ± 0.08	0.64 ± 0.14
c22:5n-3	1.58 ± 0.04	0.88 ± 0.05	1.28 ± 0.05	1.14 ± 0.28	2.86 ± 0.26
c22:6n-3	22.28 ± 1.49	11.42 ± 0.77	22.46 ± 1.00	9.47 ± 0.38	9.68 ± 1.08
c24:1n-9	2.23 ± 0.37	0.33 ± 0.02	0.92 ± 0.05	0.15 ± 0.05	0.91 ± 0.24
∑	91.30	92.16	94.87	94.66	84.85
length (cm)	20.31 ± 0.75	- - -	32.79 ± 1.59	- - -	25.02 ± 0.27
weight (g)	44.25 ± 10.01	1.21 ± 0.14	341.11 ± 46.03	0.74 ± 0.11	166.53 ± 8.07
lipid (%)	1.02 ± 0.12	2.18 ± 0.22	1.48 ± 0.11	3.20 ± 0.77	1.43 ± 0.24

Table 2.2. (cont'd)

Common name Species group	Arctic staghorn sculpin *Gymnacanthus tricuspis*	American plaice *Hippoglossoides platessoides*	Toad crab *Hyas araneus*	Shortfin squid *Illex illecebrosus*	Atlantic poacher *Leptagonus decagonus*
n	7	40	1	6	12
c14:0	2.41 ± 0.12	3.13 ± 0.19	0.65	3.14 ± 0.20	2.55 ± 0.15
c16:0	14.55 ± 0.43	14.58 ± 0.26	7.87	15.52 ± 0.41	13.10 ± 0.32
c16:1n-7	7.46 ± 1.00	7.22 ± 0.43	2.38	3.47 ± 0.46	10.27 ± 0.46
c16:3n-6	0.36 ± 0.07	0.46 ± 0.04	0.04	0.16 ± 0.02	0.42 ± 0.02
c16:4n-1	0.55 ± 0.06	0.74 ± 0.06	0.19	0.10 ± 0.03	0.23 ± 0.03
c17:0	0.39 ± 0.02	0.68 ± 0.07	0.83	0.45 ± 0.03	0.49 ± 0.05
c18:0	3.67 ± 0.26	3.25 ± 0.16	4.34	2.52 ± 0.11	2.07 ± 0.18
c18:1n-11	0.74 ± 0.13	0.56 ± 0.06	0.16	0.64 ± 0.08	1.18 ± 0.10
c18:1n-9	10.77 ± 1.38	9.26 ± 0.31	8.73	7.88 ± 0.96	13.52 ± 0.57
c18:1n-7	8.25 ± 0.27	5.49 ± 0.18	10.16	3.13 ± 0.15	8.31 ± 0.16
c18:1n-5	0.57 ± 0.04	0.38 ± 0.01	0.71	0.63 ± 0.04	0.86 ± 0.04
c18:2n-6	0.73 ± 0.03	0.77 ± 0.02	0.99	0.85 ± 0.14	1.29 ± 0.06
c18:3n-3	0.26 ± 0.02	0.17 ± 0.01	0.09	0.45 ± 0.04	0.30 ± 0.01
c18:4n-3	0.81 ± 0.09	0.59 ± 0.06	0.08	0.73 ± 0.10	0.45 ± 0.03
c20:1n-11	0.66 ± 0.04	0.86 ± 0.09	1.92	1.45 ± 0.11	0.76 ± 0.05
c20:1n-9	2.00 ± 0.24	4.58 ± 0.52	1.43	9.41 ± 0.71	4.35 ± 0.38
c20:1n-7	1.06 ± 0.08	1.55 ± 0.11	1.90	0.53 ± 0.03	1.16 ± 0.07
c20:2n-6	0.29 ± 0.03	0.25 ± 0.01	1.97	0.36 ± 0.01	0.28 ± 0.01
c20:4n-6	1.86 ± 0.16	2.37 ± 0.19	11.34	0.56 ± 0.02	1.46 ± 0.13
c20:5n-3	17.17 ± 0.65	13.53 ± 0.51	22.09	12.40 ± 0.25	12.97 ± 0.25
c22:1n-11	0.65 ± 0.13	3.48 ± 0.49	0.39	6.49 ± 0.54	2.59 ± 0.26
c22:1n-9	0.36 ± 0.04	0.59 ± 0.07	0.26	0.83 ± 0.06	0.63 ± 0.04
c22:5n-3	2.04 ± 0.19	2.23 ± 0.07	1.89	0.78 ± 0.10	1.20 ± 0.04
c22:6n-3	13.52 ± 1.15	15.03 ± 0.64	8.38	21.93 ± 1.05	12.27 ± 0.72
c24:1n-9	0.67 ± 0.11	0.87 ± 0.04	0.22	0.50 ± 0.02	0.64 ± 0.04
∑	91.25	91.80	89.23	94.44	92.74
length (cm)	13.40 ± 1.05	24.64 ± 0.90	13.10	154.40 ± 16.71	17.98 ± 0.60
weight (g)	39.35 ± 8.78	170.91 ± 19.64	172.39	97.87 ± 4.08	20.53 ± 2.23
lipid (%)	1.94 ± 0.30	1.54 ± 0.11	0.59	3.73 ± 0.23	3.85 ± 0.50

Table 2.2. (cont'd)

Common name Species group	Daubed shanny *Leptoclinus maculatus*	Yellowtail flounder *Limanda ferruginea*	Snailfish *Liparis gibbus*	Monkfish *Lophius americanus*	Checker eelpout *Lycodes vahlii*
n	6	1	18	4	8
c14:0	3.05 ± 0.11	2.42	2.51 ± 0.26	3.47 ± 0.37	2.44 ± 0.28
c16:0	16.02 ± 0.40	17.56	16.30 ± 0.26	13.88 ± 0.58	13.88 ± 0.44
c16:1n-7	13.05 ± 0.49	14.54	7.99 ± 0.95	13.05 ± 1.04	7.46 ± 0.94
c16:3n-6	0.51 ± 0.04	0.20	0.66 ± 0.11	0.34 ± 0.03	0.40 ± 0.06
c16:4n-1	0.36 ± 0.06	0.14	0.41 ± 0.02	0.21 ± 0.04	0.26 ± 0.03
c17:0	0.37 ± 0.06	0.20	1.18 ± 0.13	0.17 ± 0.01	0.44 ± 0.03
c18:0	3.18 ± 0.22	2.61	3.78 ± 0.23	2.59 ± 0.27	3.70 ± 0.48
c18:1n-11	0.24 ± 0.03	0.31	0.52 ± 0.09	1.29 ± 0.23	1.00 ± 0.10
c18:1n-9	15.43 ± 0.58	13.29	15.61 ± 0.43	13.24 ± 1.10	10.78 ± 0.22
c18:1n-7	6.16 ± 0.23	4.31	7.73 ± 0.58	4.92 ± 0.09	5.97 ± 0.18
c18:1n-5	0.55 ± 0.04	0.35	0.33 ± 0.02	0.55 ± 0.02	0.66 ± 0.04
c18:2n-6	0.59 ± 0.05	0.84	1.32 ± 0.21	0.97 ± 0.04	1.23 ± 0.07
c18:3n-3	0.23 ± 0.03	0.55	0.53 ± 0.08	0.28 ± 0.02	0.31 ± 0.04
c18:4n-3	0.57 ± 0.04	0.36	0.84 ± 0.10	0.65 ± 0.06	0.49 ± 0.07
c20:1n-11	0.79 ± 0.08	1.02	0.34 ± 0.06	0.95 ± 0.10	1.01 ± 0.09
c20:1n-9	2.10 ± 0.13	1.57	1.78 ± 0.21	8.93 ± 0.97	4.71 ± 0.51
c20:1n-7	2.47 ± 0.44	0.97	0.39 ± 0.03	0.91 ± 0.08	1.53 ± 0.19
c20:2n-6	0.27 ± 0.02	0.67	0.38 ± 0.06	0.26 ± 0.00	0.42 ± 0.03
c20:4n-6	1.04 ± 0.05	2.06	2.84 ± 0.78	1.44 ± 0.34	3.43 ± 0.53
c20:5n-3	12.21 ± 0.30	13.33	17.12 ± 0.32	5.93 ± 0.77	13.73 ± 0.39
c22:1n-11	2.21 ± 0.21	0.60	0.39 ± 0.08	5.79 ± 0.82	2.25 ± 0.39
c22:1n-9	0.37 ± 0.02	0.26	0.13 ± 0.01	1.37 ± 0.15	0.47 ± 0.06
c22:5n-3	1.50 ± 0.09	4.59	1.48 ± 0.38	1.28 ± 0.15	1.03 ± 0.07
c22:6n-3	8.00 ± 0.47	8.73	9.41 ± 0.25	9.68 ± 1.70	13.26 ± 1.04
c24:1n-9	0.39 ± 0.04	0.41	0.53 ± 0.10	0.95 ± 0.09	1.10 ± 0.21
∑	91.30	91.43	94.49	93.06	91.97
length (cm)	11.90 ± 0.06	33.80	12.04 ± 0.53	40.95 ± 2.55	25.70 ± 0.65
weight (g)	6.65 ± 0.36	529.68	30.87 ± 3.12	1789.45 ± 242.24	62.37 ± 4.68
lipid (%)	4.90 ± 0.31	2.42	2.38 ± 0.23	3.56 ± 0.90	1.59 ± 0.29

Table 2.2. (cont'd)

Common name Species-size	Capelin *Mallotus villosus*	Northern krill *Meganyctiphanes norvegica*	Haddock *Melanogrammus aeglefinus*	Silver hake *Merluccius biinearis*	Tomcod *Microgadus tomcod*
n	95	33	2	6	39
c14:0	4.15 ± 0.17	4.27 ± 0.11	3.20 ± 0.28	3.11 ± 0.12	1.98 ± 0.12
c16:0	15.53 ± 0.34	12.95 ± 0.27	13.22 ± 0.48	16.58 ± 0.45	15.27 ± 0.21
c16:1n-7	6.56 ± 0.33	8.67 ± 0.14	5.81 ± 0.06	5.31 ± 0.26	5.20 ± 0.29
c16:3n-6	0.46 ± 0.03	0.58 ± 0.03	0.29 ± 0.05	0.26 ± 0.05	0.30 ± 0.03
c16:4n-1	0.52 ± 0.05	0.58 ± 0.05	0.44 ± 0.22	0.17 ± 0.06	0.20 ± 0.03
c17:0	0.67 ± 0.07	0.37 ± 0.06	0.29 ± 0.04	0.17 ± 0.01	0.33 ± 0.03
c18:0	1.92 ± 0.07	1.24 ± 0.04	3.24 ± 0.07	2.31 ± 0.15	4.15 ± 0.15
c18:1n-11	0.61 ± 0.03	0.14 ± 0.00	2.09 ± 0.98	1.07 ± 0.10	1.16 ± 0.12
c18:1n-9	9.15 ± 0.41	8.23 ± 0.14	10.89 ± 0.63	13.27 ± 1.19	12.99 ± 0.40
c18:1n-7	4.05 ± 0.15	4.18 ± 0.08	4.15 ± 0.17	3.76 ± 0.15	5.53 ± 0.17
c18:1n-5	0.49 ± 0.01	0.51 ± 0.01	0.53 ± 0.01	0.48 ± 0.03	0.46 ± 0.02
c18:2n-6	0.70 ± 0.03	0.81 ± 0.03	0.75 ± 0.03	0.90 ± 0.06	0.87 ± 0.05
c18:3n-3	0.25 ± 0.01	0.26 ± 0.01	0.26 ± 0.05	0.42 ± 0.04	0.39 ± 0.05
c18:4n-3	0.68 ± 0.05	0.93 ± 0.05	0.68 ± 0.36	0.81 ± 0.03	0.41 ± 0.04
c20:1n-11	0.51 ± 0.02	0.61 ± 0.03	1.36 ± 0.29	1.40 ± 0.12	0.75 ± 0.06
c20:1n-9	8.42 ± 0.57	12.49 ± 0.49	6.97 ± 0.86	8.75 ± 0.80	4.15 ± 0.31
c20:1n-7	0.66 ± 0.04	0.89 ± 0.03	1.02 ± 0.22	0.64 ± 0.03	0.99 ± 0.13
c20:2n-6	0.12 ± 0.00	0.24 ± 0.01	0.31 ± 0.04	0.24 ± 0.02	0.41 ± 0.04
c20:4n-6	0.52 ± 0.03	0.46 ± 0.02	1.80 ± 0.38	0.84 ± 0.07	3.04 ± 0.27
c20:5n-3	11.94 ± 0.33	10.09 ± 0.28	12.10 ± 1.68	8.23 ± 0.68	13.54 ± 0.37
c22:1n-11	9.10 ± 0.66	15.46 ± 0.50	2.79 ± 0.24	7.47 ± 0.54	1.93 ± 0.22
c22:1n-9	0.95 ± 0.07	1.46 ± 0.06	0.74 ± 0.06	0.98 ± 0.06	0.39 ± 0.03
c22:5n-3	1.05 ± 0.04	0.39 ± 0.01	1.67 ± 0.02	0.80 ± 0.04	2.21 ± 0.13
c22:6n-3	15.82 ± 0.87	8.94 ± 0.31	17.94 ± 0.15	16.42 ± 1.35	15.31 ± 0.88
c24:1n-9	0.92 ± 0.04	0.45 ± 0.01	1.08 ± 0.18	0.83 ± 0.09	0.95 ± 0.10
∑	95.75	95.21	93.58	95.22	92.92
length (cm)	13.64 ± 0.10	4.05 ± 0.08	28.85 ± 2.95	29.18 ± 0.59	19.44 ± 0.77
weight (g)	17.37 ± 0.54	0.42 ± 0.01	216.32 ± 44.02	167.39 ± 15.55	87.38 ± 11.63
lipid (%)	3.67 ± 0.30	3.10 ± 0.15	1.22 ± 0.16	3.41 ± 0.64	2.20 ± 0.18

Table 2.2. (cont'd)

Common name	Shorthorn sculpin	Hagfish	Polychaete worm	Marlin-spike	Rainbow smelt
Species group	*Myoxocephalus scorpius*	*Myxine glutinosa*	*Nereis virens*	*Nezumia bairdi*	*Osmerus mordax*
n	8	12	12	6	127
c14:0	2.07 ± 0.33	4.43 ± 0.10	2.88 ± 0.37	2.03 ± 0.10	3.09 ± 0.09
c16:0	13.90 ± 0.36	8.24 ± 0.20	17.85 ± 0.85	12.24 ± 1.05	15.60 ± 0.19
c16:1n-7	10.01 ± 0.77	9.11 ± 0.13	2.94 ± 0.17	6.46 ± 0.39	8.04 ± 0.27
c16:3n-6	0.43 ± 0.04	0.31 ± 0.01	0.49 ± 0.13	0.23 ± 0.01	0.52 ± 0.03
c16:4n-1	0.49 ± 0.06	0.19 ± 0.02	0.28 ± 0.02	0.12 ± 0.02	0.64 ± 0.04
c17:0	0.54 ± 0.08	0.07 ± 0.00	0.65 ± 0.11	0.15 ± 0.04	0.28 ± 0.02
c18:0	2.99 ± 0.15	1.59 ± 0.07	4.56 ± 0.41	2.11 ± 0.23	2.83 ± 0.08
c18:1n-11	0.53 ± 0.21	1.60 ± 0.11	0.19 ± 0.04	5.88 ± 0.97	0.84 ± 0.07
c18:1n-9	15.08 ± 1.41	21.89 ± 0.59	4.08 ± 0.28	15.84 ± 2.15	14.32 ± 0.45
c18:1n-7	7.20 ± 0.47	3.64 ± 0.13	3.42 ± 0.27	4.22 ± 0.42	3.96 ± 0.07
c18:1n-5	0.38 ± 0.03	0.37 ± 0.01	0.43 ± 0.05	0.57 ± 0.03	0.34 ± 0.01
c18:2n-6	1.06 ± 0.14	0.78 ± 0.07	1.78 ± 0.17	0.46 ± 0.02	1.03 ± 0.04
c18:3n-3	0.44 ± 0.14	0.26 ± 0.01	1.61 ± 0.13	0.16 ± 0.01	0.48 ± 0.02
c18:4n-3	0.73 ± 0.08	0.48 ± 0.03	0.33 ± 0.05	0.26 ± 0.02	0.88 ± 0.05
c20:1n-11	0.67 ± 0.09	3.00 ± 0.19	4.42 ± 0.12	2.81 ± 0.28	0.96 ± 0.05
c20:1n-9	2.27 ± 0.70	12.40 ± 0.29	1.93 ± 0.07	14.36 ± 1.76	3.95 ± 0.33
c20:1n-7	0.97 ± 0.11	1.05 ± 0.03	1.17 ± 0.17	1.31 ± 0.11	0.77 ± 0.03
c20:2n-6	0.40 ± 0.12	0.17 ± 0.01	3.86 ± 0.35	0.21 ± 0.01	0.37 ± 0.02
c20:4n-6	2.37 ± 0.32	0.39 ± 0.02	1.91 ± 0.32	0.58 ± 0.09	1.90 ± 0.09
c20:5n-3	15.56 ± 1.18	3.79 ± 0.33	17.43 ± 1.36	5.76 ± 0.29	12.84 ± 0.23
c22:1n-11	0.92 ± 0.42	13.18 ± 0.45	0.48 ± 0.07	9.37 ± 0.92	2.50 ± 0.24
c22:1n-9	0.24 ± 0.05	1.77 ± 0.05	0.19 ± 0.04	1.78 ± 0.25	0.40 ± 0.03
c22:5n-3	1.74 ± 0.22	3.61 ± 0.13	0.27 ± 0.25	0.84 ± 0.08	1.80 ± 0.08
c22:6n-3	11.44 ± 1.04	1.86 ± 0.18	3.79 ± 0.46	6.73 ± 0.51	14.75 ± 0.54
c24:1n-9	0.35 ± 0.03	0.50 ± 0.02	0.50 ± 0.07	0.50 ± 0.04	0.66 ± 0.04
∑	92.78	94.66	77.41	94.99 9.83	93.75 3.11
length (cm)	21.55 ± 1.46	40.70 ± 0.99	20.98 ± 1.53	22.68 ± 1.08	14.97 ± 0.24
weight (g)	215.34 ± 83.96	79.41 ± 7.65	4.68 ± 0.39	35.34 ± 5.74	27.39 ± 1.64
lipid (%)	2.11 ± 0.23	15.23 ± 1.19	2.08 ± 0.33	7.51 ± 1.93	2.97 ± 0.17

Table 2.2. (cont'd)

Common name Species group	Northern shrimp *Pandalus borealis*	Striped shrimp *Pandalus montagui*	Lampreys Petromyzontidae	Winter flounder *Pseudopleuronectes americanus*	Pollock *Pollachius virens*
n	12	18	3	43	6
c14:0	2.67 ± 0.12	2.44 ± 0.23	18.68 ± 1.97	2.21 ± 0.09	2.82 ± 0.27
c16:0	14.01 ± 0.54	15.31 ± 0.28	18.60 ± 0.60	16.23 ± 0.32	14.88 ± 0.53
c16:1n-7	10.33 ± 0.53	8.53 ± 0.50	23.57 ± 0.64	7.69 ± 0.55	6.35 ± 0.50
c16:3n-6	0.50 ± 0.04	0.41 ± 0.06	0.07 ± 0.01	0.17 ± 0.01	0.41 ± 0.05
c16:4n-1	0.22 ± 0.04	0.27 ± 0.08	0.20 ± 0.07	0.10 ± 0.02	0.52 ± 0.07
c17:0	0.17 ± 0.01	0.37 ± 0.03	0.12 ± 0.03	0.43 ± 0.02	0.56 ± 0.17
c18:0	1.87 ± 0.13	2.36 ± 0.10	1.78 ± 0.30	3.68 ± 0.13	3.72 ± 0.45
c18:1n-11	0.58 ± 0.10	0.30 ± 0.04	0.41 ± 0.29	0.42 ± 0.05	1.22 ± 0.27
c18:1n-9	13.23 ± 0.44	11.86 ± 0.35	13.82 ± 0.91	9.14 ± 0.36	13.70 ± 1.79
c18:1n-7	6.27 ± 0.23	7.21 ± 0.27	2.06 ± 0.16	4.40 ± 0.14	4.43 ± 0.56
c18:1n-5	0.51 ± 0.02	0.59 ± 0.03	0.21 ± 0.01	0.48 ± 0.04	0.44 ± 0.07
c18:2n-6	0.79 ± 0.03	0.79 ± 0.04	0.35 ± 0.04	1.01 ± 0.07	0.90 ± 0.06
c18:3n-3	0.25 ± 0.01	0.23 ± 0.02	0.08 ± 0.00	0.80 ± 0.11	0.47 ± 0.03
c18:4n-3	0.53 ± 0.07	0.41 ± 0.08	0.08 ± 0.03	0.42 ± 0.04	1.18 ± 0.09
c20:1n-11	1.07 ± 0.10	0.95 ± 0.07	0.61 ± 0.09	1.16 ± 0.09	1.25 ± 0.26
c20:1n-9	5.45 ± 0.56	3.45 ± 0.62	0.69 ± 0.50	2.21 ± 0.19	6.47 ± 1.28
c20:1n-7	1.03 ± 0.06	1.39 ± 0.11	0.09 ± 0.04	1.35 ± 0.08	0.56 ± 0.05
c20:2n-6	0.31 ± 0.01	0.32 ± 0.02	0.27 ± 0.01	0.72 ± 0.07	0.29 ± 0.02
c20:4n-6	1.21 ± 0.08	1.87 ± 0.18	1.21 ± 0.30	3.83 ± 0.36	0.99 ± 0.15
c20:5n-3	15.03 ± 0.63	17.13 ± 0.72	2.81 ± 1.44	14.87 ± 0.46	12.92 ± 1.08
c22:1n-11	6.52 ± 0.77	4.69 ± 1.12	0.21 ± 0.11	0.73 ± 0.16	4.39 ± 1.09
c22:1n-9	0.92 ± 0.10	0.51 ± 0.06	0.04 ± 0.01	0.30 ± 0.04	0.65 ± 0.11
c22:5n-3	0.60 ± 0.07	0.91 ± 0.06	1.56 ± 0.08	4.79 ± 0.25	1.00 ± 0.12
c22:6n-3	10.58 ± 0.35	10.21 ± 0.51	4.26 ± 1.57	11.59 ± 0.93	14.21 ± 2.37
c24:1n-9	0.40 ± 0.03	0.43 ± 0.03	0.07 ± 0.03	0.57 ± 0.05	0.53 ± 0.09
∑	95.03	92.93	91.82	89.28	94.86
length (cm)	10.13 ± 0.70	- - -	25.50 ± 2.02	21.17 ± 0.64	31.83 ± 2.14
weight (g)	6.99 ± 1.05	3.07 ± 0.25	42.07 ± 1.49	208.29 ± 20.56	403.02 ± 64.53
lipid (%)	2.03 ± 0.14	2.01 ± 0.12	9.83 ± 3.08	2.01 ± 0.13	4.64 ± 1.10

Table 2.2. (cont'd)

Common name Species group	Greenland halibut (turbot) *Reinhardtius hippoglossoides*	Salmon-smolts *Salmo salar*	Salmon (large) *Salmo salar*-L	Scampi shrimp *Sclerocrangon boreas*	Deepwater redfish *Sebastes sp.*
n	26	12	3	6	12
c14:0	4.72 ± 0.20	1.78 ± 0.09	3.82 ± 0.38	1.54 ± 0.13	3.34 ± 0.16
c16:0	16.06 ± 0.49	16.80 ± 0.23	14.47 ± 1.20	14.30 ± 0.20	14.08 ± 0.53
c16:1n-7	9.58 ± 0.25	8.37 ± 0.68	7.10 ± 0.57	7.76 ± 0.95	6.75 ± 0.41
c16:3n-6	0.55 ± 0.04	0.66 ± 0.06	0.40 ± 0.12	0.28 ± 0.04	0.50 ± 0.07
c16:4n-1	0.30 ± 0.02	0.26 ± 0.02	0.27 ± 0.10	0.22 ± 0.03	0.33 ± 0.05
c17:0	1.11 ± 0.12	0.42 ± 0.02	0.24 ± 0.06	0.61 ± 0.06	0.13 ± 0.01
c18:0	2.70 ± 0.09	5.33 ± 0.15	2.52 ± 0.07	2.56 ± 0.21	3.64 ± 0.11
c18:1n-11	0.54 ± 0.05	0.01 ± 0.00	0.71 ± 0.19	0.11 ± 0.01	0.95 ± 0.12
c18:1n-9	13.72 ± 0.56	11.53 ± 0.60	14.59 ± 0.54	10.03 ± 0.78	11.92 ± 0.56
c18:1n-7	5.39 ± 0.19	5.43 ± 0.28	4.10 ± 0.41	9.44 ± 0.29	5.45 ± 0.32
c18:1n-5	0.40 ± 0.02	0.16 ± 0.02	0.47 ± 0.01	0.92 ± 0.09	0.46 ± 0.02
c18:2n-6	0.69 ± 0.03	3.70 ± 0.27	0.92 ± 0.15	0.91 ± 0.03	0.88 ± 0.03
c18:3n-3	0.22 ± 0.01	5.13 ± 0.36	0.41 ± 0.06	0.20 ± 0.01	0.21 ± 0.01
c18:4n-3	0.60 ± 0.04	1.14 ± 0.08	0.79 ± 0.04	0.42 ± 0.07	0.48 ± 0.06
c20:1n-11	0.86 ± 0.11	0.03 ± 0.01	1.11 ± 0.30	1.19 ± 0.13	1.10 ± 0.07
c20:1n-9	8.53 ± 0.70	0.40 ± 0.03	8.83 ± 1.03	0.79 ± 0.09	9.26 ± 0.81
c20:1n-7	1.19 ± 0.12	0.15 ± 0.01	0.60 ± 0.02	1.64 ± 0.19	0.90 ± 0.04
c20:2n-6	0.26 ± 0.02	0.29 ± 0.02	0.26 ± 0.04	0.37 ± 0.02	0.19 ± 0.01
c20:4n-6	0.70 ± 0.17	2.36 ± 0.18	0.36 ± 0.03	2.72 ± 0.32	1.24 ± 0.10
c20:5n-3	7.78 ± 0.60	10.27 ± 0.55	8.47 ± 1.05	20.76 ± 0.70	9.00 ± 0.43
c22:1n-11	8.19 ± 0.79	0.02 ± 0.00	8.92 ± 1.22	0.38 ± 0.03	9.75 ± 0.87
c22:1n-9	1.12 ± 0.09	0.06 ± 0.01	1.14 ± 0.15	0.24 ± 0.03	1.41 ± 0.11
c22:5n-3	1.22 ± 0.19	4.01 ± 0.17	2.33 ± 0.47	1.83 ± 0.14	0.59 ± 0.03
c22:6n-3	7.30 ± 0.48	14.67 ± 1.20	10.93 ± 0.74	11.03 ± 0.95	12.04 ± 0.78
c24:1n-9	0.59 ± 0.04	0.39 ± 0.06	0.77 ± 0.05	0.22 ± 0.02	0.70 ± 0.05
∑	94.31	93.36	94.51	90.45	95.32
length (cm)	25.55 ± 1.31	14.45 ± 0.25	54.97 ± 1.77	9.10 ± 0.49	16.83 ± 0.62
weight (g)	166.39 ± 23.85	26.98 ± 1.40	2285.70 ± 183.46	17.89 ± 2.59	116.74 ± 12.55
lipid (%)	5.55 ± 0.68	2.26 ± 0.26	12.62 ± 1.31	1.44 ± 0.25	2.17 ± 0.21

Table 2.2. (cont'd)

Common name Species group	Bobtail squid *Semirossia tenera*	Hyperiid amphipod *Themisto libellula*	Krill *Thysanoessa raschii*	Moustache sculpin *Triglops murrayi*	Longfin hake *Urophycis chesteri*
n	7	16	5	9	6
c14:0	2.65 ± 0.26	4.46 ± 0.14	4.80 ± 0.10	3.14 ± 0.15	3.31 ± 0.16
c16:0	14.71 ± 1.11	10.10 ± 0.59	15.29 ± 0.27	16.14 ± 0.29	10.38 ± 0.51
c16:1n-7	4.11 ± 0.91	7.91 ± 0.28	9.76 ± 0.16	11.00 ± 0.44	7.75 ± 0.27
c16:3n-6	0.09 ± 0.02	0.43 ± 0.02	1.41 ± 0.06	0.79 ± 0.05	0.46 ± 0.03
c16:4n-1	0.10 ± 0.01	0.30 ± 0.05	3.26 ± 0.20	0.77 ± 0.06	0.27 ± 0.05
c17:0	0.49 ± 0.09	0.20 ± 0.03	1.92 ± 0.13	0.83 ± 0.05	0.08 ± 0.01
c18:0	3.16 ± 0.26	0.75 ± 0.06	2.27 ± 0.07	2.68 ± 0.10	1.58 ± 0.13
c18:1n-11	0.69 ± 0.10	0.40 ± 0.03	0.08 ± 0.01	0.41 ± 0.03	5.98 ± 0.73
c18:1n-9	6.52 ± 1.00	10.21 ± 0.63	8.78 ± 0.11	15.54 ± 0.67	11.98 ± 1.56
c18:1n-7	3.80 ± 0.17	3.32 ± 0.25	4.91 ± 0.18	7.64 ± 0.12	5.08 ± 0.37
c18:1n-5	0.60 ± 0.02	0.69 ± 0.03	0.26 ± 0.01	0.35 ± 0.02	0.49 ± 0.02
c18:2n-6	0.64 ± 0.03	0.78 ± 0.03	0.55 ± 0.02	0.85 ± 0.03	0.57 ± 0.03
c18:3n-3	0.22 ± 0.03	0.29 ± 0.02	0.16 ± 0.01	0.30 ± 0.02	0.20 ± 0.01
c18:4n-3	0.30 ± 0.05	0.92 ± 0.11	2.13 ± 0.08	1.18 ± 0.07	0.49 ± 0.04
c20:1n-11	1.48 ± 0.25	3.91 ± 0.37	0.41 ± 0.03	0.36 ± 0.02	3.66 ± 0.70
c20:1n-9	8.86 ± 0.75	15.86 ± 1.14	7.78 ± 0.35	2.08 ± 0.16	14.49 ± 1.55
c20:1n-7	0.96 ± 0.17	1.14 ± 0.04	0.81 ± 0.05	0.87 ± 0.07	1.30 ± 0.07
c20:2n-6	0.57 ± 0.03	0.19 ± 0.01	0.15 ± 0.00	0.18 ± 0.01	0.15 ± 0.01
c20:4n-6	1.75 ± 0.43	0.27 ± 0.01	0.33 ± 0.01	0.89 ± 0.06	0.35 ± 0.05
c20:5n-3	16.53 ± 0.98	8.20 ± 0.58	14.62 ± 0.24	15.57 ± 0.34	5.33 ± 0.37
c22:1n-11	5.14 ± 1.02	15.61 ± 1.42	6.37 ± 0.50	1.06 ± 0.12	12.29 ± 1.63
c22:1n-9	1.20 ± 0.21	1.83 ± 0.14	0.97 ± 0.06	0.24 ± 0.01	1.97 ± 0.21
c22:5n-3	0.70 ± 0.10	0.37 ± 0.02	0.46 ± 0.01	0.94 ± 0.03	0.83 ± 0.05
c22:6n-3	16.73 ± 1.39	7.02 ± 0.34	5.96 ± 0.15	9.31 ± 0.73	6.00 ± 0.76
c24:1n-9	0.50 ± 0.04	0.41 ± 0.02	0.28 ± 0.02	0.36 ± 0.04	0.47 ± 0.03
∑	92.50	95.55	93.68	93.47	95.44
length (cm)	4.74 ± 0.69	- - -	- - -	10.41 ± 0.42	24.67 ± 1.77
weight (g)	12.17 ± 4.26	0.36 ± 0.03	0.47 ± 0.03	8.81 ± 1.24	162.96 ± 41.44
lipid (%)	1.81 ± 0.26	4.82 ± 0.51	6.47 ± 0.38	3.96 ± 0.32	8.85 ± 1.33

Table 2.2. (cont'd)

Common name Species group	White hake *Urophycis tenuis*	Ocean pout *Zoarces americanus*
n	7	1
c14:0	2.16 ± 0.09	2.15
c16:0	13.53 ± 0.13	14.09
c16:1n-7	5.26 ± 0.38	11.44
c16:3n-6	0.20 ± 0.02	0.37
c16:4n-1	0.11 ± 0.02	0.27
c17:0	0.28 ± 0.02	0.15
c18:0	4.07 ± 0.15	3.02
c18:1n-11	2.95 ± 0.16	0.92
c18:1n-9	11.74 ± 0.50	20.65
c18:1n-7	5.39 ± 0.24	5.91
c18:1n-5	0.49 ± 0.03	0.43
c18:2n-6	0.71 ± 0.03	0.81
c18:3n-3	0.23 ± 0.02	0.22
c18:4n-3	0.31 ± 0.03	0.38
c20:1n-11	1.47 ± 0.06	0.98
c20:1n-9	7.76 ± 0.42	3.76
c20:1n-7	0.83 ± 0.06	1.59
c20:2n-6	0.25 ± 0.01	0.14
c20:4n-6	1.67 ± 0.12	2.44
c20:5n-3	9.14 ± 0.29	9.68
c22:1n-11	3.82 ± 0.28	1.26
c22:1n-9	0.73 ± 0.03	0.31
c22:5n-3	1.70 ± 0.04	0.91
c22:6n-3	19.38 ± 0.93	11.17
c24:1n-9	0.72 ± 0.12	0.45
∑	94.88	93.45
length (cm)	26.17 ± 3.91	29.90
weight (g)	256.60 ± 184.97	125.51
lipid (%)	1.92 ± 0.19	1.83

Table 2.3. Principal component (PC) analysis using all specimens (n = 1028) and the set of 16 logratio fatty acids. Summary of significant (>|0.5|) factor loadings for the retained PCs (eigenvalues >1) following Varimax rotation. Maximum absolute loadings on each fatty acid are indicated in bold.

	Varimax-rotated factor			
Logratio FA	PC 1	PC 2	PC 3	PC 4
14:0		0.53	**0.58**	
16:0		**0.61**		
16:1-7		**0.71**		
16:4-1	-0.51		**0.59**	
18:1-9		**0.67**		
18:1-7		**0.57**		
18:2-6		**0.85**		
18:3-3		**0.85**		
18:4-3		0.56	**0.58**	
20:1-11				**0.87**
20:1-9				**0.70**
20:4-6			**-0.90**	
20:5-3	**-0.79**			
22:1-11			0.51	**0.69**
22:1-9				**0.70**
22:6-3	**-0.84**			
% of variance	17.66	25.17	17.91	20.92

Table 2.4. Principal component (PC) analysis using eight prey species commonly found in the Estuary in autumn and the set of 16 logratio fatty acids. Summary of significant (>|0.5|) factor loadings for the retained PCs (eigenvalues >1) following Varimax rotation. Maximum absolute loadings on each fatty acid are indicated in bold.

	Varimax-rotated factor	
Logratio FA	PC 1	PC 2
14:0	**0.74**	0.63
16:0	**0.72**	0.65
16:1-7	0.61	**0.73**
16:4-1	**0.80**	
18:1-9		**0.87**
18:1-7	0.58	**0.59**
18:2-6		**0.89**
18:3-3		**0.88**
18:4-3	**0.82**	
20:1-11	**0.69**	
20:1-9	**0.90**	
20:4-6	**-0.75**	
20:5-3	**0.79**	
22:1-11	**0.97**	
22:1-9	**0.93**	
22:6-3	**0.83**	
% of variance	50.55	29.06

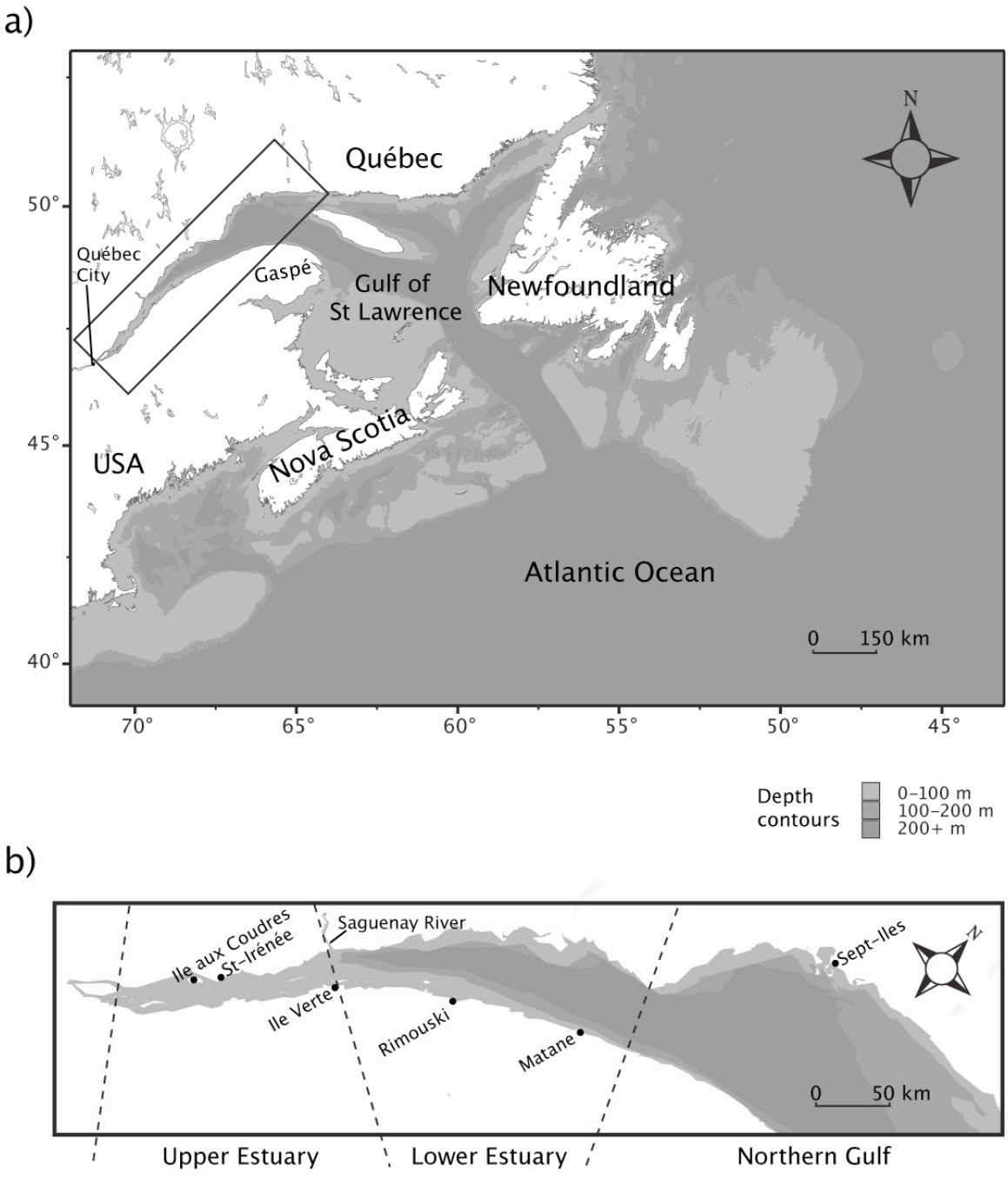


Figure 2.1. a) Study area (box) and b) sampling zones in the Estuary and Gulf of St. Lawrence, Canada.

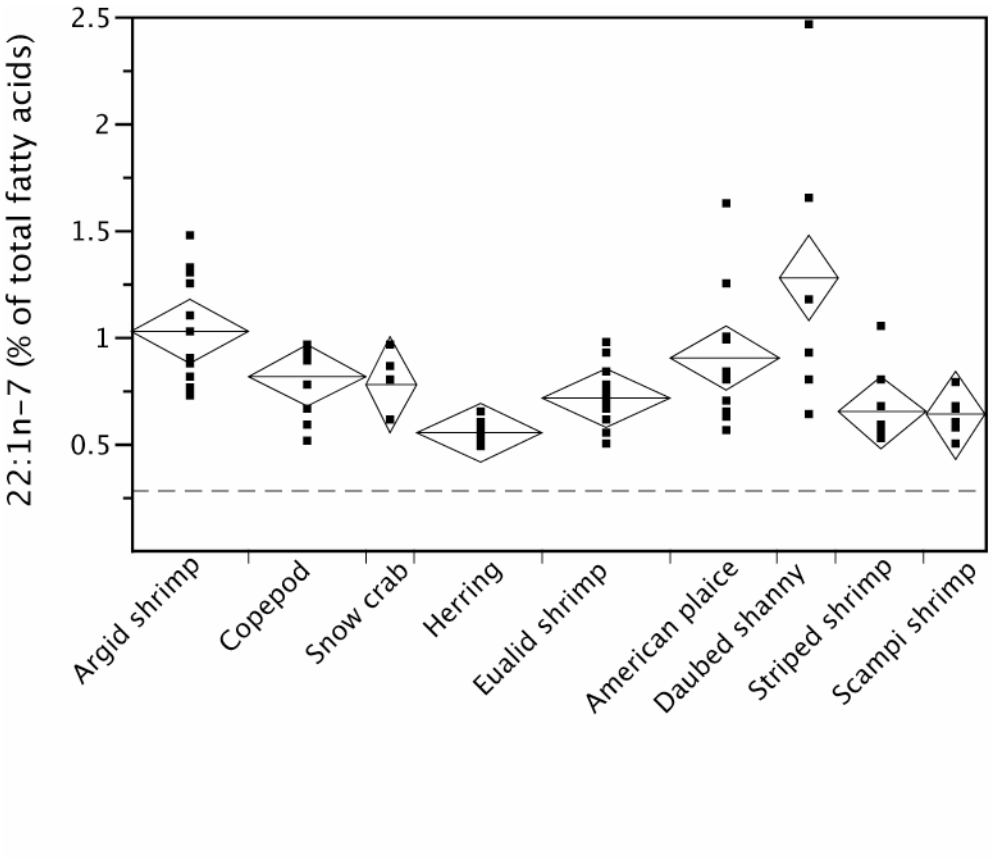


Figure 2.2. Selected species with significant amounts of the nominally 'trace-level' fatty acid 22:1n-7 not used in further evaluations. Dashed line = global mean of 0.27%. Diamonds are the horizontal spread across the mean (middle lines), with significant differences between species indicated by non-overlapping vertical apices.

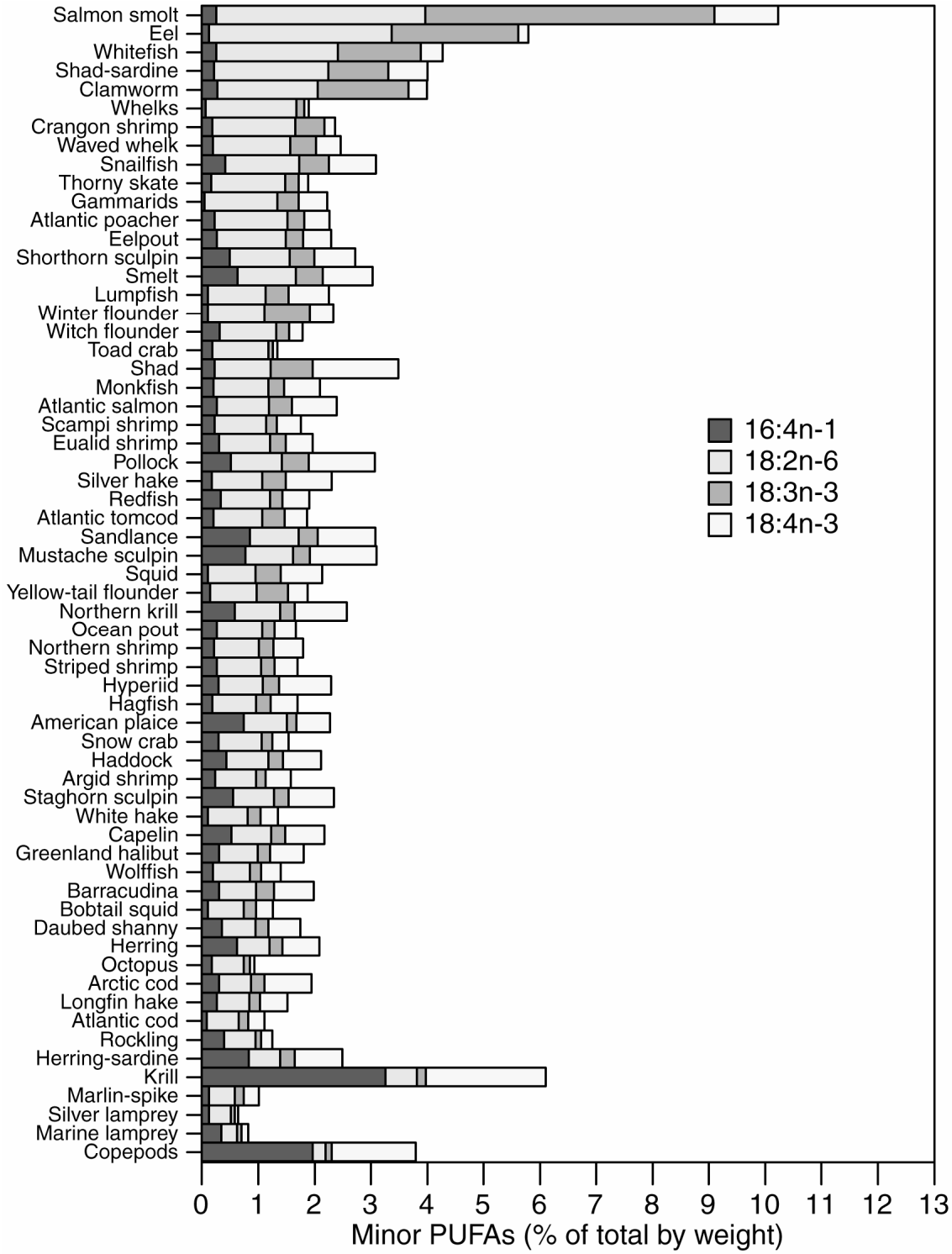


Figure 2.3. Cumulative mean abundance of four minor (global mean contribution <0.5%) polyunsaturated fatty acids. Species groups are ranked in descending order of abundance of the fatty acid 18:2n-6.

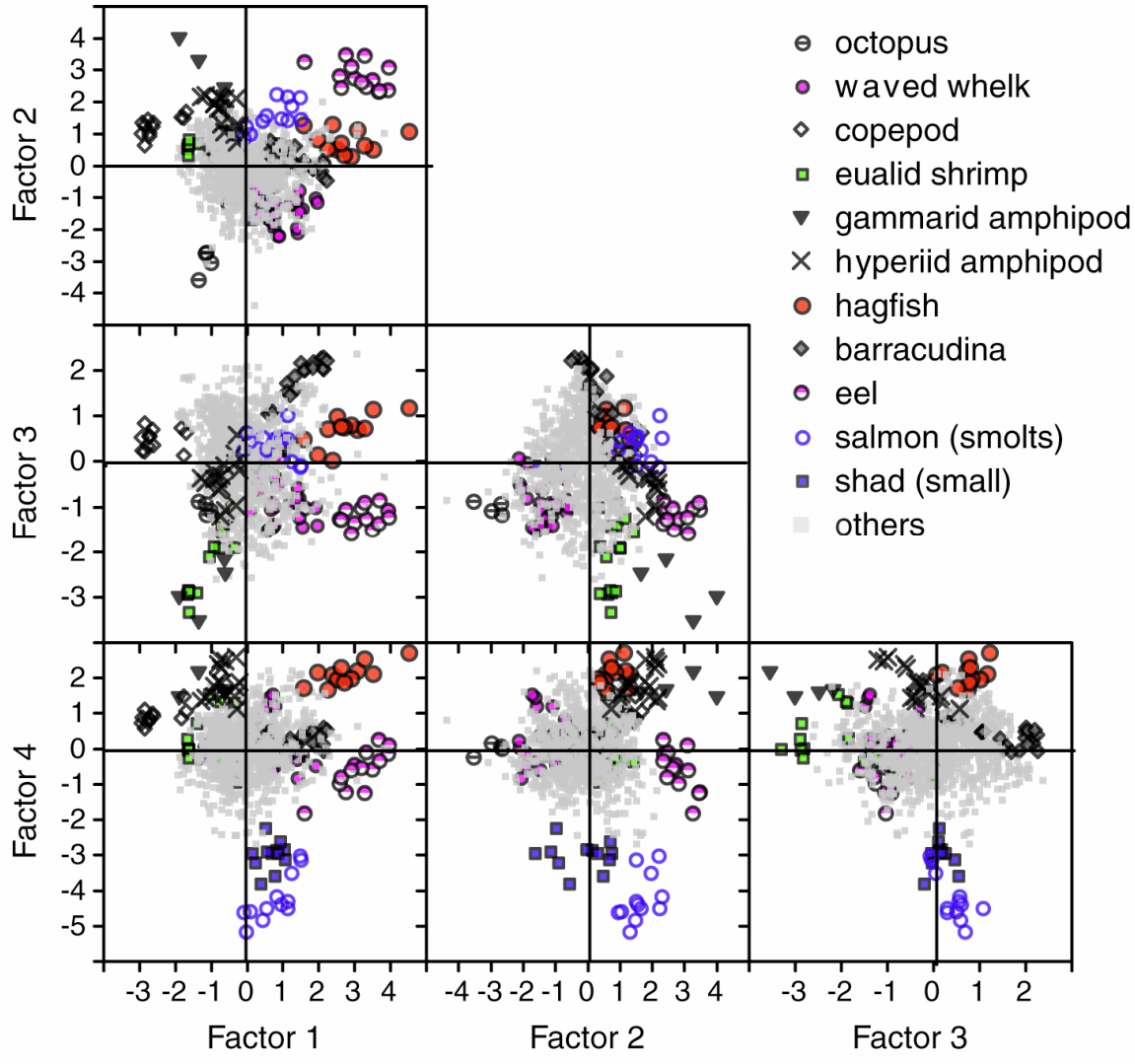
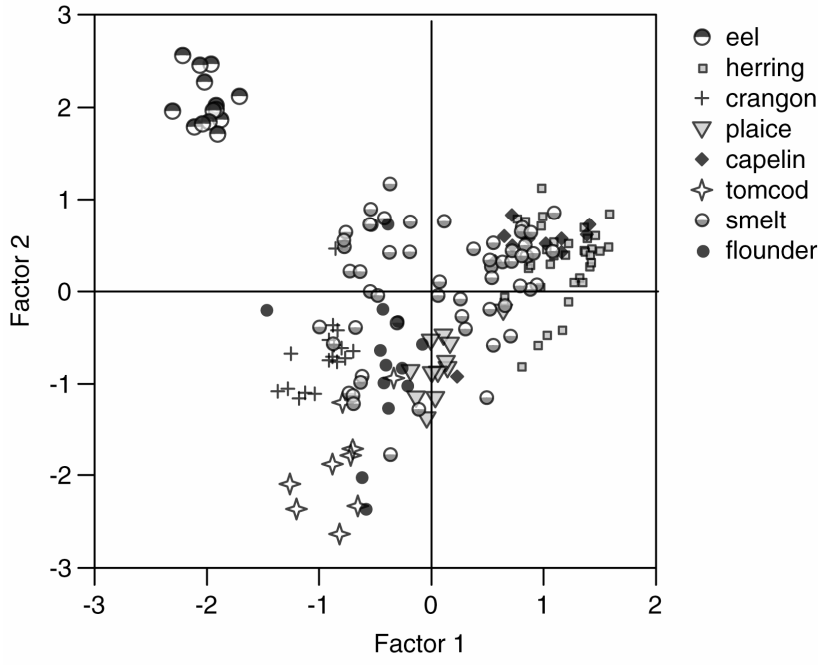


Figure 2.4. Matrix scatterplot of factor scores on the retained PC Factors 1 through 4, accounting for 81.7% of total cumulative variance in the 16 logratio fatty acid variables on all samples. Groups labelled are among those that scored farthest from the central majority.

a)



b)

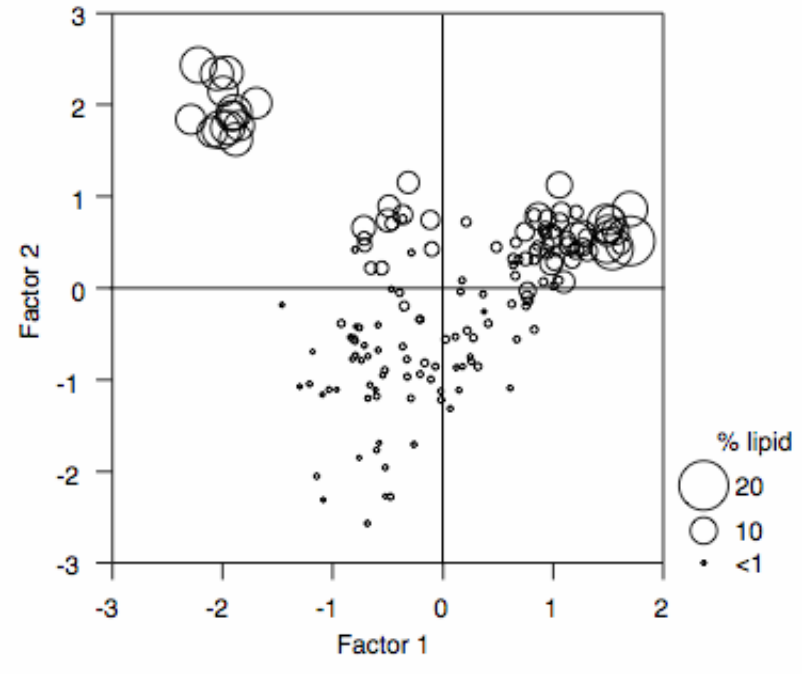


Figure 2.5. Scatterplot of scores on Factor 1 and 2 retained from a separate PC analysis conducted on eight species of prey commonly found in the Estuary in autumn. Individual scores are indicated by species name (a) and lipid content (b).

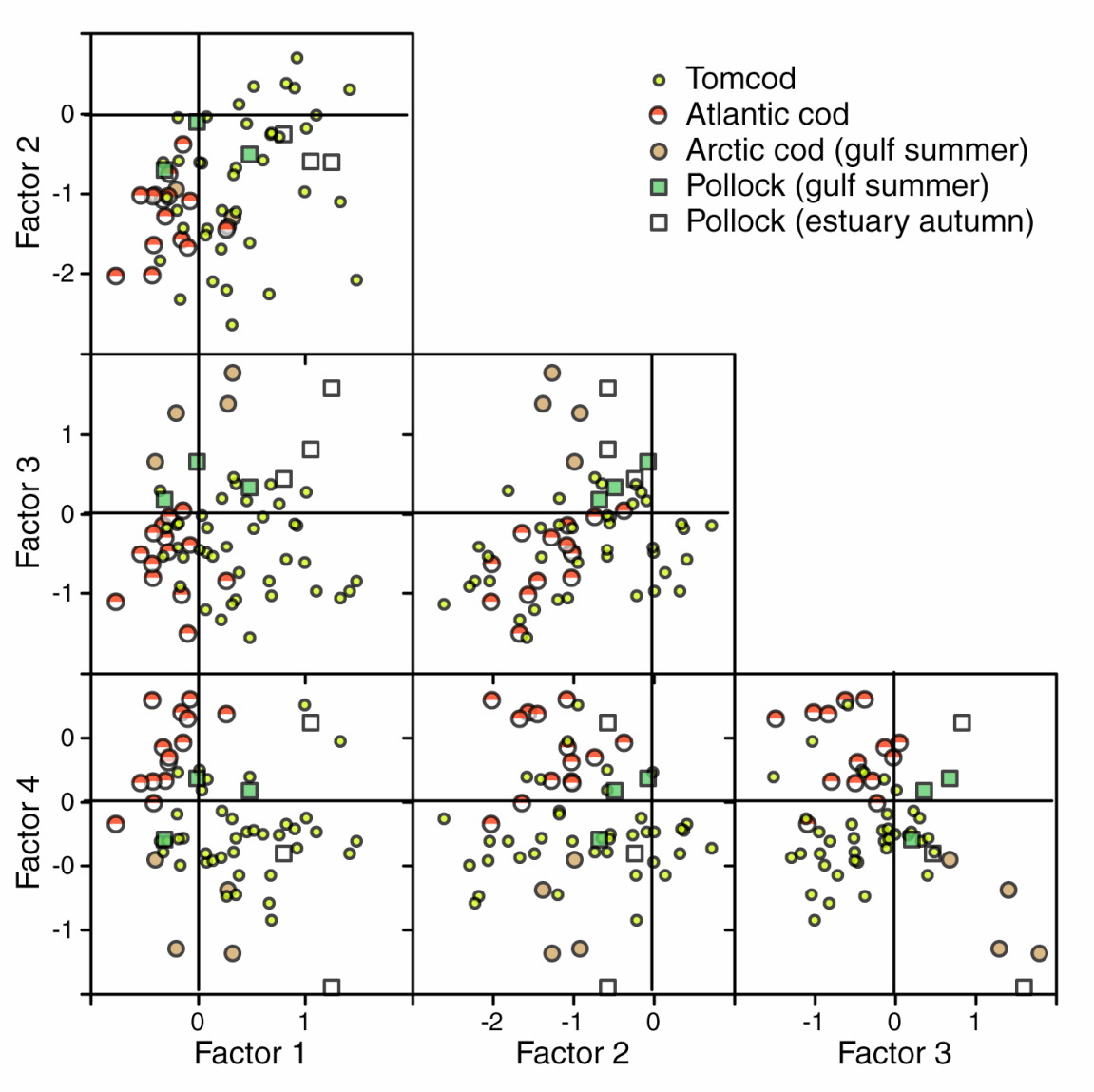


Figure 2.6. Original PC analysis showing only the scores for four cod species.

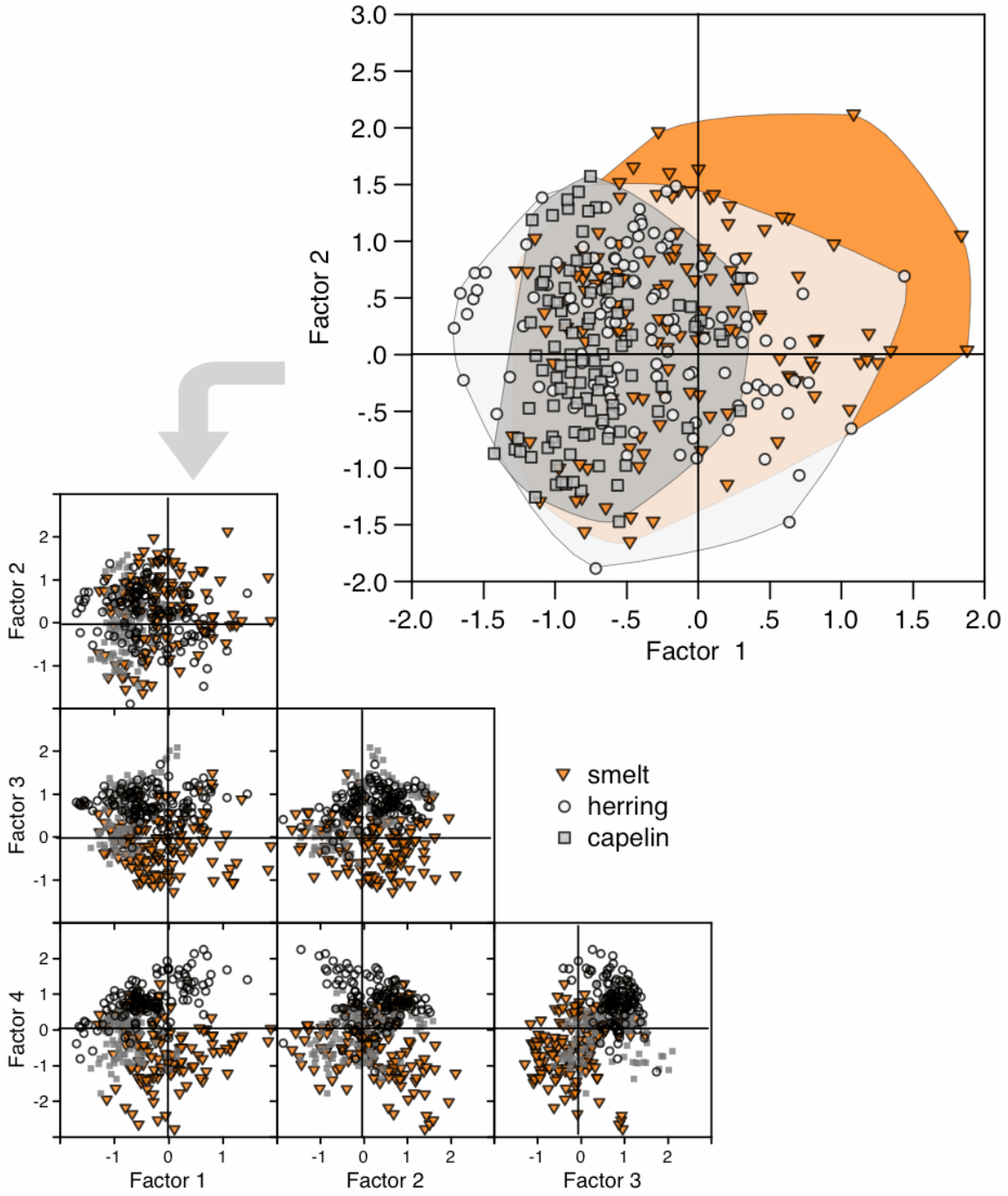


Figure 2.7. Original PC analysis showing only the scores for smelt, capelin, and herring. Overlapping scores on the first two PC factors (enlarged, in upper right, with convex area shading according to species) are in contrast to separations seen in the full matrix with Factors 3 and 4 (lower left).

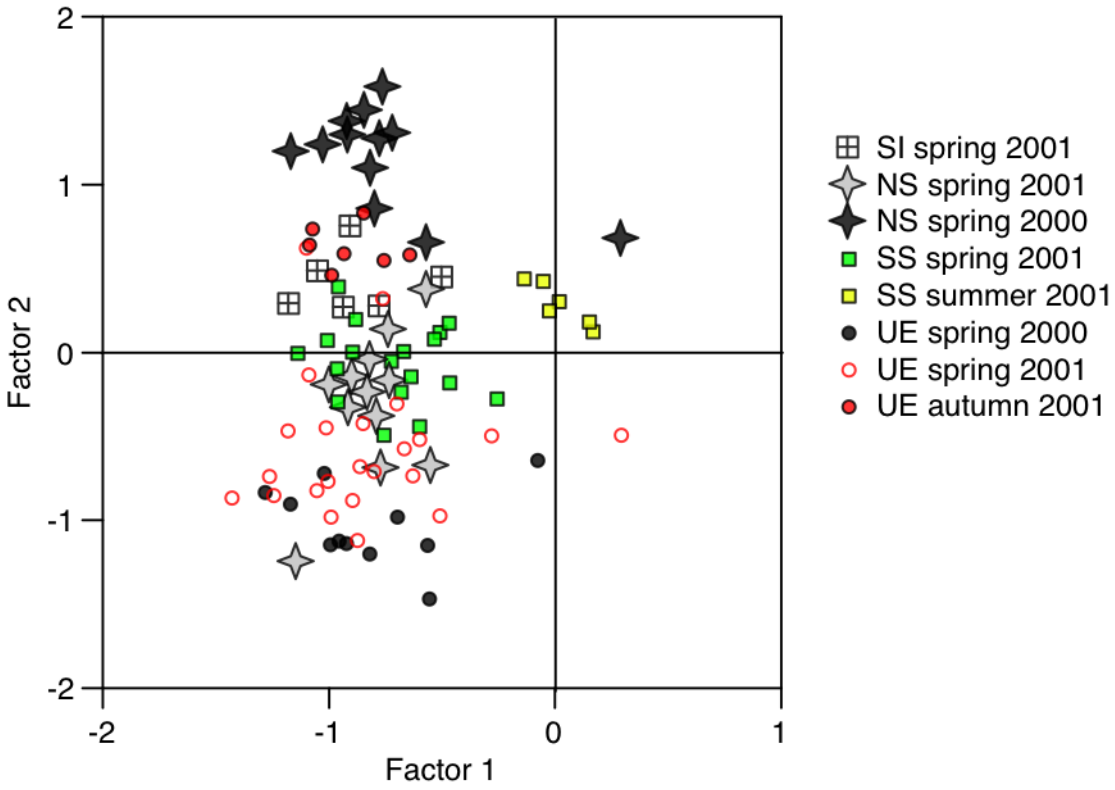


Figure 2.8. Example of within-species spatiotemporal variation, with plotted scores on Factors 1 and 2 for all capelin specimens by year, season, and locations: SI = Sept-Iles (N. Gulf), NS = North Shore (Lower Estuary), SS = South Shore (Lower Estuary), UE = Upper Estuary.

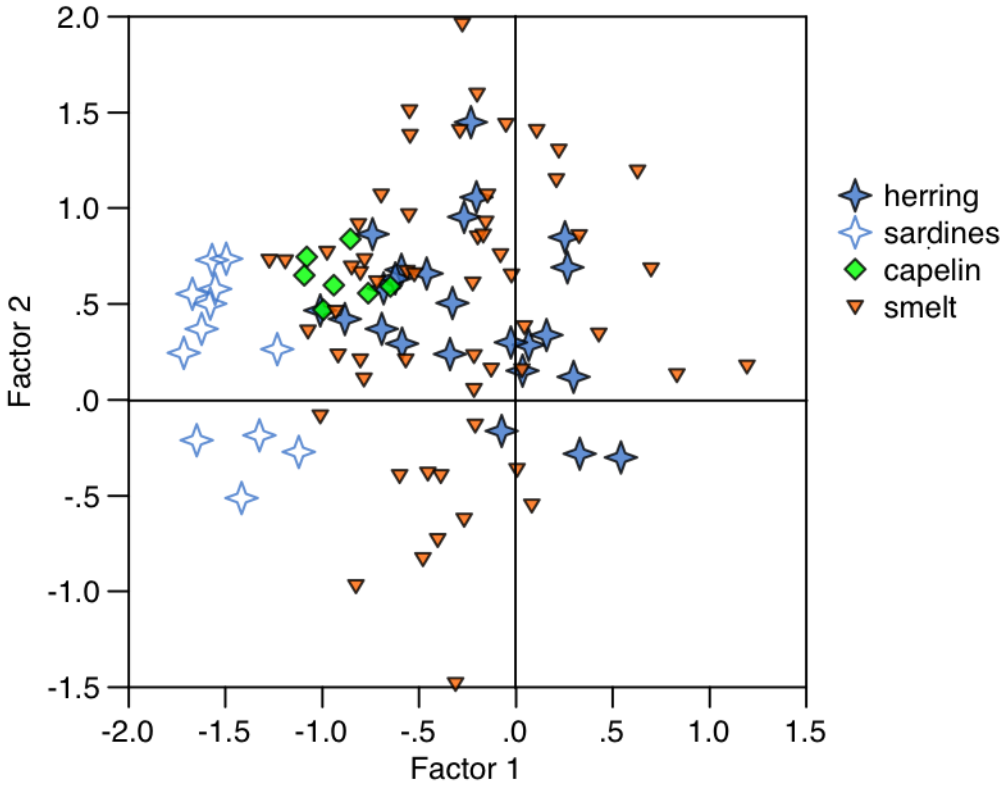


Figure 2.9. Example of between-species seasonal variability, with plotted scores on Factors 1 and 2 for autumn specimens of smelt (triangles), capelin (diamonds), herring and sardines (crosses).

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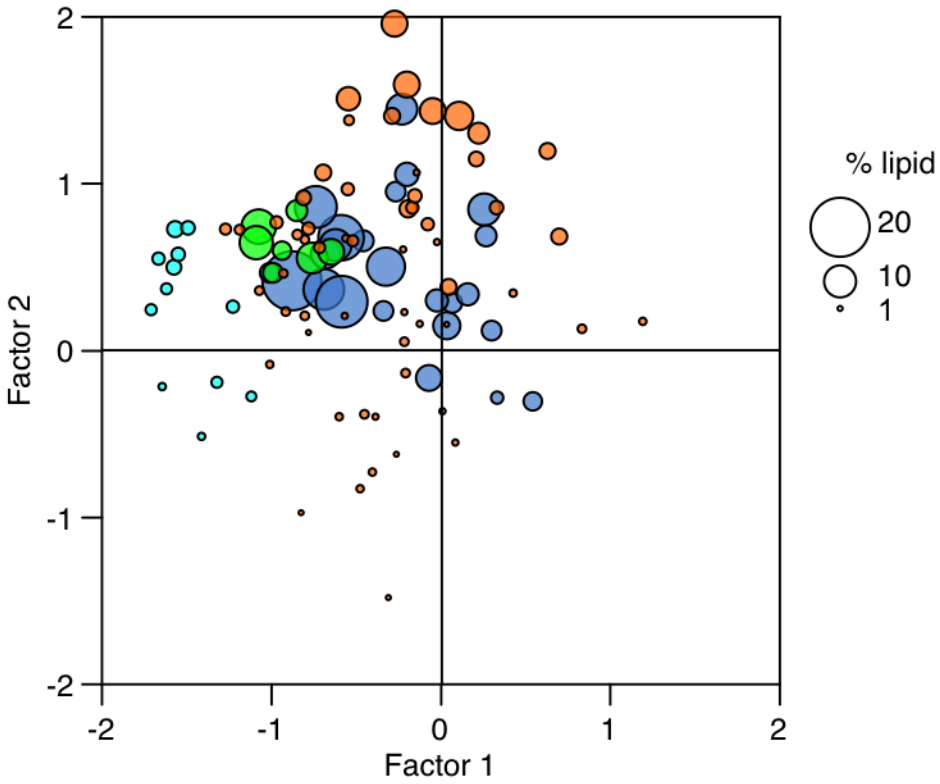


Figure 2.10. Scores on Factors 1 and 2 for autumn samples weighted by total lipid content for smelt (orange), capelin (green) and herring (blue) and herring sardines (cyan).

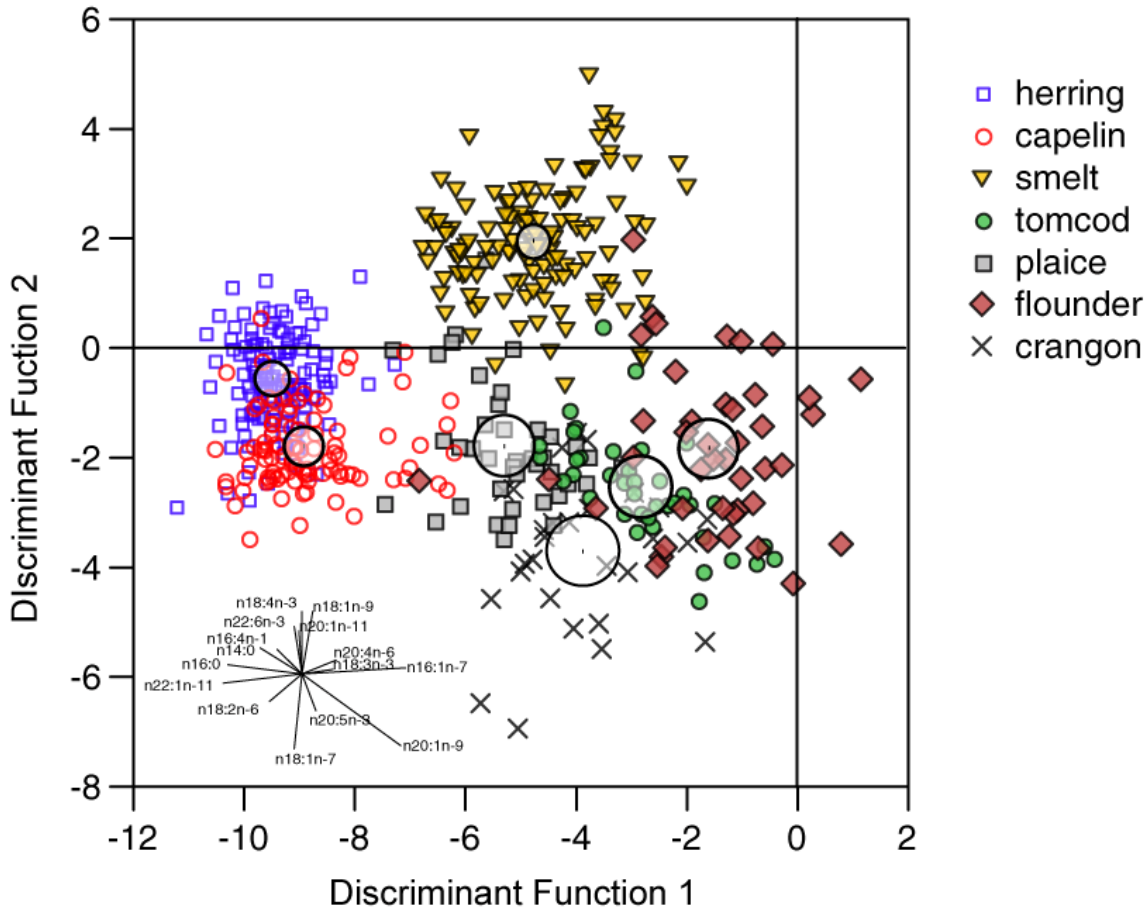


Figure 2.11. Biplot on the first two discriminant functions from an analysis classifying those prey species sampled in greatest number (range = 29–126), comprising three pelagic fishes (smelt, capelin, herring), three demersal fishes (flounder, plaice, tomcod), and one invertebrate (crangon shrimp). Circles represent 95% confidence intervals of mean scores by species. Also projected are the more-important contributions from 11 of the 16 logratio fatty acids used in the classification along with individual scores.

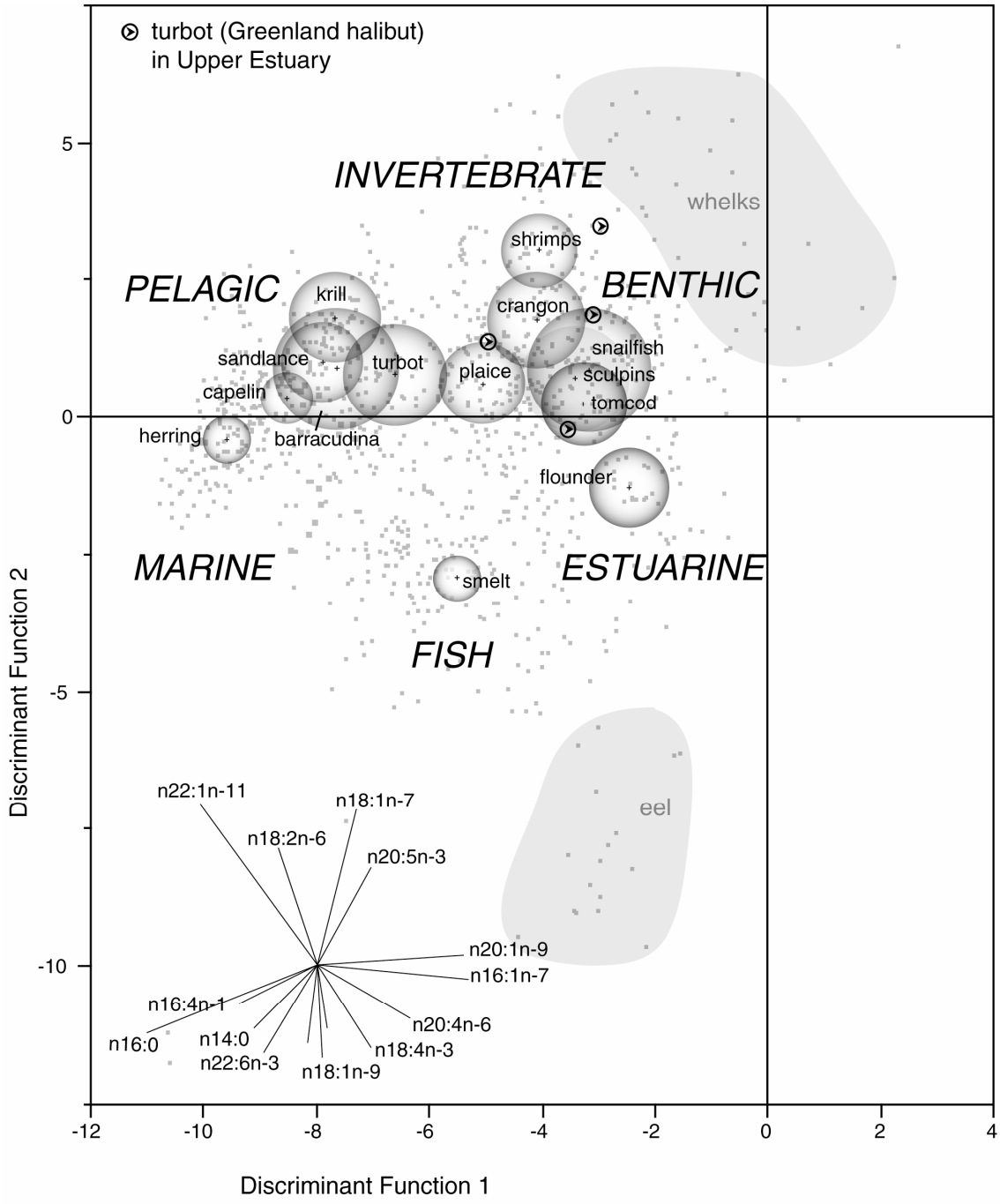


Figure 2.12. Biplot on the first two discriminant functions from an analysis classifying 15 species groups (>17 samples each, total n = 699) of potential prey of beluga, using 16 fatty acid logratios as input variables. Circles denote 95% confidence intervals for mean scores by group (sculpins and shrimps comprised three species each). Non-overlapping circles indicate significantly different groups. Oblique labels suggest species associations. For reference,

scores are plotted for all samples, including those not used in the analysis, such as eels and whelks (shaded areas). Selected individuals of Greenland halibut are marked as being distinct from their species group. Variables are projected according to their relative contributions on the two discriminant functions. For clarity, the minor contributions of fatty acids 18:3n-3, 20:1n-11, and 22:1n-9 are not included.

Chapitre 3

Comparing fatty acid signatures among marine mammals of the Estuary and Gulf of St. Lawrence: an evaluation using multivariate methods.

Résumé

Les profils en acides gras du lard chez les bélugas (*Delphinapterus leucas*) ainsi que chez les phoques communs (*Phoca vitulina*), les phoques gris (*Halichoerus grypus*), les phoques du Groenland (*Pagophilus groenlandicus*) et les phoques à capuchon (*Cystophora cristata*) ont été examinés à l'aide de méthodes d'analyse exploratoire des données. Les méthodes multivariées utilisées incluaient les arbres de classification des analyses en composantes principales, de groupements et de fonctions discriminantes. Les différences et les similarités ainsi démontrées avec 17 acides gras relativement abondants et liés à l'alimentation ont suggéré que la discrimination s'effectue entre les espèces, et, moins fortement, entre les classes incluant le sexe et l'âge. Les acides gras polyinsaturés 20:5n-3 et 22:6n-3 étaient d'intérêt spécial pour classifier le béluga à part des pinnipèdes, et les pinnipèdes juvéniles à part des adultes. La réussite de la classification souligne le potentiel des profils en acides gras pour dévoiler l'histoire alimentaire des résidents et des visiteurs de l'estuaire et du golfe du Saint-Laurent, Canada.

Abstract

Fatty acid signatures of blubber in beluga (*Delphinapterus leucas*) along with harbour seals (*Phoca vitulina*), grey seals (*Halichoerus grypus*), harp seals (*Pagophilus groenlandicus*), and hooded seals (*Cystophora cristata*) were examined using exploratory data analysis. The multivariate methods used were classification trees, along with a suite of principal component analysis, cluster analysis, and discriminant function analysis. Differences and similarities using 17 diet-linked and relatively-abundant fatty acids suggested discrimination could be achieved between these species, and to lesser extent

within classes including gender and age. Marine-linked polyunsaturated fatty acids 20:5n-3 and 22:6n-3 were of special interest in classifying belugas from pinnipeds and subadult pinnipeds from adults. The success at classification suggests the potential of fatty acid profiles to reveal the dietary history of residents and migrants for the regions of the Estuary and the Gulf of St. Lawrence, Canada.

Introduction

The Estuary and Gulf of St. Lawrence are highly productive regions of the Northwest Atlantic (El-Sabh and Silverberg 1990, Therriault 1991). These areas also represent the seasonal or permanent home to 15 species of marine mammals, including several that are of special concern (Lavigueur *et al.* 1993, Kingsley and Reeves 1998, COSEWIC 2003, Kinze 2003). The precarious status of certain populations is largely a result of historical overexploitation (Mitchell 1974, Reeves and Mitchell 1987, Kingsley 2002). However, urbanisation, fisheries, and climate changes have also occurred in a number of marine ecosystems over the last few decades, including the Estuary and Gulf of St. Lawrence (Pauly *et al.* 1998a, Hammill *et al.* 1999, Pierce *et al.* 1999, Hanson and Chouinard 2002, Zwanenburg *et al.* 2002, Law *et al.* 2003). The influence of these changes on the structure and carrying capacity of ecosystems and the rate of recovery of precarious species remains largely unknown (Bowen *et al.* 2003, Myers and Worm 2003, Trites and Donnelly 2003, Greene and Pershing 2004, Frank *et al.* 2005).

An understanding of the trophic interactions among components of food webs is necessary to appreciate the effects of ecosystem changes on species, and the role that top predators such as marine mammals may play in these ecosystems (Bowen 1997, DeMaster *et al.* 2001, Yodzis 2001, Springer *et al.* 2003, Whitehead *et al.* 2003). Diet composition has commonly been inferred from the analysis of prey remains in digestive tracts of predators (Pitcher 1980, Harvey and Antonelis 1994, Lawson *et al.* 1998a, Cherel and Duhamel 2004). This approach provides information on ingested species, but bears several important biases related mainly to the differential availability or erosion of hard parts of prey during digestion (Pierce and Boyle 1991, Burns *et al.* 1998, Staniland 2002, Arim and

Naya 2003). Other weaknesses are that digestive tracts provide information only on the last meal prior to sampling, that digestive tracts of individuals either hunted or found stranded on beaches are frequently empty, and that their collection through harvesting should not be considered for species with small populations. Visual observations and video records of feeding have also been used to obtain direct information on foraging behaviour and ingested prey items (Bowen *et al.* 2002, Born *et al.* 2003, Davis *et al.* 2003, Levermann *et al.* 2003, Williams *et al.* 2004). However, the paucity of direct observations of feeding in most species of marine mammals and the high costs of available technology to monitor feeding underwater places limits on the use of these techniques.

The limitations of direct techniques have led to interest in alternative or complementary approaches to the study of diet of marine predators. For example, stable carbon and nitrogen isotope ratios have been used to examine trophic interactions in aquatic food webs, including those of the Estuary and Gulf of St. Lawrence (Minigawa and Wada 1984, Michener and Schell 1994, Lesage *et al.* 2001). While this technique permits the general examination of carbon sources and relative trophic positions of food web components, it usually does not allow for the identification of specific prey (Gannes *et al.* 1998, Ben-David and Schell 2001). Other indirect approaches to understanding diet may use indicators of prey ingestion such as changes in stomach temperature (Hedd *et al.* 1996) and diving profiles (Folkow and Blix 1999), or information from other suitable markers such as radioisotopes (Born *et al.* 2002), synthetic contaminants (Hobbs *et al.* 2003), fecal DNA (Reed *et al.* 1997), natural pigments (Howell *et al.* 2004), and marine lipids (Dalsgaard *et al.* 2003).

The method utilising lipids to investigate diet focuses on the analysis of fatty acids contained in the fat stores of predators. Fatty acids are a component of lipids, usually

comprised of an even number of carbon atoms bonded as a linear chain of 14 to 24 carbons. A wide range of longer-chain (16-22 carbon) fatty acids with one or more unsaturated bonds have their origin in plants such as the phytoplankton in the marine environment (Jamieson and Reid 1972, Sargent *et al.* 1985, Ackman 1989). In general, species from higher trophic levels, such as fishes and mammals, are unable to produce the longer-chain, unsaturated fatty acids and therefore must obtain these elements from their diet (Greene and Selivonchick 1987, Sheridan 1994, Cook 1996, Nakamura and Nara 2003).

While it is assumed that diet plays an important role, the establishment of a direct link between a prey species and a specific fatty acid detected in predator lipids is not usually possible except in special cases. Examples of clear linkages between fatty acid compositions and diet include specialised predators such as marine turtles feeding on jellyfish which contain uncommon fatty acids (Holland *et al.* 1990), or predators that depend on environments associated with distinctive sources of fatty acids, as is seen with pinnipeds feeding in freshwater vs. marine locations (Smith *et al.* 1996, Käkelä and Hyvärinen 1998). An alternative solution to relying on specific fatty acids for the study of diet composition is the examination of the relative proportion of a range of fatty acids that may be detected in tissues of predators. This suite of fatty acids may then serve as a profile or signature that can be effective at discriminating between individual predators (Smith *et al.* 1997). In addition, the use of such signature information has been shown to potentially reveal qualitative and quantitative information on diets when employed in a powerful modelling exercise, or QFASA (Iverson *et al.* 2004). The examination of fatty acid signatures among sympatric predators might therefore provide insights into the feeding habits of the different species and their variability between regions, periods, age-classes or

genders (Iverson *et al.* 1997a, Iverson *et al.* 1997b, Brown *et al.* 1999, Walton *et al.* 2000, Dahl *et al.* 2003, Andersen *et al.* 2004).

In this study, the fatty acid signatures of one cetacean and four species of pinniped that are either resident (beluga whales, *Delphinapterus leucas*, harbour seals, *Phoca vitulina*, and some grey seals, *Halichoerus grypus*) or seasonal visitors (harp seals, *Pagophilus groenlandicus,* and hooded seals, *Cystophora cristata*) to the Estuary and Gulf of St. Lawrence were compared to gain insights into their relative degree of dietary overlap. In order to better accommodate the large number of variables (fatty acids) relative to the number of observations (individual marine mammals), fatty acid composition of individuals was examined in parallel using two exploratory, multivariate statistical approaches: 1) a series of recursive partitioning or classification trees (CART) and, 2) a combination of principal components, cluster and discriminant functions analyses.

3.2. Materials and Methods

Field collection

Blubber samples were obtained from 143 marine mammals from the Estuary and Gulf of St. Lawrence during 1996–1998: 19 beluga whales, 43 harbour seals, 31 grey seals, 31 harp seals, and 19 hooded seals (Table 3.1). Beluga samples were collected from carcasses beach-cast during the ice-free period in the lower St. Lawrence Estuary (Fig. 3.1). Samples from pinnipeds were obtained either through biopsy sampling of live-captured individuals or from sampling animals shot during scientific collections. Handling procedures for live-captured animals are presented in detail in Lesage *et al.* (2001). The pinnipeds were comprised of breeding (adult) harp, hooded, and grey seals sampled in

whelping patches in the Gulf of St. Lawrence during winter. Harbour seals, including adults (2+ years), sub-adults (yearlings) and neonates (pups), along with non-breeding adult (2+) and sub-adult (yearling) grey and harp seals were obtained from the lower St. Lawrence Estuary. All blubber samples were wrapped in aluminium foil and kept frozen in plastic bags at -20°C until processed.

Chemical analyses

For pinnipeds, the entire depth of the blubber layer from skin to muscle was used for fatty acid analyses. Owing to the more pronounced stratification of fatty acids in the blubber of toothed whales, the blubber layer of belugas was divided into tiers, and only analyses of the inner-most layer were retained (Worthy and Abend 1997, Koopman 2001, Krahn *et al.* 2004). Lipids were extracted from homogenised blubber tissue samples and esterified to fatty acid methyl esters (FAME) using a modified Folch method (Iverson *et al.* 2002). Briefly, tissue aliquots of approximately 1.5 g were solvent extracted with 2:1 chloroform-methanol. The lower lipid fraction was filtered and evaporated under nitrogen. The prepared FAME were analysed in duplicate on a Perkin-Elmer Autosystem II capillary gas chromatograph with flame ionisation detector and a flexible fused coated silica column (30 x 0.25 mm) (Iverson *et al.* 1997b). Fatty acids are presented here as mean mass percent of total fatty acids, and are designated using the standard notation X:Yn-Z, where X indicates carbon chain length, Y represents the number of double bonds and Z represents the position of the double bond nearest to the terminal methyl group.

Statistical analyses

Over 50 fatty acids and their isomers are routinely identified in quantities exceeding detection levels. Taking the smallest sample size for a species, minus one, a set of 18 fatty acids were selected for statistical analyses on the basis of their abundance and for their potential in serving as diet indicators (Iverson 1993, Kirsch *et al.* 2000).

Fatty acid data were logratio transformed before statistical treatment, as is recommended for compositional data (Aitchison 1986, Budge *et al.* 2002). Values were transformed by first normalising over 100% the proportions of the 18 selected fatty acids, then calculating the logarithm of the ratio of a given fatty acid to that of the fatty acid 18:0. The fatty acid 18:0 was selected as the standard as it is usually present in small but stable amounts (Koopman 2001, Budge *et al.* 2002). The transformation resulted in 17 logratio variables for use in statistical comparisons.

Variability in fatty acid composition between species, age classes or gender was investigated using two multivariate approaches. Fatty acid compositions were initially examined using a recursive partitioning technique, more commonly referred to as classification and regression trees (CART) (Venables and Ripley 1999). CART has the benefit that it does not require the transformation of data to meet normality assumptions, nor that the number of variables be several times lower than sample size, thus permitting the use of the original fatty acids instead of logratios (Breiman *et al.* 1984, Hastie *et al.* 2001). CART operates by minimising within-group deviations while maximising between-group variance through successive binary splits using one variable at a time (Breiman *et al.* 1984, Bakshi and Utojo 1999). CART achieves this task by dividing cases into tree nodes, or groups, that increase class homogeneity. Splitting terminates once a node contains a

pure class, or when no further splits can be made to improve class purity (Breiman *et al.* 1984, Venables and Ripley 1999). Results are presented in the form of a binary decision tree, with successive nodes indicating the value (% of mass total) of the fatty acid variable used as the splitting criterion, and terminal nodes, or leaves, representing the group composition, such as the proportion of a species, age-class or gender included in the node. In seeking to split all cases into homogenous groups, the resulting overgrown trees may become difficult to interpret. In order to minimise this effect, the tree model may then be pruned back to higher nodes to achieve an optimal tree size, which is characterised by a high purity score (R^2 of 1 = pure; 0 being poor) and relatively few branches (Breiman *et al.* 1984, Bakshi and Utojo 1999, Venables and Ripley 1999).

An initial CART analysis was produced using all 56 fatty acids. Additional analyses were made while using the reduced set of 18 fatty acids. CART models that had splits based on fatty acids in small amounts (<1%) were alternately investigated by excluding the minor fatty acid, thus inducing CART to select the next-best fatty acid for splitting (based on purity score), which may be a fatty acid found in greater amounts, and thus potentially easier to evaluate than those splits decided on trace amounts. The classification success of the CART models was evaluated using the number of misclassified individuals by class in each of the terminal nodes (Smith *et al.* 1997).

Fatty acid signatures of marine mammals were also examined using a combination of principal component and cluster analysis, followed by validation using discriminant function analysis. Initially, the set of 17 logratio fatty acids was introduced into a principal component analysis (PCA). This operation reduced the large number of correlated fatty acid variables into a smaller set of orthogonal factors while retaining most of the original variance (Hair *et al.* 1998). Factor rotation (Varimax procedure) further facilitated

interpretation of the results by emphasising the correlations of variables with a given factor (Hair *et al.* 1998). The retained factors (eigenvalues > 1) and their corresponding scores for each observation of an individual beluga or seal were then used in a hierarchical agglomerative cluster analysis with Ward's method of linkage (Martin-Fernandez and Barcelo-Vidal 1998). The classification success of the composite PC factors and derived cluster solutions were cross-validated using linear discriminant function analysis (Legendre and Legendre 1998, Field 2000). A second series of discriminant function analyses was conducted while using the 17 logratio fatty acids directly as input variables to evaluate the classification success rate when not performing a factor reduction.

All statistical analyses were performed using JMP® 5.1 (SAS Institute Inc, Cary, NC) and SPSS® 11.0 (SPSS Inc., Chicago, IL) software packages. Standard error (SEM) is reported as a measure of variability of the mean. The alpha level for statistical significance was set at 0.05.

3.3. Results

General patterns in the abundance of the different fatty acids

Approximately 56 fatty acids were identified and quantified in the blubber lipids of 143 individuals of five marine mammal species. Summary data are presented for 33 fatty acids with mean overall abundances ≥ 0.2% of the total (Table 3.2). The 18 fatty acids that were retained for statistical analyses were 14:0, 16:0, 16:1n-7, 18:0, 18:1n-7, 18:1n-9, 18:1n-11, 18:2n-6, 18:3n-3, 18:4n-3, 20:1-7, 20:1n-9, 20:1n-11 20:4n-3, 20:5n-3, 22:1n-9, 22:1n-11, 22:6n-3, which together contributed an average 86–91% (by species) of the total mass of fatty acids.

Although there were some similarities in abundances for these fatty acids, large variations in their relative quantities between marine mammal species were also readily evident. The harbour seals, which were dominated by sub-adults, had the highest amounts of fatty acid 16:1n-7 (19%) compared to the other species. Hooded seals had the highest levels of fatty acids 18:1n-9 and 20:1n-9 (22 and 14%, respectively), although the latter was also abundant in harp seals (11%). The lowest levels of 22:1n-11 were found in grey seals and harbour seals (approx. 3%), whereas the highest levels of this fatty acid were observed in belugas (9%). The abundance of each of the polyunsaturated fatty acids varied significantly across species (one-way ANOVA on logratio variables; df = 4, 138; F = 27.54-132.97; all $P < 0.05$). Beluga whales had significantly lower amounts of each of these polyunsaturated fatty acids (post-hoc Tukey's HSD pairwise means comparisons, a = 0.05), but harbour seals (all age-classes) and hooded seals also had lower average amounts, notably for the fatty acid 22:6n-3, relative to those detected in the grey seals and harp seals.

Classification models

Recursive partitioning

Classification tree analyses using 56 fatty acids produced an optimal tree model with five terminal groups, and correctly classified 133 of the 143 individuals (R^2 = 0.86) by their species (Figure 3.2a). As the initial splitting variable, the polyunsaturated fatty acid 20:4n-3 was found in lower amounts (< 0.38%) in species that are resident to the St. Lawrence Estuary (*i.e.*, beluga and harbour seals) compared to species that are likely to occur there on a seasonal basis (*i.e.*, grey seals, harp seals and hooded seals). Harbour seals were split from beluga whales on the basis of higher levels (≥ 0.07%) of a trace fatty acid (18:2n-7). In the seasonal visitor branch of the tree, harp seals were split from hooded and

grey seals on the basis of larger amounts (≥ 6.92%) of another polyunsaturated fatty acid, *i.e.*, 20:5n-3, while the latter two species were split on their levels of the metabolic intermediate fatty acid 22:5n-3.

An alternate CART analysis restricted to the subset of 18 fatty acids had 132 of 143 individuals correctly classified into five species groups (R^2 = 0.82) (Figure 3.2b). Once again, resident species to the Estuary were split from the seasonal visitors on the basis of fatty acid 20:4n-3. In this model, instead of the trace fatty acid 18:2n-7, the more abundant and polyunsaturated fatty acid 20:5n-3 was chosen to separate belugas and harbour seals. This fatty acid was also responsible for the segregation of harp seals from hooded seals and grey seals in the CART model using both the full and the reduced set of fatty acids. The fatty acid, 20:5n-3, was the least abundant in belugas (mean =1.96%, SEM = 0.67), most abundant in harp seals (mean = 8.73%, SEM = 2.06), and intermediate (mean = 4.15–5.88%) in the other three phocid species. Another difference between tree models was that diet-linked polyunsaturated fatty acid (22:6n-3), which was found in greater amounts in grey seals (mean = 9.77%, SEM = 1.43) than hooded seals (mean = 5.96%, SEM = 0.72), replaced the initial choice of the metabolic intermediate 22:5n-3 (excluded from the smaller set of fatty acids) in the partitioning between hooded seals and grey seals.

The investigation of age- and gender-class within each species suggested differences in fatty acid composition could also be revealed by CART analyses. Considering only adult individuals, males and females of the five marine mammal species were separated on the basis of different fatty acids and amounts, with varying degrees of success (Figure 3.3). Summarising the tree model results by species, we found: 1) lower amounts of fatty acid 18:2n-6 and substituted 14:0 in male belugas compared to females (splits at 0.82% and

7.06%, R^2 = 0.72 and 0.48, misclassification rate = 1/17 and 2/17, respectively); 2) lower amounts of fatty acid 14:0 and higher amounts of substituted 18:1n-7 in male hooded seals compared to females (splits at 3.64 and 4.64%, R^2 = 0.75 and 0.59, misclassification rates = 1/19 and 2/19, respectively); 3) higher amounts of fatty acid 22:1n-11 in 2 out of 7 male harp seals (split at 9.28%) and lower amounts of 18:2n-6 in 6 out of 8 male grey seals (split at 1.19%) compared to females of their respective species (R^2 = 0.54 and 0.55; misclassification rate = 2/18 and 2/16, respectively); 4) lower amounts of fatty acid 18:3n-3 and higher amounts of substituted 18:1n-11 in male harbour seals compared to females (splits at 0.38% and 5.78%, R^2 = 0.31 and 0.17, misclassification rate = 2/11 and 3/11, respectively). Fatty acid signatures were less distinct between genders when sub-adults and adults were pooled together, as indicated by high misclassification rates in the three species where more than one age class was sampled (grey seals = 9/31, harp seals = 8/31, harbour seals = 18/43). When classifying sub-adults only by gender, harbour seals and grey seals were also less distinct in their fatty acid composition (R^2 = 0.16 and 0.19; misclassification rate = 7/24 and 4/15, respectively), with greater classification success seen for sub-adult harp seals (R^2 = 0.66, misclassification rate = 1/13).

Investigation of age-class differences in fatty acid composition revealed that sub-adult harp seals and grey seals differed to some extent from the adults of their respective species. Sub-adult harp seals all had levels of fatty acid 22:1n-9 lower than 0.80%, similar to amounts observed in nearly half (8/18) of the adult harp seals (Figure 3.4a). Sub-adult grey seals had lower levels (< 0.70%) of fatty acid 20:1n-7 than adults (misclassification rate = 1/21) (Figure 3.4b). In the case of harbour seals, adults segregated from younger individuals on the basis of lower levels (<16.53%) of a commonly-biosynthesised fatty acid

(16:1n-7), whereas pups were split from sub-adults on the basis of smaller amounts (< 3.93%) of the polyunsaturated fatty acid 20:5n-3 (misclassification rate = 3/24, R^2 = 0.80) (Fig. 3.4c). The withdrawal of the fatty acid 16:1n-7 resulted in a similar tree, where harbour seal pups segregated from older individuals on the basis of lower levels of the same polyunsaturated fatty acid (20:5n-3), while greater amounts (≥ 5.1%) of 20:1n-7 was selected for segregating adults from the sub-adults (misclassification rate = 4/24, R^2 = 0.71).

Principal components and clustering analysis

Using a principal component analysis (PCA), the 17 logratio variables were reduced to four factors (eigenvalues >1), which accounted for 88% of the total variance (Table 3.3). While not shown here, the use of logratio transformed data instead of standardised fatty acids as input variables improved the output of the PCA, with an increase in the Kaiser-Meyer-Olkin measure of sampling adequacy from 0.62 to 0.77, and the total explained variance from 84% to 88%, thereby confirming that the choice of transformation and analysis was indeed appropriate for the data at hand.

The results from the PCA were also consistent with those obtained from the CART analysis since the diet-linked polyunsaturated fatty acids highlighted for the segregation by species in the CART analysis (*i.e.*, 18:2n-6, 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:6n-3) were all strongly associated with Factor 1, which explained the largest amount of variance (37%) in the data set. Factor 2 comprised several of the diet-linked monounsaturated fatty acids (*i.e.*, 22:1n-9, 22:1n-11, and 20:1n-9) and explained 19% of the total variance. Factor 3 also accounted for 19% of the total variance, and was characterised by other monounsaturated fatty acids of the 18- and 20-series. Factor 4, with 12% of the explained variance, was associated with the saturated fatty acids 14:0 and 16:0 and the

monounsaturated fatty acid 16:1n-7, all of which may be commonly acquired through the diet or produced endogenously. The abundance of the fatty acid variables associated with a given factor all covaried positively.

Significant differences were detected between species in factor scores, and thus in the abundance of the set of logratio fatty acids associated with each factor (one-way ANOVA: $F_{4,138}$ = 5.36-161.81; all $P < 0.005$) (Table 3.4). Factor 1 indicated significantly lower levels of all polyunsaturated fatty acids in belugas than in any of the pinniped species, with intermediate levels in harbour and hooded seals, and the highest values in harp and grey seals (Fig. 3.5). The diet-linked monounsaturated fatty acids that were correlated with Factor 2 were in significantly lesser amounts in harbour seals than in beluga, harp seals or hooded seals, but not in significantly different quantities than in grey seals. Harbour, hooded and grey seals had significantly higher levels of the fatty acids of the 18-series and fatty acid 20:1n-11 (Factor 3) than belugas, which in turn, had intermediate but significantly higher levels of these fatty acids than harp seals. Finally, the saturated fatty acids 14:0 and 16:0, and fatty acid 16:1n-7 characteristic of Factor 4 were significantly less abundant in hooded seals than in any other species, with intermediate values in grey seals, and higher values in beluga, harp seals and harbour seals.

A discriminant function analysis as an evaluation of using PC factors in classifying species resulted in 84% success or 120 of 143 correct assignments. The first discriminant function emphasised the strong role of Factor 1, and therefore of polyunsaturated fatty acids, in the segregation of belugas, and to a lesser extent harbour seals from the other pinniped species. The second discriminant function, which represented mainly the influence of Factor 3 with various monounsaturated fatty acids, segregated beluga and harp seals from harbour, grey and hooded seals (Fig. 3.6a). In comparison, a discriminant

functions analysis conducted using the 17 fatty acid logratios directly instead of the PC factor scores, achieved near-perfect classification success, with only 1 misclassified individual (Fig. 3.6b).

The introduction of PCA factor scores as input variables in a hierarchical cluster analysis joined the marine mammal individuals into nine groups (Fig. 3.7). Beluga whales, harbour seal pups and 21 of 24 sub-adult harbour seals, all from the St. Lawrence Estuary, segregated from adult harbour seals and from harp, hooded and grey seals to form a separate branch of three clusters. Two of these clusters were comprised of beluga whales only, whereas a third cluster consisted essentially of younger harbour seals and a few (3 of 18) sub-adult grey seals, also from the St. Lawrence Estuary. The other branch of the dendrogram consisted of six clusters, of which five were mainly mono-specific in composition and one (cluster 5) comprised a mixture of grey seals and harp seals.

The segregation of beluga whales into two clusters, one of which was dominated by females (cluster 2) and the other by males (cluster 4), was based on significantly lower levels of the fatty acids associated with Factors 3 and 4 (*i.e.*, monounsaturated fatty acids of the 18-series, fatty acids 20:1n-11 and 16:1n-7, saturated fatty acids 14:0 and 16:0) in males than females (Table 3.7; Fig. 3.8). The occurrence of harbour seal sub-adults and pups (cluster 7) in the same branch as beluga whales was related partly to generally low abundances of polyunsaturated fatty acids (Factor 1) compared to the marine mammals from the other main branch of the dendrogram, but mostly to similarities with belugas in the abundance of the monounsaturated fatty acids of the 18-series and fatty acid 20:1n-11 (*i.e.*, Factor 3), since harbour seal sub-adults and pups differed significantly from beluga whales in all other respects (*i.e.*, Factor 1, 2 and 4).

Of the 31 harp seals sampled in this study, 23 were grouped into two neighbouring clusters (clusters 3 and 6), which were similar in all respects except in the abundance of monounsaturated fatty acids associated with Factor 2; these monounsaturated fatty acids of dietary origin were significantly less abundant in harp seals from cluster 3 than cluster 6 (or 5). A group of grey seals resembled hooded seals in their levels of monounsaturated fatty acids of the 18-series and 20:1n-11 (Factor 3) and saturated fatty acids 14:0 and 16:0 and fatty acid 16:1n-7 (Factor 4). However, these grey seals formed a separate cluster (cluster 8) from hooded seals on the basis of significantly lesser amounts of monounsaturated fatty acids (Factor 2) and to a lesser extent, higher levels of polyunsaturated fatty acids compared to hooded seals. The segregation of these two groups from adult harbour seals (cluster 9) and the other grey seals and remaining eight harp seals (cluster 5) was essentially based on their significantly lower levels of the saturated fatty acids 14:0 and 16:0, and fatty acid 16:1n-7 compared to other pinnipeds. The significantly higher abundance of monounsaturated fatty acids of the 18-series and fatty acid 20:1n-11 in adult harbour seals compared to the other pinnipeds, and their lower levels of polyunsaturated fatty acids compared to grey seals and harp seals, resulted in their classification as a separate group (cluster 9). For comparative purposes, the means (± SEM) by cluster for each fatty acid are presented in Table 3.8.

Cross-validation of cluster memberships using a discriminant function analysis indicated that the nine-cluster solution as presented here was optimal, having the lowest rate of misclassifications (4/143 individuals) compared to cluster solutions with 7, 8, 10, 11 or 12 clusters. Repeating the discriminant function analysis on the nine-cluster solution while using the original 17 logratio fatty acids as input variables produced similar results to those

obtained using the factor scores, even though the success rate declined slightly from 97% to 93%, with 10 individuals misclassified (Fig. 3.9).

In both sets of discriminant analyses, cross-validating results from either PCA or cluster analyses produced similar, general patterns of projected groups, with the exception of harbour seals and hooded seals. These two groups would switch positions when using PCA factors as input variables, as compared to the discriminant projections with the original logratio fatty acids. Hence, when classifying groups using either factors or clusters, harbour seals scored closer to belugas than did the hooded seals on the first function (Figs. 3.6a, 3.9a). Conversely, when using the set of fatty acids, both discriminant analyses had hooded seals rather than harbour seals being projected closer to the belugas (Figs. 3.6b, 3.9b)

3.4. Discussion

The comparative analysis of fatty acid composition of beluga whales and four pinniped species of the Estuary and Gulf of St. Lawrence revealed differences in fatty acid composition between species, age-classes and, to some extent, genders of these marine mammals. However, the multivariate approaches used in this study could also vary in the manner with which they revealed patterns between classes. Therefore, the exploratory mechanics of the methods used will be reviewed prior to further interpretation of the biological significance of the results.

Methodological considerations

Classification and Regression Tree (CART) and Principal Components/Cluster analysis (hereafter called 'PC/Cluster') were both successful at classifying species, age-

classes and, to some extent, genders of the different marine mammals, and did so primarily on the basis of the same groups of diet-linked fatty acid, usually the polyunsaturated fatty acids. The PC/Cluster analysis also revealed some similarities in fatty acid composition among groups from different species (*e.g.*, adult grey seals and harp seals) as well as a substructure within certain groups, such as with adult or juvenile harp seals and grey seals. Meanwhile, CART segregated individuals by pre-defined groups, for example by gender, even when no significant differences could be established between groups on the chosen fatty acid.

The differences observed in the patterns issued from the two approaches may be better understood by examining the characteristics inherent to these methods. CART examined all fatty acids at once, then proceeds to partition the cases into the defined classes based on decisions using one fatty acid at a time. By comparison, the PC/Cluster analyses used all significant information (retained factors) simultaneously in order to reveal associations (*i.e.*, clusters) among all cases at once, and did so without reference to the classes involved. This difference in approaches between the two data exploratory methods was reflected in our study, where the cluster analysis classified adult males and females of hooded seals and harbour seals as part of the same cluster (*i.e.*, clusters 1 and 9, respectively), whereas CART identified the specific fatty acids that would achieve segregation of cases by species, or by gender within each species when directed to classify these classes.

One benefit of CART over other multivariate analyses is the relaxed restrictions on the number of entry variables, allowing the inclusion of fatty acids that might be strongly correlated and whose relevance is not known beforehand. By comparison, PCA usually benefits from some restriction in the number of variables used, although this is not a strict

requirement provided other conditions are met, *e.g.* with > 100 samples and loadings > 0.6 (Field 2000, Osborne and Costello 2004). Cluster analysis is particularly sensitive to correlations among variables, and thus can benefit from the use of factors following a PCA or other means of transforming data and preselecting independent variables. Despite the flexibility of exploratory methods such as PCA and CART in particular with regards to the number and correlation among variables, some degree of variable selection is often warranted. The selection of a reduced set of diet-linked fatty acids has obvious benefits since some of the fatty acids available to conduct exploratory analyses might be of lesser value, either because they occur in 'trace' (usually < 0.5%) amounts or because they are not of strong interest for the question being addressed, such as associations with diet sources vs. environmental or genetic influences.

Initially, some of the classification trees developed using CART were based on fatty acids found in trace amounts or suspected to be intermediate metabolites, such as 18:2n-7 and 22:5n-3 for the species classifications (Fig. 3.2). The use of 18 instead of 56 fatty acids as variables resulted in similar classifications (*i.e.*, from 20/143 to 21/143). This pattern indicated a certain robustness of the CART approach, namely that the patterns revealed when using the initial database were essentially repeated when using only the subset of fatty acids. Thus, the similar groupings in both CART models may be due to underlying factors associated with these fatty acids, rather than to the high variability (and thus potentially high discriminatory power) inherent to trace fatty acids. These results also brought confidence that significant patterns of interest in the total database could be revealed during the variable reduction process of PC/Cluster analysis.

While valuable for exploring among all potential variables, CART can be less useful for understanding individual cases because these are not identified in the analysis during

their placement in the pre-defined classes. PC/Cluster analyses can be better suited for exploring group associations because they maintain the identity of cases when evaluating them on the available variables, in this case, their similarity in fatty acid composition. In addition, PC/Cluster analyses have no requirement to establish classes prior to analysis. This feature of the PC/Cluster analysis provides it with an advantage over supervised classifications, such as CART and discriminant function analyses, particularly in the context of studies whose aim is to reveal unknown and shared classes, as might occur with dietary overlap among species. When examining this question, the investigator is interested as much in the similarities as in the differences in fatty acid composition between species or other classes.

In this study, the PC/Cluster analysis permitted the segregation of a group of harp seals including adults and sub-adults (cluster 3) and a group of grey seals (cluster 8), also including both age-classes, from other harp seals (clusters 5 and 6) and grey seals (cluster 5) on the basis of much lower levels of some fatty acids of dietary origin, *i.e.*, monounsaturated fatty acids associated with Factor 2 (Table 3.6). In the CART analysis, the overlap of cases due to their similarities in fatty acid composition was not actually specified, but rather was implied from those cases that were 'misclassified' within the prior-defined classes such as species and gender (Walton *et al.* 2000). The 'failure' of CART to correctly classify cases in specific tree models then becomes useful information, as the 'misclassified' cases reveal the potential inadequacies of the defined classes. One possible interpretation is that the misclassifieds in classification treees represent individuals with similar trophic histories, for example between species, or between genders and age-classes. In these instances, it may be interesting to evaluate for these hidden (i.e, not pre-defined), 'functional' groups.

The value of interpreting groupings as suggested from cluster analysis and as compared to testing for pre-defined classes was revealed through the cross-validations using discriminant functions analyses. For example, the position of harbour seals and hooded seals along the discriminant function was inverted when classifications (for both species and clusters) were performed using PC factors (Figs. 3.6a, 3.9a) or fatty acids (Figs. 3.6b, 3.9b) as classification variables. This reversal revealed the utility of classifying subgroups such as the harbour seal subadults (seen elsewhere as Cluster 7), apart from the adult harbour seals whose mean scores were closer to that of the hooded seals (Clusters 9 and 1, respectively) when using the PC factors. Alternatively, this reversal in position may indicate a greater sensitivity to a loss of information through factor reduction in these two species. Indeed, the polyunsaturated fatty acids that contributed largely to PC factor 1 were all positively correlated among each other in the PCA, but some fatty acids were ascribed differing values (opposing vectors) in discriminant analysis when they were used separately, *i.e.*, fatty acids 20:4n-3 and the 22:6n-3. A similar situation was observed for the fatty acids 14:0, 16:0 and 16:1n-7, which all loaded positively on Factor 4, but which differed in the vector of their contributions in the discriminant functions analysis (Figs. 3.6 and 3.9). These differences in the visualisation of contributing fatty acids vs. PC factors are of potential consequence in trophic interpretations, as will be discussed later.

The similarity in general patterns detected by CART and PC/Cluster analyses in this study indicated that the two approaches were useful for the study of fatty acid composition of marine predators. In some instances, the two techniques provided complementary information, which emphasised the power of these tools when used in combination. The strength and weaknesses of the two approaches in their capacity to answer questions of

interest such as dietary overlap must be borne in mind while searching for biologically-meaningful patterns among individuals.

Biological significance of patterns in fatty acid composition

Throughout this investigation, diet-linked fatty acids were consistently chosen as the primary factor for grouping cases, even when these fatty acids were not necessarily the most abundant nor the most variable. Among those fatty acids of dietary origin, polyunsaturated and monounsaturated fatty acids were the most successful variables at classifying species or age- and gender-classes in CART and constituted the two main principal components and segregation criteria in the PC/Cluster analysis.

Polyunsaturated fatty acids were used in both CART and the PC/Cluster analysis (through Factor 1) to broadly discriminate between marine mammal species that reside or visit the Estuary and Gulf of St. Lawrence, thereby separating resident harbour seals and belugas from Gulf visitors such as grey, harp, and hooded seals. In general, it might be proposed that the lower levels of polyunsaturated fatty acids observed in beluga and harbour seals (especially sub-adults) reflected their history in the estuary while higher levels in harp and grey seals (especially adults) revealed presumably greater dietary inputs of marine origin (Ackman 1989, Budge 1999).

This trophic-residency hypothesis is supported by the results from an earlier investigation, where the same individual marine mammals used in this study were analysed for their stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios (Lesage *et al.* 2001). The $\delta^{13}C$ values of seasonal visitors to the Estuary and Gulf of St. Lawrence were generally lower than those of species resident to the Estuary, and were also suggestive of seasonal movements outside of the Estuary by grey seals and harp seals that were captured within

the Estuary. These differences were suspected to reflect the characteristics of carbon sources of the food webs from the two environments, a larger enrichment in ^{13}C in the Estuary resulting from a greater productivity and input of particulate matter of terrigenous origin (Lesage *et al.* 2001). The large number of prey of that may enter the diet of beluga and pinnipeds of the St. Lawrence, and the limitations in the number of potential prey that can be entered in prey source mixing models to calculate diet composition using isotope ratios (Phillips and Gregg 2001) limited the ability of the authors to evaluate the specific prey inputs from estuarine vs marine origins, or combinations thereof, using these markers.

Without greater specificity or external validation of inferred diets when using either stable isotopes or fatty acids, both as individual tracers or combined as a profile, the trophic-related factors responsible for the general pattern differentiating estuarine residents and Gulf visitors must await confirmation. Meanwhile, in order to assist with present and future interpretations, other question of proximate causes might be examined with the current, limited set of fatty acid data. For example, one of the most obvious findings in this study was the strong segregation of beluga from the pinniped species on the basis of low amounts in polyunsaturated fatty acids. While the main hypothesis was to find species-related, diet-linked differences, we could not discount the uncontrolled factors of sampling procedure and sample classes, in particular body size, as also affecting the observed fatty acid compositions.

Samples of beluga blubber were all collected from stranded carcasses and not live animals. Unlike the present situation with pinnipeds, or even birds and fish, local sampling and controlled diet studies in captivity have thus far not been possible with belugas. This is due to logistics, conservation issues, but also biological differences, *i.e.*, blubber stratification and metabolic specialisation, more-invasive biopsying of thicker dermis or

deeper blubber, and the difficulty to control regularity of feeding in belugas. The problems with sampling stranded animals are not yet well-documented, despite their value as an alternate and relatively-available source of information, especially for restricted populations. Documenting comparisons of carcasses for several species of cetaceans and pinnipeds might be especially valuable as there may be species-specific patterns in the stratification of fatty acid compositions in blubber tissues (Käkelä *et al.* 1993, Koopman *et al.* 2002, Best *et al.* 2003b, Olsen and Grahl-Nielsen 2003, Arnould *et al.* 2005). Documenting similarities and differences will then assist the application of knowledge gained from model organisms in captivity (*e.g.*, rats, seals, mink, and fishes) towards calibrating diet models and will likely result in more accurate as well as more precise estimations.

Apart from unknown species-based differences, there is also the issue of comparing samples from studies. The manner of tissue sampling is sometimes suspected to have unforeseen effects on detected compositions, as in the case of the leaching of fats when sampling in small amounts, especially when using biopsies (Krahn *et al.* 2004, Thiemann *et al.* 2004b). Leaching may also be of concern when considering the role of poor health or post-mortem changes on blubber tissues. In these cases, tissue quantity is not the issue so much as determining the quality and the degree to which further analysis may still be useful relative to biopsying live or fresher individuals. (Hooker *et al.* 2001, Evans *et al.* 2003, Krahn *et al.* 2003, Mau 2004). The result is that different pictures may arise during comparisons. Thus, one study on pinnipeds (harbour seals) suggested relative compositions went unchanged despite lower absolute levels in emaciated individuals (She *et al.* 2002). Similarly, studies of feeding in seabirds and pinnipeds revealed fine-scale, diet-linked changes in blood composition reflecting the signature of prey species consumed regardless

of total lipid content (Cooper *et al.* 2005, Käkelä *et al.* 2005). In contrast, a broader study of blubber compositions in a species of cetacean (harbour porpoise) concluded that diet-based fatty acid signatures were diminished in emaciated compared to normal individuals, thereby making it unlikely for longer-term information on diet to be obtained in the poor-condition individuals (Koopman *et al.* 2002).

The condition of blubber or other fat stores can be evaluated by measures such as gross physical signs of tissue decay or an increase in the proportion of free fatty acids in lipid classes (Ohman 1996, Krahn *et al.* 2003, Mau 2004). In our study, no general trend in fatty acid compositions was detected from sampled belugas that were classed as 'good' (fresh carcass collected for necropsy) vs. 'poor' (tissues collected from individuals in advanced decomposition), even when examining additional samples that were obtained and processed more recently (1999-2002, not shown here). One possible explanation for the similarity in compositions from both 'good' and' poor' samples is that they were all of the same type (obtained from carcasses), and thus equally 'degraded' relative to what might be seen in live individuals. Support for this idea may be seen with selected cases. For example, four male belugas (three adults and one newborn) scored closer to pinnipeds in the PC/Cluster analysis than they did to all other belugas, mainly because of their higher abundances in polyunsaturated fatty acids (*i.e.*, Factor 1). Three belugas (not identified) were also 'misclassified' among the seals on this basis in the CART species models. These individuals may have been in relatively better condition, that is, were sampled more recently following death, than the other belugas, and possibly more similar to the other marine mammals sampled in this region or with live-sampled belugas sampled elsewhere (Dahl *et al.* 2000). An alternate solution would be to evaluate the fatty acid scores of the stranded belugas relative to those of normal and stranded cetaceans obtained in one region,

for example, by using available data on harbour porpoises (Koopman 2001). In this latter case, the belugas from this study would score closer to stranded, emaciated porpoises than to those sampled in normal condition (bycaught in fisheries) or pinnipeds (not shown).

Setting aside the question of sample condition and its effect on the beluga signatures, it remains of interest that lower amounts of polyunsaturated fatty acids were observed at once in the largest (hooded seals) and smallest pinniped species (harbour seals) examined here (Table 3.2). In the case of the harbour seals, this similarity with belugas in general (*i.e.*, in PCA and CART, but not cluster analysis) suggests that residency in the estuary is the common factor in their polyunsaturated fatty acid compositions. The closer proximity of harbour and grey seal sub-adults (cluster 9) further suggests estuarine diet sources, perhaps as neonates and yearlings did not experience foraging outside of the estuary relative to adults that may travel farther in the winter months (Lesage 2004).

Interestingly, the relatively low amounts of 'marine-linked' fatty acids such as 20:5n-3 and 22:6n-3 in the resident marine mammals from the estuary was not necessarily counter-balanced by higher amounts of more 'estuarine-linked' n-6 series fatty acids. This could be taken to suggest that resident prey species did not make significant contributions of lipids to the diet of belugas or sub-adult seals. For example, fatty acids 18:3n-3 and 18:2n-6 may be derived from terrigenous inputs (plants and detritus), and are expected in greater amounts in freshwater prey including American eels (*Anguilla rostrata*), salmonids, or common estuarine invertebrates such as sand shrimps (*Crangon septemspinosa*) (Ackman and Hooper 1973, Smith 1999, McKenzie 2000).

Eels are a special case, as they represent a large and fatty prey item (averaging 14% lipid by wet weight, see Chapter 2) and even their occasional consumption would represent a significant source of lipid-soluble pollutants such as Mirex and other organochlorine

pesticides that are detected in substantial concentrations in the St. Lawrence beluga (Hickie *et al.* 2000, Falandysz *et al.* 2002). However, detecting evidence for eels in diet is greatly diminished by historical and seasonal factors. Firstly, the eel population of the St. Lawrence watershed has undergone a drastic decline in recent decades, thereby likely reducing their potential as prey (Bérubé and Lambert 1999, Wirth and Bernatchez 2003). More importantly, eels spend their adult lives in streams (hence the 'estuarine-linked' diet and contamination) and are only present in the Estuary during a short period in October, at which time they undertake their final spawning migration to the ocean. While reports are rare, belugas have been observed feeding on eels in the Saguenay River during this brief window of opportunity. However, as the samples from belugas and seals presented here were obtained in spring and summer, it is not expected that these individuals would show any evidence of eel consumption dating from the preceding autumn.

Normally, fatty acid signatures in blubber as related to diet are assumed to act as 'medium-term' tracers that remain in evidence for some weeks and months after assimilation (Kirsch *et al.* 2000, Iverson *et al.* 2004). More-recalcitrant tracers may be necessary in order to reveal the diet history of past seasons and years in a sampled predator. Synthetic contaminants are one example, as certain organisms (*e.g.*, mammals) may have a limited capability to modify or eliminate such compounds. In a related study, samples from the same series of prey and predators were examined for organochlorine contaminants. The results suggested that the 'estuarine' (stream- and shore-based) eels, smelt (*Osmerus mordax*), and smooth flounder (*Pleuronectes putnami*) were associated with a separate food web from that shared by other fishes or harbour seals and beluga whales residing in the St. Lawrence Estuary (Law *et al.* 2003). Such a finding, if confirmed with other tracers, might

account for the relatively low amounts of 'estuarine-linked' fatty acids in belugas and harbour seals.

After the polyunsaturates, the monounsaturated fatty acids were selected as the next-most important variables by CART and the PC/Cluster analysis (*i.e.*, Factor 2) to discriminate between classes including species, age, and gender. Specifically, 18- and 20-series mononunsaturated fatty acids were found in higher amounts (and conversely, lower amount of polyunsaturated fatty acids) in the larger species such as the beluga (22:1n-11) and hooded seal (18:1n-9) than in the smaller species. Similarly, diet-linked monounsaturates (Iverson *et al.* 2004) were more abundant in adults relative to juveniles, as in the cases of harp seals (22:1n-9) and grey seals (20:1n-7).

However, the effects of diet or physiological processes on the abundance of monounsaturated fatty acids are usually more difficult to isolate as they are both abundant and amenable to modification in adipose tissues in comparison to many polyunsaturates (Lin and Conner 1990). Harbour seals were perhaps the clearest example of this difficulty, as the optimal fatty acid selected in CART for segregating adults from subadults was the common and abundant fatty acid 16:1n-7 (Bremer and Noren 1982, Ackman 1989, Iverson 1993, Leonard *et al.* 2003, Soriguer *et al.* 2003). Unfortunately, only harbour seals were all sampled from the same period and region for all age-classes of their species. Harp seal sub-adults were sampled in the Estuary, whereas all but three adults came from the Gulf. For grey seals, yearlings were sampled in the estuary over the summer while adults were obtained from winter breeding grounds. However, across all three pinniped species, a common trend appears to be the reduced mean amounts of 20-series mononunsaturates, most often for 20:1n-9 and 22:1n-11, in sub-adults compared to adults, even when this was not highlighted from the multivariate analyses, as in the case of harbour seals here (not

shown). In other studies, when comparing individuals within a specific region, similar differences for the two age-classes using 20-series fatty acid compositions were evident in a large-scale study of grey seals, but were not seen in smaller studies of harbour seals or harp seals (Andersen *et al.* 2004, Falk-Petersen *et al.* 2004, Beck *et al.* 2005).

One possibility for the observed differences in this and other study is that larger individuals may have relatively greater deposits of fat compared with smaller individuals. Such a trend has been suggested to occur in large fish such as salmon, herring, turbot, and cod, as these species are often fattier and enriched in either polyunsaturated or monounsaturated fatty acids relative to smaller individuals of their species (Iverson *et al.* 2002, Veefkind 2003).

Along with the influence of physiology and related to this trend in allometry is the potential influence on fatty acid patterns from foraging behaviour associated with dive profiles (Schreer *et al.* 2001, Beck *et al.* 2005). Behaviour and size may play a role in fatty acid compositions if larger individuals forage differently, consuming prey of lesser quality or greater size and species diversity relative to smaller predators (Beck *et al.* 2003). By consuming larger fish specimens which have themselves accumulated more of certain fatty acids, as in the examples described above of herring with 22:1n-11 and cod with 22:6n-3, large predators such as adult grey seals will then also reflect this accumulation with their body size relative to smaller marine mammals. Deep-diving by larger predators may also provide increased access to monounsaturated-rich (*i.e.*, 20:1n-9, 22:1n-11) prey including squid, redfish, and Greenland halibut (see Chapter 2), which might account for the elevated levels of these fatty acids detected in hooded seals.

In addition to biology, behaviour then might be involved with the recurring trends of small, but at times significant, differences in diet-linked fatty acids (polyunsaturates and

monounsaturates) in sexually-dimorphic species (Beck *et al.* 2005). Thus, size-linked diving and foraging behaviour might then be involved in the differences seen in this study between the males and females of beluga whales, hooded and grey seals, and to a lesser extent harbour and harp seals. Alternately, gender-based differences in metabolism rather than diet might be involved. Unfortunately, there is a tendency in biology to assume that gender has no role, or that the male organism can serve as unspoken reference for the physiology of a given species. A recent diet study on human nutrition revealed similar patterns as seen in this study, with female metabolic patterns resulting in slightly higher levels of certain minor fatty acids (*e.g.*, 14:0, 18:2n-6) than males (Burdge and Calder 2005). Separating foraging history from physiological allometric- and gender-based influences will continue to present challenges towards generalising diet-linked signatures of fatty acids, but also opportunities to further discriminate among identified classes (Andersen *et al.* 2004, Beck *et al.* 2005).

Finally, apart from the trends explored here for individuals of different classes and species, there is also a need to consider the type of samples used, and the way fatty acid compositions are investigated in general, in order to draw the most information from such studies. Firstly, the blubber samples analysed in this study were almost exclusively composed of neutral lipids (triacylglycerols) which are comprised of fatty acids as assimilated from the total lipids of ingested prey (Ackman 1989, Christie 2003). Secondly, the use of blubber tissue highlighted dietary differences through the multivariate treatments used in this and other fatty acid signature studies (Walton *et al.* 2000, Budge *et al.* 2002, Iverson *et al.* 2002). Comparisons using a set of diet-linked fatty acids distinguishes these results from studies examining fatty acids which focused on the success of categorising individuals, even when this was performed on the basis of lesser-known fatty acids in trace

amounts, such as the initial CART model (see also Iverson *et al.* 1997b, Møller *et al.* 2003, Krahn *et al.* 2004).

As an example of the pitfalls with interpreting only trace-level fatty acids, we may look at the polyunsaturate 20:4n-6, a fatty acid which is useful as a diet tracer at the planktonic level (Dunstan *et al.* 1993). In mammals, however, 20:4n-6 is principally associated with metabolic functions and is conserved in phospholipids of cell membranes, including those of adipose tissues (Durnford and Shahidi 2002, Lapillonne *et al.* 2002, Best *et al.* 2003a, Rapoport 2003, Marangoni *et al.* 2004). The result is that 20:4n-6 is often only detected in very small, stable amounts relative to other diet-linked fatty acids in the blubber, and was subsequently not considered in the subset of fatty acids for statistical analyses. Similarly, while some studies have successfully used monousaturated fatty acids (*e.g.*, copepod-derived 20:1n-9) to infer specific plankton contributions in lower-level predators (Falk-Petersen *et al.* 2000, Dahl *et al.* 2003), their abundance in mammalian fats is not necessarily a reflection of the importance of plankton as direct prey inputs to tertiary-level predators (Dahl *et al.* 2000, Andersen *et al.* 2004).

While our understanding of lipid biology and digestive assimilation from observing free-living individuals is still being developed, ecological inferences are possible because of the insights obtained from empirical investigations (Kirsch *et al.* 1998, Kirsch *et al.* 2000), along with feeding observations (Bowen *et al.* 2002), and the results of diet modeling (*i.e.*, QFASA, Iverson *et al.* 2004). Controlled feeding trials using captive animals, and an increasing number of studies on species in the wild, have demonstrated the value of fatty acid profiles in discriminating regional dietary patterns among populations of marine mammals, including those of fishes, molluscs and other invertebrates (Skerratt *et al.* 1995, Cripps and Atkinson 2000, Freites *et al.* 2002, Lea *et al.* 2002a, Bradshaw *et al.* 2003,

Walton and Pomeroy 2003). Identifying differences with which to distinguish populations is a reliable function of analysing fatty acids, among other biomarkers (Westgate and Tolley 1999, Møller *et al.* 2003, Olsen and Grahl-Nielsen 2003). In some cases, this relative ease with which patterns of differences are seen among samples may in fact become a hindrance. This is because success at detecting differences may make it easy to overlook the difficulties at elucidating the influences such as diet, and the necessity to compensate for factors including the assimilation of prey fatty acids in predator tissues (Kirsch *et al.* 1998, Iverson *et al.* 2004, Thiemann *et al.* 2004a).

Summary of potential diets

While this survey of the patterns and influences on fatty acid compositions is neither exhaustive nor conclusive, the results can be summarised given what is generally believed regarding the diet of these marine mammals, principally from existing knowledge on foraging behaviour and digestive tract contents. For example, the classifications presented here are not inconsistent with belugas foraging predominantly on small marine pelagics, linked to the copepod food web on the basis of their amounts in 20-series monounsaturated fatty acids. These pelagics may include sand lance (*Ammodytes* sp.) and capelin (*Mallotus villosus*), which are species that have been previously identified as among the dominant contributions to foraging in sampled belugas from the St. Lawrence during the 1930s (Vladykov 1946).

Harbour, harp, and grey seals are another group that are consumers of small forage species, however their relatively higher concentrations of marine and demersal species-linked fatty acids (including 20:1n-9, 20:5n-3 and 18.1n-7) relative to the belugas might point towards to their consumption of additional species, or perhaps larger specimens, as

was further witnessed by higher amounts of these fatty acids in the clusters of adults relative to juvenile harbour seals. Marine pelagic and demersal fishes such as herring, cod, and flatfishes are reported to be among the principal prey shared by these pinnipeds (Bowen and Harrison 1994, Hammill and Stenson 2000). Support for the suggested prey may also be seen from stomach contents which had samples dominated by crustaceans and capelin in harp seals, while other samples from grey seals had mostly herring, cod, turbot, and plaice (Hammill et al, unpubl.).

Unlike the considerable overlap in fatty acid compositions, and presumed diet, between several groups of pinnipeds, hooded seals were notably classified apart from the other seals. However, hooded seals may also represent a special case, as their biology and behaviour is arguably the least-understood of all the predators examined in this study (Hammill *et al.* 1995, Folkow and Blix 1999, Schreer *et al.* 2001). Nonetheless, using strictly a diet-based approach, it can be postulated that the low levels of polyunsaturated and elevated levels of monousaturated fatty acids observed for this large-bodied predator were consistent with greater inputs of deepwater rather than coastal or epipelagic prey, relative to the other pinnipeds sampled here. Prey species available offshore and in deeper waters include redfish (*Sebastes* sp.), boreal squid (*Gonatus fabricii*), and Greenland halibut (*Reinhardtius hippoglossoides*), as has been previously reported in preliminary analyses of some stomach content samples of hooded seals (Hammill *et al.* 1995, Hauksson and Bogason 1997, Kapel 2000, Potelov *et al.* 2000).

3.5. Conclusion

In this study, the examination of similarities and differences in the fatty acid composition of blubber sampled from beluga and four species of pinnipeds from the Estuary and Gulf of St. Lawrence resulted in several groupings of individuals suggesting the influence of dietary factors. The parallel use of several analytical approaches helped in the interpretation of the results, as they variously highlighted consistencies, but also inconsistencies, in the patterns observed in fatty acid compositions for these diverse groups. These findings emphasize the benefit of using a combination of approaches not only in the exploration of the data, but also in the validation of the classification results, especially in the absence of direct confirmation of diets from observations and stomach contents. The comparative approach is particularly important in multivariate analyses for which several options exist involving the use of cases and variables, but also for viewing output, whose suitability in revealing patterns may vary between studies. It also suggested, however, that careful data analysis was by itself insufficient to provide a detailed understanding of predator fatty acid profiles as they related to the consumption of prey species. As has often been the case with related groups that are believed to share diets, specific dietary inferences will first require more analyses of the differing predator classes, such as gender and size. Also of direct benefit would be additional information on the factors involved, including the condition of tissue samples, the metabolism of different classes of predators, and the evaluation of the variability in prey species signatures from the region under investigation. Ultimately, 'calibration' from such complementary investigations using fatty acid signatures as trophic biomarkers will lead to increased knowledge of the different and shared dietary sources among marine mammals. This knowledge will be especially

valuable with regards to understanding trophic interactions between the more-abundant harp and grey seals as compared with at-risk populations in the case of the belugas of the St. Lawrence Estuary.

Table 3.1. Marine mammals sampled in the Estuary and Gulf of St. Lawrence between 1996–1998.

Species	Region	Age class	Total	Male	Female
Phoca vitulina	Estuary	Adult	11	5	6
Harbour seal		Sub-adult	24	12	12
		Newborn	8	3	5
Pagophilus groenlandicus	Gulf	Adult	15	7	8
Harp seal	Estuary	Adult	3	0	3
		Sub-adult	13	6	7
Halichoerus grypus	Gulf	Adult	16	8	8
Grey seal	Estuary	Sub-adult	15	7	8
Cystophora cristata	Gulf	Adult	19	10	9
Hooded seal					
Delphinapterus leucas	Estuary	Adult	17	9	8
Beluga whale		Sub-adult	1	[a]	[a]
		Newborn	1	1	0

[a]Gender unknown

Table 3.2. Fatty acid compositions of five marine mammal species sampled in the Estuary and Gulf of St. Lawrence. Values are means by mass % (± SEM) for 33 fatty acids with overall mean abundances ≥ 0.2%. The 18 fatty acids selected for use in statistical analyses are indicated in bold.

		Hooded seal (n = 19)		Beluga (n = 19)		Grey seal (n = 31)		Harp seal (n = 31)		Harbour seal (n = 43)	
Groups	Fatty acid	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Saturated	C5:0	0	0	0.87	1.83	0	0	0	0	0	0
fatty acids	C12:0	0.06	0.02	0.72	0.61	0.11	0.02	0.1	0.04	0.14	0.03
(SFA)	**C14:0**	**3.58**	**0.59**	**6.76**	**1.52**	**4.43**	**0.51**	**5.42**	**0.74**	**4.02**	**0.48**
	C15:0	0.2	0.02	0.3	0.05	0.26	0.03	0.25	0.04	0.23	0.02
	ISO15:0	0.12	0.01	0.34	0.12	0.15	0.02	0.18	0.04	0.12	0.02
	C7ME16:0	0.23	0.03	0.21	0.04	0.28	0.05	0.32	0.05	0.25	0.03
	C16:0	**6.49**	**0.85**	**8.73**	**1.84**	**8.06**	**1.28**	**7.79**	**1.6**	**8.84**	**1.46**
	C18:0	**1.07**	**0.15**	**1.53**	**0.57**	**0.9**	**0.16**	**0.95**	**0.22**	**0.86**	**0.13**
Mono-	C14:1n-9	0.14	0.01	0.44	0.43	0.14	0.03	0.19	0.04	0.15	0.03
unsaturated	C14:1n-7	0.04	0.01	0.36	0.32	0.07	0.01	0.05	0.02	0.09	0.02
fatty acids	C14:1n-5	0.68	0.07	0.93	0.89	1.04	0.23	0.71	0.33	1.53	0.45
(MUFA)											
	C16:1n-11	0.56	0.09	0.61	0.68	0.46	0.08	0.44	0.1	0.6	0.11
	C16:1n-9	0.41	0.04	1.24	0.68	0.4	0.09	0.25	0.07	0.58	0.1
	C16:1n-7	**12.55**	**1.26**	**12.69**	**4.86**	**13.94**	**1.61**	**14.85**	**3.19**	**18.93**	**3.66**
	C18:1n-11	**4.09**	**1.1**	**3.88**	**1.33**	**4.08**	**0.97**	**2.58**	**1**	**4.03**	**1.53**
	C18:1n-9	**22.48**	**3.15**	**17.64**	**2.5**	**15.02**	**3.1**	**10.48**	**2.33**	**17.5**	**1.49**
	C18:1n-7	**4.75**	**0.47**	**3.54**	**0.59**	**4.71**	**0.9**	**3.57**	**1.01**	**4.47**	**0.31**
	C18:1n-5	0.45	0.03	0.32	0.06	0.49	0.07	0.48	0.09	0.4	0.03
	C20:1n-11	**2.37**	**0.41**	**4.29**	**1.12**	**2.18**	**0.58**	**1.62**	**0.62**	**2.16**	**0.64**
	C20:1n-7	**0.95**	**0.17**	**0.61**	**0.22**	**0.86**	**0.26**	**0.75**	**0.33**	**0.49**	**0.09**
	C20:1n-9	**13.56**	**1.45**	**8.79**	**2.2**	**8.77**	**2.29**	**11**	**3.83**	**7.94**	**1.79**
	C22:1n-11	**5.09**	**1.13**	**8.79**	**3.46**	**3.32**	**1.84**	**5.69**	**3.12**	**2.97**	**1.23**
	C22:1n-9	**1.09**	**0.26**	**0.95**	**0.43**	**0.65**	**0.28**	**0.89**	**0.5**	**0.41**	**0.12**
Poly-	C16:3n-4	0.26	0.04	0.21	0.06	0.26	0.06	0.19	0.05	0.27	0.06
unsaturated	C16:3n-6	0.43	0.1	0.6	0.13	0.55	0.14	0.71	0.12	0.56	0.11
fatty acids											
(PUFA)	**C18:2n-6**	**1.11**	**0.16**	**0.86**	**0.13**	**1.24**	**0.16**	**1.19**	**0.29**	**0.85**	**0.09**
	C18:3n-3	**0.34**	**0.07**	**0.29**	**0.1**	**0.46**	**0.07**	**0.49**	**0.13**	**0.32**	**0.09**
	C18:4n-3	**0.71**	**0.17**	**0.27**	**0.09**	**1.09**	**0.27**	**1.86**	**0.87**	**0.66**	**0.13**
	C20:4n-3	**0.47**	**0.07**	**0.28**	**0.07**	**0.54**	**0.08**	**0.53**	**0.14**	**0.28**	**0.06**
	C20:5n-3	**4.15**	**0.78**	**1.96**	**0.67**	**5.88**	**0.88**	**8.73**	**2.06**	**4.89**	**1.13**
	C20:4n-6	0.25	0.07	0.25	0.06	0.39	0.14	0.29	0.08	0.47	0.15
	C22:5n-3	2.3	0.33	2.59	1.19	5.23	0.57	4.43	0.93	4.22	0.76
	C22:6n-3	**5.96**	**0.72**	**4.75**	**1.53**	**9.77**	**1.43**	**8.91**	**1.78**	**7.06**	**1.21**

Table 3.3. Significant loadings (>0.5) of 17 fatty acid logratio variables on the four principal components (PC) that were retained (Eigenvalues > 1). The maximum loading for each fatty acid variable is highlighted in bold. Also shown is the % of variance (Varimax-rotated) accounted for by each PC. Kaiser-Meyer-Olkin measure of sampling adequacy was 0.772.

	Principal component			
Normalized logratio	1	2	3	4
18:4n-3	**0.938**			
20:5n-3	**0.931**			
20:4n-3	**0.916**			
22:6n-3	**0.878**			
18:2n-6	**0.834**			
18:3n-3	**0.829**			
20:1n-7	**0.582**			
22:1n-9		**0.921**		
22:1n11		**0.903**		
20:1n-9		**0.786**		
18:1n-9			**0.907**	
18:1n-11			**0.775**	
18:1n-7	0.559		**0.726**	
20:1n-11		0.587	**0.653**	
14:0				**0.831**
16:0				**0.693**
16:1n-7			0.548	**0.560**
% variance explained	36.84	19.91	19.04	12.18
% cumulative variance	36.84	56.75	75.79	87.97

Table 3.4. Results of one-way ANOVAs ($F_{4,\ 138}$, $P < 0.005$) for differences among species groups for each PC factor. Values in the same column not sharing a common superscript letter are significantly different (post hoc Tukey HSD multiple comparison of means, overall $a = 0.05$). Species groups are: hooded seals (Cc), beluga whales (Dl), harp seals (Pg), grey seals (Hg), and harbour seals (Pv).

		Factor			
Species group	*N*	1	2	3	4
Pg	31	0.87^{a}	0.32^{a}	-1.12^{c}	0.35^{a}
Hg	31	0.71^{a}	-0.04^{ab}	0.27^{a}	-0.28^{b}
Cc	19	-0.08^{b}	0.46^{a}	0.42^{a}	-1.44^{c}
Pv	43	-0.23^{b}	-0.50^{b}	0.63^{a}	0.45^{a}
Dl	18	-2.03^{c}	0.25^{a}	-0.45^{b}	0.33^{ab}

Table 3.5. Results of ANOVA ($F_{8, 134}$, $P < 0.001$) to test differences among clusters on each PC factor. Mean values in the same column not sharing a common superscript letter are significantly different (post hoc Tukey HSD multiple comparison of means, overall $a = 0.05$). The number of members for each cluster is indicated following each species: hooded seals (Cc), beluga whales (Dl), harp seals (Pg), grey seals (Hg), and harbour seals (Pv).

		Factor			
Cluster	Members (*n*)	1	2	3	4
1	Cc (17)	-0.14^{bc}	0.37^{ab}	0.46^{b}	-1.54^{c}
2	Dl (10)	-1.73^{d}	0.35^{ab}	0.22^{b}	1.43^{a}
3	Pg (6), Hg(1)	1.10^{a}	-2.06^{d}	-1.43^{c}	0.08^{b}
4	Dl (9)	-2.29^{d}	0.08^{b}	-1.20^{c}	-0.90^{c}
5	Hg (17), Pg (8), Cc (2)	0.83^{a}	0.73^{ab}	0.14^{b}	0.18^{b}
6	Pg (17)	0.64^{a}	0.96^{a}	-1.59^{c}	0.36^{b}
7	Pv (29), Hg (3)	-0.30^{c}	-0.81^{c}	0.36^{b}	0.53^{b}
8	Hg (8), Pv (1)	0.80^{a}	-1.19^{cd}	0.55^{ab}	-1.15^{c}
9	Pv (13), Hg (2)	0.13^{b}	0.29^{b}	1.19^{a}	0.13^{b}

Table 3.6. Means (±SEM) for the subset of 18 fatty acids (normalized over 100% for each individual) on the nine marine mammal cluster groups resulting from the hierarchical cluster analysis on 4 PC factors derived from the 17 logratios.

Cluster	1		2		3		4		5		6		7		8		9	
n	*17*		*10*		*7*		*9*		*27*		*17*		*32*		*9*		*15*	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:0	3.84	0.15	8.82	0.51	6.44	0.44	6.71	0.45	5.48	0.10	6.27	0.19	4.91	0.08	4.55	0.20	4.23	0.11
16:0	7.21	0.23	9.48	0.70	11.35	0.86	10.67	0.44	8.40	0.30	9.03	0.28	10.96	0.21	9.34	0.45	8.28	0.22
16:1n-7	13.55	0.27	19.01	1.92	20.98	1.74	10.28	0.85	16.31	0.37	15.33	0.63	23.16	0.68	16.49	0.72	17.64	0.51
18:0	1.21	0.04	1.27	0.12	1.21	0.10	2.27	0.09	0.94	0.03	1.16	0.05	1.06	0.02	1.12	0.08	0.87	0.03
18:1n-11	4.53	0.31	5.07	0.43	1.47	0.20	3.86	0.55	4.88	0.18	2.88	0.11	3.96	0.21	3.68	0.38	6.45	0.29
18:1n-9	25.48	0.67	21.55	1.33	13.84	1.17	19.26	0.72	15.27	0.33	10.10	0.31	19.69	0.31	22.39	0.81	20.55	0.41
18:1n-7	5.34	0.10	3.78	0.18	6.24	0.32	4.41	0.15	4.55	0.10	3.34	0.16	5.26	0.08	6.55	0.22	5.11	0.11
18:2n-6	1.22	0.05	1.00	0.03	1.69	0.18	0.98	0.07	1.40	0.03	1.22	0.05	1.00	0.03	1.52	0.08	1.06	0.03
18:3n-3	0.38	0.02	0.36	0.03	0.58	0.05	0.30	0.05	0.55	0.02	0.53	0.05	0.36	0.02	0.55	0.03	0.43	0.03
18:4n-3	0.73	0.04	0.27	0.03	2.07	0.29	0.34	0.04	1.49	0.07	2.28	0.30	0.79	0.04	1.04	0.07	0.81	0.03
20:1n-11	2.66	0.11	4.27	0.33	0.83	0.09	5.66	0.29	2.66	0.13	1.98	0.08	2.17	0.09	2.08	0.12	3.21	0.15
20:1n-9	14.70	0.35	8.88	0.77	6.09	1.03	11.40	0.37	12.44	0.45	14.79	0.80	8.44	0.28	7.74	0.33	10.90	0.37
20:1n-7	1.04	0.05	0.54	0.05	0.70	0.09	0.88	0.05	0.94	0.05	0.95	0.11	0.54	0.02	1.12	0.13	0.71	0.03
20:4n-3	0.51	0.02	0.29	0.03	0.55	0.03	0.35	0.02	0.61	0.02	0.64	0.05	0.34	0.02	0.64	0.05	0.38	0.02
20:5n-3	4.43	0.19	2.34	0.22	12.93	1.18	2.19	0.28	7.56	0.29	9.09	0.35	5.52	0.24	6.69	0.44	6.10	0.25
22:1n-11	5.42	0.27	7.95	1.23	2.26	0.70	12.34	0.65	5.11	0.30	9.09	0.51	3.14	0.22	1.34	0.15	4.06	0.38
22:1n-9	1.18	0.07	0.77	0.11	0.45	0.12	1.43	0.12	0.91	0.06	1.35	0.12	0.44	0.02	0.53	0.08	0.57	0.03
22:6n-3	6.59	0.20	4.36	0.43	10.32	0.58	6.65	0.33	10.49	0.35	9.98	0.64	8.26	0.28	12.63	0.51	8.62	0.46

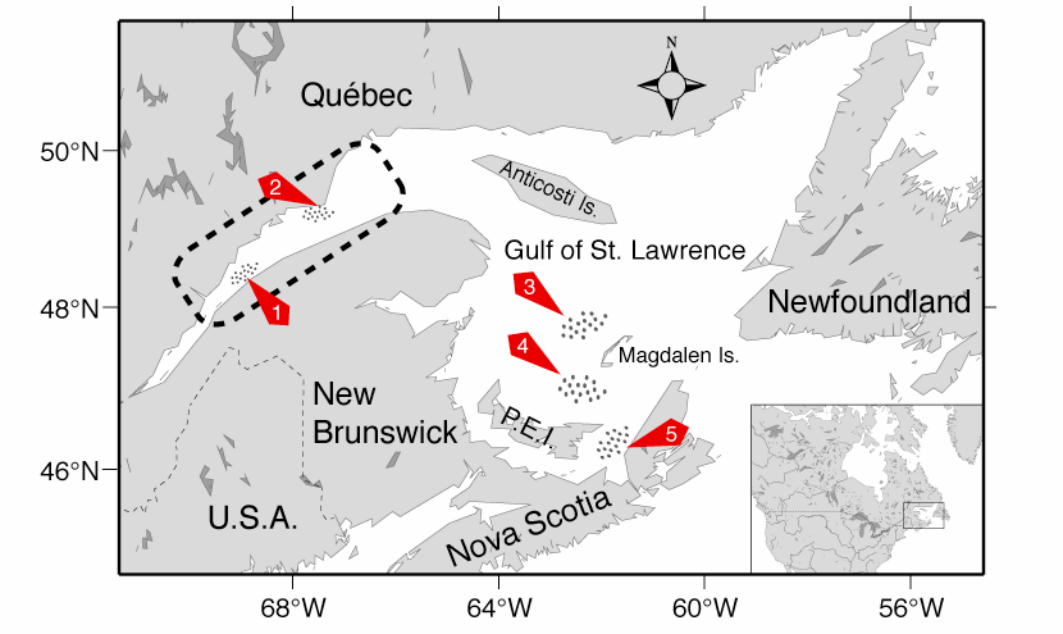


Figure 3.1. Approximate sampling locations in the Estuary and Gulf of St. Lawrence. Beluga carcasses were recovered along the shores of the lower Estuary (dashed outline). Seals were from (1) Bic, for harbour and grey seals (Estuary), (2) Godbout, for harp seals (Estuary), (3) near the Magadalen Islands for breeding harp seals (Gulf), (4) adjacent to Prince Edward Island (P.E.I.) for breeding hooded seals (Gulf), and (5) near Port Hood, Nova Scotia for breeding grey seals (Gulf).

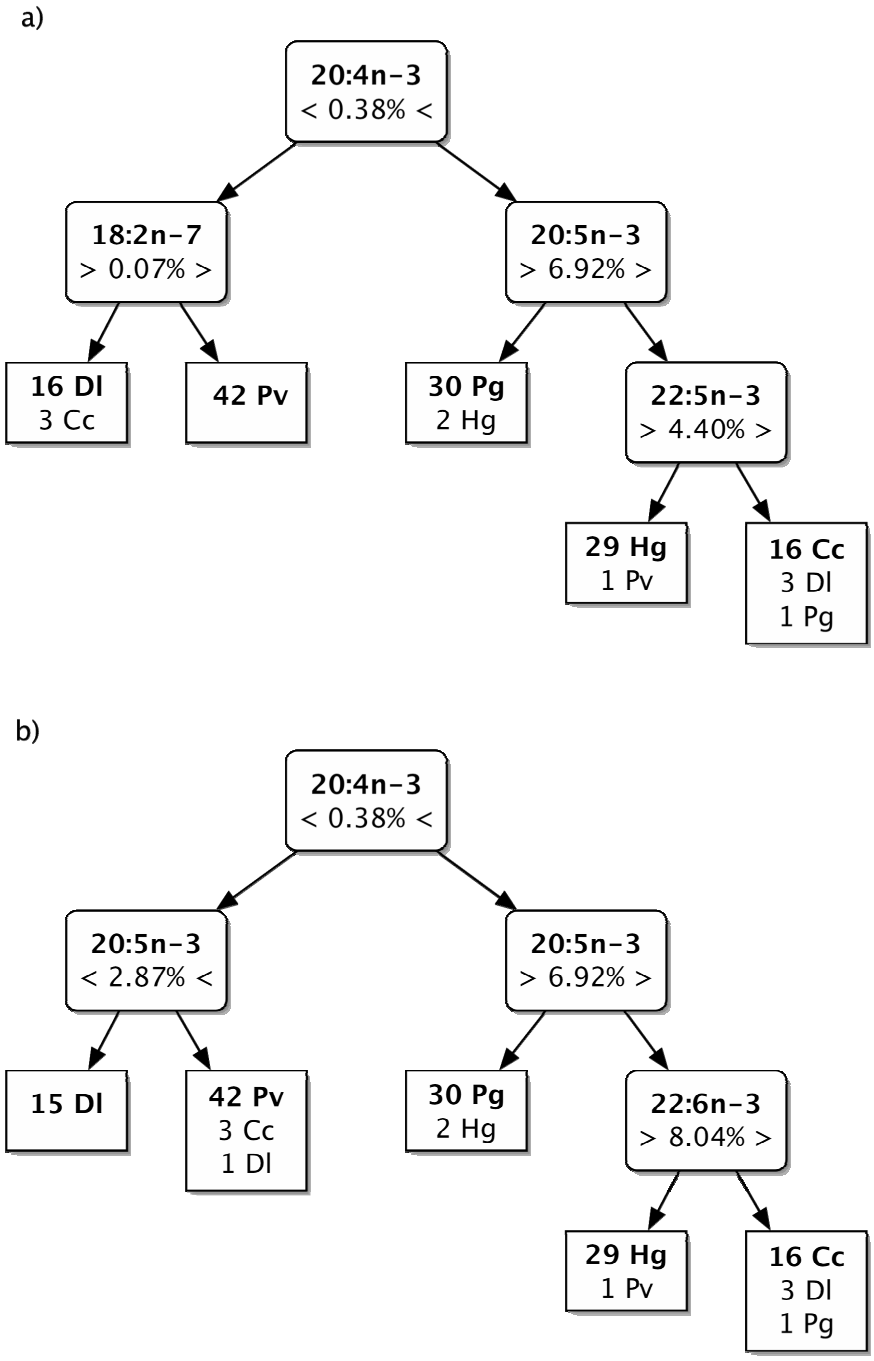


Figure 3.2. Classification trees for five different species of marine mammals (n = 143) collected in the Estuary and Gulf of St. Lawrence grouped by species: (a) initial classification using all available (56) fatty acids, (b) classification produced while using the reduced set of 18 fatty acids. Fatty acids in rounded boxes represent variables and the level (% amount) chosen to split cases. The number of individuals of each species are listed in the terminal

nodes (squared boxes), with the numerically dominant species in bold and the ‘misclassified’ individuals in normal type. Species classes are beluga (Dl), harbour seals (Pv), harp seals (Pg), grey seals (Hg), and hooded seals (Cc). Total misclassification rate was 10 and 11 of 143 individuals for (a) and (b) respectively.

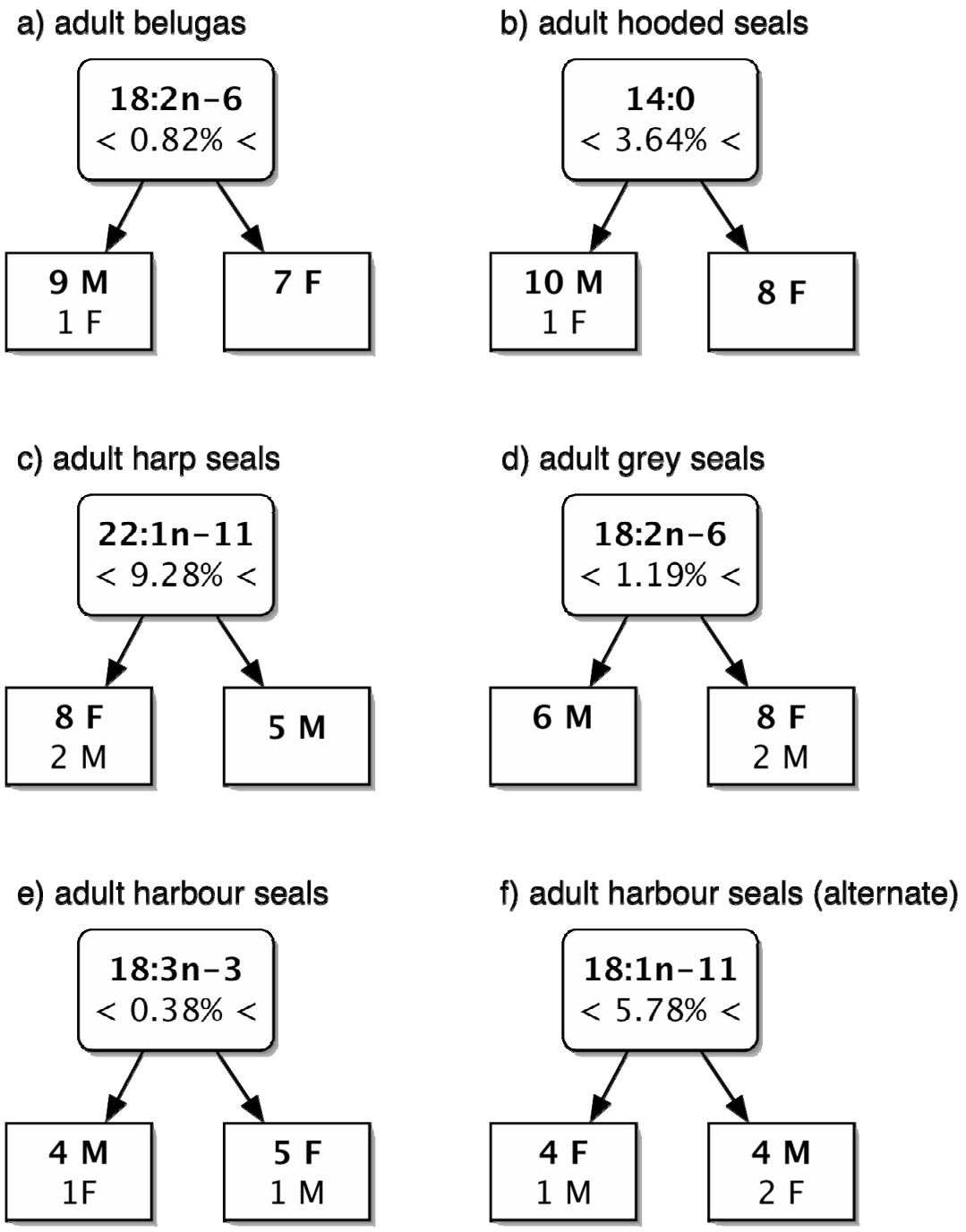


Figure 3.3. Classification trees by gender for adult male (M) and female (F) beluga (a), hooded seals (b), harp seals (c), grey seals (d) and harbour seals (e and f) as determined by CART while using the set of 18 fatty acids.

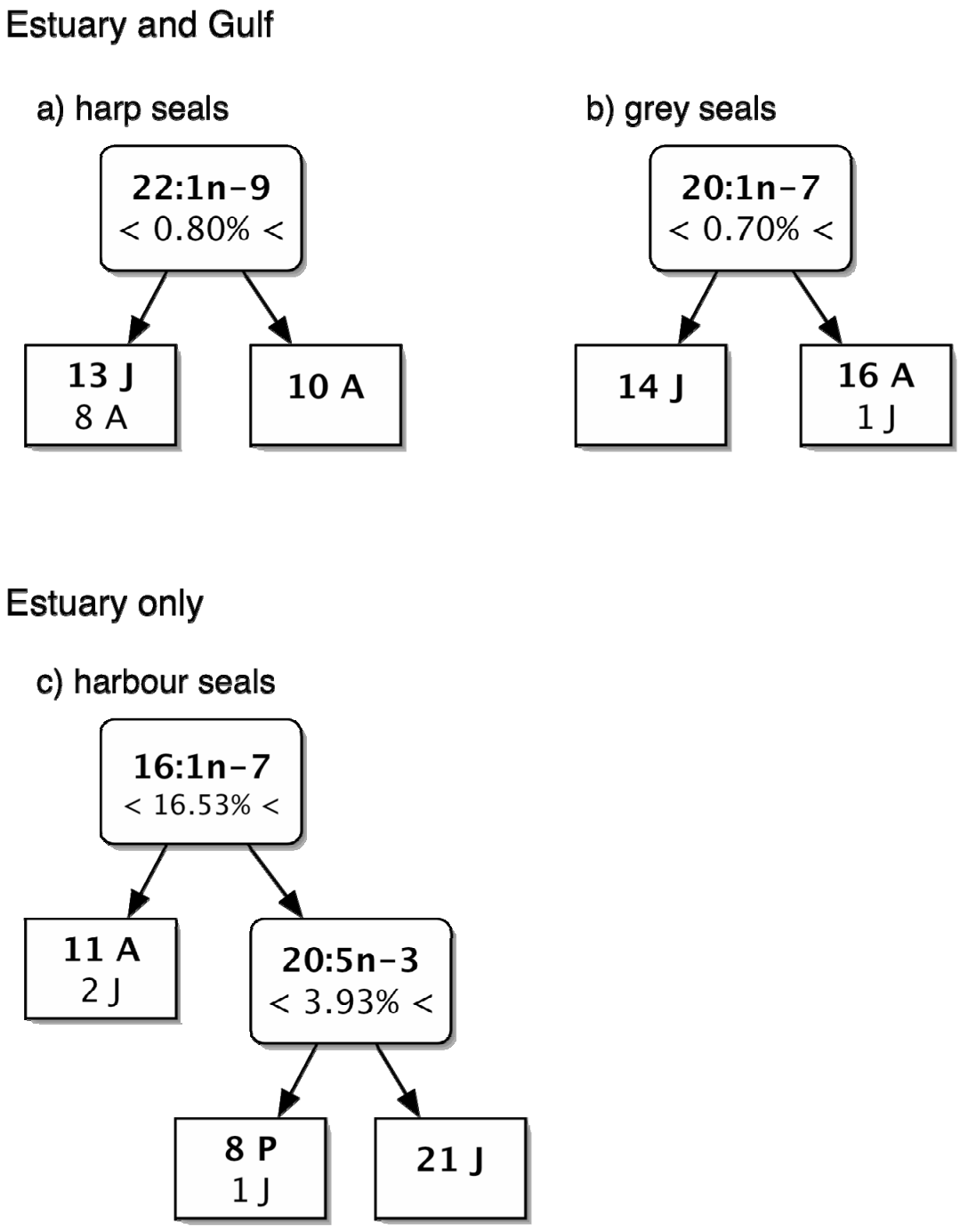


Figure 3.4. Classification trees by age-class (P = neonates; J = sub-adults; A = adults) for (a) harp seals, (b) grey seals, and (c) harbour seals, as determined by CART while using the set of 18 fatty acids.

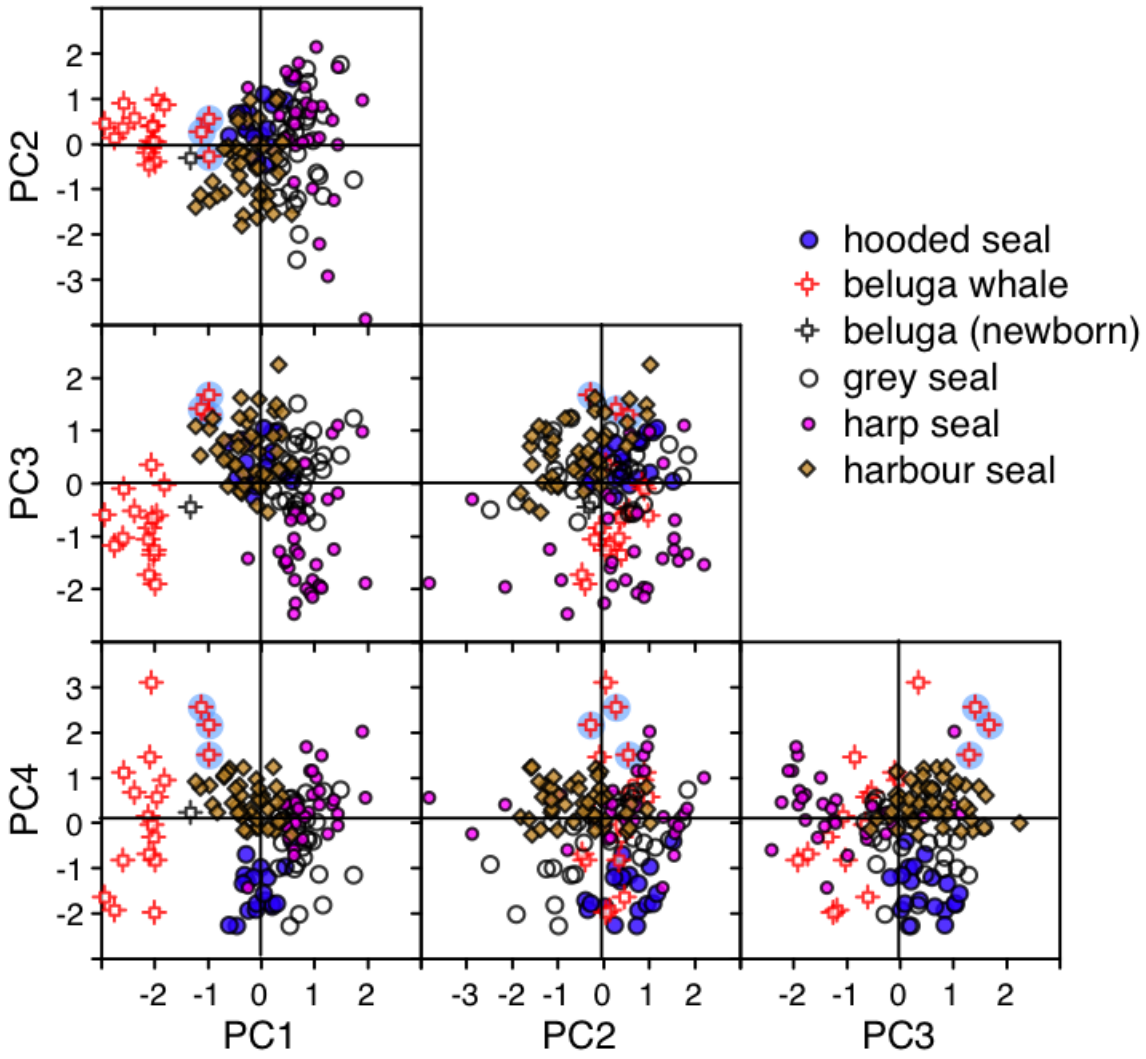
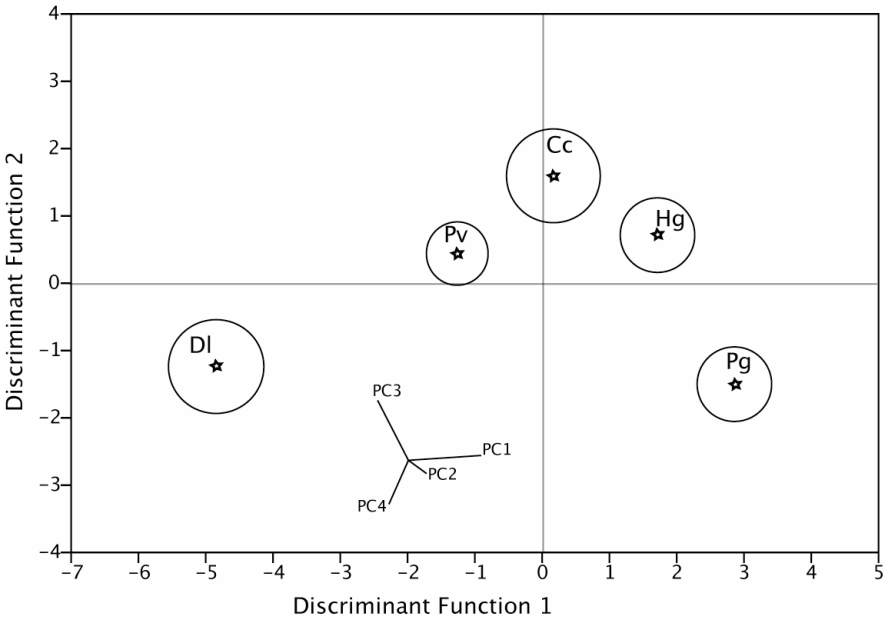


Figure 3.5. Varimax-rotated PC factor scores of the five marine mammal species on a matrix of of 4 PCs obtained using 17 fatty acid logratio variables, with species groups as marked. A separate label was added for a newborn beluga (black cross) and three beluga males (blue halos) that scored away from other belugas and towards the pinnipeds on PC 1.

a)



b)

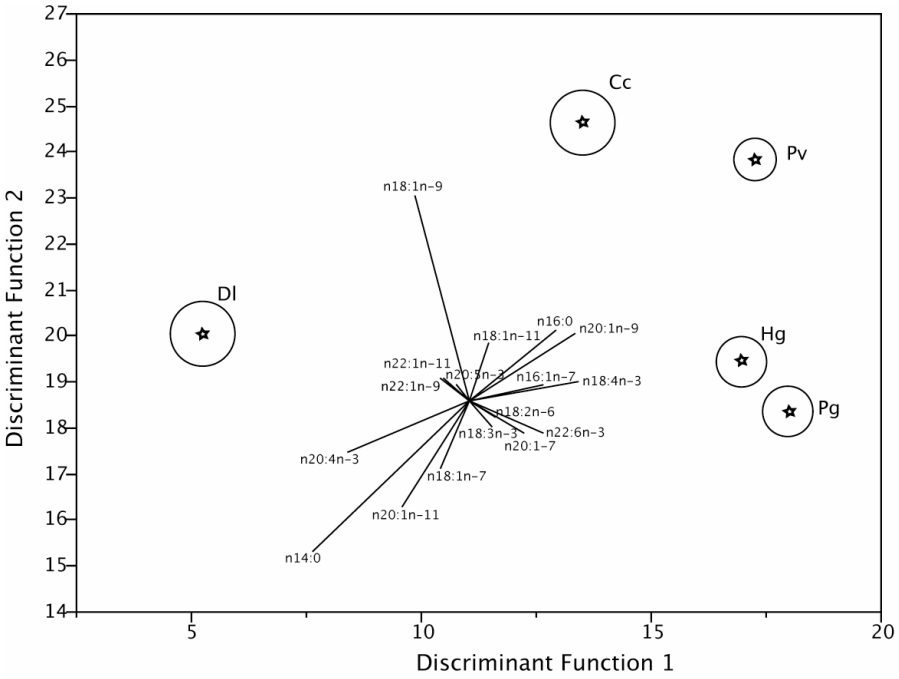


Figure 3.6. Discriminant functions analysis of the five species of marine mammals using (a) scores on the four factors retained from the principal components (PC) analysis, and (b) the 17 fatty acid logratios as input variables. Group centroids (star) and 95% confidence intervals are indicated for each species.

Variables (PCs or fatty acids) are projected (rescaled to 200% for visibility) according to their relative contributions on the two discriminant functions. The cross-validated classification success rate was 84% and 99% (120/143 and 142/143 individuals correctly classified) for (a) and (b), respectively.

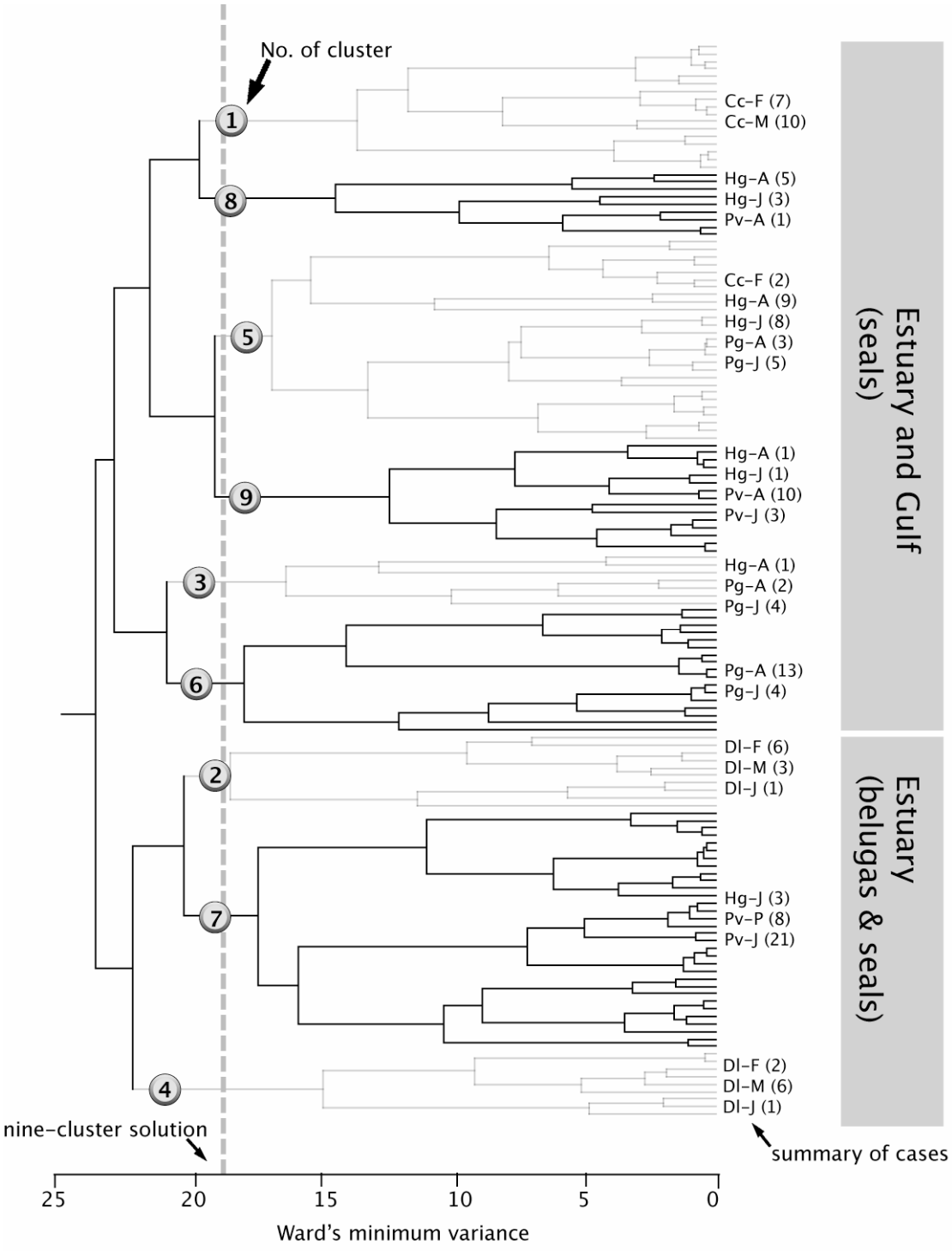


Figure 3.7. Dendrogram of hierarchical clustering with Ward's method using the four principal component factors as input variables. Horizontal scale denotes the increase in within-cluster's variance, rescaled from 0 to 25. Vertical dashed

line highlights the joining of cases into a nine-cluster solution. Individual members are summarised in each cluster by their species: hooded seals (Cc), beluga (Dl), grey seals (Hg), harp seals (Pg), and harbour seals (Pv). Hooded seals comprised only adult individuals. Belugas were all adults except for one individual each in clusters 2 and 4. Beluga and hooded seals are sub-labelled by gender: female (F), and male (M). Other pinnipeds are sub-labelled by age-class: adult (A), sub-adults (J), and neonates or pups (P). The highest aggregation into two clusters is suggested by the labelled boxes for a grouping of belugas and principally sub-adults seals from the Estuary relative to all other pinnipeds from the Estuary and the Gulf.

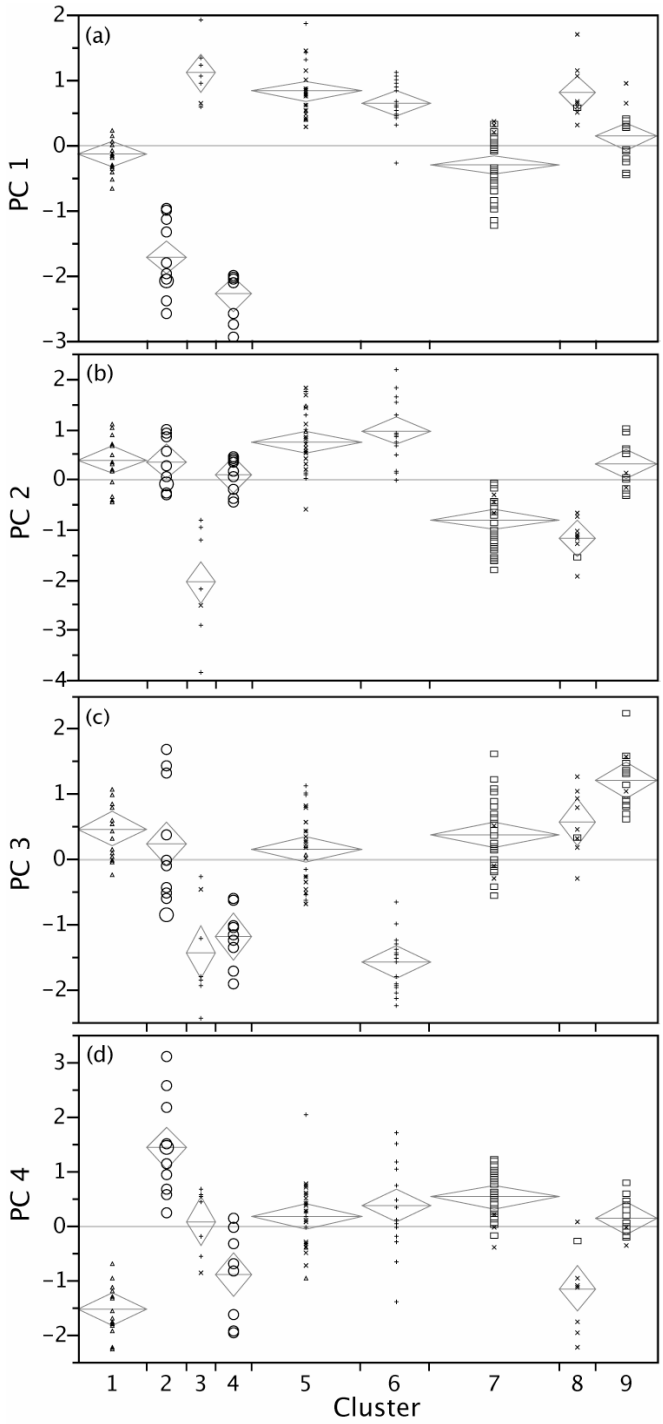
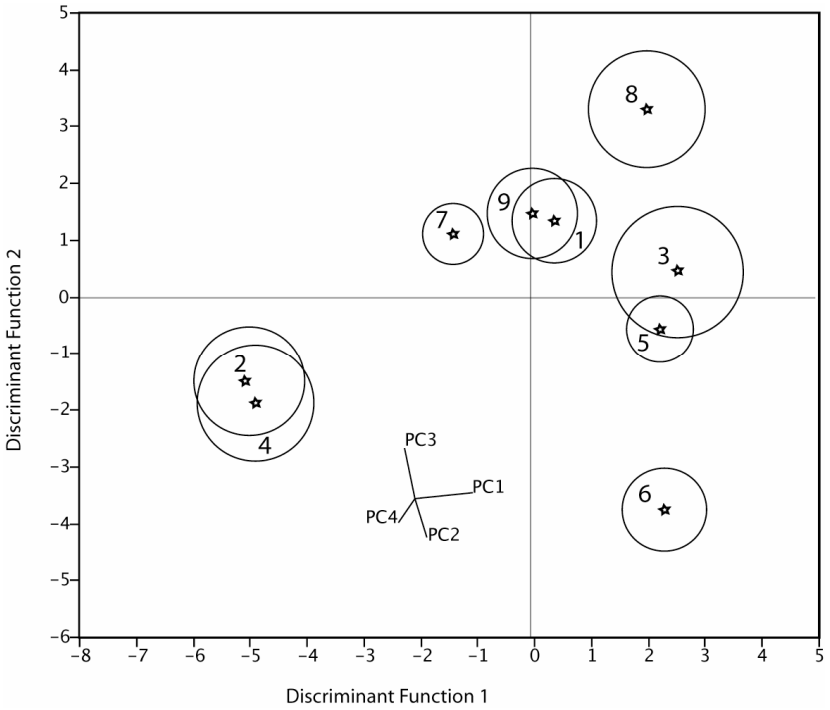


Figure 3.8. Mean diamond scatterplots of scores for all individuals by cluster number on PC factors 1 through 4 (a-d). Diamonds indicate cluster group means ± 95% confidence intervals (vertically) in proportion to group size (horizontally). Scores are marked by species: beluga (open circles), hooded seals (triangles), harbour seals (open boxes), grey seals (x), and harp seals (crosses).

a)



b)

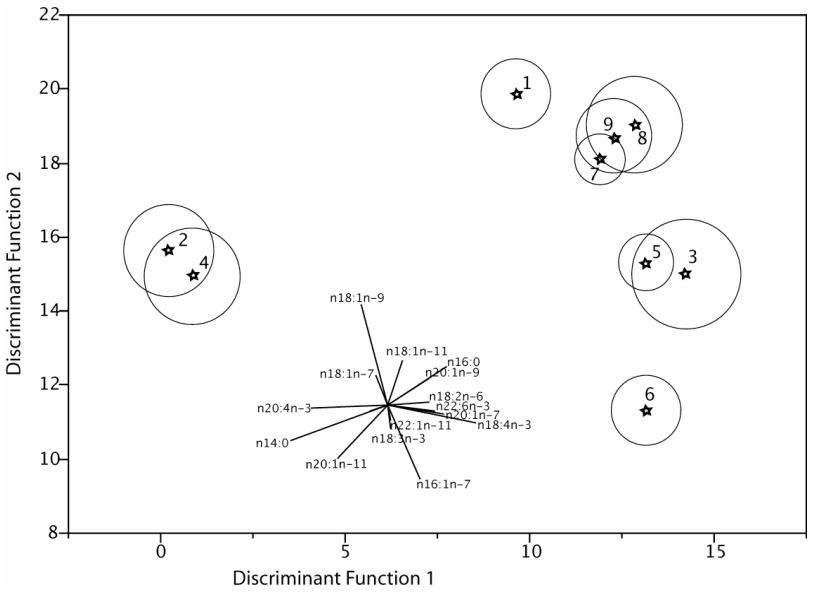


Figure 3.9. Discriminant function analysis of the classification results for the nine clusters using (a) the four principal components (PC) or (b) the set of 17 fatty acid logratios as input variables. Large circles denote the 95% confidence interval centroids of mean scores (stars) for clusters 1 through 9. Variables

are projected (rescaled to 200% for visibility) according to their relative contributions on the two discriminant functions. The overall classification success rate was (a) 97% (139/143 individuals correctly classified) and (b) 93% (133/143 correct assignments).

Chapitre 4

Conclusions générales

4.1. Sommaire des résultats

Comparaisons de proies potentielles

L'étude du régime alimentaire des espèces à l'aide des signatures d'acides gras est un objectif intéressant mais qui comporte plusieurs étapes dont les conclusions sont sensibles à l'interprétation. Une telle compréhension approfondie des profils d'acides gras de l'ensemble des proies potentielles des prédateurs bénéficiera de l'identification des signatures d'acides gras alimentaires des prédateurs. Dans une étape importante vers la construction de modèles de régime alimentaire, plus de 60 espèces de proies potentielles des mammifères marins (baleine blanche, ou béluga), ont été analysées pour leur teneur en lipides et leur composition en acides gras.

En plus d'identifier les profils d'acides gras caractéristiques des espèces, la variabilité intraspécifique a également été étudiée. Plusieurs espèces-clés, particulièrement les espèces pélagiques, ont démontré de fortes variations en termes de contenu et de qualité des acides gras selon la taille dans le cas du hareng et du poulamon, et selon la saison ou le lieu d'échantillonnage dans le cas de l'éperlan et du capelan. Pour ces espèces, un échantillonnage répété est donc indiqué afin de capter ces fortes variations qui se cachent dans les valeurs moyennes par espèce. D'autre part, plusieurs espèces de fond, telles que les crevettes et les poissons plats, démontraient une variabilité beaucoup moindre. Ainsi, un échantillonnage exhaustif chez ces espèces n'est pas nécessairement utile afin d'améliorer les données déjà compilées. Néanmoins, les résultats présentés ici restent provisoires compte tenu des difficultés d'échantillonnage et de l'absence d'expériences de manipulation alimentaire.

Comparaisons de mammifères marins

En se basant sur les profils d'acides gras, nous avons réussi une classification efficace des prédateurs en haut de la chaîne trophique (quatre espèces de pinnipèdes et une de cétacé). Ces prédateurs partagent au moins en partie leurs aires de distribution et d'alimentation dans les écosystèmes marins du Saint-Laurent. Certains acides gras d'origine alimentaire se sont révélés des facteurs importants pour faciliter la discrimination des espèces, et même des classes de taille, de sexe ou d'âge. Les différences d'alimentation des individus seraient donc un facteur important dans l'établissement de ces patrons. La comparaison d'un nombre plus élevé d'individus des différentes classes pourrait aider à établir l'importance relative de l'influence du régime alimentaire, de la génétique et de l'environnement dans les profils d'acides gras observés.

En plus de confirmer la pertinence de certains acides gras comme indicateurs de groupes trophiques de ces mammifères marins, cette étude a également mis en évidence certains avantages mais aussi des défis dans l'exploration des données. Ainsi, l'application judicieuse d'une série de traitements mathématiques (transformations en ratios logarithmiques des acides gras généralement les plus abondants) et de méthodes exploratoires des données (arbres de classification, analyses en composantes principales et de groupements, validées par des analyses discriminantes) a permis d'obtenir des résultats robustes. L'obtention de résultats semblables suite à l'application des diverses méthodes était encourageante, surtout considérant la diversité des résultats possibles lors de

l'exploration des signatures d'acides gras des mammifères marins, basée sur un si grand nombre de variables.

4.2. Approches futures

Il existe actuellement un grand intérêt vers l'utilisation des biomarqueurs pour apporter des informations sur la diète selon plusieurs échelles et scénarios, où l'information s'avère difficile à obtenir autrement, comme c'est le cas chez le béluga du Saint-Laurent. Avec cet intérêt, il demeure utile de dénombrer les voies qui aideront à augmenter l'information vers l'objectif ultime d'identifier plus définitivement les patrons alimentaires parmi les espèces de mammifères marins dans le temps et l'espace. En continuant le thème des travaux présentés ici et par d'autres recherches dans le domaine des lipides marins, on pourrait proposer trois axes de recherche promettant de répondre aux lacunes afin de nous acheminer vers cet objectif. En somme, ces axes sont 1) la continuation d'une approche à plusieurs facettes utilisant des biomarqueurs d'acides gras, 2) le développement de techniques analytiques complémentaires en traceurs trophiques et 3) la validation généralisée des modèles tels que QFASA. Aucune de ces approches ne guarantit l'obtention des solutions désirées, mais leur application soignée demeure notre meilleur espoir pour raffiner notre compréhension, et est en même temps probablement plus efficace que de suivre le mantra (ou la plainte), peut-être trop commun en écologie, de faire appel à des études avec 'encore plus d'échantillons'.

L'approche à plusieurs facettes

Cette approche est presque une philosophie : logique à recommander et à considérer importante même si ce n'est pas toujours évident de la pratiquer (Iverson *et al.* 2004,

Thiemann *et al.* 2004a). Pour paraphraser Iverson *et al.* (2004), on pourrait la résumer ici comme l'acquisition de l'information écologique par les outils vidéo ou le repérage par satellite (Bowen *et al.* 2002), l'emploi des techniques chimiques pour les analyses et les tissus appropriés (*e.g.*, Budge et Iverson 2003, Cooper *et al.* 2005) et l'analyse statistique robuste, pour balancer les explorations de données avec les confirmations. Ce chemin servira à mieux cibler les informations utiles à d'éventuels tests d'hypothèses. Les raisons pour lesquelles cette approche n'est pas plus répandue jusqu'à présent sont liées aux coûts et au temps nécessaire pour entreprendre une étude ayant plusieurs phases d'analyse, mais aussi à la difficulté qu'il y a à se familiariser avec les multiples domaines qui touchent un tel sujet 'écosystémique'. Pourtant, toutes les études ne pourraient pas être de grands survols (*i.e.*, Budge *et al.* 2002, Iverson *et al.* 2002), et il demeurera que des avancées seront possibles avec la continuation de petites études examinant des aspects spécifiques du métabolisme et de l'écologie lipidique, si elles sont menées dans l'esprit de garder en tête le contexte de l'organisme et son environnement.

Les traceurs complémentaires à venir

Comme c'était suggéré dans l'introduction générale, il y a une histoire et de l'intérêt à développer des méthodes qui se serviront de traceurs complémentaires (Volkman *et al.* 1998, Ackman 2002, Christie 2003). Il y a lieu de mettre au point de nouveaux éléments de retraçage, comme via les isotopes stables et les radioactifs moins connus. Une autre possibilité est de combiner des isotopes communs, tels que ceux du carbone, de l'azote et du soufre, avec l'intention de produire une 'signature' élémentale comme on a vu se faire avec les acides gras. Leur potentiel demeurera le produit des raffinements de la technologie analytique, lesquels permettront de souligner de nouvelles sources pour les différences de

rapports dans les éléments individuels, ou dans une suite, retrouvés dans un environnement précis ou dans un tissu spécifique d'organisme.

La validation des modèles : un leçon contemporaine

Après avoir écarté ci-dessus le potentiel de recherche de l'information reflétant la diète qui est promulgué par les holistes des systèmes d'une part, et les réductionnistes des éléments d'autre part, il nous reste finalement les simulistes et leurs modèles. L'identification et même la quantification du régime alimentaire par la modélisation des relations entre les profils d'acides gras des proies et des prédateurs comme ça se fait en QFASA est un objectif direct et ne fait pas partie des étapes 'préliminaires' qui sont issues des analyses lipidiques. En disant ceci, il reste à valider en général quelques aspects du comportement dans le processus de modélisation pour produire des résultats satisfaisants. Plus précisement, après avoir écarté des questions d'écologie, de chimie et de statistiques, il demeure nécessaire de compenser pour le sujet, c'est-à-dire l'espèce et la classe de prédateur (taxonomie, maturité, sexe, ou santé), qui pourrait avoir une influence dans la modélisation des profils en acides gras. Des exemples de catégories incluent vertébrés et invertébrés, carnivores terrestres et marins, cétacés et pinnipèdes, alimentés en captivité ou libres en nature.

Pour la validation des modèles incluant QFASA, on doit alors faire appel à deux démarches, soit la comparaison avec un indicateur externe et la compensation pour mieux refléter ce qui est connu avec cet indicateur. Comme dans le cas des isotopes stables, le produit des modèles de profils en acides gras reste provisoire en l'absence de confirmation des résultats en se référant à des paramètres connus comme ceux fournis par d'autres indicateurs bien établis (*e.g.*, traces de proies non-digérées), les observations d'alimentation

ou les manipulations de régime alimentaire en captivité. En ce faisant, on pourrait établir des facteurs de calibration, afin d'ajuster les profils des prédateurs pour compenser l'assimilation différentielle des acides gras provenant des proies consommées (Iverson *et al.* 2004, Thiemann *et al.* 2004a). La nécessité en même temps que la réussite de telles approches de modèle 'calibré' est bien mise en évidence dans le cadre des études présentement en cours chez les oiseaux et les pinnipèdes par les pionniers du laboratoire Iverson et leur modèle QFASA. Mais, comme dans le cas de l'établissement d'une banque de données de proies, il reste toujours à savoir à quel point la variabilité, ou plutôt la généralisation des facteurs de calibration produits avec certains individus pourrait être appliquée à d'autres espèces d'intérêt, notamment aux cetacés (Santos et Pierce 2003, Krahn *et al.* 2004, Samuel et Worthy 2004).

Jusqu'à présent, il commence à y avoir des indications qu'il y aura des exceptions, ou au moins des subtilités, aux généralités établies avec les phocidés juvéniles en captivité en comparaison avec les autres scénarios (Grahl-Nielsen *et al.* 2003, Andersen *et al.* 2004, Beck *et al.* 2005, Käkelä *et al.* 2005, Staniland et Pond 2005). Cela n'est pas tout à fait surprenant, vu la variété souvent inattendue des stratégies physiologiques de l'exploitation des lipides (Käkelä et Hyvärinen 1996, Pond 2000, Sprecher 2000, Hulbert *et al.* 2002, Tocher 2003). La variété peut être regardée comme un obstacle initialement décourageant, surtout pour les gestionnaires des projets de recherche, mais elle pourrait aussi présenter des opportunités qui ne sont pas encore imaginées par les chercheurs. C'est l'exploitaton des différences et 'défauts' qui propulse l'évolution, dont on est le produit, selon les idées prémonitoires de Darwin. Néanmoins, les remarques aux détails et aux exceptions peuvent rendre le discours sensible aux critiques au sujet du potentiel des acides gras comme

marqueurs trophiques en modélisation. Ce qui risque d'inutilement nuire au développement des modèles, du côté des cyniques par leurs échecs et manque de rigueur en réplication, autant que par les promoteurs présentant des résultats difficiles à simuler.

Un rappel de l'histoire et des débats autour de l'évolution pourrait nous servir comme un dernier point pour conclure. Aujourd'hui, en 2005, la théorie de l'évolution est toujours contestée dans l'éducation des élèves dans certains districts aux Etats-Unis. Ce qui pourrait nous faire penser encore aux mots d'un des grands supporteurs de l'évolution, Stephen Jay Gould, qui parmi plusieurs scientifiques s'intéressait aux phénomènes naturels autant qu'à notre capacité à accepter des idées utiles à la science. En effet, leurs essais littéraires nous aident à voir qu'il y a deux façons d'attaquer un problème quelconque : l'approche *a priori* et l'approche historique. L'approche 'en avant' est celle qui est mieux connue par les chercheurs, les ingénieurs et les gestionnaires des parcs naturels à la navette spatiale parce que c'est ce qu'on fait tous les jours : identifier un problème et proposer des causes et des réponses logiques et raisonables à tester selon l'évidence acquise. Dans cette étude, on pouvait observer des patrons en acides gras et proposer des liens possibles. Sauf qu'il est difficile de savoir ce qui est la solution unique, c'est-à-dire parcimonieuse. Mais, comme se lamente le généticien Dobzhansky, « tout se comprend mieux dans le contexte de l'évolution » ('*Nothing makes sense except in the light of evolution*'). Ainsi, l'histoire, tant dans l'évolution des espèces que dans les interactions écologiques des populations et les activités d'un individu, pouvait nous fournir des idées auxquelles des explications *a priori* sont correctes ou non, à cause du chemin pris par un organisme pour démontrer les patrons observés. Dans cette étude, l'histoire est proposée comme ultimement responsable pour les différences dans les patrons, mais on n'a à présent que des outils tels que QFASA, qui nous

offre ce qu'il voit analytiquement et propose les solutions probables. Quand la question à répondre est bien délimitée, ce serait suffisant, mais en écologie on peut poser bien des questions encore pour l'histoire à venir.

En août 2005, l'exploration de l'espace par les humains a fait les manchettes des nouvelles pour ses difficultés. On a cherché des causes pour les échecs des programmes extraterrestres et les gaffes d'aujourd'hui. Une exercise intéressant est de regarder le cas du fameux réservoir externe de la navette spatiale. Ostensiblement, ses dimensions et propriétés sont bien conçues pour répondre aux besoins des propulseurs de la navette. Mais aussi il réflète l'histoire des matériaux et des transports en communs, comme le fait d'être transportable par chemin de fer via des tunnels dont les dimensions étaient définies par les normes européennes, avec leur origine ultime dans la taille des chemins construits par les romains pour leurs chariots de guerre. Si vous proposez que le réservoir est conçu pour la navette, vous avez raison autant que si vous proposez d'établir des liens entre diète et la détection d'acides gras chez les proies et les prédateurs. Et si vous savez qu'en partie le design réflète une contrainte historique qui suit la logique pas-à-pas, mais qui ultimement n'a pas de sens et était peu probable à prédire, vous avez gagné un peu d'information potentiellement utile. Je pense que Darwin et Gould auraient dû être bien curieux de savoir quelles autres histoires on raconterait autour des lipides dans une domaine d'étude qui sort de l'ennui d'être un simple tissu d'isolant et d'énergie, pour sortir avec les autres molécules renommées, telles que l'ADN et les protéines, au bal des finissants dans les années à venir (Pond 2000).

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ANNEXES

Annexe I.

Examination of extra fish specimens for digestive tract contents.

Species	Region[1]	Month	TL[2] (mm)	Wt.[3] (g)	Digestive tract contents
Am. shad	UE	Aug.	111	11	insect larva, insect eyes
Am. shad	UE	Aug.	117	10.5	insect larva, insect eyes
Am. shad	UE	Aug.	112	11.5	empty
Am. shad	UE	Aug.	110	9	empty
Sand lance	NG	April	100	N/A	copepods
Sand lance	NG	April	140	N/A	copepods
Barracudina	LE	May	242	25	1 whole krill, 6 pairs eyes (*T.r.*)[4]
Barracudina	LE	May	250	25.5	10 krill, 5 pairs eyes, (*T.r.*)
Barracudina	LE	May	242	26.5	14 whole krill (*T.r.*)
Barracudina	LE	May	256	28.5	8 krill
Barracudina	LE	May	256	27.5	15 krill (*T.r.*), 1 krill (*M.n.*)[5]
Barracudina	LE	July	254	23.6	1 krill (*M.n.*)
Barracudina	LE	July	280	29	11 krill (*M.n.*)
Barracudina	LE	July	245	26.3	7 krill (*M.n.*)
Arctic cod	NG	July	110	6	large copepods (*Euchaeta*)
Arctic cod	NG	July	102	5.5	a few large copepods (*Euchaeta*)
Arctic cod	NG	July	160	24	1 *Pandalus* shrimp
Snailfish	LE	May	185	29.8	krill (full)
Snailfish	NG	Aug.	156	66.7	amphipods (not themisto)
Poacher	LE	July	205	N/A	1 krill, many copepods
Poacher	LE	July	175	N/A	1 isopod, many copepods
Eelpout	NG	Aug.	425	458	1 brittle starfish
Eelpout	NG	Aug.	246	N/A	crab legs
Tomcod	UE	May	146	22.7	copepods
Tomcod	UE	May	146	24	copepods
Tomcod	UE	May	134	18.5	copepods
Tomcod	UE	May	140	21.5	green detritus
Tomcod	UE	May	136	21.5	green detritus, nereids
Tomcod	UE	May	142	22.5	copepods, nereids
Tomcod	UE	May	222	66.5	green detritus
Tomcod	UE	May	210	74.5	nereids, copepods
Tomcod	UE	May	187	46	1 crangon
Tomcod	UE	May	189	42.5	1 gammarid, 1 nematode, copepods
Tomcod	UE	May	204	68.5	detritus, 1 small gammarid

[1] UE = Upper Estuary, LE = Lower Estuary, NG = Northern Gulf

[2] TL = Total length of fish specimen

[3] Wt. = Wet weight of fish specimen

[4] *T.r.* = *Thysanoessa raschii* (krill)

[5] *M.n.*= *Meganyctiphanes norvegica* (northern krill)

Species	Region[1]	Month	TL[2] (mm)	Wt.[3] (g)	Digestive tract contents
Tomcod	UE	May	215	79	1 capelan (gravid)
Tomcod	UE	May	181	40	1 crangon
Tomcod	UE	May	203	84	2 crangons, 1 gammarid, 1 nereid
Tomcod	UE	May	180	46.5	2 crangons
Tomcod	UE	May	182	42	1 crangon, 1 large amphipod
Tomcod	UE	May	177	36.5	40 small amphipods, 1 small nereid
Tomcod	UE	May	210	78	1 capelan, 3 mysids, 1 crangon
Tomcod	UE	May	187	57	1 capelin
Tomcod	UE	May	187	54.5	5 copepods, nereids
Tomcod	UE	May	192	61.5	1 capelin
Tomcod	UE	May	186	55.5	20 copepods, 1 amphipod
Tomcod	UE	May	181	46	40 copepods, 1 nereid, 1 crangon
Tomcod	UE	Aug.	182	45	1 gammarid
Tomcod	UE	Aug.	N/A	N/A	2 gammarids, nereid pincers, caddisfly
Tomcod	UE	Aug.	N/A	N/A	1 large gammarid
Tomcod	UE	Aug.	N/A	N/A	1 large nereid
Tomcod	UE	Aug.	N/A	N/A	4 gammarids, 1 small nereid, 1 slug
Tomcod	UE	Aug.	167	25.5	1 gammarid
Tomcod	UE	Aug.	151	25	green detritus, large nereid
Tomcod	UE	Aug.	151	24.5	1 large, small gammarids; 1 small nereid
Tomcod	UE	Aug.	160	30	1 nereid, few small gammarids
Tomcod	UE	Aug.	156	23.5	1 small gammarid
Smelt	UE	May	156	24.5	empty
Smelt	UE	May	140	17.8	copepods
Smelt	UE	May	136	14.5	copepods
Smelt	UE	May	145	8	copepods
Smelt	UE	Aug	148	14.7	empty
Smelt	UE	Aug	145	18.5	1 crangon, few copepods
Smelt	UE	Aug	134	13.5	empty
Smelt	UE	Aug	126	12	empty
Smelt	UE	Aug	141	14.5	1 crustacean, 1 krill (*M.n.*)
Smelt	UE	Aug	136	13.5	1 large, few small gammarids
Smelt	UE	Aug	131	14.5	empty
Smelt	UE	Aug	145	19	gammarid, nereid, themisto, insect
Smelt	UE	Aug	125	12.5	empty
Smelt	UE	Sept.	190	36	empty, yellow eggs
Smelt	UE	Sept.	179	33.5	empty, yellow eggs
Smelt	UE	Sept.	194	46	empty
Smelt	UE	Sept.	144	19	small gammarids & copepods, eggs
Smelt	LE	Oct.	147	23.5	empty, yellow eggs
Smelt	LE	Oct.	175	32	many small gammarids
Smelt	LE	Oct.	165	28.5	many small gammarids
Smelt	LE	Oct.	166	36	small gammarids
Smelt	LE	Oct.	108	7	empty

Species	Region[1]	Month	TL[2] (mm)	Wt.[3] (g)	Digestive tract contents
Smelt	LE	Oct.	121	11.5	few small gammarids, yellow eggs
Smelt	LE	Oct.	123	9	1 gammarid, 1 fly
Smelt	LE	Oct.	119	13.5	1 large, some small gammarids
Smelt	LE	Oct.	118	9.5	4 gammarids, yellow eggs
Smelt	LE	Oct.	105	7	empty

Annexe II

List of available specimens for species and spatiotemporal comparisons.

Sampling of species groups by region, year, and season (1 = winter, 2 = spring, 3 = summer, 4 = autumn). L = larger specimens (> 25 cm).

Subregion	Upper Estuary						Lower Estuary									Northern Gulf				
Year	2000			2001			1999	2000				2001			2002	2001			2002	
Season	2	3	4	2	3	4	2	1	2	3	4	2	3	4	2	2	3	4	2	3
Arctic cod												4								
argid shrimp										12										
Atlantic cod												5	9							
Atlantic poacher										8	4									
barracudina													12		6					
bobtail squid																7				
capelin		11			23		7		12			30	6			6				
clamworm					6		6													
copepods												5	7							
crangon shrimp			11	6	6	6														
daubed shanny													6							
eel			14																	
eelpout										1	7									
eualid shrimp												6	3	6	3					
gammarids																			4	
Greenland halibut										9										
Greenland halibut L		4								6	1	6								
haddock												2								
hagfish													12							
herring		12			12	12	24					12					12			
herring-sardine		6			12		12								12					
hyperiid													3	5	3				5	
krill														5						
lampreys						1	1							1						
longfin hake													6							
lumpfish												9					3			
marlin-spike													6							
monkfish																	4			
moustache sculpin										9										
northern krill								12				6	6		3	6				
northern shrimp										6	6									
ocean pout			1																	

Subregion	Upper Estuary						Lower Estuary									Northern Gulf				
Year	2000			2001			1999	2000				2001			2002	2001			2002	
Season	2	3	4	2	3	4	2	1	2	3	4	2	3	4	2	2	3	4	2	3
cctopus																			5	
plaice		5								9	10	4				2	8	2		
pollock						3						1					2			
smelt	12	12	9	24		36	6						16	12						
redfish												12								
rockling											4		3							
salmon												3								
salmon-smolt												12								
sand lance		12						12				6				6			6	
scampi shrimp																6				
shad	1																			
shad-sardine		12																		
shorthorn sculpin										6		1	1							
silver hake												3	3							
snailfish				6						1	5		6							
snow crab															6					
shortfin squid																				6
staghorn sculpin										1						6				
striped shrimp												6	3	6	3					
thorny skate		5		6						1										
toad crab										1										
tomcod				9	12	12														
tomcod L				6																
waved whelk				6								6				12				
whelk (*Colus* sp.)											2									
white hake												3	4							
whitefish				2	2	1														
winter flounder		4	11	12	12	1					1		2							
witch flounder		5																		
wolffishes																	9			
yellowtail flounder										1										

Annexe III

Extra figures from Chapter 2 (Prey base)

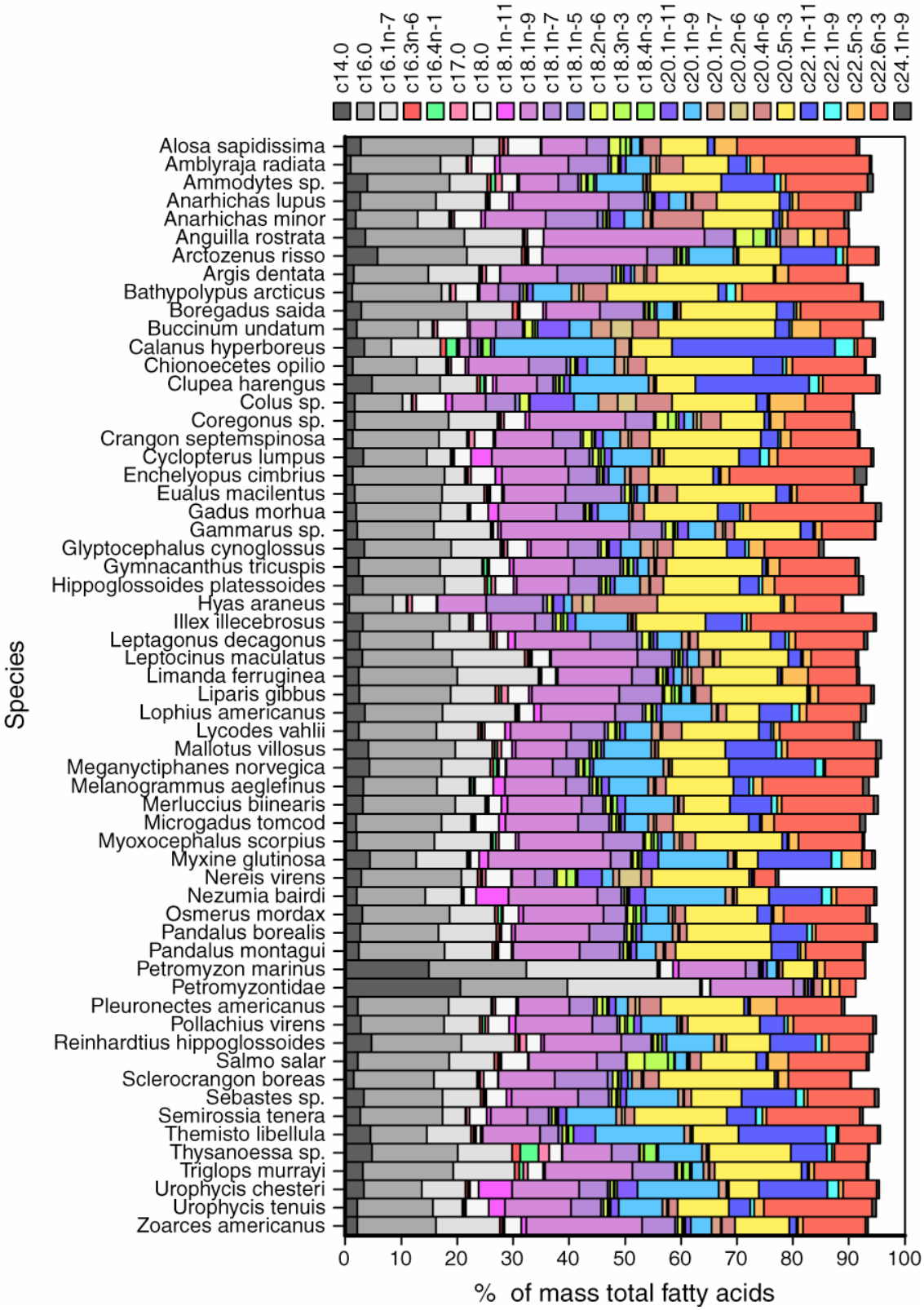

Figure III a). Cumulative mean abundance for 25 fatty acids as ranked by species.

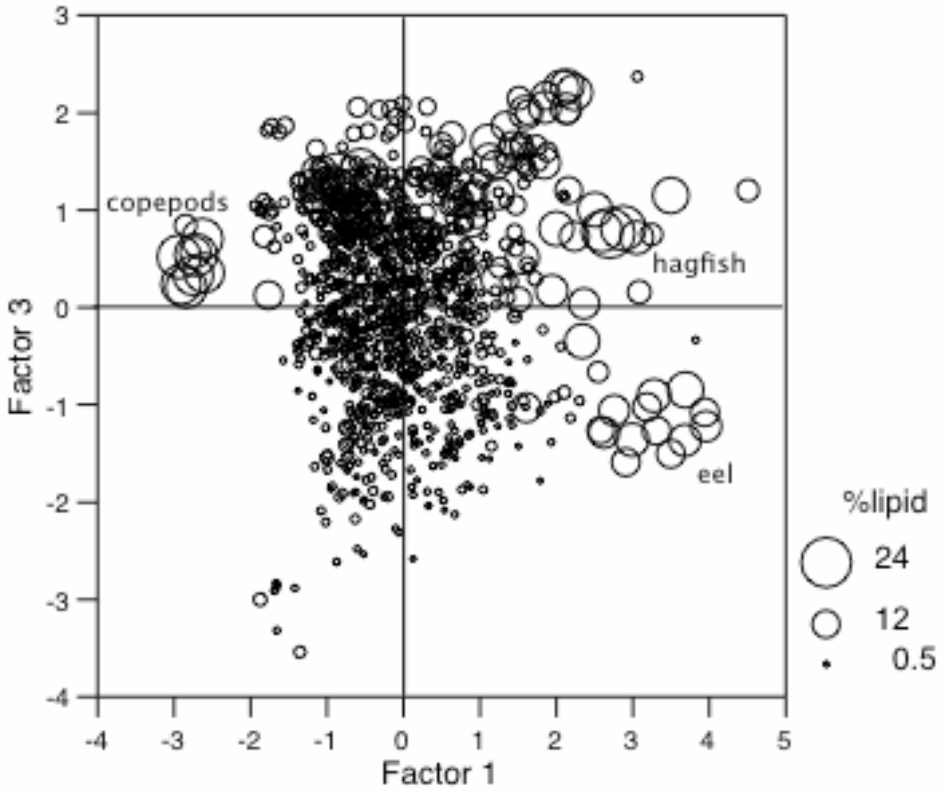

Figure III b). Scatterplot of all scores on PC Factors 1 and 3 when weighted by total lipid content. Labels indicate examples of three distinctive groups (scoring apart from majority) that also had high lipid content.

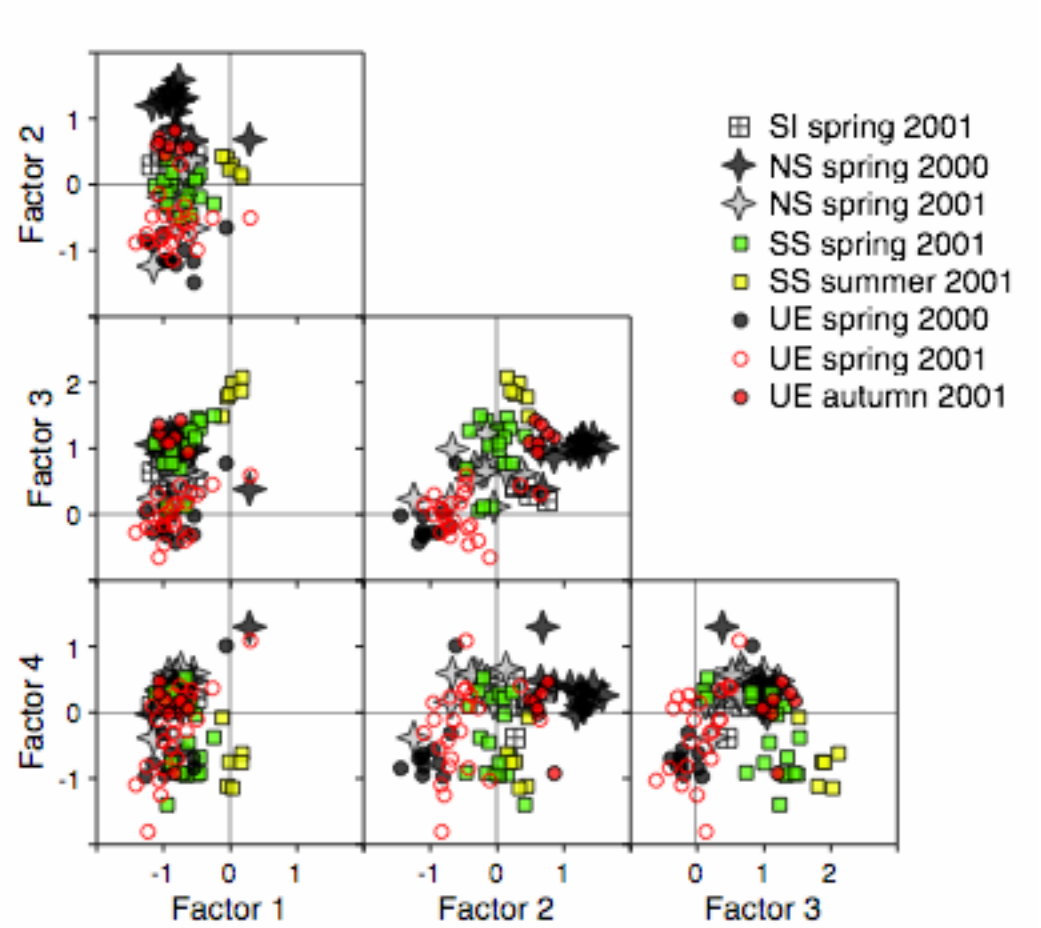

Figure III c) Full matrix for PC 1 through 4, showing individual scores of capelin only. Subregions were for SI = Sept-Iles, NS = North Shore and SS = South Shore of Lower Estuary, respectively, and UE = Upper Estuary. Seasons were spring (April-June), summer (July-August), and autumn (Sept.-Nov.).

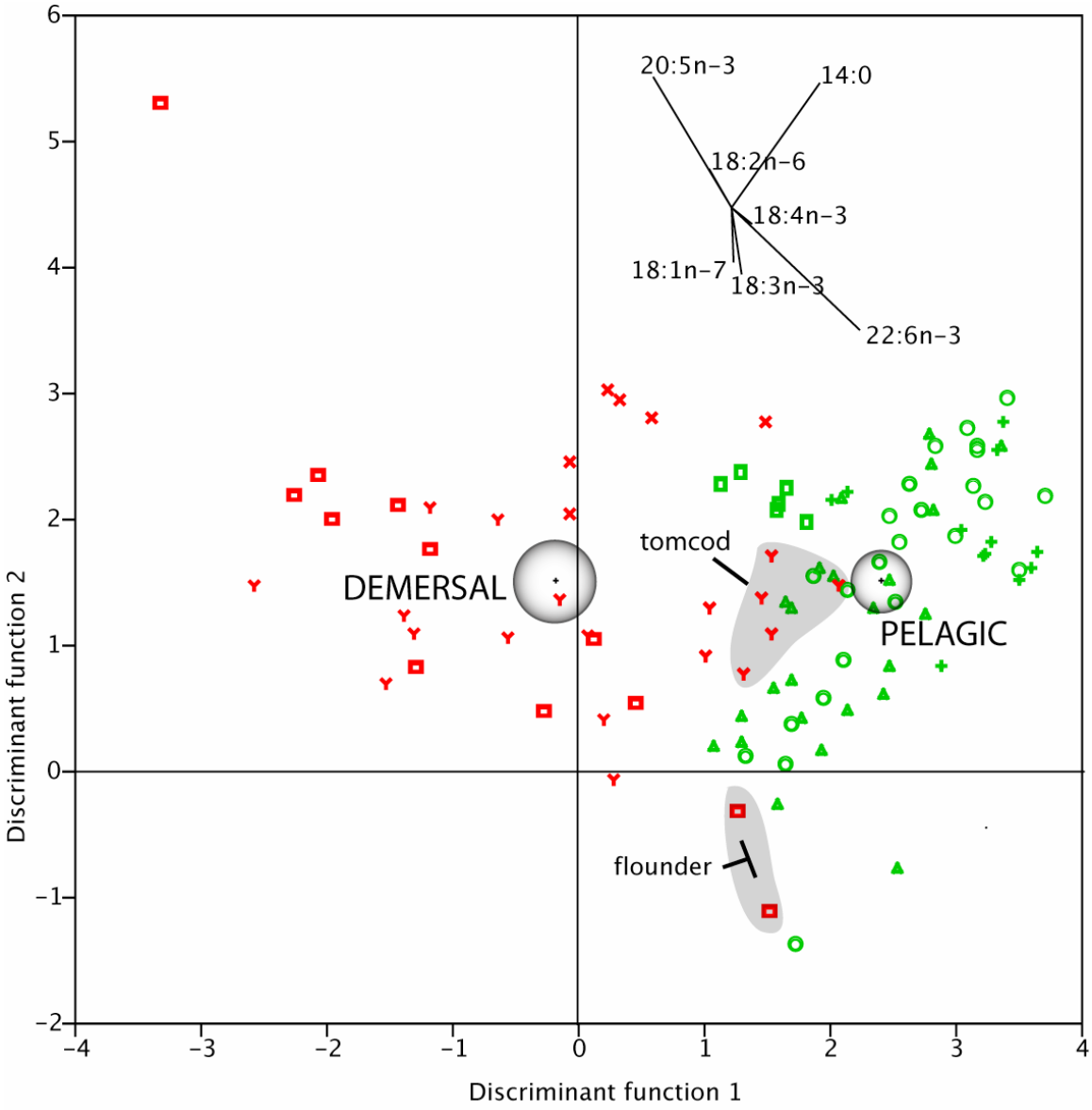

Figure III d) Biplot of first two Discriminant Functions classifying specimens of common species from the spring of 2001 into two groups: PELAGIC (smelt, capelin, herring, and turbot) with green markers, and DEMERSAL (tomcod, flounder, and crangon shrimp) with red markers. Circles indicate 95% confidence intervals for group means. Shading indicates notable misclassified specimens (tomcod = Y and flounder = open squares).

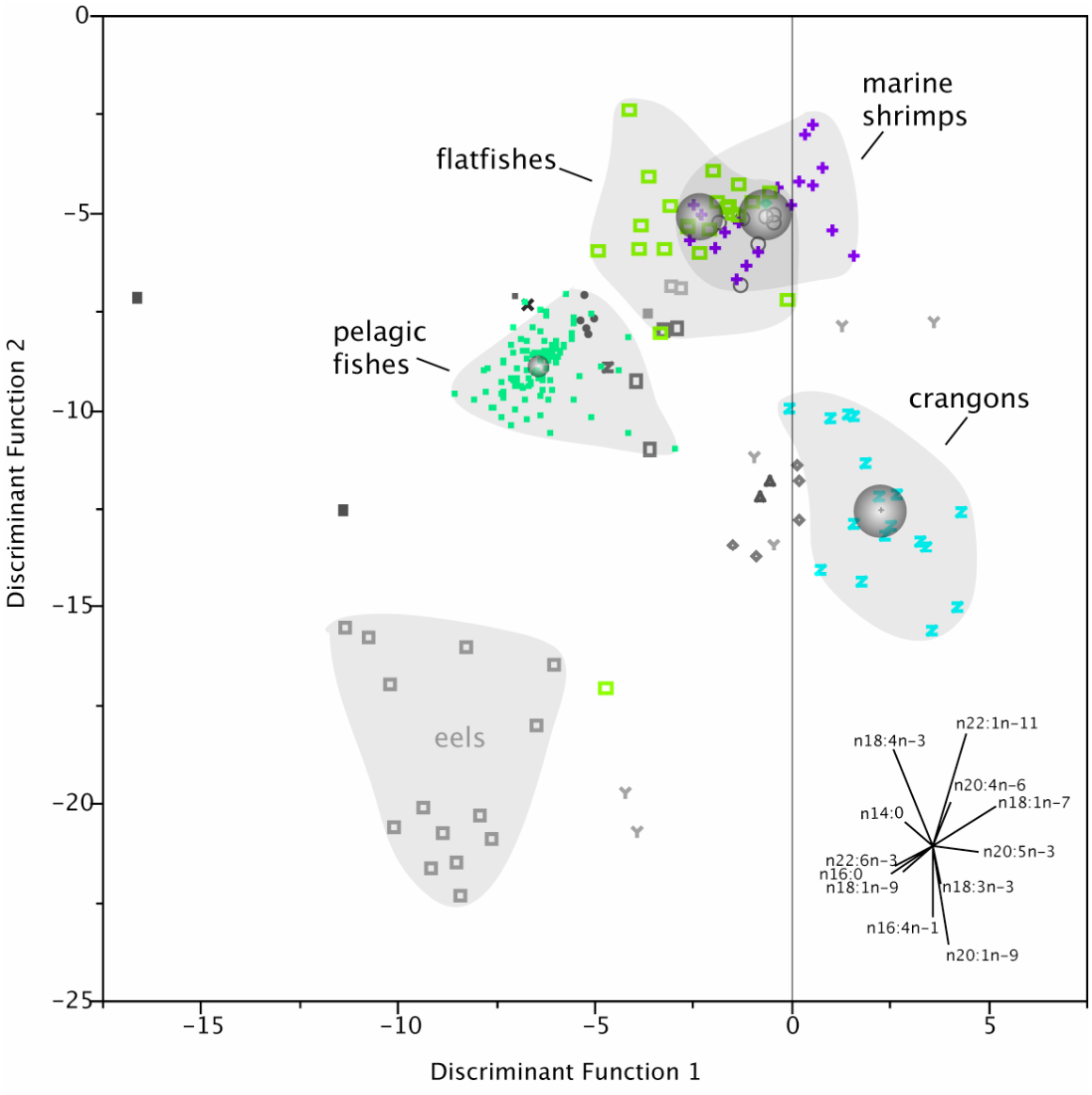

Figure III e) Biplot of the first two discriminant functions classifying autumn samples (2000-2001) into four composite species groups when using the set of 16 fatty acid variables. The groupings were for flatfishes (flounder and plaice: open squares), marine shrimps (eualids and pandalids: +), pelagic fishes (smelt, herring, and capelin: small squares) and crangons (estuarine sand shrimp: z). Shading indicates the spread of samples from the group mean scores in the darker circles (= 95% confidence intervals). Also plotted were

those species sampled in lesser abundance and not used to calculate the functions (greyed-out symbols). 12 of the 16 logratio fatty acid variables used as input variables are labelled with their relative projected contributions.

Annexe IV

Alternate presentations of figures in Chapter 3

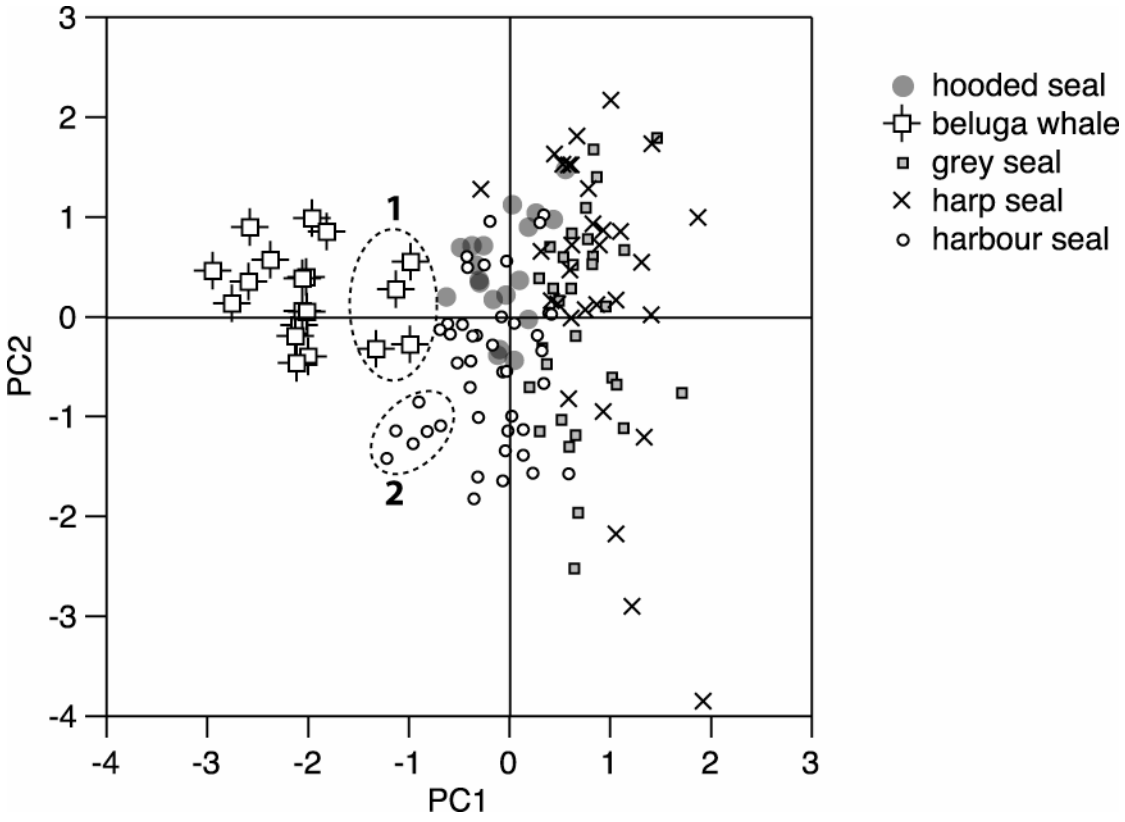

Figure IV a) Alternate view of Fig. 3.5a, with outlines for three males and one newborn beluga (1), and harbour seal neonates (2).

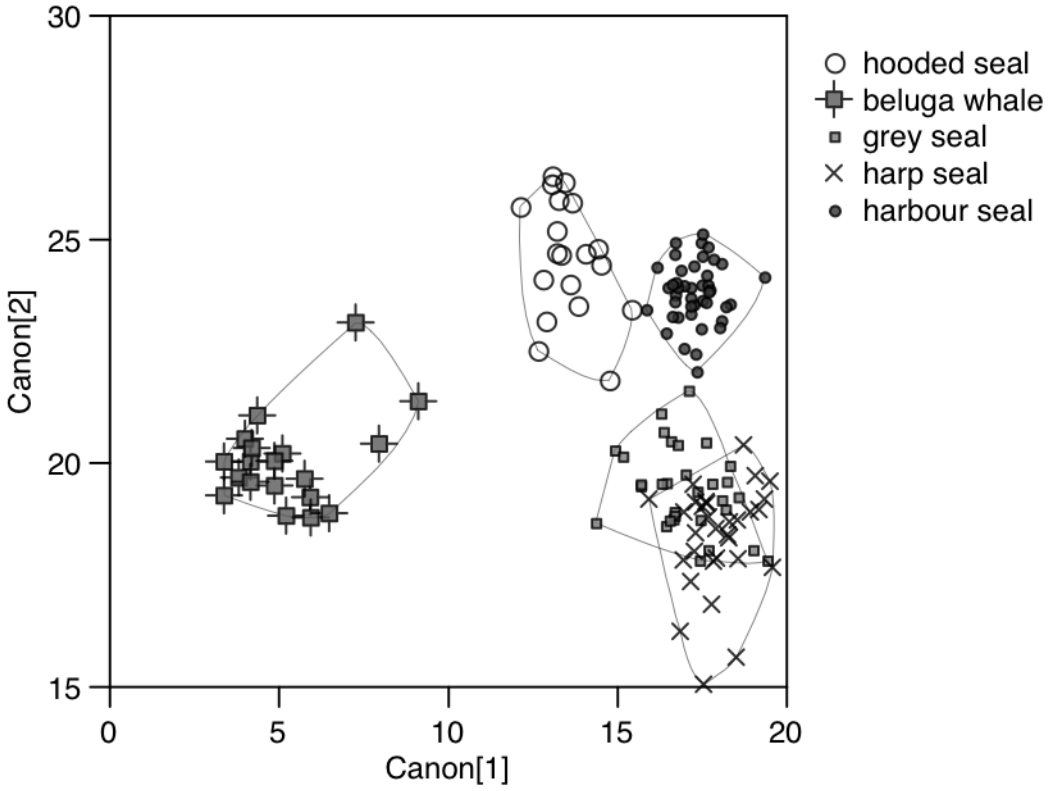

Figure IV b) Alternate view of Fig. 3.6b, for a "traditional' presentation as seen in most publications. All individual scores are plotted on the first 2 DFs (canonical variates), without projections of fatty acid variables.

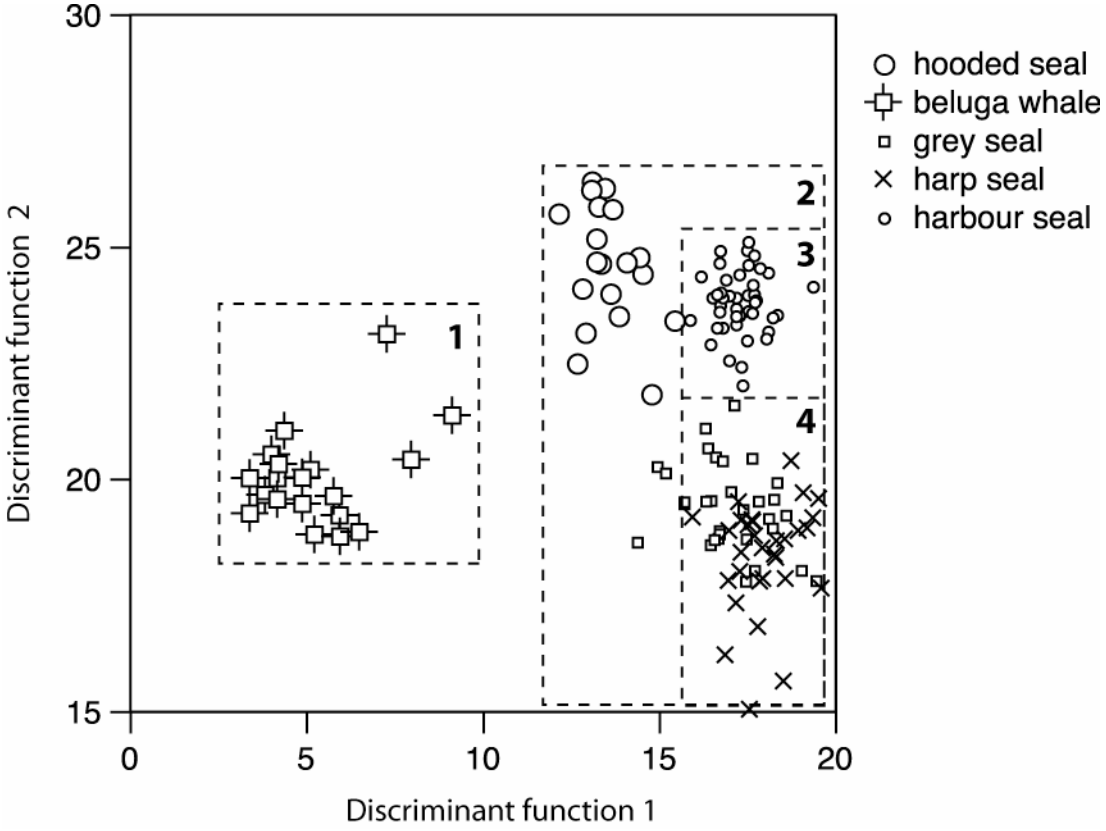

Figure IV c) Second alternate view of Fig. 3.6b, with outlined boxes to suggest nested 'metapatterns' of groupings discriminating belugas (1) vs. seals (2), then hooded vs. other seals (3) along the first DF, then harbour seals vs. greys and harps along the second DF (4).

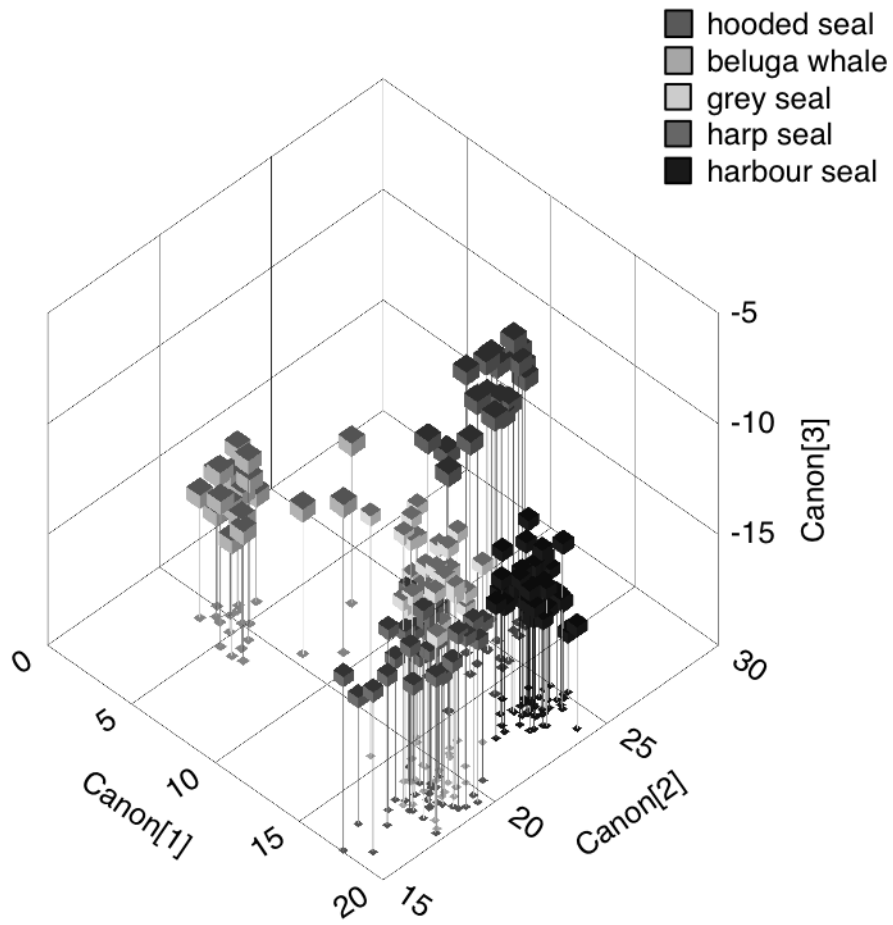

Figure IV d) Third alternate view of Fig. 3.6b when using three DFs, or canonical variates. The third DF (Canon[3]) enhanced discrimination of hooded seals and grey seals, and to a lesser extent, harp seals or harbour seals. Belugas and hooded seals are represented here by the larger cubes relative to the other species.