UNIVERSITÉ DU QUÉBEC À RIMOUSKI

UTILISATION DES ISOTOPES STABLES DANS L'ÉVALUATION DE L'ORIGINE DES POPULATIONS ZOOPLANCTONIQUES D'UNE AIRE MARINE PROTÉGÉE

LE CAS DES *CALANUS* SPP. DANS LE PARC MARIN DU SAGUENAY-SAINT-LAURENT, QUÉBEC, CANADA

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PAR

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AVANT-PROPOS

Le sujet de cette étude découle de l'un des buts du projet Canadian Healthy Oceans Network (CHONe). L'idée principale de ce partenariat entre les scientifiques des universités et du gouvernement est l'approfondissement des connaissances et la compréhension de l'environnement océanique et de sa santé globale dans un but de protection et de gestion. Pour ce faire, trois principaux thèmes de recherche ont été définis : caractérisation de la biodiversité marine, fonction et santé des écosystèmes et connectivité des populations. C'est sous ce troisième aspect qu'est utilisé le Parc Marin du Saguenay-St-Laurent comme modèle d'étude d'une grande zone protégée où la dispersion planctonique et la dynamique source-puits demandent encore à être approfondis pour soutenir les efforts de conservation.

RÉSUMÉ

Le Parc Marin du Saguenay-Saint-Laurent (PMSSL) est une région riche en biodiversité et comporte une population zooplanctonique abondante, formant ainsi une biomasse importante de proies pour les poissons, les oiseaux marins et les baleines. Le but de cette étude était d'évaluer la connectivité et l'origine des populations de zooplancton du PMSSL en utilisant les isotopes stables comme marqueur d'origine de *Calanus finmarchicus* et *C. hyperboreus*, deux espèces dominantes et ubiquitaires dans les systèmes du Saguenay et du St-Laurent. L'hypothèse de départ était que considérant la variabilité des conditions environnementales de l'aire d'étude (fjord du Saguenay, estuaire du St-Laurent et nord-ouest du golfe du St-Laurent), la signature isotopique de carbone ($\delta^{13}C$) des *Calanus* spp. en début de diapause dans les eaux profondes serait suffisamment différente entre les sous-régions pour discriminer l'origine des *Calanus* spp. dans le PMSSL au printemps suivant, après leur transport au cours de l'hiver. Pour évaluer la stabilité de la signature isotopique ($\delta^{13}C$ et $\delta^{15}N$) des *Calanus* spp. en diapause, une expérimentation en conditions contrôlées a été effectuée durant 4 mois sur des copépodites pré-adultes (CV) échantillonnés le 24 septembre 2009 en profondeur (100-320 m) au large de Rimouski. Le $\delta^{13}C$ des CV non-alimentés et dont les lipides ont été extraits s'est révélé être une variable conservative (stable) alors que le $\delta^{15}N$ variait dans le temps. Pour chaque espèce, une analyse discriminante quadratique utilisant les variables conservatives ($\delta^{13}C$ et pourcentage de carbone (%C)) fut effectuée pour discriminer les régions d'origine potentielles des *Calanus* spp. CV en diapause échantillonnés à la fin juillet 2009, au début de leur période d'hivernage. Par la suite, les CV de chaque espèce échantillonnés dans le PMSSL en profondeur (>125 m) avant leur sortie de diapause (11 mai 2010) ont été assignés à leur région d'origine la plus probable. Pour les deux espèces, les résultats suggèrent que les populations du PMSSL à la fin du printemps 2010 provenaient à la fois du Saguenay (source locale : 23%) et des régions de l'estuaire maritime et du nord-ouest du golfe du St-Laurent démontrant ainsi une grande connectivité dans le système mais aussi un potentiel pour une production (recrutement) locale de *Calanus* spp.. Cette étude a démontré l'efficacité du $\delta^{13}C$ comme marqueur pour retracer l'origine d'organismes en phase de diapause sur une période relativement longue.

Mots clés : Isotopes stables, *Calanus* spp., Parc Marin du Saguenay-Saint-Laurent, connectivité, origine, diapause.

ABSTRACT

The Saguenay-St. Lawrence Marine Park (SSLMP) is a region sustaining high biodiversity and abundance of zooplanktonic organisms which are important prey for fish, marine birds and whales. The aim of this study was to assess the connectivity and the origin of zooplankton populations in the SSLMP using stable isotopes as a marker of origin of *Calanus finmarchicus* and *C. hyperboreus*, two ubiquitous and dominant species in the Saguenay and the St. Lawrence systems. We hypothesized that the carbon stable isotope signature ($\delta^{13}C$) of *Calanus* spp. would be different among sub-regions of the study area (Saguenay fjord, St. Lawrence estuary and Northwest Gulf of St. Lawrence) in response to variable environmental conditions, meaning that region-specific $\delta^{13}C$ could be used to discriminate among different sources/origins of deep-dwelling diapausing *Calanus* spp. in the SSLMP the next spring after their transport during overwintering. To evaluate the stability of the isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) of *Calanus* spp. in diapause, a laboratory experiment under controlled conditions was conducted over 4 months on diapausing late copepodite stages (CV) sampled on 24 September 2009 in deep waters (100-320 m) next to Rimouski. The $\delta^{13}C$ of lipid extracted non-feeding CV have been identified as a conservative (stable) variable while the $\delta^{15}N$ was variable with time. For each species, quadratic discriminant function analyses using conservative variables ($\delta^{13}C$ and carbon percentage (%C)) of lipid extracted CVs were performed to discriminate different potential regions of origin of diapausing *Calanus* spp. CV sampled in late July, at the beginning of their overwintering period. Then, diapausing CV of both species sampled at depth (>125 m) in the SSLMP at the end of their overwintering period (11 May 2010) were assigned to their most probable region of origin. For both species, results suggested that the *Calanus* spp. population in the SSLMP in late spring 2010 originated from the Saguenay Fjord (local source: 23%) and from regions outside the SSLMP, the lower St. Lawrence estuary and the Northwest Gulf of St. Lawrence, showing high connectivity among the system but also revealing the potential for local production/recruitment of *Calanus* spp. in the Saguenay Fjord. This study showed the effectiveness of using $\delta^{13}C$ as a marker of origin of zooplankton in diapause on a relatively long period.

Keywords: Stable isotopes, *Calanus* spp., Saguenay-St. Lawrence Marine Park, connectivity, origin, diapause.

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INTRODUCTION GÉNÉRALE

Le Parc Marin du Saguenay-Saint-Laurent

Les aires marines protégées sont des zones d'importance biologique généralement déterminées pour assurer la viabilité d'espèces, de populations ou d'écosystèmes marins dont le statut est soit précaire, d'importance écologique, économique et/ou culturel. Créé en 1998, le Parc Marin du Saguenay-Saint-Laurent (PMSSL) est encore aujourd'hui le seul parc national au Canada ayant pour but la conservation d'une aire marine. Représentatif de plusieurs régions du système du St-Laurent, il fut à l'origine créé pour protéger l'habitat du béluga dont la situation était devenue précaire. Depuis, le parc est devenu un symbole dans le domaine de la conservation d'écosystèmes marins. Cette zone de 1245 km^2 est localisée à la confluence des eaux de la rivière Saguenay et du fleuve St-Laurent et est caractérisée par une communauté biologique diversifiée et abondante suscitant l'intérêt du public et des scientifiques. Certaines études ont expliqué cette richesse biologique par la combinaison de la circulation estuarienne et des migrations verticales nycthémérales et saisonnières du zooplancton favorisant l'agrégation des proies zooplanctoniques à la tête du chenal laurentien (Fig. 7) (Runge & Simard, 1990; Zakardjian *et al.*, 1999; Lavoie *et al.*, 2000; Sourisseau *et al.*, 2006; Simard, 2009; Maps *et al.*, 2011). De plus, la circulation des eaux profondes vers l'amont ainsi que leur résurgence à la tête du chenal permettent de maintenir

un apport constant en éléments nutritifs et une bonne oxygénation des eaux, favorisant la production primaire (Therriault & Lacroix 1976, Ingram 1983, Therriault et al. 1990) et contribuant ainsi à la richesse biologique du PMSSL. La connectivité entre les régions du Saguenay et du St-Laurent ainsi que les processus permettant le maintien d'une biomasse importante de proies zooplanctoniques pour les poissons pélagiques, les oiseaux marins et les baleines requièrent toutefois une documentation plus approfondie pour soutenir les efforts de conservation de ces espèces.

Le PMSSL chevauche trois régions aux caractéristiques océanographiques distinctes: le fjord du Saguenay, l'estuaire supérieur et l'estuaire maritime du St-Laurent. Ces trois régions sont fortement inter-reliées bien qu'elles soient séparées les unes des autres par des seuils aussi peu profonds que 20 m près de Tadoussac. En effet, l'échange d'une grande quantité d'eau s'effectue à la confluence des eaux du Saguenay et du St-Laurent. En période estivale, la colonne d'eau de l'estuaire maritime et du golfe du St-Laurent est stratifiée en 3 couches distinctes : les eaux de surface d'une profondeur d'environ 20 à 30 m, la Couche Intermédiaire Froide (CIF; 30-100m; $< 1°C$) et la couche plus profonde aux eaux denses, salées et relativement chaudes ($< 6°C$) (Galbraith et al. 2010). Les eaux douces de surface s'écoulent dans l'estuaire du St-Laurent et le fjord du Saguenay vers l'aval. En dessous, les couches plus profondes entrent dans l'estuaire par le chenal laurentien, une vallée sous-marine de plus de 300 m de profondeur qui origine à la marge du plateau continental. La circulation du système du St-Laurent permet aux eaux denses et

profondes de l'Atlantique de circuler vers l'amont à l'intérieur du chenal laurentien en passant par le golfe et l'estuaire maritime jusqu'à la tête du chenal, près de l'embouchure du Saguenay (Koutitonsky & Bugden 1991). La circulation estuarienne, les ondes internes ainsi que les fortes marées diurnes à la tête du chenal permettent l'infiltration des eaux salées de la CIF au-dessus des seuils peu profonds vers l'estuaire supérieur et le Saguenay, entraînant les organismes zooplanctoniques vers l'amont et contribuant aux caractéristiques des eaux profondes du fjord (Drainville 1968, Rainville 1979, De Ladurantaye et al. 1984, Therriault et al. 1984, Saucier & Chassé 2000, Stacey & Gratton 2001, Bélanger 2003, Bourgault et al. 2012).

La connectivité des populations du PMSSL

Peu d'études ont jusqu'à maintenant évalué la connectivité entre les populations animales vivant dans les eaux du parc marin et des régions avoisinantes. Une étude récente utilisant des marqueurs génétiques sur des poissons de fonds ainsi que des crustacés benthiques retrouvés dans le Saguenay et le St-Laurent suggère qu'ils appartiendraient tous à la même population (Sévigny et al. 2009). En ajoutant d'autres paramètres tels que des mesures morphologiques, l'analyse élémentaire des otolithes et la composition parasitaire, les poissons démersaux du Saguenay se sont révélés différents de ceux échantillonnés dans le St-Laurent. Il en fut conclu que cette différence devait être principalement due à l'environnement où l'organisme passe la plus grande partie de son cycle de vie et que le Saguenay jouerait surtout le rôle de puits. En analysant la phase larvaire des populations de

poissons, Sirois et al. (2009) confirmèrent les résultats de Sévigny et al. (2009) pour les espèces démersales mais découvrirent que la production et la rétention des larves à l'intérieur du fjord favorisaient le recrutement local d'espèces anadromes telles que le capelan et l'éperlan.

L'ichtyoplancton et certaines larves d'espèces méroplanctoniques ont des habiletés natatoires leur permettant de se diriger horizontalement contrairement à la majorité des espèces zooplanctoniques qui dépendent majoritairement des courants et de leur position verticale dans la colonne d'eau pour leur dispersion. Les études réalisées jusqu'à aujourd'hui tendent à conclure que l'introduction d'espèces zooplanctoniques marines telles que *Calanus* spp. dans l'estuaire supérieur et le fjord, résulterait du passage des eaux de la CIF vers l'amont au rythme des marées semi-diurnes (Rainville 1979, De Ladurantaye et al. 1984, Laprise & Dodson 1994, Lavoie et al. 2000, Saucier & Chassé 2000, Bélanger 2003, Saucier et al. 2009). Le fjord du Saguenay est formé de 3 bassins dont les deux plus petits situés en aval subissent d'importants mélanges turbulents causés entre autre par l'entrée des eaux denses de l'estuaire (Seibert et al. 1979, Stacey & Gratton 2001, Bélanger 2003). Le bassin intérieur, plus long (80 km) et plus profond (260 m), est soumis à moins de perturbations. Selon les données obtenues par Bélanger (2003), ses eaux profondes sont renouvelées à un rythme plus lent qu'en aval, soit à peine plus qu'une fois par mois, sur une période de 9 mois et principalement durant la période automne/hiver. Dans le bassin intérieur, Rainville (1979) et De Ladurantaye et al. (1984) identifièrent une population

zooplanctonique spécifique à cet environnement et comprenant des espèces probablement endogènes telles que *Microcalanus pygmaeus* et *Oncaea borealis*, ainsi qu'une importante production de nauplii de copépodes en amont du fjord du Saguenay où les conditions océanographiques sont plus stables. En plus des intrusions d'organismes provenant de l'estuaire, les conditions plus stables ainsi que la présence d'espèces endogènes en amont du bassin interne suggèrent un environnement propice au recrutement local d'espèces zooplanctoniques.

Calanus finmarchicus et *Calanus hyperboreus* sont deux espèces présentes dans tout le système du St-Laurent ainsi que dans le Saguenay (Runge & Simard 1990, Plourde et al. 2002). Ensemble, ils représentent jusqu'à 95% de la biomasse du mésozooplancton de l'estuaire maritime (Ouellet et al. 2008). Leur cycle de vie se caractérise par une période active (alimentation, reproduction, ponte et développement) au printemps et au début de l'été dans les eaux de surface (i.e. 0-100 m). Au milieu (*C. hyperboreus*) et à la fin de l'été (*C. finmarchicus*), les stades de développement copépodites pré-adultes/adultes descendent en eaux profondes (>100 m) pour y entrer en diapause jusqu'au printemps suivant (Plourde et al. 2001, 2003). La circulation générale du système du St-Laurent et l'afflux d'eau vers l'amont à 5-10 cm/s en profondeur du côté nord du chenal laurentien (Simard et al. 1986, Saucier et al. 2003, 2009, Maps et al. 2011) suggèrent le transport passif potentiel des stades pré-adultes de *Calanus* spp. en diapause hivernale vers l'amont jusque dans le PMSSL. Ils pourraient ainsi provenir d'aussi loin que le nord-ouest du golfe du St-Laurent

(Plourde et al. 2001, Sourisseau et al. 2006). En outre, rien n'exclu la possibilité d'un recrutement local d'une partie de la population de *Calanus* spp. à l'intérieur du fjord du Saguenay bien qu'aucune étude ne l'aie démontré.

L'isotope stable du carbone ($\delta^{13}C$) comme marqueur d'origine

À grande échelle, spatiale et temporelle, les marqueurs génétiques neutres tels que les microsatellites peuvent être des outils efficaces pour différentier les populations de *Calanus* spp. (Bucklin & Kocher 1996, Bucklin et al. 1996, Kann & Wishner 1996, Unal & Bucklin 2010). Jusqu'à aujourd'hui, aucune étude n'a pu utiliser les analyses moléculaires pour pour évaluer la connectivité du PMSSL avec les possibles régions sources. En effet, cette méthode est peu adaptée puisqu'elle implique l'isolement d'une population sur une longue période pour créer une différentiation génétique. Les isotopes stables pourraient servir de marqueur à petite échelle et permettre de retracer l'origine des populations de *Calanus* spp. se trouvant dans le PMSSL grâce à la variabilité spatiale du ratio $^{13}C/^{12}C$ ($\delta^{13}C$) dans l'environnement. En effet, les proportions isotopiques varient selon la source de carbone du milieu (Fry 1981, Deegan & Garritt 1997, Perry et al. 1999). Dans les régions fortement influencées par un apport d'eau douce contenant du carbone d'origine terrestre et anthropique tels que le Saguenay et l'amont du St-Laurent, le carbone inorganique dissous ainsi que le phytoplancton peuvent avoir une signature isotopique distincte des milieux aux caractéristiques plus océaniques comme le golfe et l'estuaire maritime (Tan & Strain 1979, Martineau et al. 2004, Tremblay & Gagné 2009). En considérant la variabilité des

conditions environnementales dans le système du St-Laurent, nous pouvons supposer que ces variations se traduisent dans la composition isotopique de la source de carbone (dissous et phytoplanctonique) et par conséquent, sur les *Calanus* spp. s'en nourrissant (Peterson & Fry 1987, Perry et al. 1999, Post 2002). La signature isotopique spécifique à chaque région pourrait donc permettre d'identifier l'origine des *Calanus* spp. retrouvés dans le PMSSL au printemps après leur transport vers l'amont par les masses d'eaux profondes durant sa période d'hivernage.

Utiliser les isotopes comme marqueur d'origine spatiale sur une période relativement longue implique par contre le recrutement d'individus physiologiquement stables. En effet, la signature isotopique varie selon le $\delta^{13}C$ de la ressource alimentaire, le taux de renouvellement cellulaire, les mues, le stade de développement et la composition lipidique (Peterson & Fry 1987, Schmidt et al. 2003). Le cycle de vie de *C. finmarchicus* et *C. hyperboreus* est propice à retracer leur origine au travers de la phase d'hivernage puisqu'ils cessent de se nourrir et que leur métabolisme est réduit (Hirche 1983, 1996). La signature isotopique des individus en hivernation dans la couche profonde pourrait donc potentiellement demeurer relativement stable et conserver la signature de la source de carbone alimentaire caractéristique de la région d'origine au moment de l'entrée en diapause.

L'objectif principal de cette étude est d'évaluer l'origine des populations de *Calanus* spp. présentes dans le PMSSL en utilisant les isotopes stables comme marqueur, ce qui permettra d'améliorer la compréhension de la connectivité des populations zooplanctoniques de cette région. Au cours du premier chapitre, la stabilité de la signature isotopique de *Calanus* spp. en diapause durant l'hiver sera évaluée lors d'un suivi en laboratoire pour tester l'hypothèse selon laquelle le $\delta^{13}C$ est susceptible de demeurer stable dans le temps du au métabolisme réduit et à l'absence de prise alimentaire. La compréhension de la dynamique des isotopes stables des *Calanus* spp. durant leur période d'hivernation et de transport en profondeur permettra d'atteindre l'objectif principal de cette étude discuté dans le second chapitre, soit d'évaluer si la population de *Calanus* spp. occupant le PMSSL est de source locale et/ou externe. La discrimination des sources (origines) probables des *Calanus* spp. se fera tout d'abord à partir d'échantillons recueillis entre le 18 et le 25 juillet 2009 au début de la phase d'hivernation (diapause) dans l'aire d'étude couvrant le nord-ouest du golfe, l'estuaire maritime et supérieur du St-Laurent et le fjord du Saguenay. Par la suite, les *Calanus* spp. échantillonnés dans le PMSSL le 11 mai 2010, à la fin de leur phase de diapause, seront classés selon leur origine probable. Considérant la circulation générale du système du St-Laurent, les échanges d'eaux entre le Saguenay et la tête du chenal Laurentien ainsi que l'environnement relativement stable et biologiquement riche de l'amont du bassin intérieur du fjord, la population des *Calanus* spp. du PMSSL pourrait être composée d'un mélange d'individus recrutés localement et à l'extérieur du PMSSL (estuaire maritime et/ou nord-ouest du golfe du St-Laurent).

CHAPITRE 1
EVALUATION EN LABORATOIRE DE LA DYNAMIQUE DES ISOTOPES STABLES CHEZ *CALANUS FINMARCHICUS* ET *C. HYPERBOREUS* EN DIAPAUSE HIVERNALE

1.1 RÉSUMÉ EN FRANÇAIS DU PREMIER ARTICLE

Cette étude avait pour but de décrire les variations de la composition en isotopes stables chez les stades copépodites 5 (CV) d'espèces marines subarctiques (*Calanus finmarchicus* and *C. hyperboreus*) au cours de la période de jeun hivernal. Un suivi des stades CV en diapause échantillonnés dans les eaux profondes de l'estuaire maritime du St-Laurent (Québec, Canada) à la fin de septembre 2009 fut effectué durant 4 mois sous conditions contrôlées. La signature en $\delta^{13}C$ et $\delta^{15}N$ ainsi que le contenu en lipides, carbone et azote ont été analysés chez les CVs et les adultes ayant mué durant l'expérimentation. Les lipides ont été extrait de la moitié des échantillons pour comparer le $\delta^{13}C$ des individus avec et sans lipides ainsi que pour évaluer l'efficacité du modèle mathématique basé sur l'équilibre des masses pour corriger l'influence des lipides sur la signature en $\delta^{13}C$. Le contenu lipidique a généralement diminué dans le temps chez les deux espèces, provoquant une augmentation du $\delta^{13}C$ chez les CVs mais une valeur constante chez les adultes. De plus, l'extraction des lipides a eu pour effet d'augmenter le $\delta^{13}C$ pour tous les échantillons. La signature isotopique $\delta^{13}C$ moyenne des individus dont les lipides ont été extraits est restée stable dans le temps pour les CVs des deux espèces et chez les adultes *C. finmarchicus*. La signature isotopique $\delta^{15}N$ a également augmenté suite à l'extraction des lipides mais les valeurs ne sont pas demeurées stables dans le temps, ce qui suggère que plusieurs processus métaboliques endogènes ont affectés le $\delta^{15}N$ des individus. L'efficacité du modèle mathématique de correction du $\delta^{13}C$ fut différente entre les espèces

et les stades de développement suggérant que l'extraction des lipides devrait toujours être effectuée avant d'appliquer la correction mathématique

Mots clés : *Calanus* spp., isotopes stables, diapause, lipides, model de correction mathématique basé sur l'équilibre des masses.

Cet article fut corédigé par moi-même, le professeur Claudio DiBacco, mon directeur Stéphane Plourde et ma codirectrice Gesche Winkler. Il fut soumis à la revue *Journal of Plankton Research* en avril 2012 sous le titre «Assessing stable isotopes dynamics of *Calanus finmarchicus* and *C. hyperboreus* during the overwintering period : a laboratory experiment. » après une première révision par les pairs, fut accepté en mai puis publié dans le volume 34(8) du mois d'août 2012. Ma contribution en tant que premier auteur fut l'essentiel de l'échantillonnage et du suivi des animaux durant l'expérimentation, de la préparation des échantillons pour les dosages isotopiques, de l'analyse des données ainsi que de la rédaction. Les coauteurs Dr. Claudio DiBacco, Dr. Stéphane Plourde et Dr. Gesche Winkler ont contribué à la révision de cet article. Les résultats de cette étude ont été présentés dans le cadre du *Canadian Healthy Ocean Network (CHONe) Meeting* à Montréal (Québec, Canada) en avril 2011 ainsi que lors de la *10^{ième} Assemblée Générale Annuelle de Québec-Océans* au Lac Delage (Québec, Canada) en novembre 2011.

1.2 ASSESSING STABLE ISOTOPES DYNAMICS OF DIAPAUSING *CALANUS FINMARCHICUS* AND *C. HYPERBOREUS* DURING THE OVERWINTERING PERIOD: A LABORATORY EXPERIMENT

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1.2.1 ABSTRACT

This study aimed at describing changes in the stable isotopic composition of subarctic marine copepods (*Calanus finmarchicus* and *C. hyperboreus*) during overwintering non-feeding periods as late copepodites stage V (CV). Diapausing stage CVs sampled in deep waters of the Lower St. Lawrence Estuary (Québec, Canada) in late-September 2009 were monitored for 4 months under controlled laboratory conditions. CVs and newly moulted adults were analyzed for $\delta^{13}C$ and $\delta^{15}N$ signatures as well as lipid, carbon and nitrogen content. Lipids were extracted in half of the samples to compare $\delta^{13}C$ of individuals with and without lipids and to evaluate the accuracy of mass balance correction models for $\delta^{13}C$ under lipid influence. Lipid content generally decreased with time for both species, which was reflected in an increase of $\delta^{13}C$ values of CVs but a constant $\delta^{13}C$ in newly moulted adults. Accordingly, lipid extraction resulted in an increase of $\delta^{13}C$ in CVs and adults. The mean $\delta^{13}C$ signature of lipid-extracted individuals remained constant through the time for CVs of both species and for *C. finmarchicus* adults. $\delta^{15}N$ signatures of individuals increased after lipid extraction, but this did not result in a constant value over time, suggesting that several endogenous metabolic processes affected nitrogen isotopic content. The accuracy of the mass balance model differed between species and stages, suggesting that lipid extraction should always be performed prior to applying mathematical corrections.

Keywords: *Calanus*; stable isotopes; diapause; lipids; mass balance correction model

1.2.2 INTRODUCTION

Over the last 30 years, the application of stable isotopic tracers to identify sources of energy at the base of food webs and to describe trophic pathways in aquatic systems has become prevalent (Martinez Del Rio *et al.*, 2009; McConnaughey & McRoy, 1979; Peterson & Fry, 1987; Post, 2002; Vander Zanden & Rasmussen, 2001). The principle is based on the assumption that stable isotopes are transferred in predictable ratios between food and consumers. Stable isotope signatures are affected by cell turnover rate (Fry & Arnold, 1982; Graeve *et al.*, 2005; Lorrain *et al.*, 2002; Tieszen *et al.*, 1983), diet (Vander Zanden & Rasmussen, 2001) and selective isotope distribution in different tissues (routing) (DeNiro & Epstein, 1978; DeNiro & Epstein, 1981; Gannes *et al.*, 1998; Martinez del Rio *et al.*, 2009; Vander Zanden & Rasmussen, 2001). A good example of these isotopic processes can be observed in lipid signatures, which results in ^{13}C depletion of up to 6 - 7‰ relative to proteins (DeNiro & Epstein, 1977; Melzer & Schmidt, 1987).

A few experimental studies have attempted to describe specific factors influencing isotopic signatures in zooplankton. Most studies have characterized turnover rates and isotopic fractionation in zooplankton reared on a controlled diet (Fry & Arnold, 1982; Minagawa & Wada, 1984; Frazer *et al.*, 1997; Gorokhova & Hansson, 1999; Graeve *et al.*, 2005; Tamelander *et al.*, 2006; Saage *et al.*, 2008). Mayzaud (1976) found that dry weight (DW) and C:N steadily decreased in *C. finmarchicus*, while the rate of ammonia excretion oscillated throughout long periods of starvation. These results indicate that lipid reserves were used in conjunction with periods of high and low protein catabolism. However, the impact of basal metabolic rate on whole-organism stable isotope signature is still unknown. Unfed zooplankton signatures remained relatively constant during 1- to 4-week experiments (Frazer *et al.*, 1997; Gorokhova & Hansson, 1999; Tamelander *et al.*, 2006). Nevertheless, at high latitudes, the potential duration of the fasting period of *Calanus* spp. is longer. After active growth in surface waters (reproduction, spawning and development) in spring and summer, *C. finmarchicus* and *C. hyperboreus* enter diapause to overwinter as

deep-dwelling late copepodite stages for up to 8 months (Plourde *et al.*, 2001, 2003; Johnson *et al.*, 2008). During this period, metabolism is reduced and thought to be mainly supported by lipid reserves, mainly wax ester stores in oil sacs (Conover, 1988; Dahms, 1995; Hirche, 1996; Ohman *et al.*, 1998). Until now, a few studies attempted to measure the *in situ* evolution of isotopic signatures with time in these diapausing zooplanktonic organisms (Forest *et al.* 2011; Wold *et al.* 2011).

High-latitude marine zooplankton is generally rich in lipids (Lee *et al.*, 2006; Kattner *et al.*, 2007), which can represent as much as 85% of the body weight in late copepodite stages (Vogedes *et al.*, 2010). Lipid content also varies among species, developmental stages (ontogeny) and season (Søreide *et al.*, 2008). These factors contribute to variations in the relative amount of lipids in copepods and can lead to misinterpretation of stable isotope signatures. To control for this potential bias, lipids are usually extracted prior to stable isotope analyses so the signature reflects more conservative body constituents such as proteins and exoskeleton. The removal of lipid increases the ^{13}C:^{12}C while the magnitude of this effect is positively correlated with lipid content (McConnaughey & McRoy, 1979; Smyntek *et al.*, 2007; Logan *et al.*, 2008). Unexpected effects on nitrogen isotopic signatures ($\delta^{15}N$ enrichment) have been reported with extraction techniques that used polar solvents (not lipid specific) that removed lipid-bound proteins (Sotiropoulos *et al.*, 2004; Murry *et al.*, 2006; Søreide *et al.*, 2006; Sweeting *et al.*, 2006). An alternative to lipid extraction is to employ a lipid normalization correction model for carbon signatures. Since lipid content is positively correlated with C:N (Smyntek *et al.*, 2007; Logan *et al.*, 2008; Syväranta & Rautio, 2010), this relationship can be used to estimate a mathematical correction. These models performed well for zooplankton samples when taxon-specific parameters such as lipid percentage and differences in carbon isotopic signatures between proteins and lipids were applied to bulk zooplankton from freshwater lakes (Smyntek *et al.*, 2007; Syväranta & Rautio, 2010). However, no study has evaluated the effectiveness of these correction models for different zooplankton species, species-specific stages of development or individuals in marine environments.

This study is the first part of a broader project aiming at using stable isotopes signature as a marker of origins for *Calanus* spp. in a Marine Protected Area, the Saguenay-St. Lawrence Marine Park (SSLMP), Canada (Perrin *et al.* in prep.). The general circulation in the area is hypothesized to favour the transport of diapausing *Calanus* spp. from eastern regions toward the marine park during their overwintering period (Runge & Simard 1990; Plourde *et al.*, 2001; Maps *et al.*, 2011). The objective of the present study was to describe and understand changes in stable isotopic signatures of *C. finmarchicus* and *C. hyperboreus* during overwintering, non-feeding periods in their development as late copepodites stages (CV), in order to validate the effectiveness of using stable isotopes as a marker to assess the origin of *Calanus* spp. throughout their transport during the overwintering period. The null hypothesis was that stable isotope signatures of lipid-extracted individuals would not change during diapause because animals do not feed. Our expectation of individuals with lipids was that isotopic signatures would increase (become enriched) as lipid reserves were consumed during diapause. Differences in stable isotope signatures between the two species and different development stages as well as the impact of lipid content and fasting duration (time) on whole-body isotope signatures were evaluated. In order to test these objectives, lipid content as well as carbon and nitrogen stable isotope signatures were monitored in animals maintained under laboratory controlled conditions for a 4-month period. Isotopic signatures of lipid-extracted animals were compared with whole animals to document the effect of lipid content. A second objective was to compare mass balance correction models for lipid content (Smyntek *et al.*, 2007; Syväranta & Rautio, 2010) on our data at the species and development stage level. These laboratory experiments intended to document the impact of time, development stage, species, lipid content, lipid extraction and mathematical corrections on stable isotopes signatures in order to improve efficiency of future stable isotopes studies.

1.2.3 METHODS

1.2.3.1 Sampling and laboratory conditions

Zooplankton was sampled on 24 September 2009 at a fixed station near Rimouski (48°40N, 68°35W) located in the middle of the Laurentian Channel (340m) of the St. Lawrence Estuary (Québec, Canada) (see Plourde et al. 2001). Deep-dwelling zooplankton (100-320m) were collected with a vertical plankton tow using a 0.75-m diameter plankton net, 202-µm Nitex net equipped with an opening-closing device. The sampled plankton was diluted in 4-L containers filled with 0.2-µm filtered sea water maintained at ambient temperature in coolers upon their transport to the laboratory (~ 3 h).

Stage V copepodites (CV) of *Calanus finmarchicus* (N= 280) and *C. hyperboreus* (N= 280) were sorted. *C. finmarchicus* was distinguished from *C. glacialis* using prosome length limits of 2.9 mm (Plourde et al. 2001). Recent molecular analyses on samples collected in 2008 and 2009 indicated that this size limit minimized the potential of classifying *C. glacialis* as *C. finmarchicus*, the latter being generally one order of magnitude more abundant in the region (Parent *et al.*, 2011). Copepods were maintained in fourteen 10-L plastic containers (40 CVs container^{-1}) filled with salinity 34, 0.2 µm-filtered and UV sterilized sea water. Each container held four cylinders (10 CVs cylinder^{-1}) with a 202-µm Nitex bottom net which allowed transferring copepods with a minimum of stress in another container with clean sea water. Culture containers were held in the dark in a walk-in cold room at 5 °C, mimicking *in situ* environmental conditions at depths where the bulk of the *Calanus* spp. population overwinters (Plourde et al. 2001, 2003). On September 25 (day 0), October 26 (day 32), December 8 (day 75) and January 19 (day 117), 30 *C. finmarchicus* and 30 *C. hyperboreus* were sub-sampled for analysis. Animals were collected from one container unless the number of copepods was insufficient. Since a majority of CVs had moulted to the adult stage by 19 January, all remaining CVs (5 *C. finmarchicus*, 10 *C. hyperboreus*) were sampled on this date (day 117). Dead animals

were counted on a weekly basis and removed during water changes to estimate mortality. The development stages of dead copepods were not considered.

1.2.3.2 Laboratory analysis

All analyses were performed on individual copepods (e.g. stable isotopes, C:N, carbon and nitrogen percentages, lipid index). Each animal was photographed and examined with BIOQUANT NOVA Advanced Image Analysis software (ver. 5.50.8, R&M Biometrics, Nashville, TN, USA) to confirm developmental stage, assess the presence or absence of material in the gut and measure oil sac (Ao) and prosome (Ap) areas subsequently used to calculate a lipid index ($\%Lip_{Ao/Ap}$). Afterward, animals were rinsed with millipore water, placed in pre-weighed 5 by 9 mm tin capsules and freeze-dried for 48 h before being weighed using a microscale (Mettler MT5; ± 0.001mg). In general, lipids were extracted from half (15) of the individuals for each species to compare results among animals with and without lipids. All copepodites sampled on day 117 had lipids extracted due to the limited sample size. Lipids were extracted without homogenization by soaking individual copepods in a chloroform:methanol (2:1) solution for 24 h at room temperature and maintained in an atmosphere saturated with chloroform:methanol to prevent evaporation of the solution. This lipid extraction protocol is based on Bligh & Dyer's (1959) method adapted to Ohman's (1988) passive procedure to prevent the material loss. Extracted samples were freeze-dried for 48 h and reweighed. The difference in DW before and after extraction was used to estimate a second percent lipid index ($\%Lip_{DW}$). Dry samples were maintained in a dessicator until isotopic analysis.

Isotope samples were analyzed on an elemental analyzer COSTECH ECS 4010 coupled to an Isotope Ratio Mass Spectrometer (IRMS) DeltaPlus XP with a ConFlo III interface (Thermo Electron Co.). System control, acquisition and data treatments were accomplished with Isodat NT software (vers. 2). Isotopic composition is expressed in parts per thousand (‰) relative to C and N standards:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right] * 1000 \quad (1)$$

where δX (i.e. $\delta^{13}C$, $\delta^{15}N$) is the relative depletion or enrichment of ^{13}C to ^{12}C or ^{15}N to ^{14}N and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of sample and standard (Peterson & Fry, 1987). Analysis were performed using whole animals if DW was <1 mg (i.e. *C. finmarchicus*) or on 1 mg of freeze-dried homogenate if animals were >1 mg in the case of *C. hyperboreus*. Working laboratory standards were Caffeine (Sigma Chemical Co., St Louis, USA), Mueller Hinton Broth (Becton Dickinson, Franklin Lakes, New Jersey, USA) and Nannochloropsis (freeze-dry microalgae paste produced at ISMER) and were first calibrated against primary reference standards with certified ^{15}N and ^{13}C value relative to atmospheric N_2 and Vienna Pee Dee Belemnite (VPDB) from the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland, USA).

A mass balance correction model (Smyntek *et al.*, 2007; Syväranta & Rautio, 2010) was used to correct for the influence of $\delta^{13}C$ depleted lipids ($\delta^{13}C_{Lip}$) on samples with lipids ($\delta^{13}C_W$) and to obtain a corrected $\delta^{13}C_C$ which should represent values of lipid-free constituents.

$$\delta^{13}C_C = \delta^{13}C_W + D * \left(\frac{C:N_W - C:N_{LE}}{C:N_W} \right) \quad (2)$$

where $C:N_W$ and $C:N_{LE}$ are the C:N ratios of samples with lipids and lipid-extracted samples, respectively. D is the $\delta^{13}C$ differences (‰) between proteins (lipid extracted values; $\delta^{13}C_{LE}$) and lipids ($\delta^{13}C_{lip}$).

$$D = \delta^{13}C_{LE} - \delta^{13}C_{Lip} \quad (3)$$

Lipid isotopic signatures ($\delta^{13}C_{lip}$) were estimated from

$$\delta^{13}C_{Lip} = \frac{\delta^{13}C_W - \left[\delta^{13}C_{LE} * (1 - f_{Lip})\right]}{f_{Lip}} \quad (4)$$

where f_{Lip} is the lipid fraction calculated from oil sac (Ao) and prosome (Ap) areas ratio ($f_{Ao/Ap}$) or from DW of lipids and whole animal ratio (f_{DW}).

1.2.3.3 Data analysis

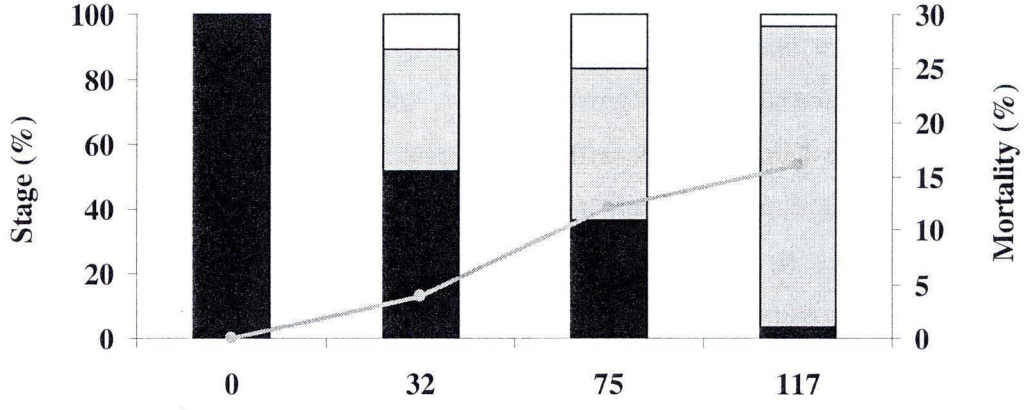
Data analyses were performed with JMP (vers. 7). One-way analysis of variance (ANOVA) was used to test for mean differences (independently of the time of the experiment) in %lipid, C:N, %C, %N, $\delta^{13}C$, and $\delta^{15}N$ between copepod species and developmental stages that contained lipids, had lipids extracted and lipid corrections estimated ($\delta^{13}C$ only). Further, these variables were compared among sampling dates with ANOVA followed by *a posteriori* HSD Tukey's test. Linear regressions analysis were used to assess correlations between lipid content indicators, namely C:N, $\%Lip_{Ao/Ap}$ and $\%Lip_{DW}$. Species and stage specific estimates of lipid content that fell outside 1.5 times the inter-quartile range were identified as outliers (JMP, vers. 7) and omitted from further analysis. Assumptions of normality and homogeneity of variances was tested for each variable with the Shapiro-Wilk test and a visual evaluation of residual distributions, respectively.

1.2.4 RESULTS

1.2.4.1 General findings

Copepods were maintained in the laboratory for almost 4 months (117 days). During this time, >90% of CVs moulted into adult stages, which were dominated by females (93%) in *C. finmarchicus* (Fig. 1 A) and nearly equal percentages of males (48%) and females (44%; including 14% gravid females with eggs visible inside the prosome) in *C. hyperboreus* (Fig. 1 B). Gonad maturation was observed in ~20% of *C. hyperboreus* females. Despite maintaining copepods in filtered seawater, ~57% of *C. finmarchicus* adults and 41% of *C. hyperboreus* adults had few visible materials in their digestive tract. Less than 3% of CVs had materials in their guts. A total of 16% of *C. finmarchicus* and 25% of *C. hyperboreus* died over the course of the incubation (Fig. 1), resulting in a mortality rate of 0.4 and 0.6 copepods day^{-1}, respectively.

A. *C. finmarchicus*



B. *C. hyperboreus*

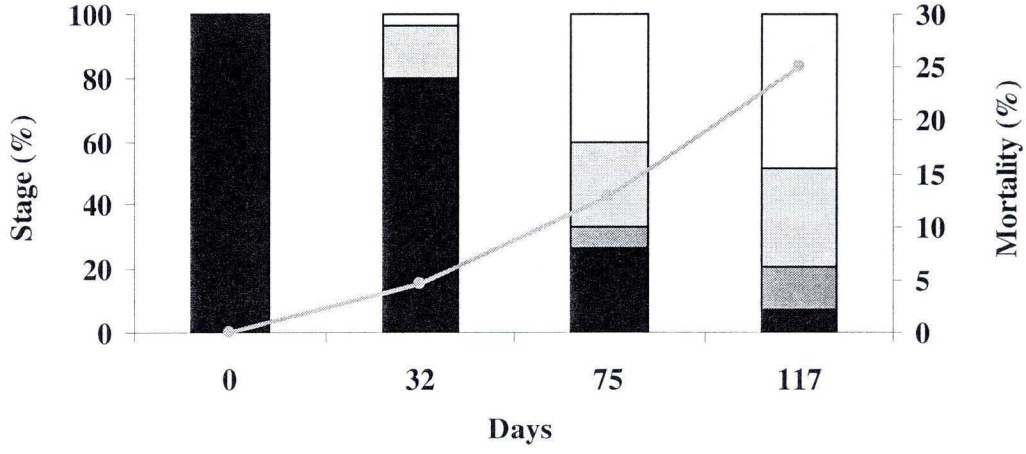


Figure 1: Percentage of different development stages (black: CVs, dark grey: gravid females, light grey: females, white: males) and cumulative mortality (grey line) of *Calanus finmarchicus* (A) and *C. hyperboreus* (B) throughout the experimental period

1.2.4.2 Lipid, carbon and nitrogen content

Overall, the lipid index ($\%Lip_{Ao/Ap}$) was positively correlated to C:N of *Calanus* spp. when lipids had not been extracted ($R^2 = 0.76$, $F_{2,114} = 180.4$, $p < 0.0001$) (Fig. 2 A). However, this relationship differs between species and stages (Fig. 2 B and C). The C:N ratio for individuals that had lipids extracted revealed a constant C:N of 4.1 ± 0.0 (±SE) (see arrow, Fig. 2 A). Lipid-extracted samples were used to measure lipid content via DW estimates before and after extraction ($\%Lip_{DW}$). Since lipids were lost during this extraction process, it was not possible to measure both lipid content and C:N for the same individuals or to correlate $\%Lip_{DW}$ to C:N. Overall, both estimates of percent lipid content ($\%Lip_{Ao/Ap}$ and $\%Lip_{DW}$) were significantly correlated for all data combined ($R^2 = 0.45$, $F_{1,124} = 103.4$, $p<0.0001$) (Fig. 2 D) as well as for both stages of *C. finmarchicus* and for *C. hyperboreus* CV (Fig. 2 E and F). There was no significant relationship between both lipid indices in *C. hyperboreus* adults (Fig. 2 F).

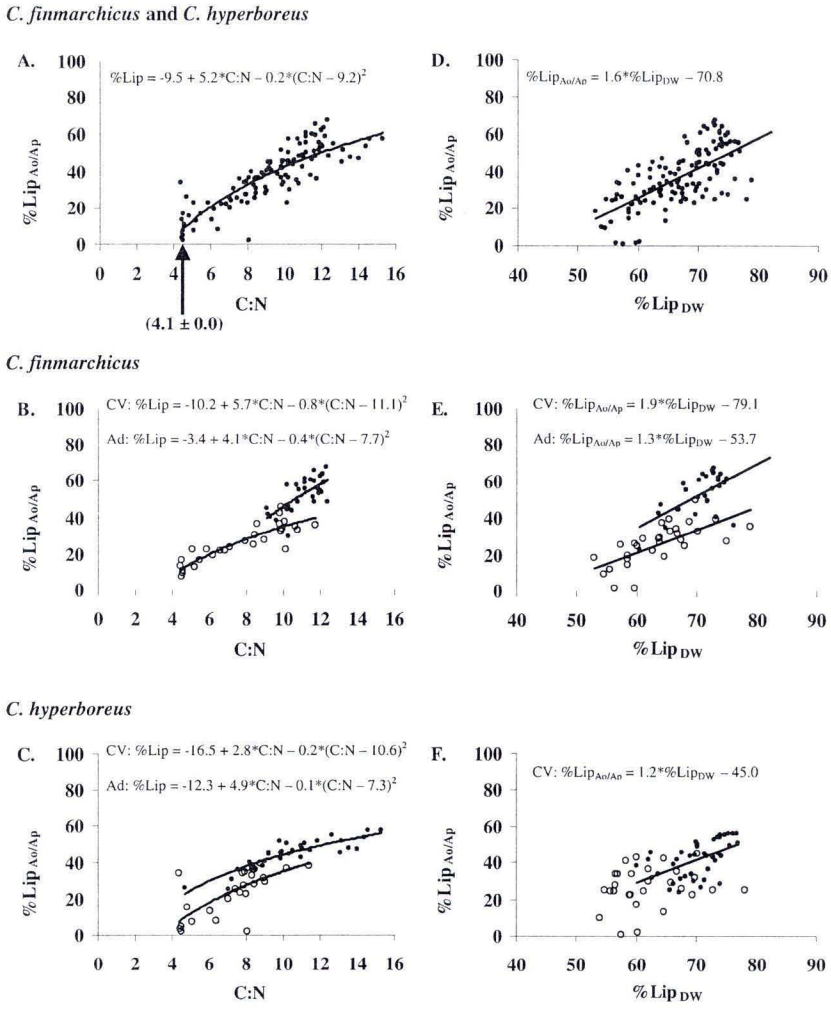


Figure 2: Relationship between the lipid index ($\%Lip_{Ao/Ap}$) and C:N in animals with lipids (A) overall ($R^2 = 0.76$, $p < 0.0001$), (B) *C. finmarchicus* (CV: $R^2 = 0.47$, $p < 0.0001$; Ad: $R^2 = 0.80$, $p < 0.0001$) and (C) *C. hyperboreus* (CV: $R^2 = 0.78$, $p < 0.0001$; Ad: $R^2 = 0.53$, $p < 0.0001$) where the arrow represent the measured C:N of lipid extracted samples. The relationship between the $\%Lip_{Ao/Ap}$ and $\%Lip_{DW}$ index of lipid extracted animals (D) overall ($R^2 = 0.45$, $p < 0.0001$), (E) *C. finmarchicus* (CV: $R^2 = 0.37$, $p = 0.0004$; Ad: $R^2 = 0.46$, $p < 0.0001$) and (F) *C. hyperboreus* (CV: $R^2 = 0.28$, $p = 0.0003$; Ad: $R^2 = 0.0$, $p = 0.3034$). In B, C, E and F, solid circles represent stages CV and open circles represent Adults

Lipid reserves presented in the remainder of this paper are $\%Lip_{Ao/Ap}$ estimates while C:N were determined from non-lipid-extracted copepod samples. Percent carbon (%C) and nitrogen (%N) are estimated from lipid-extracted copepods since this alleviates the variation in lipid content of individual copepods. In general, the mean $\%Lip_{Ao/Ap}$ and C:N estimates for CV and adult stages combined did not differ significantly between *C. finmarchicus* and *C. hyperboreus* (Table 2, Fig. 3 A and B). However, the overall mean $\%Lip_{Ao/Ap}$ of CVs differed between *C. finmarchicus* (mean ± SE; 52.3% ± 1.4) and *C. hyperboreus* (43.2% ± 1.1) while $\%Lip_{Ao/Ap}$ of adults was similar, respectively, 25.5 ± 1.4% and 25.4 ± 1.7% (Table 2; Fig. 3 A). The C:N ratio did not differ significantly among species for either CVs or adults (Table 2; Fig. 3 B). When combining CVs and adults, the overall %C was significantly higher for *C. finmarchicus* (49.5% ± 0.3) relative to *C. hyperboreus* (47.9% ± 0.5), but CVs were similar and adults were significantly different (Fig. 3 C). The overall mean %N was similar between species for stages combined for CVs and for adults. See Table 1 for statistics.

For both species, CVs had significantly higher overall mean lipid content, C:N, %C and %N than adults (Table 2, Fig. 3). These variables were not significantly different between *C. finmarchicus* sexes (data not shown). However, the mean $\%Lip_{Ao/Ap}$ was different between males (29.4% ± 2.0) and females (21.4% ± 2.5) ($F_{1,54} = 6.3$, $p = 0.0149$) while the mean C:N, %C and %N did not differ significantly between *C. hyperboreus* sexes (data not shown). See Table 1 for statistics.

The mean $\%Lip_{Ao/Ap}$ and C:N decreased significantly over the course of the experiment for CV and adult stages of both species (Fig. 3 A and B; Fig. 4). The %C was generally stable throughout the experiment with only a slight decrease of 2.5% ± 0.4 (±SE) between Day 75 and 117 in *C. finmarchicus* adults (Fig. 3 C). %N showed a similar pattern for both species (Fig 3 D). There was only a small difference in %N between species CV stages, which increased between Day 0 and 32 (1.0% ± 0.1% for *C. finmarchicus* and 1.9% ± 0.3% for *C. hyperboreus*) and then decreased slightly for *C. finmarchicus*, but steadily for *C. hyperboreus*. All adults showed a significant reduction in %N from the beginning to the end of the culture period (Fig. 3 D).

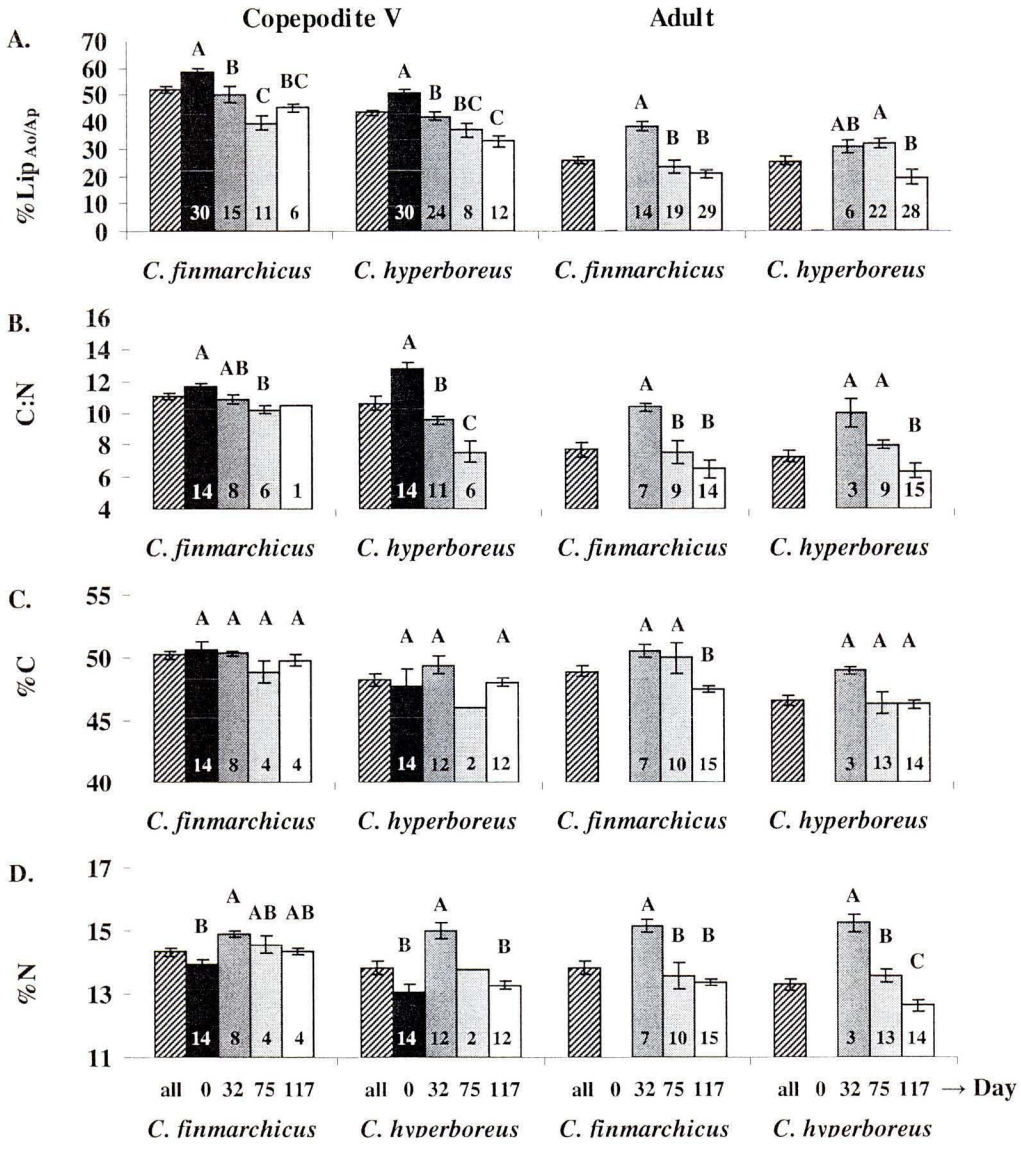
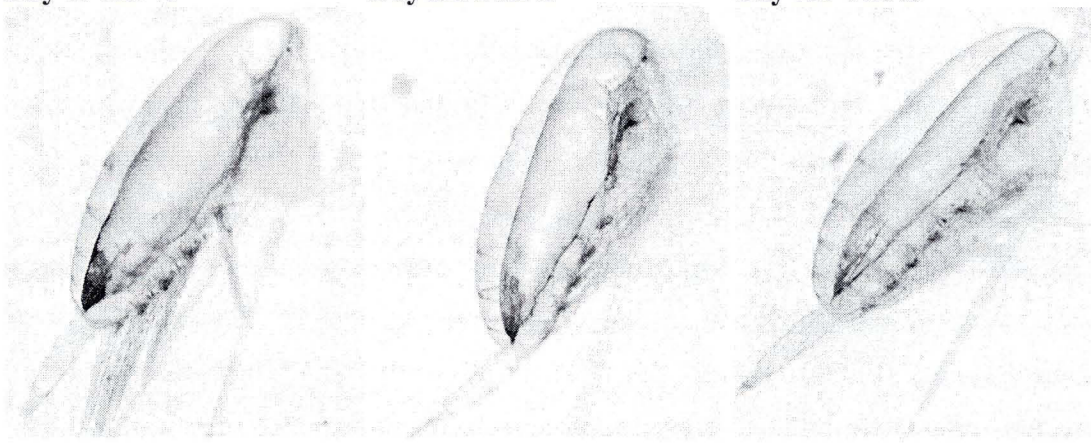


Figure 3: Overall (oblique lines bars) and time evolution (Days 0: black, 32: dark grey, 75: light grey and 117: white bars) of mean (± standard error) lipid index ($\%Lip_{Ao/Ap}$) (A), the C:N ratio in animals with lipids (B), and carbon (C) and nitrogen (D) percentage (%) in lipid-extracted animals. N is represented by numbers inside columns and letters above are results of HSD Tukey's comparisons ($\alpha = 0.05$) among sampling days in each species and stages

C. finmarchicus

Day 0: 65.7% **Day 32: 56.2%** **Day 75: 44.0%**



C. hyperboreus

Day 0: 61.1% **Day 32: 43.0%** **Day 117: 26.0%**

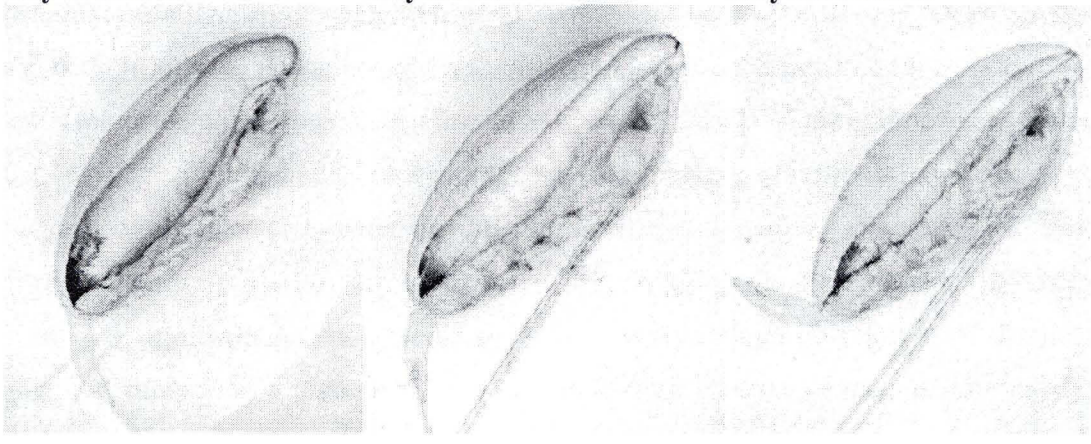


Figure 4: Time evolution of the oil sac area and $\%Lip_{Ao/Ap}$ for *Calanus* spp. CVs

1.2.4.3 Stable isotopes

Lipid extraction increased $\delta^{13}C$ and $\delta^{15}N$ signatures for both species and stages of development. After extraction, the overall means of $\delta^{13}C$ and $\delta^{15}N$ (CV + adults) increased by 3.0 and 1.4‰, respectively, in *C. finmarchicus* and 2.3 and 1.6‰ in *C. hyperboreus* (Fig. 5, 6). CV stages had the highest overall increases in $\delta^{13}C$ (*C. finmarchicus*: +4.1‰; *C. hyperboreus*: +2.4‰) (Fig. 5) and the lowest shift in $\delta^{15}N$ (*C. finmarchicus*: +1.1‰; *C. hyperboreus*: +1.3‰) (Fig. 6). Differences in the overall $\delta^{13}C$ and $\delta^{15}N$ before and after lipid extraction were similar in adults of both species: $\delta^{13}C$ (*C. finmarchicus*: +1.9‰; *C. hyperboreus*: +2.0‰) (Fig. 5) and $\delta^{15}N$ (*C. fin.*: +1.8‰; *C. hyp.*: +2.0‰) (Fig. 6). Standard errors were omitted in this paragraph for clearness since they were <0.1‰ for every mean difference between isotopic signatures. See Table 1 for statistics.

Estimates of overall mean $\delta^{13}C$ (CVs and adults combined) were significantly depleted for *C. finmarchicus* than for *C. hyperboreus* whether lipids had been extracted or not (Table 2). When comparing species-specific developmental stages with lipids (CVs or adults), the overall mean $\delta^{13}C$ of *C. finmarchicus* was significantly depleted compared with *C. hyperboreus*, but differences decreased when lipids were extracted (Fig. 5; Table 2). The mean $\delta^{15}N$ estimates were not significantly different between species (CVs and adults combined) with or without lipids (Fig. 6). However, the overall mean $\delta^{15}N$ of lipid-extracted *C. hyperboreus* adults (9.5‰ ± 0.2) was slightly, but significantly enriched than *C. finmarchicus* adults (9.0‰ ± 0.2) while other stage-specific comparisons had similar mean $\delta^{15}N$ signatures (Fig. 6). Between males and females, there was a significant difference only for *C. finmarchicus* $\delta^{15}N$ with lipids ($F_{1,28} = 6.7$, $p = 0.0150$) but there was any other significant differences in isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of males vs. females for either species whether lipids had been extracted or not (data not shown); therefore, all adults we grouped for future comparisons. See Table 1 for statistics.

The mean $\delta^{13}C$ of *C. finmarchicus* adults were significantly enriched compare with CVs if lipids were not extracted, but conversely adults had depleted values compare with CVs when lipids were extracted (Fig. 5 A). The mean $\delta^{13}C$ of CV and adult *C. hyperboreus* were similar whether lipids were retained or removed from samples (Fig. 5 B). The mean $\delta^{15}N$ differed significantly between stages (CV < Adult) for both species when lipids had been extracted but there were no differences between stages when lipids were not extracted (Fig. 6). See Table 1 for statistics.

Species exhibited similar variations in $\delta^{13}C$ and $\delta^{15}N$ during the course of the experiment. In samples with lipids, the mean $\delta^{13}C$ of *C. finmarchicus* CVs increased significantly by 1.0‰ (± 0.2; ± SE) between Day 0 to 75 (Fig. 5 A) while *C. hyperboreus* CVs showed a significant increase of 1.3‰ (± 0.0) after 32 days followed by a significant decrease of 1.0‰ (± 0.1) by Day 75 (Fig. 5 B). However, $\delta^{13}C$ was stable for adults and in lipid-extracted CVs, except for adult *C. hyperboreus* without lipids (Fig. 5). The mean $\delta^{15}N$ showed similar changes during culture experiments independent of species, development stages or sample treatments (lipid extraction). Mean $\delta^{15}N$ in all stages and species reached a maximum around Day 75 followed by a decrease (Fig. 6).

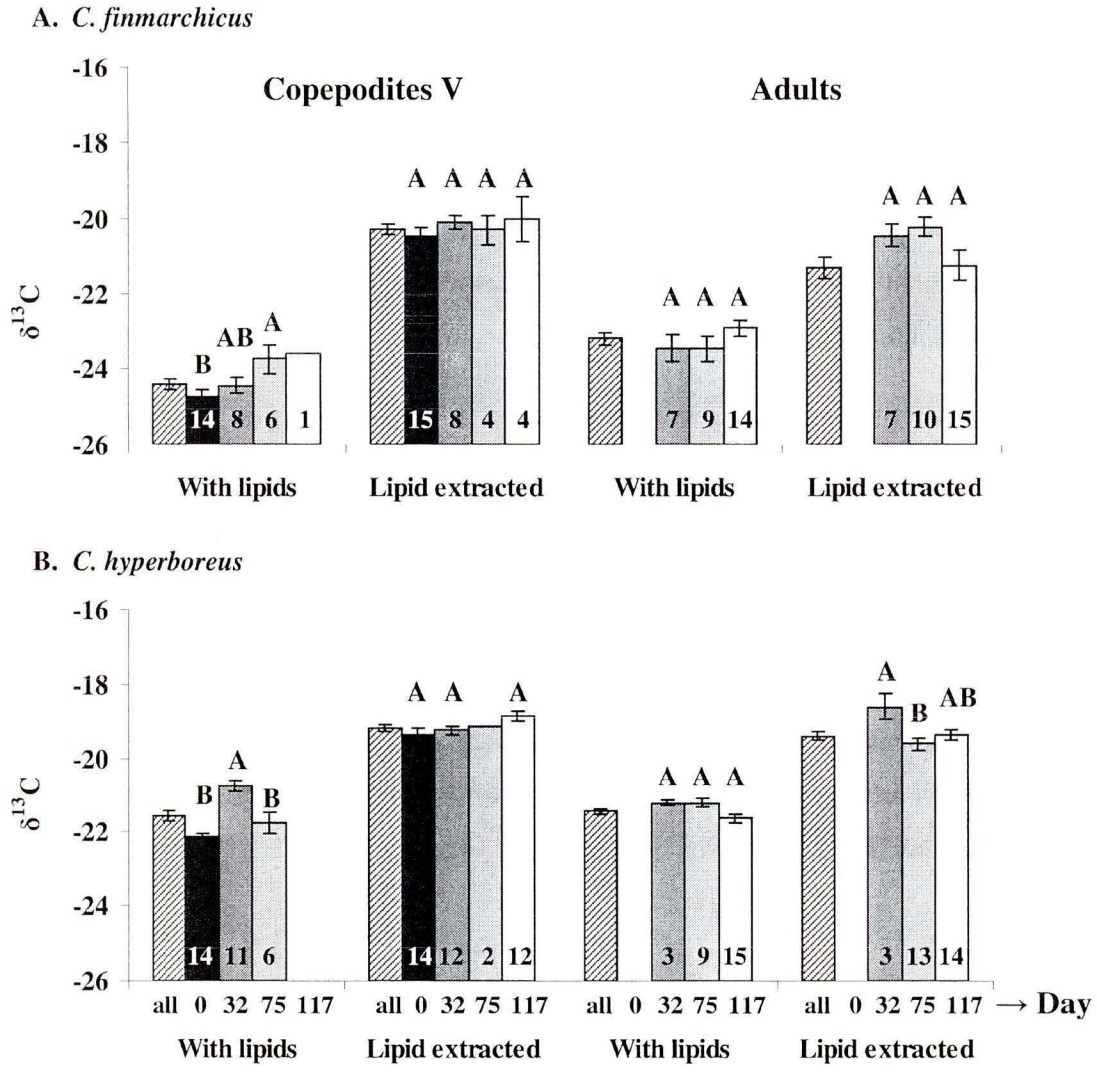


Figure 5: Overall (oblique lines bars) and time evolution (Days 0: black, 32: dark grey, 75: light grey and 117: white bars) of mean carbon isotopic signature ($\delta^{13}C$) (± standard error) of *C. finmarchicus* (A) and *C. hyperboreus* (B) copepodites V and adults, with and without lipids. N is represented by numbers inside columns and letters above are results of HSD Tukey's comparisons ($\alpha = 0.05$) among sampling day in each species, stage and different lipid treatment (extraction or not)

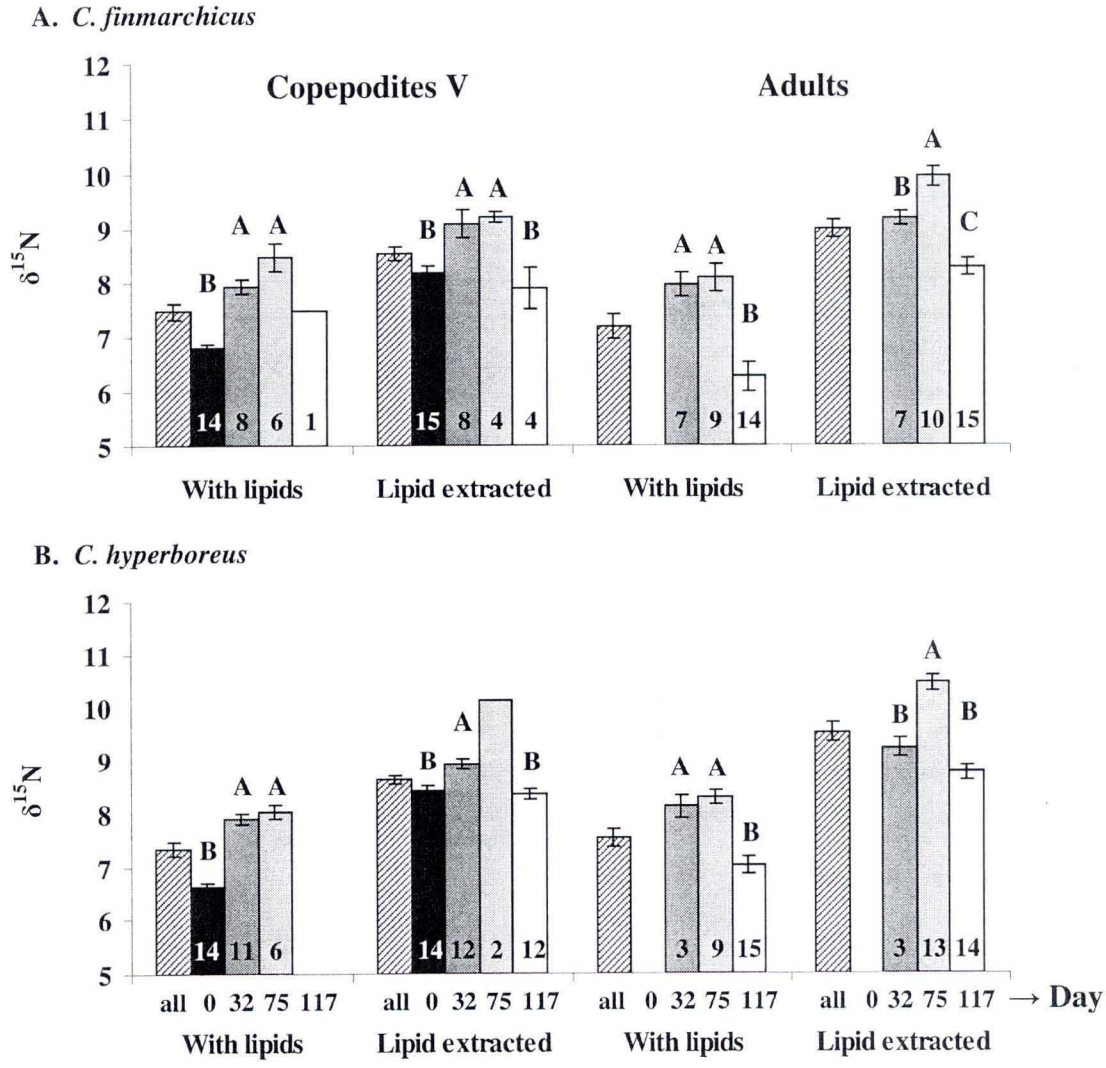


Figure 6: Overall (oblique lines bars) and time evolution (Days 0: black, 32: dark grey, 75: light grey and 117: white bars) of mean nitrogen isotopic signature ($\delta^{15}N$) (± standard error) of *C. finmarchicus* (A) and *C. hyperboreus* (B) copepodites V and adults, with and without lipids. N is represented by numbers inside columns and letters above are results of HSD Tukey's comparisons ($\alpha = 0.05$) among sampling day in each species, stage and different lipid treatment (extraction or not)

Table 1: F-ratio and p-value for the analyses of variance (ANOVA) conducted with different variables ($\%lipid_{Ao/Ap}$, C:N, %C, %N, $\delta^{13}C$ and $\delta^{15}N$) between *Calanus finmarchicus* and *C. hyperboreus* and between development stages (CV and Adults) of each species for lipid extracted samples (LE) and samples with lipids (W). Significant results are bold typed

		$\%Lip_{Ao/Ap}$	C:N	%C	%N	$\delta^{13}C$	$\delta^{15}N$
C. finmarchicus vs C. hyperboreus							
CV + Ad	**W**		$F_{1,115} = 0.4$	-	-	**$F_{1,115} = 201.0$**	$F_{1,115} = 0.4$
		$F_{1,248} = 2.6$	$p = 0.5262$	-	-	**$p < 0.0001$**	$p = 0.5456$
	LE	**$p = 0.1062$**	-	**$F_{1,130} = 7.8$**	$F_{1,130} = 3.5$	**$F_{1,131} = 70.5$**	$F_{1,131} = 2.9$
			-	**$p = 0.0059$**	$p = 0.0635$	**$p < 0.0001$**	$p = 0.0936$
CV vs CV	**W**		$F_{1,58} = 1.0$	-	-	**$F_{1,58} = 200.8$**	$F_{1,58} = 0.5$
		$F_{1,130} = 24.9$	$p = 0.3184$	-	-	**$p < 0.0001$**	$p = 0.4699$
	LE	**$p < 0.0001$**	-	$F_{1,68} = 2.1$	$F_{1,68} = 1.4$	**$F_{1,69} = 46.0$**	$F_{1,69} = 0.5$
			-	$p = 0.1526$	$p = 0.2444$	**$p < 0.0001$**	$p = 0.4945$
Ad vs Ad	**W**		$F_{1,55} = 0.5$	-	-	**$F_{1,55} = 81.1$**	$F_{1,55} = 1.6$
		$F_{1,116} = 0.001$	$p = 0.4803$	-	-	**$p < 0.0001$**	$p = 0.2097$
	LE	$p = 0.9778$	-	**$F_{1,60} = 14.0$**	$F_{1,60} = 3.9$	**$F_{1,60} = 35.5$**	**$F_{1,60} = 5.1$**
			-	**$p = 0.0004$**	$p = 0.0525$	**$p < 0.0001$**	**$p = 0.0276$**
Calanus finmarchicus							
CV vs Ad	**W**		**$F_{1,57} = 50.2$**	-	-	**$F_{1,57} = 30.0$**	$F_{1,57} = 1.2$
		$F_{1,122} = 183.2$	**$p < 0.0001$**	-	-	**$p < 0.0001$**	$p = 0.2857$
	LE	**$p < 0.0001$**	-	**$F_{1,60} = 5.4$**	**$F_{1,60} = 4.6$**	**$F_{1,61} = 9.8$**	**$F_{1,61} = 5.1$**
			-	**$p = 0.0234$**	**$p = 0.0352$**	**$p = 0.0027$**	**$p = 0.0273$**
CV + Ad	**W vs LE**	-	-	-	-	**$F_{1,120} = 176.6$**	**$F_{1,120} = 69.9$**
		-	-	-	-	**$p < 0.0001$**	**$p < 0.0001$**
CV	**W vs LE**	-	-	-	-	**$F_{1,58} = 397.3$**	**$F_{1,58} = 29.3$**
		-	-	-	-	**$p < 0.0001$**	**$p < 0.0001$**
Ad	**W vs LE**	-	-	-	-	**$F_{1,60} = 30.2$**	**$F_{1,60} = 43.3$**
		-	-	-	-	**$p < 0.0001$**	**$p < 0.0001$**
Calanus hyperboreus							
CV vs Ad	**W**		**$F_{1,56} = 31.9$**	-	-	$F_{1,56} = 0.7$	$F_{1,56} = 1.0$
		$F_{1,128} = 87.2$	**$p < 0.0001$**	-	-	$p = 0.3991$	$p = 0.3126$
	LE	**$p < 0.0001$**	-	**$F_{1,68} = 5.6$**	**$F_{1,68} = 4.4$**	$F_{1,68} = 2.8$	**$F_{1,68} = 24.7$**
			-	**$p = 0.0207$**	**$p = 0.0388$**	$p = 0.0980$	**$p < 0.0001$**
CV + Ad	**W vs LE**	-	-	-	-	**$F_{1,126} = 431.0$**	**$F_{1,126} = 114.4$**
		-	-	-	-	**$p < 0.0001$**	**$p < 0.0001$**
CV	**W vs LE**	-	-	-	-	**$F_{1,69} = 235.5$**	**$F_{1,69} = 73.3$**
		-	-	-	-	**$p < 0.0001$**	**$p < 0.0001$**
Ad	**W vs LE**	-	-	-	-	**$F_{1,55} = 206.0$**	**$F_{1,55} = 68.3$**
		-	-	-	-	**$p < 0.0001$**	**$p < 0.0001$**

1.2.4.4 Mass balance correction model

Differences (D_{DW} and $D_{Ao/Ap}$; Eq. 3) in the mean $\delta^{13}C$ signatures of proteins and lipids (calculated from lipid fractions f_{DW} and $f_{Ao/Ap}$ respectively; Eq. 4) were calculated for CV and adult stages of each *Calanus* species as well as global values for combined species and stages (Table 2). Since extractions were performed on individuals, comparison of $\delta^{13}C$ signature for each sample before and after extraction was not possible as well as correlations between C:N and $\Delta\delta^{13}C$. The mean C:N of lipid-extracted animals ($C:N_{LE}$) was 4.1 ± 0.02 for both species (Table 2, Fig. 2 B and C). Corrections ($\delta^{13}C_C$) with D_{DW} values obtained from $\%Lip_{DW}$ estimates (Table 2, Eqs. 3 and 4) were significantly lower than $\delta^{13}C$ measurements from lipid-extracted animals ($\delta^{13}C_{LE}$) ($p < 0.0001$; Table 3). Using $D_{Ao/Ap}$ values calculated from $\%Lip_{Ao/Ap}$ (Table 2, Eqs. 3, 4) resulted in more accurate $\delta^{13}C_C$ values depending on the development stage and species being considered (Table 3). In fact, the mean $\delta^{13}C_C$ of *C. finmarchicus* CV were quite accurate when stage-specific $D_{Ao/Ap} = 7.9$ was used while accurate adult $\delta^{13}C$ corrections resulted from all $D_{Ao/Ap}$ estimates/corrections (i.e. $D_{C.fin.\ Ad} = 7.4$, $D_{C.fin.} = 7.8$, $D_{Calanus\ spp.} = 7.2$). For adult *C. finmarchicus* and both stages of *C. hyperboreus*, the mass balance correction model resulted in realistic corrections with every $D_{Ao/Ap}$. On the other hand, when the development stage was not considered, corrections with $D_{Ao/Ap}$ were inappropriate (Table 3). Smyntek's *et al.* (2007) model (D = 6.3) lead to accurate corrections for *C. finmarchicus* (HSD Tukey's test, $p > 0.05$) but not for *C. hyperboreus* while Syväranta & Rautio's (2010) correction (D = 9.1) overestimated every $\delta^{13}C_C$ compared with lipid extracted samples (HSD Tukey's test, $p < 0.05$; Table 3).

Table 2: Mass-balance correction model parameters: measured C:N ratio (mean ± SD) of copepods with lipids ($C:N_W$), mean measured $\delta^{13}C$ of animals with lipids ($\delta^{13}C_W$) and lipid-extracted ($\delta^{13}C_{LE}$; $\delta^{13}C$ of proteins), calculated lipid fractions from dry weight (DW) ratios before and after extraction (f_{DW}) or lipid fraction from oil sac and prosome areas ratios (Ao/Ap; $f_{Ao/Ap}$), $\delta^{13}C$ of lipids calculated with different lipid fraction ($\delta^{13}C_{Lip\text{-}DW}$, $\delta^{13}C_{Lip\text{-}Ao/Ap}$; Eq. 4) and differences in $\delta^{13}C$ between protein and lipid (D_{DW}, $D_{Ao/Ap}$; Eq. 3). (Smyntek *et al.*, 2007; Syväranta & Rautio, 2010)

Species	Stages	$C:N_W$*	$\delta^{13}C_W$ (‰)	$\delta^{13}C_{LE}$ (‰)	f_{DW}	$\delta^{13}C_{Lip\text{-}DW}$ (‰)	D_{DW} (‰)	$f_{Ao/Ap}$	$\delta^{13}C_{Lip\text{-}Ao/Ap}$ (‰)	$D_{Ao/Ap}$ (‰)
C. finmarchicus	CV	11.1 ± 0.2	-24.4	-20.3	0.71	-26.0	5.7	0.52	-28.2	7.9
	Ad	7.7 ± 0.4	-23.2	-21.3	0.62	-24.3	3.0	0.25	-28.7	7.4
	all	9.4 ± 0.3	-23.8	-20.8	0.67	-25.3	4.5	0.38	-28.6	7.8
C. hyperboreus	CV	10.6 ± 0.4	-21.6	-19.2	0.70	-22.6	3.5	0.43	-24.8	5.6
	Ad	7.3 ± 0.4	-21.4	-19.4	0.61	-22.7	3.3	0.25	-27.4	8.0
	all	9.0 ± 0.4	-21.5	-19.3	0.66	-22.6	3.4	0.35	-25.6	6.4
Calanus spp.	all	9.2 ± 0.2	-22.7	-20.0	0.66	-24.0	4.0	0.36	-27.2	7.2

*C:N of lipid-extracted samples ($C:N_{LE}$) was constant: 4.1 ± 0.0 for both *C. finmarchicus* and *C. hyperboreus*.

Table 3: Mean carbon isotope signature ($\delta^{13}C$ (‰) ± standard deviation) of lipid extracted animals (LE) compare to corrected values with different $D_{Ao/Ap}$ values (calculated with $\%Lip_{Ao/Ap}$; Eq. 3) for lipids mass balance correction model. $D_{Ao/Ap}$ calculated for stages per species (*C. fin.* CV = 7.9, Ad = 7.4, *C. hyp.* CV = 5.6, Ad = 8.0), $D_{Ao/Ap}$ for each species (*C. fin.* = 7.8, *C. hyp.* = 6.4), a general $D_{Ao/Ap}$ = 7.2 for *Calanus* spp., D = 6.3 (Smyntek *et al.*, 2007) and D = 9.1 (Syväranta & Rautio, 2010). For each line, values associated to different letters are significantly different (HSD Tukey's test, $p > 0.05$) and corrected values not significantly different to $\delta^{13}C$ of lipid extracted samples are bold typed

Species	Stage	Group comparisons					
		LE	$D_{Ao/Ap}$ (stage/species)	$D_{Ao/Ap}$ (species)	$D_{Ao/Ap}$ (*Calanus* spp.)	D = 6.3	D = 9.1
C. finmarchicus	CV	**-20.3 ± 0.2 B**	**-20.8 ± 0.1 B**	-21.6 ± 0.1 C	-21.9 ± 0.1 C	**-20.5 ± 0.1 B**	-18.7 ± 0.1 A
	Adult	**-21.3 ± 0.3 BC**	**-22.0 ± 0.2 C**	**-21.4 ± 0.2 BC**	**-21.6 ± 0.2 BC**	**-20.6 ± 0.2 B**	-19.5 ± 0.3 A
	CV + Ad	**-20.8 ± 0.2 B**	-21.4 ± 0.1 C	-21.5 ± 0.1 C	-21.7 ± 0.1 C	**-20.5 ± 0.1 B**	-19.1 ± 0.2 A
C. hyperboreus	CV	**-19.2 ± 0.1 C**	**-19.5 ± 0.1 C**	**-19.6 ± 0.1 C**	**-19.2 ± 0.1 C**	-17.9 ± 0.1 B	-16.2 ± 0.2 A
	Adult	**-19.4 ± 0.1 BC**	**-20.1 ± 0.2 C**	**-20.1 ± 0.2 C**	**-19.9 ± 0.2 C**	**-19.0 ± 0.2 B**	-17.9 ± 0.3 A
	CV + Ad	**-19.3 ± 0.1 C**	-19.8 ± 0.1 D	-19.8 ± 0.1 D	**-19.5 ± 0.1 CD**	-18.4 ± 0.2 B	-17.0 ± 0.2 A
Calanus spp.	all	**-20.0 ± 0.1 B**	-20.6 ± 0.1 C	-20.7 ± 0.1 C	-20.6 ± 0.1 C	**-19.5 ± 0.1 B**	-18.1 ± 0.2 A

1.2.5 DISCUSSION

Results from this study have improved our description of changes in major body constituents of *Calanus* spp. during diapause. Three factors were considered for their potential in affecting stable isotope signature: moulting, body constituents (lipids, nitrogen and carbon) and time.

1.2.5.1 Moulting

Calanus spp. CV stages overwinter in diapause for several months in the stable conditions of the St. Lawrence Estuary deep water layer, with *C. finmarchicus* overwintering at shallower depths than *C. hyperboreus* (Plourde *et al.*, 2001, 2003). The dormancy duration has been hypothesized to be controlled by environmental and biological factors. Light (Miller *et al.*, 1991; Speirs *et al.*, 2005) and the quantity of lipid reserves (Johnson *et al.*, 2008; Maps *et al.*, 2010) are two elements that could influence diapause dynamics and duration. For further recent consideration of the role of lipids, see Pond (2012). In addition to the stress associated with their capture and displacement from overwintering depths to the surface, experimental copepods in this study also were disturbed by the weekly exchange of culture water. Capture, associated turbulence and exposure to elevated light levels "awakened" some CVs and stimulated observed moulting to adult stages, gonad maturation and enhanced metabolic rates (Conover & Corner, 1968; Ingvarsdottir *et al.*, 1999; Auel *et al.*, 2003; Plourde *et al.*, 2003). Increased activity accelerates energy utilization and some CVs could have reached a lipid reserve threshold that could stimulate moulting to the adult stage (Maps *et al.*, 2010).

Calanus finmarchicus CVs with lipids had a lower mean $\delta^{13}C$ than adults, which reflects their higher content of $\delta^{13}C$-depleted lipids. This trend was reversed in non-lipid structures of CVs, which were enriched in $\delta^{13}C$ (Fig. 5A). Further, moulting resulted in

depleted $\delta^{13}C$ signatures in non-lipid adult structures (Fig. 5 A). Depleted carbon isotopic signatures of lipid-extracted adults may arise from the removal of heavy stable isotopes in $\delta^{13}C$-enriched exuviae (DeNiro & Epstein, 1978; Gorokhova & Hansson, 1999; Dean *et al.*, 2011). This is relevant in our unfed cultures where CVs likely assimilate carbon from $\delta^{13}C$-depleted lipid reserves to produce new structural molecules resulting in depleted adult structures. Conversely, moulting did not alter *C. hyperboreus*'s $\delta^{13}C$ (Fig. 5 B). It is recognized that *Calanus* spp. utilize lipid reserves for metamorphosis (Sargent & Falk-Petersen, 1988). Lipid reserves (wax esters) as a source of carbon could be less important for building adults structures of *C. hyperboreus* than for *C. finmarchicus* since the $\%Lip_{Ao/Ap}$ of *C. finmarchicus* CVs was significantly higher than that of *C. hyperboreus*, but adults of both species had similar $\%Lip_{Ao/Ap}$ (Fig. 3 A). The utilization of carbon from lipid sources by *C. hyperboreus* may not have been large enough to significantly reduce the $\delta^{13}C$ in adults. This could be related to the different reproductive strategy with *C. hyperboreus* mainly reproducing from its body reserves and *C. finmarchicus* from external food sources (Conover, 1988; Plourde & Runge, 1993; Plourde *et al.*, 2003). Therefore, *C. hyperboreus* would save its lipid reserve for reproduction and use a smaller lipid fraction than *C. finmarchicus*.

The difference in $\delta^{15}N$ among development stages of both species appeared only in lipid-extracted animals. The effect of moulting was opposite to the $\delta^{13}C$ trends as the mean $\delta^{15}N$ was more enriched in adults than CVs. It is unlikely that these enriched $\delta^{15}N$ values are due to moulting since lipids are nitrogen free. Murry et al. (2006) hypothesized that nitrogen waste would be removed with lipid extraction. Metabolism tends to retain heavy isotopes and produces isotopically light urea and ammonia (-3‰ relative to non-lipid body constituents) (Checkley & Miller, 1989), which are soluble in organic solvents (Murry *et al.*, 2006). Taking into account that newly moulted adults would be more metabolically active than diapausing CVs, they should have produced more nitrogen waste-depleted $\delta^{15}N$. Greater $\delta^{15}N$ of adults relative to CVs after lipid extraction (Fig. 6) could have resulted from larger amounts of light metabolic products removed in solvents.

1.2.5.2 Lipid, carbon and nitrogen content

Lipid content had a consistent effect on $\delta^{13}C$ signatures. Since lipids are depleted in $\delta^{13}C$, a greater amount of lipid, as indicated by high $\%Lip_{Ao/Ap}$ and C:N, correspond with depleted $\delta^{13}C$ signatures of the whole body. For example, CVs with larger oil sacs were depleted in $\delta^{13}C$ compared with adults with lower lipid content (Fig. 3 and 5). As demonstrated in many studies, lipid extraction caused a significant increase in $\delta^{13}C$ and the effect is correlated with lipid amount (Logan *et al.*, 2008; McConnaughey & McRoy, 1979; Smyntek *et al.*, 2007). This effect was greatest in the $\delta^{13}C$ signature of *C. finmarchicus* CVs, which typically had the highest lipid content (Fig. 3 A and B) and became enriched by 4.1‰ after lipid extraction (Fig. 5 A). Overall, *C. finmarchicus* had depleted $\delta^{13}C$ values compare with *C. hyperboreus*, a difference caused by $\delta^{13}C$ depleted lipid content. In fact, the $\%Lip_{Ao/Ap}$ in CVs was generally higher in *C. finmarchicus* than in *C. hyperboreus.* Additionally, even if the overall $\%Lip_{Ao/Ap}$ and C:N ratio were similar between species (CV and adults combined), species-specific discrepancy was higher in animals with lipids (2.3‰) than without lipids (1.5‰), which reflects the influence of lipid content.

There were significant differences in the carbon content (%C) of lipid extracted tissues measured for different *Calanus* species and stages; however, differences between overall means (≤1.6%) were relatively small (Fig. 3 C). Stage-specific similarities in %C support the previous hypothesis that carbon from lipid reserves could be used to build $\delta^{13}C$ depleted structures to replace exuviae after moulting. Since N content of tissues is correlated with protein content (Michener & Lajtha, 2007) and since proteins are metabolized during periods of starvation (Mayzaud, 1976; Hirche, 1996; Mayor *et al.*, 2009) we estimated changes in %N in lipid-extracted tissues to determine whether copepods were assimilating proteins during culture. The increase in %N between Day 0 and 32 in unfed CVs was unexpected because %N could increase if there was a nutrient uptake or if a large amount of nitrogen-free molecules (e.g. carbohydrates) were metabolized and excreted. CVs analyzed here did not have food in their digestive tract, suggesting that they were fasting and probably remained in diapause throughout the experiment. Even if

carbohydrates consumption was plausible, the reduced metabolism was not prone to burn significant amounts of these molecules. While some adults likely fed on eggs (Ohman & Hirche, 2001; Bonnet *et al.*, 2004; Plourde & Joly, 2008), exuviae or dead animals (Ikeda, 1971), their %N decreased instead of increasing with this source of protein. After Day 32, however, the %N in lipid-extracted tissues declined in both species and all stages of development, likely reflecting protein metabolism. Even if some adults fed, only half of them had food in their digestive track and the amount was low. Since detritus and dead animals were removed weekly and because *Calanus* spp. are recognized as principally herbivorous (Søreide *et al.*, 2008; Falk-Petersen *et al.*, 2009; Forest *et al.*, 2011), active copepods likely needed to support their metabolism by using proteins as source of energy to compensate for food scarcity.

1.2.5.3 Time evolution of stable isotopes

Characterizing the effect of time on adult $\delta^{13}C$ or $\delta^{15}N$ signatures was not feasible, because individual-specific moult dates were not monitored. Nevertheless, adult results were generally consistent with CV patterns. *Calanus* spp. use their reserves, including lipids and proteins, for basal metabolism when starved (Mayzaud, 1976; Hirche, 1983, 1996; Helland *et al.*, 2003; Lee *et al.*, 2006;). Observed reductions in the oil sac area through time in stage CV *Calanus* spp. indicate the consumption of wax esters which is supported by observed decreases in both $\%Lip_{Ao/Ap}$ and C:N (Fig. 3 A and B) and the observed increase in the $\delta^{13}C$ signature of copepods with lipids (Fig. 5). However, the reduction of $\delta^{13}C$ in *C. hyperboreus* CVs after Day 32 was surprising and hard to explain especially since time had no significant influence on either $\delta^{13}C$ or %C when lipids were extracted, suggesting that little or no non-lipid carbon was consumed. Moreover, lipid and protein metabolism did not alter isotopic carbon ratios of non-lipid structural compounds. Variations in $\delta^{15}N$ with time were similar between species and stages even though moulting time could not be determined. Similar patterns in $\delta^{15}N$ signatures between species, stages and lipid treatment, whether lipids were extracted or not, was shown in this experiment,

suggesting that consistent and coherent endogenous molecular processes play different roles in nitrogen metabolism.

Results of the time evolution of the stable isotopes signatures show that $\delta^{13}C$ of lipid extracted CVs was conservative contrary to the $\delta^{15}N$. This validated the potential of using $\delta^{13}C$ as a marker in determining origins of diapausing *Calanus* spp. stage CV over at least 4 months in future studies.

1.2.5.4 Mass balance correction model

Mathematical normalization to correct for $\delta^{13}C$ signatures affected by lipid content involves identifying the most accurate method to compensate and explain for the effects of lipid content. Here, lipid weights estimated from differences between dry mass before and after extraction (f_{DW}, $\%Lip_{DW}$) overestimated lipid percentage probably due to the removal of proteins in addition to targeted lipids. Therefore, calculation of lipid signatures ($\delta^{13}C_{Lip}$) were overestimated (Eq. 4) which underestimated D_{DW} (Eq. 3). Thus, $\delta^{13}C$ corrected values from $\%Lip_{DW}$ remained lower than lipid-extracted values. $\%Lip_{Ao/Ap}$ gave better estimates of lipid content since corrections resulted in $\delta^{13}C$ values closer to the lipid extracted ones. However, $\%Lip_{Ao/Ap}$ represents only the wax esters in the oil sac and did not consider other lipids (e.g. structural phospholipids) considered in Figure 2D. The $\%Lip_{DW}$ is expected to be higher than 0% even if an empty oil sac was observed ($\%Lip_{Ao/Ap} = 0$). The estimate of adult lipid content based on the oil sac area could be skewed by lipid-rich eggs in gravid females. This likely helps to explain the lack of a relationship between $\%Lip_{Ao/Ap}$ and $\%Lip_{DW}$ in *C. hyperboreus* adults as some of the females examined contained eggs (Fig. 1 A and 2 F). A more accurate methodology to estimate the lipid fraction of individuals is required to improve $\delta^{13}C$ lipid signature (Eq. 4) and the difference between protein and lipid specific $\delta^{13}C$ signatures, or D (Eq. 3). In the present study, samples were soaked 24h in a chloroform:methanol polar solution for complete lipid extraction without homogenization. This passive lipid extraction technique avoids material lost in small

samples like *Calanus* spp. individuals but soaking for a long period could have contributed to extract proteins as well. Indeed, as in Syväranta & Rautio (2010) and Smyntek et al. (2007) studies, a more rapid extraction technique could have reduced the protein extraction problem, leading to a more accurate $\%Lip_{DW}$ estimation. When Ohman (1988) tested the efficiency of passive extraction, he noted that after 12h at room temperature (23°C) more than 80% of phospholipids and 100% of other lipids were extracted. Since polar solvent is likely to remove proteins bounded with lipids as phospholipids, a shorter soaking duration (<12h) could be tested to extract enough lipids for accurate estimation of $\%Lip_{DW}$ and to reduce the protein removal in future studies.

Few studies have compared the predictability of mathematical lipid correction models for zooplankton $\delta^{13}C$. Smyntek et al. (2007) reported that an average D value of 6.3‰ lead to a strong correlation ($R^2 = 0.95$) between lipid-extracted versus mathematically corrected $\delta^{13}C$ values, especially for copepods with C:N between 5.0 to 7.0. Recently, Syväranta & Rautio (2010) calculated a higher D value (9.1‰; $R^2 = 0.93$) from analyses of calanoid copepods with a larger range of C:N values (ca. 4-17) caused by a different timing of sampling during the year. Many other factors could have contributed to differences in the accuracy of the model. For example, both of these studies were conducted in lakes and on bulk zooplankton homogenates (mixed development stages) while our data were based on individual marine calanoid copepods of CV or adult developmental stages. Moreover, even if lipid content is considerable in all copepod species, their fatty acid constituents are not necessarily the same chemically. For example, lipids in freshwater calanoid copepods analyzed in Smyntek et al. (2007) were mainly triacylglycerols (Ventura, 2006) while wax esters dominate in marine *Calanus* spp. (Lee *et al.*, 2006; Ventura, 2006). Further, forms of fatty acids have been shown to differ in $\delta^{13}C$ by up to 10‰ (Abrajano *et al.*, 1994). In this study, adults had significantly lower $\%Lip_{Ao/Ap}$, which represents a lower content of wax esters than CVs. Thus, the relative difference in the abundance of fatty acids types in these development stages could help explain observed differences in the effectiveness of applied correction factors.

Mathematical correction of $\delta^{13}C$ for lipid influence could be an interesting alternative of lipid extraction when the $\delta^{15}N$ have to be considered as well. However, considering differences in lipid content and correction effectiveness between stages and species, lipid extraction should always be performed prior to the mathematical corrections to evaluate equations parameters (lipid percentages, D values and C:N ratios) specific to the analysis level (development stage, species, genus, taxa, etc).

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CHAPITRE 2
ÉVALUATION DE LA SOURCE DES ESPÈCES DE *CALANUS* D'UNE AIRE MARINE PROTÉGÉE À L'AIDE DE MARQUEURS ISOTOPIQUES : APPLIQUÉ À *C. FINMARCHICUS* ET *C. HYPERBOREUS* DANS LE PARC MARIN DU SAGUENAY-SAINT-LAURENT

2.1 RÉSUMÉ EN FRANÇAIS DU DEUXIÈME ARTICLE

Le Parc Marin du Saguenay-St-Laurent (PMSSL) est une région supportant une grande biodiversité et une forte abondance d'organismes zooplanctoniques. La connectivité entre les populations de zooplancton du PMSSL est des régions autour fut étudiée pour *Calanus finmarchicus* et *C. hyperboreus*. Les stades copépodites V (CV) furent échantillonnés en eaux de surface (0-100 m) et profondes (>100 m), respectivement lors de leur phase active et de diapause. Les échantillons ont été recueillis en juillet 2009 à l'intérieur du PMSSL ainsi que dans les régions de source potentielles du système du St-Laurent. Une Fonction d'Analyse Discriminante Quadratique (FADQ) a été produite pour discriminer les origines des CVs de chaque espèce en diapause dans les eaux profondes. L'algorithme de classification produite à partir de la FADQ basée sur 2 variables reconnues comme étant conservatives dans le temps lorsque les lipides sont extraits ($\delta^{13}C$, %C) (Perrin *et al.*, 2012) fut utilisée pour prédire la région d'origine la plus probable des CVs échantillonnés dans le PMSSL à la fin de leur période d'hivernation (mai 2010). Les résultats suggèrent qu'environ 23% de la population de *Calanus* spp. échantillonnée dans le PMSSL à la fin du printemps 2010 était originaire du fjord du Saguenay alors que les autres provenaient de régions extérieures au PMSSL telles que l'estuaire maritime du St-Laurent et même plus loin à l'est dans le nord-ouest du golfe du St-Laurent. Ces résultats révèlent une grande connectivité entre les systèmes du Saguenay et du St-Laurent ainsi qu'un potentiel de recrutement local *Calanus* spp. à l'intérieur du fjord du Saguenay. L'étude

démontre l'efficacité du $\delta^{13}C$ en tant que marqueur pour déterminer l'origine d'espèces de *Calanus* hivernant durant une période relativement longue sans se nourrir leur permettant de conserver leur signature isotopique.

Mots clés : *Calanus*, $\delta^{13}C$, origine, connectivité, circulation, Parc Marin du Saguenay-St-Laurent.

Cet article fut corédigé par moi-même, mon directeur Stéphane Plourde, le professeur Claudio DiBacco, ma codirectrice Gesche Winkler ainsi que le professeur Pascal Sirois. Il sera soumis prochainement à la revue *Journal of Plankton Research* sous le titre «Assessing stable isotopic markers to source *Calanus* species origins in a Marine Protected Area: Application to *C. finmarchicus* and *C. hyperboreus* in the Saguenay-St. Lawrence Marine Park (Québec, Canada)». Ma contribution en tant que premier auteur fut l'essentiel de l'échantillonnage dans l'estuaire maritime et le nord-ouest du golfe du St-Laurent en 2009 et dans le PMSSL en 2010, de la préparation des échantillons pour les dosages isotopiques, de l'analyse des données ainsi que de la rédaction. L'échantillonnage des régions du PMSSL en 2009 fut réalisé par l'équipe du Dr. Pascal Sirois. L'idée originale du projet découle du programme Canadian Healthy Ocean Network (CHONe) sous le thème de la connectivité des populations, et fut crée par les coauteurs Dr. Stéphane Plourde, Dr. Claudio DiBacco et Dr. Pascal Sirois. Dr. Gesche Winkler ainsi que les autres coauteurs cités plus tôt on contribués à la révision de cet article. Les résultats de cette étude ont été présentés dans le cadre du *Canadian Healthy Ocean Network (CHONe) Meeting* à Montréal (Québec, Canada) en avril 2011 ainsi que lors de la *10^{ième} Assemblée Générale Annuelle de Québec-Océans* au Lac Delage (Québec, Canada) en novembre 2011.

2.2 Assessing stable isotopic markers to source *Calanus* species origins in a Marine Protected Area: Application to *C. finmarchicus* and *C. hyperboreus* in the Saguenay-St. Lawrence Marine Park (Quebec, Canada)

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2.2.1 ABSTRACT

The Saguenay-St. Lawrence Marine Park (SSLMP) is a region sustaining high biodiversity and abundance of zooplanktonic organisms. The connectivity between zooplankton populations within the SSLMP and surrounding areas was investigated for *Calanus finmarchicus* and *C. hyperboreus*. Stage V copepodite (CV) were sampled in surface (0-100 m) and deep waters (>100 m) during active and diapause phases, respectively. Samples were collected in July 2009 from the Marine Park as well as possible source regions in the St. Lawrence system. To discriminate origins of deep-dwelling diapausing CVs of both *Calanus* spp., a Quadratic Discriminant Function Analysis (QDFA) have been performed. Classification algorithm developed from QDFA based on 2 variables proved to be conservative with time when lipids were extracted ($\delta^{13}C$, %C) (Perrin *et al.*, 2012) was used to predict probable regions of origin for CVs sampled within the SSLMP at the end of their overwintering period (May 2010). Our results suggested that about 23 % of the *Calanus* spp. population sampled in SSLMP in late spring 2010 originated from the Saguenay Fjord while the remainder originated from regions outside the SSLMP, including the Lower St. Lawrence Estuary and likely further east in the Gulf of St. Lawrence. Our results revealed high connectivity across the Saguenay and the St. Lawrence systems as well as potential for significant local production and recruitment of *Calanus* spp within the Saguenay Fjord. This study revealed the effectiveness of using $\delta^{13}C$ as a marker in delineating the origin of *Calanus* species with relatively long non-feeding overwintering periods that were amenable to the conservation of isotopic signature.

Keywords: *Calanus*, $\delta^{13}C$, origin, connectivity, circulation, Saguenay-St. Lawrence Marine Park

2.2.2 INTRODUCTION

The Saguenay-St. Lawrence Marine Park (SSLMP) is a Marine Protected Area (MPA) primarily designed to protect representative habitat of the endangered St. Lawrence beluga whale population. This 1245 km^2 area encompasses three distinct oceanographic regions: the Saguenay Fjord, the brackish Upper St. Lawrence Estuary and the marine Lower St. Lawrence Estuary including the head of the Laurentian channel, a deep and continuous marine valley (>300 m) extending from the edge of the continental shelf and covering a large area of the Gulf of St. Lawrence (Fig. 7). The Marine Park is an important feeding ground for pelagic fishes, marine birds and whales, and the important biomass of zooplanktonic prey in the SSLMP are highly dependent on the complex water circulation of the region and surrounding areas. Comprehension of the bio-physical processes favouring the biodiversity richness and abundance is essential to support management efforts in the SSLMP. However, the connectivity between zooplankton populations within the Marine Park as well as with other potential source regions is unclear as our understanding of zooplankton exchange dynamics in the region is based on conceptual and coupled bio-physical models (Lavoie *et al.*, 2000; Plourde *et al.*, 2001; Sourisseau *et al.*, 2006; Simard, 2009; Maps *et al.*, 2011).

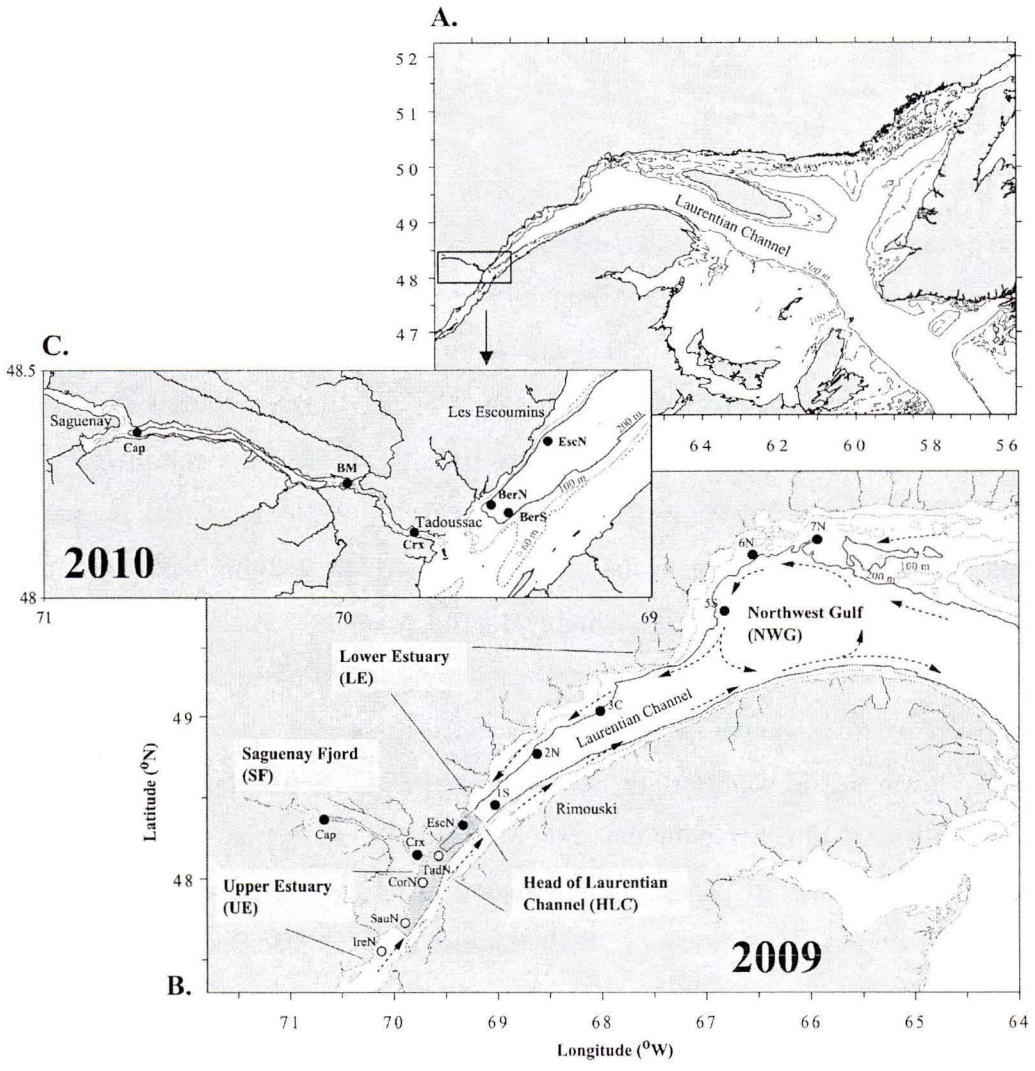

Figure 7: Map showing the St. Lawrence Estuary and Gulf (A), different stations and regions of the study area (Saguenay Fjord (SF), Head of the Laurentian Channel (HLC), Upper and Lower St. Lawrence Estuary (UE, LE) and Northwest Gulf of St. Lawrence (NWG)) in July 2009 (B) and sampling stations of the Saguenay St. Lawrence Marine Park (SSLMP) in May 2010 (C). Solid circles represent deep stations sampled in two layers (0-100 m, >100 m) and open circles stations sampled only in the surface layer, shallower than 100 m. The dark grey area represents boundaries of the SSLMP and dashed lines arrows resume the general circulation of the water column (adapted from Saucier et al. 2003, 2009)

The two-layer estuarine circulation in the St. Lawrence system combines with the diel and seasonal vertical migrations of zooplankton has been hypothesized to favour the transport and aggregation of deep dwelling zooplankton as *Calanus* spp. at the head of the Laurentian Channel in the SSLMP (Runge & Simard, 1990; Zakardjian *et al.*, 1999; Lavoie *et al.*, 2000; Sourisseau *et al.*, 2006; Simard, 2009; Maps *et al.*, 2011). The similarity in zooplankton species composition among different regions of the SSLMP and recurrent advection of deep water from the deep Channel Head region over the shallow sills (<20 m) prompted the mostly accepted concept that the head of the Laurentian Channel represent the main source of *Calanus* species to the Saguenay Fjord and the Upper Estuary (Rainville, 1979; De Ladurantaye *et al.*, 1984; Laprise & Dodson, 1994; Lavoie *et al.*, 2000; Saucier & Chassé, 2000; Bélanger, 2003; Saucier *et al.*, 2009). This concept proposes that *Calanus* species would be a 'non-resident' species advected in the Saguenay fjord in contrast to smaller neritic and 'resident' estuarine species (Runge & Simard, 1990). However, the observation of early development stages of C. finmarchicus at the upstream end of the Saguenay Fjord in summer suggests potential for local production and recruitment of large marine zooplankton (Rainville, 1979).

Since *Calanus finmarchicus* and *C. hyperboreus* are key components of the zooplankton population in the Saguenay and St. Lawrence systems (Runge & Simard, 1990; Plourde *et al.*, 2002; Harvey & Devine, 2009), they were chosen to test if stable isotopes could be used to determine potential sources of zooplankton in the MPA. This idea is based on the geographic variability in ^{13}C:^{12}C of representative carbon sources along the axial gradient of the estuary and its imprint on organisms feeding on it (Fry, 1981; Deegan & Garritt, 1997; Perry *et al.*, 1999). The St. Lawrence system encompasses a wide range of environmental conditions. The Saguenay Fjord and the Upper St. Lawrence Estuary are highly influenced by freshwater runoff, which supplies these regions with carbon from terrestrial and anthropogenic origins (Martineau *et al.*, 2004; Tremblay & Gagné, 2009). Therefore, these upstream regions could have a different stable isotopic ratio ($\delta^{13}C$) of the carbon source compare to more marine regions characterized by higher salinity with lower terrestrial carbon loads (Tan & Strain, 1979; Tremblay & Gagné, 2009). Considering the

anticipated spatial variability of the $\delta^{13}C$ in the food source of *Calanus* spp. in the St. Lawrence system, the isotopic composition of copepods is likely to reflect these differences between regions. *Calanus* species could acquire a region-specific isotopic signature during their active growth phase in the surface layer (0-100m) in spring and summer. Afterward, they enter diapause as deep-dwelling (>100 m) non-feeding late stage copepodite in early (*C. hyperboreus*) and late summer (*C. finmarchicus*) until the following spring (Conover, 1988; Plourde *et al.*, 2001; Falk-Petersen *et al.*, 2003, 2009). These non-feeding deep-dwelling *Calanus* spp. are subjected to prevailing deep upstream circulation in the Laurentian channel toward the SSLMP, representing potential transport of several hundreds of kilometres (Sourisseau et al. 2006; Maps et al. 2011). A laboratory study has shown that non-feeding diapausing CVs of *C. finmarchicus* and *C. hyperboreus* captured in early fall conserved their $\delta^{13}C$ signature in their lipid extracted body structures over 4 months (Perrin *et al.*, 2012). This study indicated that the signature at the time of the entry in diapause is conservative, confirming the potential of using stable isotopes in determining origins of *Calanus* spp. overwintering population in the SSLMP.

The aim of this study was to use conservative markers (e.g., $\delta^{13}C$) to evaluate if *Calanus* spp. in the SSLMP originate from local or external sources to help better define zooplankton connectivity in the Marine Park. We hypothesized that the conservative $\delta^{13}C$ of *Calanus* spp. would differ among sub-regions of the St. Lawrence system and the SSLMP in response to variable environmental conditions and therefore useful to discriminate among different sources/origins of diapausing *Calanus* spp. in the SSLMP the proceeding spring. In order to achieve our main objective, the spatial characteristics of the St. Lawrence system in late summer 2009 was described based on temperature and salinity and different variables including $\delta^{13}C$, $\delta^{15}N$, lipid-extracted dry weight (DW_{LE}), percent carbon (%C) and lipid content ($\%Lip_{DW}$) measured on active (0-100 m) and diapausing (> 100 m) CVs of *C. finmarchicus* and *C. hyperboreus*. Then, diapausing CVs of both species sampled in the SSLMP at the end of their overwintering period (spring 2010) were assigned to their most probable region of origin using conservative variables, which included $\delta^{13}C$ and %C. Considering the general water circulation in the Saguenay and the

St. Lawrence systems, we proposed that the population of *Calanus* spp. in the SSLMP originated from local and external sources as far away as the eastern Gulf of St. Lawrence.

2.2.3 METHOD

2.2.3.1 Study site

The stations distribution and the study area were determined to cover major potential sources regions of *Calanus* spp. to the SSLMP (Fig. 7). The two-layer estuarine circulation typical of the Gulf of St. Lawrence is characterized by upstream transport of Cold Intermediate Layer (CIL; 30-100 m) and deep Atlantic waters from the Gulf to the head of the Laurentian channel in the Lower St. Lawrence Estuary (Koutitonsky & Bugden, 1991). General circulation in the St. Lawrence system is cyclonic and upstream deep water currents (0.01-0.02 m s^{-1}) mostly restricted along the northern flank of the Laurentian Channel (Saucier *et al.*, 2003, 2009). This circulation likely represents the predominant pathway for the transport and supply of deep-dwelling zooplankton as *Calanus* spp. to the SSLMP from eastern downstream sources (Plourde *et al.*, 2001; Sourisseau *et al.*, 2006; Simard, 2009). The study area included the SSLMP region comprising the Saguenay Fjord (SF), the Upper Estuary (UE) and the Head of the Laurentian Channel (HLC) as well as the Lower St. Lawrence Estuary (LE) and the northwest Gulf of St-Lawrence (NWG) (Fig. 7).

2.2.3.2 Sampling

In 2009, sampling was carried out between July 18th and 25th at 13 stations on the Research Vessel BOREALIS (Université du Québec à Chicoutimi) in the SF and in the UE, the RV ALLIANCE (Marine Park) at the HLC and the RV FREDERICK G. CREED (Fisheries and Oceans Canada) in the LE and NWG (Fig. 7). The zooplankton sampling

strategy aimed at separating actively growing *Calanus* spp. mainly occurring in the upper 100 m of the water column and diapausing individuals at depths greater than 100 m (Plourde *et al.*, 2001, 2003). Each station sampled consisted of a Seabird CTD profile from bottom to surface and a stratified vertical tow for zooplankton (0-100 m and >100 m, 0.75 m diameter and 202 µm mesh plankton net equipped with an opening-closing device). On May 11th 2010, deep-dwelling, diapausing CV *Calanus* spp. (>125m) were collected at 6 stations on the RV BOREALIS to identify *Calanus* spp. sources in deep waters of the marine park (SF and HLC). All zooplankton samples were frozen in liquid nitrogen or on dry ice immediately after collection and stored at -80°C until analyzed.

2.2.3.3 Laboratory analysis

Zooplankton frozen samples were gently soaked in cold filtered sea water allowing the sorting of individual CVs of *C. finmarchicus* and *C. hyperboreus* within a few minutes. Only *C. finmarchicus* CVs smaller than 2.9 mm in prosome length were selected as this size criterion minimizes the potential for identification errors with *C. glacialis* (Parent *et al.*, 2011). A preliminary analysis showed that twelve individuals were sufficient to minimize the within station variability in CV characteristics and was therefore targeted for the sorting of CVs of both species from deep layer samples. Three replicates of 1 to 10 CVs, depending on their availability in the sample, were prepared to represent the isotopic signature of active CVs in surface waters (0-100 m). CVs were rinsed in millipore water, lyophilised for 48 h in a pre-weighed 5 x 9 mm tin capsule and weighed using a microscale (Mettler MT5; ± 0.001mg). Lipids were extracted to minimize their influence on the isotopic signature associated with depleted $\delta^{13}C$ values in lipids (DeNiro & Epstein, 1977; McConnaughey & McRoy, 1979; Post *et al.*, 2007) and their proportion of total weight that may vary among individuals (Kattner *et al.*, 1989; Søreide *et al.*, 2008). Animals were individually soaked in a chloroform:methanol (2:1) solution in air saturated with chloroform:methanol vapours to reduce evaporation of the solution. The technique was based on Bligh and Dyer's (1959) method adapted to the Ohman's (1988) passive

procedure since avoiding homogenisation prevented material loss for small samples (individuals). After 24 h, copepods were rinsed twice with the chloroform: methanol (2:1) solution and then twice with millipore water before being placed in a pre-weighed 5 x 9 mm tin capsule and lyophilised again for 48 h. Dry samples were reweighed and maintained in a dessicator until isotope analysis. Percent lipid content of individuals ($\%Lip_{DW}$) was estimated as dry weight difference before (DW) and after lipid extraction (DW_{LE}).

Stable isotope analyses were performed on a Thermo Electron Delta Plus XP isotope ratio mass spectrometer interfaced to a Costech ECS4010 Elemental Analyzer via a Conflo III (University of New Hampshire, Stable Isotope Laboratory). The isotopic composition (δX) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) was expressed in parts per thousand (‰) relative to C and N standards as the relative proportion of ^{13}C to ^{12}C or ^{15}N to ^{14}N:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] * 1000$$

where R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the sample and a standard (Peterson & Fry, 1987). Standard for $\delta^{13}C$ was Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen for $\delta^{15}N$. Copepods from deep water samples (>100 m) were analysed individually (17.0 to 446.0 µg) and analyses of surface samples (0-100 m) was on a maximum of 200 µg of homogenized pooled CVs.

2.2.3.4 Data analysis

Salinity was used as an indicator of the level of terrestrial influence in the region in summer 2009, a key factor in determining $\delta^{13}C$. Salinity and temperature data at each station were averaged in the 0 to 10 m depth layer and plotted in a T-S diagram to visually identify regions with potentially distinct $\delta^{13}C$ signature (Fig. 8). Four sample regions were selected based on a surface (0-10 m) salinity gradient that increased from the (i) Saguenay

Fjord (SF), (ii) Upper St-Lawrence Estuary (UE), Lower St-Lawrence Estuary (LE) including the (iii) Head of the Laurentian Channel (HLC) and the salty (iv) Northwest Gulf of St-Lawrence (NWG). The HLC was considered as a distinct region since it is situated within the boundary of the SSLMP (Fig. 1B). These regions were used as *a priori* grouped stations to discriminate the origin of deep-dwelling *Calanus* spp. CVs sampled in 2009 in the following analyses.

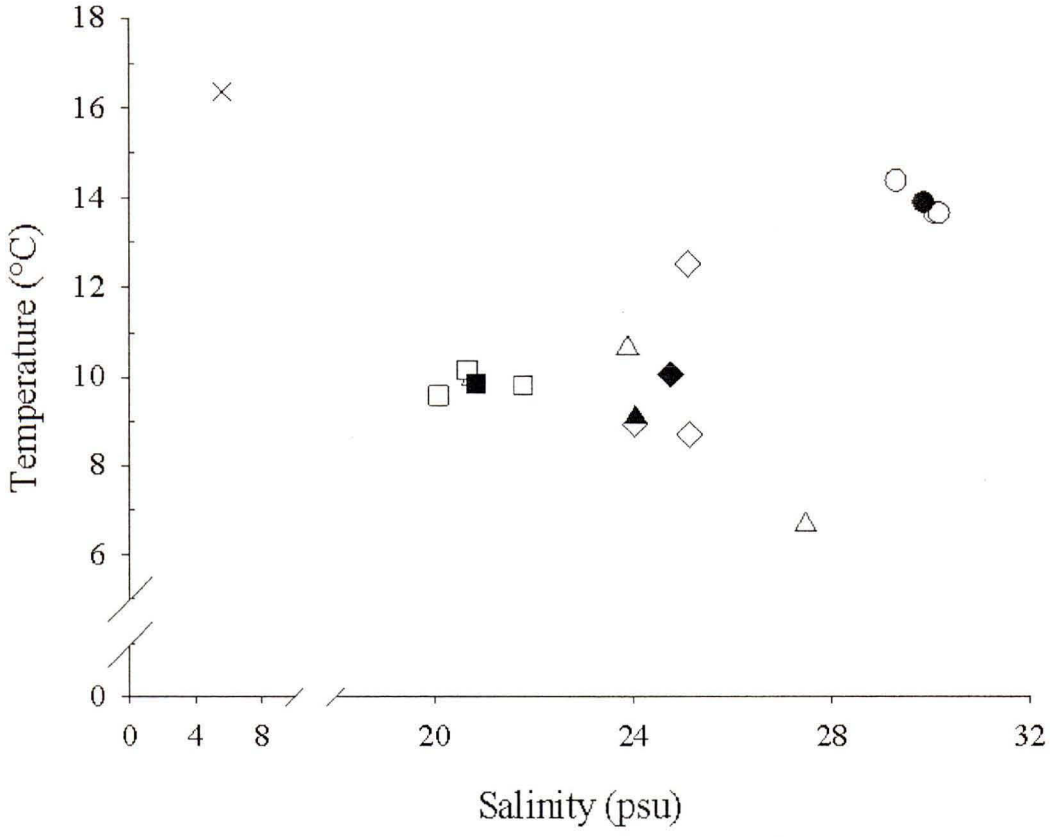

Figure 8: Diagram of mean temperature and salinity of the 10 m top layer (SE ≤ 0.2) of each station (open symbols) sampled in July 2009 including overall means (solid symbol) of different regions (Saguenay Fjord (×), Upper (□) and Lower (◊) St. Lawrence Estuary, Head of Laurentian channel (Δ) and Northwest Gulf of St. Lawrence (○)) identified based on environmental data (temperature and salinity)

One way ANOVAs were performed for each reliable variable measured on deep-dwelling CVs in 2009 ($\delta^{13}C$, $\delta^{15}N$, %C, DW, DW_{LE} and $\%Lip_{DW}$) to test differences between regions of the study area. In order to compare the $\delta^{13}C$ of active surface-dwelling and overwintering deep-dwelling CVs between depth and among regions, the analysis was performed between regions including the shallow UE; deep and surface layers in each region were considered separately. Every analysis of variance was followed by *a posteriori* HSD Tukey's test. Nitrogen weight, nitrogen percentage and C:N ratio were not included in the analyses because of low accuracy due to frequent critically low nitrogen content in samples (<30 µg dry weight). These statistical tests were carried out with JMP (vers. 7, SAS Institute).

A Quadratic Discriminant Function Analysis (QDFA) with Jackknifed validations were performed with SYSTAT (vers. 13) to classify deep-dwelling (>100 m) individual *C. finmarchicus* and *C. hyperboreus* in the different regions based on individual characteristics (variables measured on CVs). DW of individuals before lipid extraction was not included in these analyses since DW is dependant of DW_{LE} and $\%Lip_{DW}$. Data collected in the 0-100 m water layer, and therefore in the shallow UE, were not included in these analyses due to animal pooling and because these data did not represent characteristics of the diapausing component of the population. We analysed data following three different steps aiming at optimizing the classification success of individuals and to compare if the QDFA conducted with a maximum of variable is more effective than QDFA using only two conservative variables during the overwintering period (Perrin *et al.*, 2012), $\delta^{13}C$ and %C. First, the QDFA analysis considered all reliable variables ($\delta^{13}C$, $\delta^{15}N$, %C, DW_{LE} and $\%Lip_{DW}$). Second, a QDFA with Jackknifed validations was conducted using only $\delta^{13}C$ and %C. Because QDFAs are multivariate analysis, we included the %C even if this variable did not show significant differences between regions (see results section). Third, the analysis was rerun with conservative variables ($\delta^{13}C$ and %C) and after grouping some regions characterized by low classification success due to reciprocal misclassifications.

These analysis using only conservative variables was necessary to use the QDFA for CVs at the end of their overwintering to make sure that these markers will represent their original values. Pillai's trace was used to test the null hypothesis for QDFA analysis. Variance of each variable was tested for normality with Shapiro-Wilk's test and for homogeneity via visual evaluation of residual distributions. Grouped regions and assignment algorithms from the last QDFA were used to classify animals collected in the SSLMP at the end of their overwintering period (i.e., May 2010, see below).

Deep-dwelling *C. finmarchicus* and *C. hyperboreus* CVs sampled in the SSLMP in May 2010 were assigned to their potential regions of origin using the QDFA produced with conservative variables ($\delta^{13}C$ and %C) and newly grouped regions. The relative contribution of different origin in each station in the Marine Park in 2010 was evaluated using the Mahalanobis distances (Mahalanobis, 1936) where the highest p-value represents the highest probability of region membership for each individual.

2.2.4 RESULTS

2.2.4.1 Spatial characterization of the study area in July 2009

The July 2009 sampling period preceded peak abundance of diapausing CV *C. finmarchicus* in deep waters (Fig. 9 A). However, the relatively equal proportion of *C. finmarchicus* CVs in surface (0-100m) and deep (100-320 m) layers indicated that we sampled this species at the beginning of the entry into diapause (Fig. 9 B). In contrast, nearly 100% of *C. hyperboreus* CVs were in the deep layer, indicating that the population was already in diapause (Fig. 9 D). In 2010, field sampling was accomplished on May 11th, which corresponded with the end of the overwintering (diapausing) period and resulted in low sample densities of deep-dwelling CVs of both species in central LE (Fig. 9).

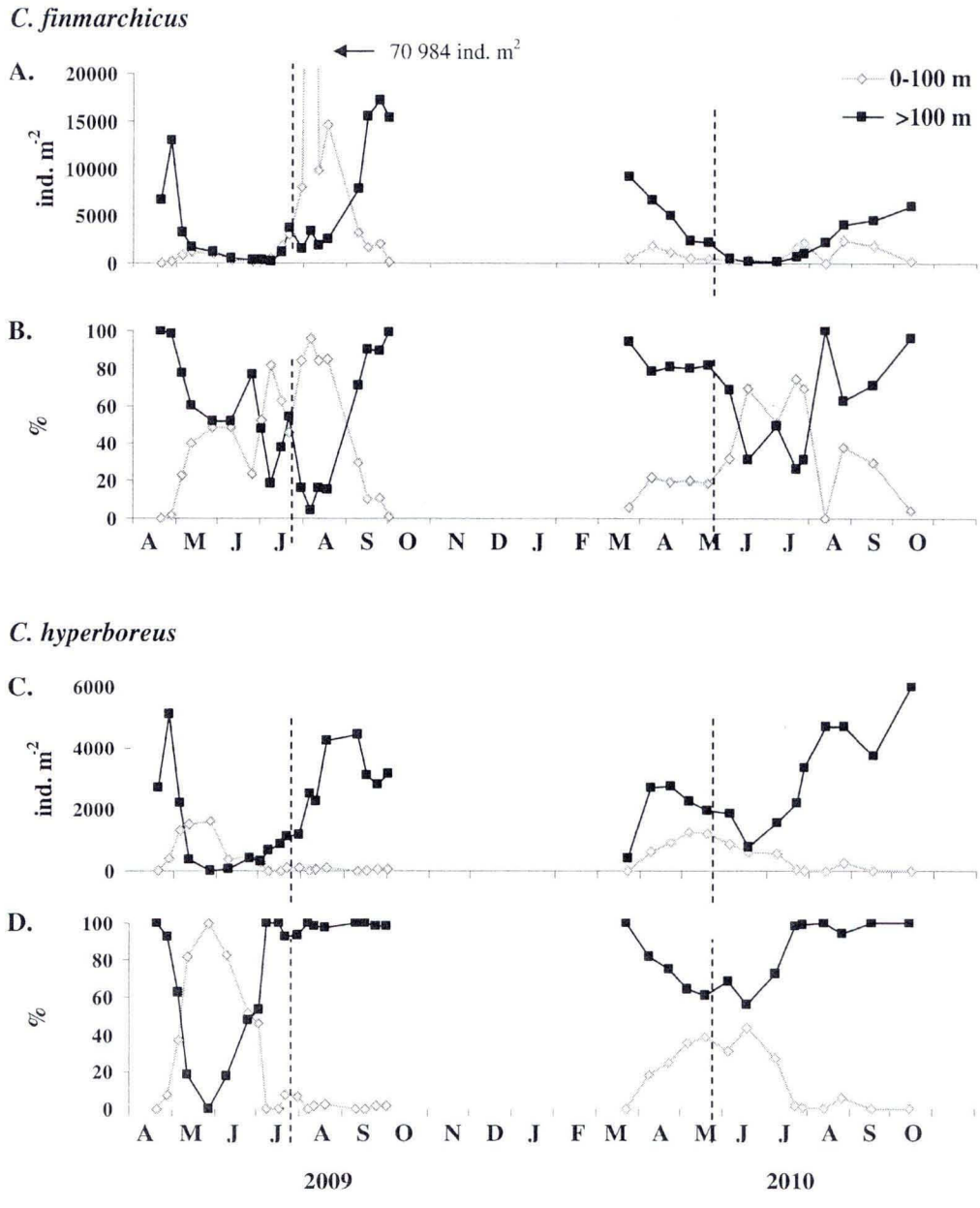

Figure 9: Abundance and proportion of *C. finmarchicus* (A, B) and *C. hyperboreus* (B, C) stage CV sampled in 2009 and 2010, in surface (0-100 m) and depth (>100 m), at a fixed station near Rimouski (48°40N, 68°35W) located in the middle of the Laurentian Channel (340m) in the Lower St. Lawrence Estuary (Québec, Canada) (Plourde *et al.* unpublished data). Dotted lines: sampling events

Region-specific averaged values and significance of comparisons among regions for each variables ($\delta^{13}C$, $\delta^{15}N$, %C, DW, DW_{LE} and $\%Lip_{DW}$) measured on individual *Calanus* spp. CV collected in the deep layer and $\delta^{13}C$ in surface layer are presented in Table 4. There were significant differences in $\delta^{13}C$ between depth and regions for *C. finmarchicus*. The post-hoc Tukey's HSD test revealed significant differences in $\delta^{13}C$ among regions and between surface and depth in the NWG but not in the SF, the HLC and the LE. The $\delta^{13}C$ in the shallow UE was not significantly different with the $\delta^{13}C$ in surface and depth of the HLC and the LE. For *C. hyperboreus* CV, the $\delta^{13}C$ in surface and depth was similar in each region but there was significant difference between regions (Table 4). The percentage of carbon (%C) of lipid extracted animals was the only variable similar across the study area, reflecting the constant contribution of carbon to the structural weight (DW_{LE}). DW and DW_{LE} of *C. finmarchicus* and *C. hyperboreus* were generally lower in the SF and HLC, with *C. finmarchicus* showing a particular small size (DW_{LE}) in HLC (Table 4).

Table 4: Measured variables (mean ± standard error; stable isotope signature of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), carbon percentage (%C), dry weight (DW), lipid extracted dry weight (DW_{LE}) and lipid percentage calculated from dry weight before and after extraction ($\%Lip_{DW}$)) on deep-dwelling (>100 m) and $\delta^{13}C$ on surface-dwelling (0-100 m) *C. finmarchicus* (A) and *C. hyperboreus* (B) CVs in different regions of the study area in July 2009 (the Upper Estuary (UE), the Saguenay Fjord (SF), the Head of the Laurentian Channel (HLC), the Lower St. Lawrence Estuary (LE) and the Northwest Gulf of St. Lawrence (NWG)) and overall for 2009 and the Saguenay St. Lawrence Marine Park in May 2010. Results of one way ANOVAs among regions in July 2009 are shown for each variable (surface and depth together for $\delta^{13}C$), values associated to different letters (columns) are significantly different (HSD Tukey's test, $p > 0.05$)

A. *C. finmarchicus*

	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	%C	DW (µg)	DW_{LE} (µg)	$\%Lip_{DW}$
	0-100 m	>100 m	>100 m	>100 m	>100 m	>100 m	>100 m
2009							
UE	-18.7 ± 0.1 A						
SF	-21.5 ± 0.6 BC	-23.1 ± 0.5 C	9.3 ± 0.2 A	47.9 ± 0.3 A	287.9 ± 16.7 A	77.5 ± 3.6 A	72.7 ± 1.1 B
HLC	-19.2 ± 0.1 A	-19.1 ± 0.1 A	8.0 ± 0.2 B	46.1 ± 2.4 A	154.6 ± 11.1 B	35.3 ± 2.2 B	75.9 ± 1.5 A
LE	-18.9 ± 0.1 A	-19.1 ± 0.1 A	9.1 ± 0.2 A	44.6 ± 0.4 A	292.4 ± 28.3 A	67.0 ± 7.7 A	76.9 ± 0.8 A
NWG	-22.0 ± 0.2 C	-20.6 ± 0.1 B	9.3 ± 0.2 A	46.3 ± 0.3 A	281.9 ± 26.8 A	80.0 ± 8.8 A	71.9 ± 1.1 B
	$F_{8,138} = 34.96$		$F_{3,104} = 13.8$	$F_{3,104} = 1.2$	$F_{3,104} = 5.3$	$F_{3,104} = 6.8$	$F_{3,104} = 5.7$
	$p < 0.0001$		$p < 0.0001$	$p = 0.3028$	$p = 0.0019$	$p = 0.0003$	$p = 0.0012$
Overall							
2009		-20.1 ± 0.1	9.0 ± 0.1	45.9 ± 0.5	258.5 ± 14.5	65.8 ± 4.3	74.6 ± 0.6
2010		-20.5 ± 0.1	10.2 ± 0.1	47.6 ± 0.2	223.8 ± 9.7	72.3 ± 3.1	66.6 ± 1.0

B. *C. hyperboreus*

	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	%C	DW (µg)	DW_{LE} (µg)	$\%Lip_{DW}$
	0-100 m	>100 m	>100 m	>100 m	>100 m	>100 m	>100 m
2009							
UE	-18.8 ± 0.2 A						
SF	-20.9 ± 0.5 BC	-20.8 ± 0.5 B	9.4 ± 0.2 AB	48.7 ± 0.2 A	466.6 ± 39.5 C	119.6 ± 12.8 C	73.7 ± 2.1 A
HLC	-19.2 ± 1.2 ABC	-19.5 ± 0.2 AC	9.0 ± 0.2 B	47.8 ± 0.5 A	441.9 ± 29.9 C	159.8 ± 15.9 C	63.8 ± 2.3 B
LE	-19.5 ± 0.5 ABC	-19.2 ± 0.1 A	9.4 ± 0.1 A	48.3 ± 0.3 A	1058.3 ± 56.8 B	237.7 ± 14.9 B	77.6 ± 1.0 A
NWG	-19.8 ± 0.2 ABC	-19.3 ± 0.1 AC	9.6 ± 0.1 A	48.3 ± 0.1 A	1234.0 ± 56.2 A	287.6 ± 13.7 A	76.5 ± 0.7 A
	$F_{8,113} = 6.54$		$F_{3,92} = 3.2$	$F_{3,92} = 0.7$	$F_{3,92} = 35.4$	$F_{3,92} = 15.5$	$F_{3,92} = 13.8$
	$p < 0.0001$		$p = 0.0275$	$p = 0.5781$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Overall							
2009		-19.5 ± 0.1	9.4 ± 0.1	48.3 ± 0.1	969.5 ± 44.1	231.2 ± 09.9	74.9 ± 0.7
2010		-19.9 ± 0.1	9.3 ± 0.1	48.0 ± 0.1	646.6 ± 41.6	160.3 ± 10.0	74.5 ± 0.8

2.2.4.2 Origin discrimination of *Calanus* spp. in summer 2009

C. finmarchicus – The Jackknifed validation of the classification matrix showed that the QDFA conducted with 5 variables ($\delta^{13}C$, $\delta^{15}N$, %C, DW_{LE} and $\%Lip_{DW}$; Table 5 A) correctly discriminated the origins of 70% of animals tested on average, with maximum and minimum classification success of 91% vs. 47% in the SF and the LE respectively (Table 5 A). Misclassified animals in the LE were distributed almost equally between HLC and NWG, but not in SF (Table 5 A). The classification success decreased by only 2% when using only $\delta^{13}C$ and %C, the two conservative variables with time (Table 5 B). The LE and the HLC showed lower assignment success (58% and 52% respectively) with a high proportion of reciprocal misclassification (Table 5 B), suggesting a high degree of exchange or homogeneity of carbon signatures between these two regions. This was not surprising since the HLC constitutes a sub-region of the LE based on bathymetry, circulation and surface salinity (Saucier *et al.*, 2009) (Fig. 7, Fig. 8). We therefore combined these 2 regions (HLC/LE) and recalculated classification matrices, which improved our overall classification success from 70 % to of 84 % and with similar classification success among regions (Table 5 C).

C. hyperboreus – The classification matrix with Jackknifed validations conducted with the 5 variables resulted in an overall classification success of 67% for *C. hyperboreus* CVs (Table 6 A). The lowest assignment success (54%) occurred with the LE animals, whereas about 75% of individuals were correctly assigned in other regions. Reciprocal errors with the NWG explained most the misclassification in the LE (Table 6 A). When only $\delta^{13}C$ and %C were employed, the overall classification success decreased by 2 % (to 52%) with a relatively high classification success in the SF (67%) and NWG (71%) and a much lower success in the HLC (25 %) and LE (37%) regions (Table 6 B). The overall assignment accuracy was optimized at 81 % after grouping the NWG, LE and HLC regions based on the reciprocal classification error (Table 6 B) leading to 2 remaining regions, the SF and the St. Lawrence (SL) (Table 6 C).

Table 5: Jackknifed classification matrices from the QDFA model conducted with (A) 5 measured variables on *C. finmarchicus* ($\delta^{13}C$, $\delta^{15}N$, %C, dry weight (DW) and $\%Lip_{DW}$) and (B) 2 conservatives variables ($\delta^{13}C$ and %C) in 4 geographic regions (the Saguenay Fjord (SF), the Head of the Laurentian Channel (HLC), the Lower St. Lawrence Estuary (LE) and the Northwest Gulf of St. Lawrence (NWG)) and (C) with 2 conservatives variables ($\delta^{13}C$ and %C) in newly grouped regions. Pillai's trace results below tables

C. finmarchicus

A. 5 variables

Origin	N	Classified region				% correct
		SF	HLC	LE	NWG	
SF	11	10	0	0	1	91
HLC	23	0	17	5	1	74
LE	36	0	10	17	9	47
NWG	36	1	1	4	30	83
Total	106	11	28	26	41	70

$F_{15,300} = 9.9$; $p < 0.0001$

B. 2 conservative variables

Origin	N	Classified region				% correct
		SF	HLC	LE	NWG	
SF	11	9	0	0	2	82
HLC	23	0	12	11	0	52
LE	36	0	6	21	9	58
NWG	36	2	0	4	30	83
Total	106	11	18	36	41	68

$F_{6,204} = 17.9$; $p < 0.0001$

C. Grouped regions (2 variables)

Origin	N	Classified region			% correct
		SF	HLC/LE	NWG	
SF	11	9	0	2	82
HLC/LE	59	0	48	11	81
NWG	36	2	2	32	89
Total	106	11	50	45	84

$F_{4,206} = 26.6$; $p < 0.0001$

Table 6: Jackknifed classification matrices from the QDFA model conducted with (A) 5 measured variables on *C. hyperboreus* ($\delta^{13}C$, $\delta^{15}N$, %C, dry weight (DW) and $\%Lip_{DW}$) and (B) 2 conservatives variables ($\delta^{13}C$ and %C) in 4 geographic regions (the Saguenay Fjord (SF), the Head of the Laurentian Channel (HLC), the Lower St. Lawrence Estuary (LE) and the Northwest Gulf of St. Lawrence (NWG)) and (C) with 2 conservatives variables ($\delta^{13}C$ and %C) in newly grouped regions (SF and St. Lawrence (SL)). Pillai's trace results below tables

C. hyperboreus

A. 5 variables

Origin	N	Classified region				% correct
		SF	HLC	LE	NWG	
SF	12	9	3	0	0	75
HLC	12	2	9	1	0	75
LE	35	1	2	19	13	54
NWG	35	0	0	9	26	74
Total	94	12	14	29	39	67

$F_{15,264} = 11.4$; $p < 0.0001$

B. 2 conservative variables

Origin	N	Classified region				% correct
		SF	HLC	LE	NWG	
SF	12	8	1	1	2	67
HLC	12	2	3	2	5	25
LE	35	3	8	13	11	37
NWG	35	0	7	3	25	71
Total	94	13	19	19	43	52

$F_{6,180} = 6.1$; $p < 0.0001$

C. Grouped regions (2 variables)

Origin	N	Classified region		% correct
		SF	SL	
SF	12	9	3	75
SL	82	15	67	82
Total	94	24	70	81

$F_{2,91} = 20.4$; $p < 0.0001$

2.2.4.3 Potential origin of deep-dwelling CV in the Marine Park in spring 2010

Last algorithms produced with $\delta^{13}C$ and %C in grouped regions was used to assign *Calanus* spp. sampled in May 2010 to their potential regions of origins. Our results showed that deep-dwelling CVs sampled at the end of the overwintering period within the SSLMP (SF and HLC sub-areas) likely originated from both local and external sources (Table 7). At the scale of the SSLMP, 23.6% of *C. finmarchicus* collected at the six stations sampled in the SSLMP (SF and HLC) were re-assigned (were similar) to the SF group characterized with July 2009 data, suggesting that 76% of the population originated from the HLC/LE region (23.6%) and the NWG (52.8%) (Table 7 A). The pattern for *C. hyperboreus* was highly similar (Table 7 B). At the scale of the SSLMP sub-regions, SF and HLC, our results suggest that a larger proportion (43.8%) of the *C. hyperboreus* population in the SF was produced and retained locally relative to *C. finmarchicus* (19.4%) (Table 7). Animals likely originating from the LE and NWG were observed in the SF, representing 56% (*C. hyperboreus*) and 80% (*C. finmarchicus*) of the population (Table 7). Likewise, *Calanus* spp. CVs ascribed to the SF were observed in the HLC region in spring 2010. The low station-based sample size precluded any analysis at a smaller scale.

Table 7: Relative contribution of deep-dwelling CV of *C. finmarchicus* (A) and *C. hyperboreus* (B) classified as originating from regions of the study area (the Saguenay Fjord (SF), the Head of the Laurentian Channel grouped with the Lower St. Lawrence Estuary (HLC/LE), the Northwest Gulf of St. Lawrence (NWG) or the entire St. Lawrence system (SL)) to the SSLMP population in May 2010, including its two sub-regions (SF and HLC) and results for each sampled stations (Cap, BM, Crx, BerN, BerS, EscN) (Fig. 7 C)

A. *C. finmarchicus*

		SF		HLC/LE		NWG		
	Station	**n**	**%**	**n**	**%**	**n**	**%**	**N**
SF	Cap	3	25.0	6	50.0	3	25.0	12
	BM	3	25.0	4	33.3	5	41.7	12
	Crx	1	8.3	2	16.7	9	75.0	12
	total	7	**19.4**	12	**33.3**	17	**47.2**	36
HLC	BerN	6	50.0	0	0.0	6	50.0	12
	BerS	1	8.3	2	16.7	9	75.0	12
	EscN	3	25.0	3	25.0	6	50.0	12
	total	10	**27.8**	5	**13.9**	21	**58.3**	36
SSLMP	all	17	**23.6**	17	**23.6**	38	**52.8**	72

B. *C. hyperboreus*

		SF		SL		
	Station	**n**	**%**	**n**	**%**	**N**
SF	Cap	3	100.0	0	0.0	3
	BM	4	50.0	4	50.0	8
	Crx	0	0.0	5	100.0	5
	total	7	**43.8**	9	**56.3**	16
HLC	BerN	0	0.0	12	100.0	12
	BerS	3	25.0	9	75.0	12
	EscN	2	16.7	10	83.3	12
	total	5	**13.9**	31	**86.1**	36
SSLMP	all	12	**23.1**	40	**76.9**	52

2.2.5 DISCUSSION

2.2.5.1 Spatial characterization and origin discrimination of *Calanus* spp. in July 2009

Determination of *a priori* sample regions for the QDFA based on the salinity gradient ultimately reflected areas with different $\delta^{13}C$ signatures for two *Calanus* species. Discrimination of *Calanus* spp. CV origins was mostly attributable to regional differences in their $\delta^{13}C$ signatures (Table 4), while the other conservative variable (i.e., %C) was similar between regions. Other variables differed somewhat among some regions (Table 4), but they contributed little to discriminating CV origins. The lack of predictability of $\delta^{15}N$, DW_{LE} and $\%Lip_{DW}$ was confirmed by the lack of contribution in the discriminatory power via the QDFA (Table 5 and 2.3). Additionally, their non-conservative nature during diapause (Perrin *et al.*, 2012) made them poor indicators of CV origin. DW and $\%Lip_{DW}$ of deep-dwelling (overwintering) CV *Calanus* spp. sampled in summer 2009 were lower than typically observed in the region. This was probably due to observed damage to the oil sac, while animals collected in spring 2010 had undamaged oil sac and showed characteristics $\%Lip_{DW}$ of late stage overwintering individuals (Plourde *et al.*, 2003; Maps *et al.*, 2010) (Table 4). Region-specific differences in DW and DW_{LE} could be related to spatial differences in the environmental growth condition (temperature, food availability: Campbell *et al.*, 2001) and in potential region-specific differences in the timing of arousal and entry in diapause (Johnson *et al.* ; 2008).

Differences in the $\delta^{13}C$ of CV *Calanus* spp. among regions could be related to biochemical and oceanographic processes across the study area. For both species in 2009, animals from the Saguenay Fjord (SF) had the most depleted $\delta^{13}C$ signature (Table 4). The low $\delta^{13}C$ signature might represent a greater proportion of terrestrial material via freshwater inputs (Fry & Sherr, 1984; Michener & Kaufman, 2007). The St-Lawrence Estuary receives freshwater from the Great Lakes, rivers and direct runoff from land. Sources of carbon originating from the marine environment are typically enriched in ^{13}C compared to

freshwater sources where terrestrial material is more abundant (Fry & Sherr, 1984; Michener & Kaufman, 2007). However, $\delta^{13}C$ signatures of *C. finmarchicus* in the Upper and Lower St. Lawrence Estuary (UE and HLC/LE) had higher values than in the Northwest Gulf (NWG) (Table 4 A) where water had more marine characteristics (higher salinity and lower temperature; Fig. 8). In the St. Lawrence Estuarine Transition Zone (ETZ) located upstream of the SSLMP, Martineau *et al.* (2004) estimated the $\delta^{13}C$ signature of phytoplankton (as in Riera & Richard, 1997) to be around -18 ‰ based on the stable isotopic signature of the dissolved inorganic carbon (Helie *et al.*, 2002). Moreover, Barnard *et al.* (2006) measured an average of -19‰ for *Thalassiosira* sp., an estuarine diatom found in the ETZ. The enriched $\delta^{13}C$ of the phytoplankton is therefore likely to influence the stable isotopic signatures downstream in the UE and the LE. High algal growth rates have been observed to increase the $\delta^{13}C$ signature of the carbon resource (i.e., dissolved inorganic carbon, phytoplankton) and its zooplankton consumers (Fry & Wainright, 1991; Laws *et al.*, 1995; Fry, 1996; Perry *et al.*, 1999). The LE is characterized by nutrient rich waters sustained by upwelling along the north coast of the LE and at the HLC, which support successive algal blooms during summer (Ingram & El-Sabh, 1990; Therriault *et al.*, 1990). The higher primary productivity in the HLC/LE throughout summer compared to regions in the gulf (Harvey & Starr, 2005) combine with enriched $\delta^{13}C$ signature of phytoplankton in the ETZ could help to explain why the $\delta^{13}C$ of *C. finmarchicus* was higher in the St. Lawrence Estuary (UE and LE) than in the NWG.

Species- and region-specific timing of diapause could have contributed to differences in classification assignments reported here for *Calanus* species (Fig. 9, Table 5 and 2.3). $\delta^{13}C$ signatures reported for *C. hyperboreus* CVs sampled from HLC/LE and NWG could not be discriminated as they were for *C. finmarchicus*. Since *C. hyperboreus* enters diapause earlier in the NWG than in the LE, a process occurring much earlier than in *C. finmarchicus* (Plourde *et al.*, 2001, 2003; Fig 2.3), diapausing *C. hyperboreus* CVs from the NWG may already have been transported to the estuary in deep waters, thereby influencing mean $\delta^{13}C$ signatures of deep-dwelling populations in the HLC/LE. The QDFA conducted with separated regions (Table 6 B) showed relatively high assignment success in

the NWG (71%) compared to the LE (37%) and the HLC (25%) where a high proportion of misclassifications were attributed to the NWG, likely reflecting the advection of deep-dwelling *C. hyperboreus* CVs from the NWG in St-Lawrence estuary. Such region-specific timing of entry into diapause could also explain part of the uncoupling between the $\delta^{13}C$ signatures of *C. finmarchicus* CVs in surface and depth in the SF and the NWG in late summer (Table 4 A).

2.2.5.2 Potential origin of deep-dwelling CV in the Marine Park in spring 2010

This study showed that in May 2010 the population of *Calanus* spp. in the SSLMP had multiple sources even if the sample period was late and not optimal for sampling the overwintering population. The arousal from diapause was well underway in both species as inferred from the relative abundance of CVs in the deep sample layer (Fig. 9). However, we are confident that our sampling strategy captured mainly overwintering animals that had initiated diapause the year before. DW and $\%Lip_{DW}$ was typical of CVs at the end of the overwintering period (Plourde *et al.*, 2003; Maps *et al.*, 2010) (Table 4). The increase in weight (and lipid content) of *C. hyperboreus* CVs recently moulted from overwintering CIV is observed in June with an entry in diapause in early July (Plourde *et al.*, 2003) while the production of the new cohort of *C. finmarchicus* occurs in response to the phytoplankton bloom in late June and early July with an entry in diapause in August (Plourde *et al.* 2001; Johnson *et al.* 2008). In addition to the timing of sampling, proportions of origin classification could not have been interpreted as an exact representation of the entire 2009 overwintering cohort because the sample sizes were relatively small and because similarities in *C. finmarchicus* $\delta^{13}C$ between surface and depth among the SF and NWG (Table 4 A) make re-assignment to population origin difficult. Nevertheless, the distribution of different origins was consistent with what could be expected, e.g. origin gradients inside the SF, important import of *Calanus* spp. from the HLC/LE and the NWG and possible recruitment of local CVs in the SF.

We found strong empirical evidence supporting a high connectivity among sub-areas of the LE-NWG region through the residual estuarine circulation. Our results have shown that the general circulation of the Saguenay and the St. Lawrence systems could be an important factor sustaining *Calanus* spp. populations in the SSLMP. For example, the high contribution of individuals attributed to the HLC, LE and NWG to the SSLMP *Calanus* spp. population implies an upstream transport up to 500 km in the deep waters of the Laurentian Channel during the overwintering period. Our data support previous field and modeling studies describing the St. Lawrence system as a *Calanus* pump which transport deep-dwelling copepodites stages upstream (mainly in the Cold Intermediate Layer (CIL) during summer and deeper in autumn and winter) while younger stages as eggs and nauplii are flushed downstream in surface waters (Plourde & Runge, 1993; Plourde *et al.*, 2001, 2002, 2003; Maps *et al.*, 2011).

In addition to the identification of distant locations as important sources supporting the *Calanus* spp. populations in the SSLMP, our study provided for the first time evidence for local recruitment of these marine species in the SF. Deep-dwelling CVs ascribed to the SF due to their distinct $\delta^{13}C$ signature represented an important component of the *Calanus* spp. CVs population in the SF but also in another region of the SSLMP, the HLC (Table 7). Previous studies suggested that marine *Calanus* spp. observed in the SF were most likely to originate from the LE, acting as 'drifting species' while more neritic and estuarine species such as *Eurytemora* spp. and *Acartia* spp. would be endemic to the SF (De Ladurantaye *et al.*, 1984; Rainville, 1979; Schafer *et al.*, 1990). However, these studies were not designed to detect local recruitment. A survey of zooplankton across the entire Saguenay Fjord from July to October 2004 showed relatively high abundance of early copepodite stages of *C. finmarchicus* and late overwintering stages of *C. hyperboreus* in the inner basin more than 170 km upstream of the fjord mouth (Plourde et al. unpublished data). The presence of early copepodites stages at the head of the fjord indicates local reproduction but also a high potential for local development and entry into diapause. Our results could not prove that *Calanus* spp. could complete its whole life cycle inside the fjord, however the significant difference of the $\delta^{13}C$ signature in the SF compare to adjacent estuarine regions combined

with observations of early development stages and accumulation of deep-dwelling *Calanus* spp. CVs in the upper part of the SF inner basin suggest local development and recruitment of *Calanus* spp..

2.2.5.3 $\delta^{13}C$ as a marker for origin tracking

The efficiency of $\delta^{13}C$ as a marker to track the origin of zooplankton was based on two main characteristics: the stability of the $\delta^{13}C$ signature of lipid extracted tissues in time and the spatial variability in $\delta^{13}C$ signatures of CVs within sampling regions of this study area. Although the laboratory experiments by Perrin's *et al.* (2012) lasted 4 months (Chapter 1), a period much shorter than the estimated duration of the overwintering period of 200-300 days in the region (Johnson *et al.*, 2008), we are confident that our experimental results showing a stability in $\delta^{13}C$ signatures over time were representative of the *in situ* dynamics. We recognize the shorter duration of the overwintering period in the laboratory reflected a higher metabolism due to experimental conditions (e.g. weekly water changes, turbulence, light). However, our experiments were ran until almost of CV had used their lipid reserves and moulted to adult stages (*C. finmarchicus*) or lipid reserves were exhausted in females (*C. hyperboreus*), these characteristics being similar to body conditions observed in the field at the end of the overwintering period in the region (Plourde *et al.*, 2003; Johnson *et al.*, 2008; Maps *et al.*, 2010). Additionally, the jackknifed classification success was high (overall >80%) and differences in the $\delta^{13}C$ signatures of CVs among regions of the study area were large enough to support a small variation in $\delta^{13}C$ signatures over the overwintering period. Because of its conservative property during a relatively long period of time, the $\delta^{13}C$ allowed characterizing the exchange of planktonic organisms in water masses. However, the origin discrimination could not have been possible if $\delta^{13}C$ signatures of the carbon source and *Calanus* spp. were not significantly different among regions.

Other variables could have been used to discriminate the origins of *Calanus* spp. and possibly improve QDFA-based classification success. Fatty acid content (Kattner *et al.*, 1989, 2007; Falk-Petersen *et al.*, 2009; Petursdottir *et al.*, 2010) and other stable isotopes

such as $\delta^{34}S$ (Peterson & Fry, 1987; Michener & Lajtha, 2007; Godbout *et al.*, 2010) could potentially be region specific and discriminate the origin of *Calanus* spp., but since their evolution during diapause is unknown they would have to be assessed as it was done for $\delta^{13}C$ and $\delta^{15}N$ (Perrin *et al.*, 2012). The viability of natural and anthropogenic variations of trace element composition also could be tested. This approach was used to delineate the origin and associated bay-ocean exchange dynamics of crustacean larvae (DiBacco & Levin, 2000; DiBacco & Chadwick, 2001). In this case, the elemental composition of the exoskeleton of brachyuran larvae reflected the trace elemental composition of ambient seawater where the exoskeleton was formed. In the case of diapausing *Calanus* spp. that do not moult during the overwintering period, the elemental fingerprint of its exoskeleton could serve as additional and complementary variables to delineate the origins of diapausing zooplankton.

To our knowledge, this study was the first to use stable isotopes to identify origins of planktonic organism over several months. The stable isotopic marker would be an interesting tool in future studies to improve the understanding of the source-sink dynamics of zooplankton prey and their associated species, which has important implications in management and identification of Marine Protected Areas. To increase the efficiency of this technique, it would be profitable to add conservative variables to the QDFA, increase sample sizes and enhance the time scale analysis with additional sampling sessions.

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CHAPITRE 3
CONCLUSION

L'origine des *Calanus* spp. a pu être évalué principalement grâce à deux propriétés importantes de la signature isotopique de carbone : la stabilité dans le temps du $\delta^{13}C$ des CV en diapause et la variabilité spatiale. Plusieurs facteurs sont susceptibles d'influencer la dynamique de la signature isotopique des organismes dans le temps. Au cours du suivi hivernal des CV en diapause (chapitre 1), le contenu lipidique s'est révélé être le facteur le plus influent sur le $\delta^{13}C$ et le pourcentage de carbone (%C). Après extraction des lipides, le $\delta^{13}C$ et le %C sont par contre demeurés stables durant les 4 mois d'expérimentation en conditions contrôlées.

Pour sa part, le $\delta^{15}N$ ne s'est pas révélé être sous l'influence de la composition lipidique mais plusieurs mécanismes internes semblent faire fluctuer le $\delta^{15}N$. L'instabilité du $\delta^{15}N$ dans le temps rend cette variable inapte à être utilisée dans l'analyse discriminante. Par contre, la technique d'extraction a augmenté significativement le $\delta^{15}N$. Pour cette raison, une correction mathématique du $\delta^{13}C$ peut-être une alternative utile à l'extraction lipidique lorsque les signatures isotopiques de carbone et d'azote sont analysés

conjointement. Suite aux résultats obtenus, nous concluons que pour corriger adéquatement le $\delta^{13}C$, les paramètres de l'équation mathématiques (pourcentage de lipides, valeur de la constante D et le ratio C :N) doivent d'abord être évalués à l'aide d'une extraction lipidique. En effet, ces paramètres peuvent varier selon la quantité et la nature des lipides, l'espèce, le stade de développement, etc.

L'hétérogénéité des conditions environnementales retrouvée dans l'aire d'étude a permis d'attribuer une signature de carbone distincte aux individus se développant et entrant en diapause dans les différentes régions échantillonnées. Les résultats de cette étude suggèrent que les populations de *Calanus finmarchicus* et de *C. hyperboreus* présentes dans le PMSSL en mai 2010 avaient une origine multiple. Au cours de la période d'hivernage, les courants des eaux profondes auraient transporté dans le parc marin une importante quantité de *Calanus* spp. en diapause en provenance des régions de l'estuaire et du nord-ouest du golfe du St-Laurent, impliquant ainsi un transport sur plus de 500 km. De plus, à l'intérieur du PMSSL, le fjord du Saguenay semble être un environnement propice au recrutement d'une cohorte locale qui pourrait s'y développer, acquérir un $\delta^{13}C$ distinct des régions avoisinantes, entrer en diapause, hiverner et s'y reproduire. La circulation estuarienne et les processus océanographiques se produisant à la tête du chenal Laurentien permettent une grande connectivité entre les populations du PMSSL et les régions environnantes ainsi qu'à l'intérieur même du parc où des échanges de CV de *Calanus* spp. s'opèrent au-dessus des seuils peu profonds à l'embouchure du Saguenay.

Les résultats obtenus au cours de cette étude apportent des éléments nouveaux en ce qui concerne l'utilisation des isotopes stables en écologie et la compréhension de la dynamique des populations de *Calanus* spp. dans le PMSSL. En effet, aucune étude publiée jusqu'à aujourd'hui ne semble avoir utilisé les isotopes stables comme marqueur pour retracer l'origine d'organismes planctoniques après leur transport sur une période relativement longue. Le suivi hivernal en laboratoire de la dynamique des isotopes stables a permis d'identifier les propriétés conservatives du $\delta^{13}C$ dans les structures non-lipidiques des CV ne se nourrissant pas et de permettre l'utilisation du $\delta^{13}C$ dans l'analyse discriminante. De plus, l'analyse des résultats suggère la possibilité d'un recrutement local de *Calanus* spp. à l'intérieur du fjord du Saguenay alors que les études précédentes suggéraient plutôt que leur présence dans le PMSSL et le fjord du Saguenay était tributaire des apports externes.

Pour approfondir et renforcer l'évaluation de l'origine des populations de *Calanus* spp. dans le PMSSL, quelques améliorations pourraient être ajoutées au plan d'étude. Tout d'abord, la discrimination des CV provenant des différentes régions ainsi que l'assignation à l'origine des individus retrouvés dans le PMSSL à la fin de la phase de diapause hivernale pourraient être précisés par l'addition de variables conservatives à l'analyse discriminante. Augmenter le nombre de sessions d'échantillonnages pourrait aussi aider à clarifier l'impact de la composante temporelle sur les résultats. À l'intérieur du PMSSL (fjord du

Saguenay et tête du chenal Laurentien), la circulation de l'eau et les échanges au-dessus des seuils à l'embouchure du Saguenay sont très complexes et requièrent une étude plus approfondie, particulièrement durant l'hiver. La distribution des organismes planctoniques étant dépendante de la circulation des masses d'eau, la compréhension des processus océanographiques est nécessaire pour interpréter la connectivité à l'intérieur du PMSSL et avec ses régions avoisinantes.

Notre étude est un pas de plus dans la compréhension de la dynamique des populations des *Calanus* spp. permettant de supporter les efforts de protection et de conservation des écosystèmes du PMSSL ainsi que les impacts de perturbations anthropiques et/ou climatiques. En effet, différents stress tels qu'une variation de l'intensité de la circulation estuarienne sujette à être modifiée par les changements du climat pourraient avoir un impact important sur le maintien de l'importante biomasse zooplanctonique à la base d'une chaîne alimentaire si chère à l'écosystème et à la biodiversité du PMSSL.

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