

Tracing the origins of *Calanus* sp. in the Saguenay-St. Lawrence Marine Park (Québec, Canada) using $\delta^{13}\text{C}$ as a marker

G. Perrin^{1,*}, S. Plourde², C. DiBacco³, G. Winkler¹, P. Sirois⁴

¹Institut des Sciences de la Mer, Université du Québec à Rimouski, Rimouski, Québec G5L 3A1, Canada

²Maurice-Lamontagne Institute, Fisheries and Oceans Canada, Mont-Joli, Québec G5H 3Z4, Canada

³Bedford Institute of Oceanography, Fisheries and Oceans Canada, Dartmouth, Nova Scotia B2Y 4A2, Canada

⁴Laboratoire des sciences aquatiques, Université du Québec à Chicoutimi, Chicoutimi, Québec G7H 2B1, Canada

ABSTRACT: The Saguenay-St. Lawrence Marine Park (SSLMP) is a region that sustains a high abundance of zooplankton. The connectivity between zooplankton populations within the SSLMP and the surrounding areas was investigated for *Calanus finmarchicus* and *C. hyperboreus*. Deep-dwelling stage V copepodites (CVs) were collected in the Marine Park as well as in putative source regions in the St. Lawrence system in July 2009 (a time when they were entering into diapause). In May 2010, at the end of the overwintering period, diapausing CVs were sampled again in the Marine Park. To discriminate the origins and to predict the probable regions of origin of these deep-dwelling diapausing CVs in the SSLMP, a quadratic discriminant function analysis (QDFA) was performed. The classification algorithm was based on the carbon isotopic composition ($\delta^{13}\text{C}$) and percent carbon (%C) of individual copepods, as these variables are conservative when lipids are extracted prior to analysis. Our results suggest that about 23 % of the *Calanus* spp. population sampled in SSLMP in late spring 2010 originated from the Saguenay Fjord (inside the SSLMP). The remainder of this population 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 the potential for significant local production and recruitment of *Calanus* spp. within the Saguenay Fjord. This study also revealed the effectiveness of using $\delta^{13}\text{C}$ as a marker in delineating the origin of *Calanus* spp., which has a relatively long non-feeding overwintering and diapausing period making it amenable to the conservation of isotopic signatures.

KEY WORDS: *Calanus* · $\delta^{13}\text{C}$ · Origin · Connectivity · Circulation · Saguenay-St. Lawrence Marine Park

Resale or republication not permitted without written consent of the publisher

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 *Delphinapterus leucas* population. This 1245 km² area encompasses 3 distinct oceanographic regions: the Saguenay Fjord, the brackish Upper St. Lawrence Estuary and the marine

Lower St. Lawrence Estuary. The SSLMP also includes 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. 1). The Marine Park is an important feeding ground for pelagic fishes, marine birds and whales, while the biomass of zooplanktonic prey in the SSLMP is highly dependent on the complex water circulation of the

*Corresponding author: genperrin@hotmail.com

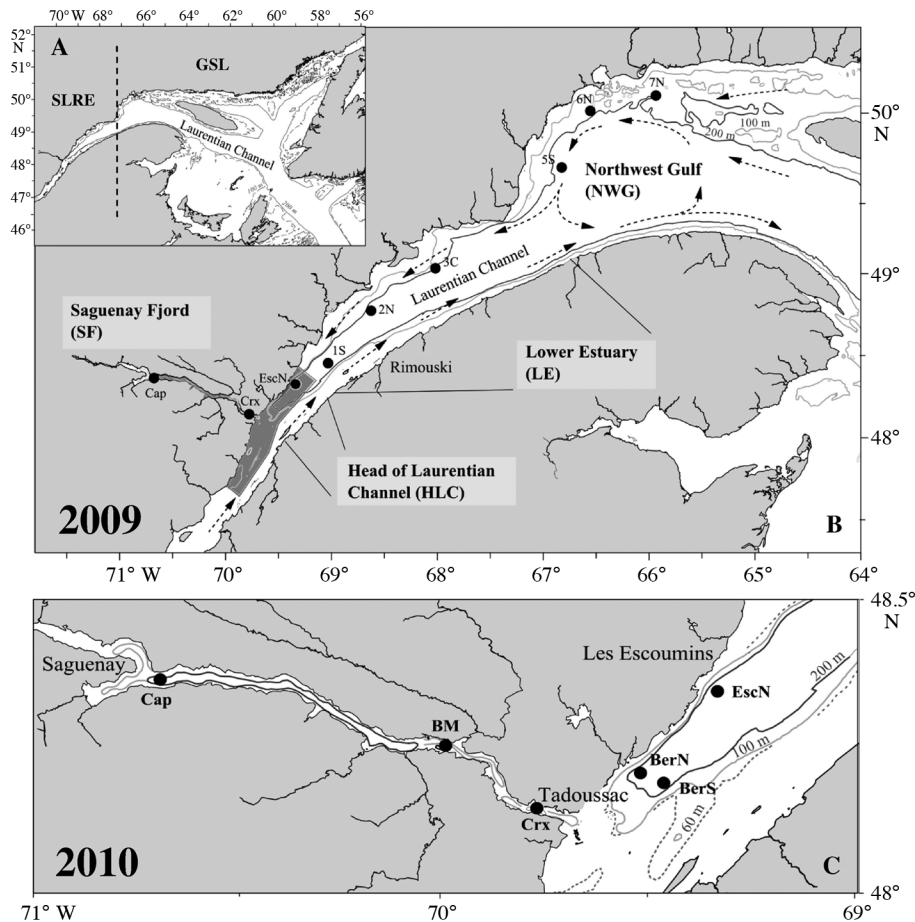


Fig. 1. Study area showing (A) the St. Lawrence River Estuary (SLRE) and Gulf of St. Lawrence (GSL) on Canada's east coast, (B) different stations (solid circles) and regions of the study area: Saguenay Fjord (SF), Head of the Laurentian Channel (HLC), Lower St. Lawrence Estuary (LE) and Northwest Gulf of St. Lawrence (NWG) in July 2009, and (C) sampling stations of the Saguenay-St. Lawrence Marine Park (SSLMP) in May 2010. The dark grey area in (B) represents the boundaries of the SSLMP, and dashed arrows show general circulation integrated from the entire water column (adapted from Saucier et al. 2003, 2009)

region and surrounding areas (Simard 2009). Comprehension of the bio-physical processes favouring the rich locally produced and advected biodiversity and species abundance is essential to support management efforts in the SSLMP, since the water circulation (and therefore zooplankton transport) could be affected by climate changes (Zakardjian et al. 2003, Saucier et al. 2009). However, the connectivity among zooplankton populations within the Marine Park as well as with other potential source regions is unknown, as our understanding of zooplankton exchange dynamics in the region is based only 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, 2013).

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). The interaction between the 2-layer estuarine circulation and the diel and seasonal vertical migrations of *Calanus* spp. has been hypothesized to favour their transport and aggregation at the head of the Laurent-

ian Channel in the SSLMP (Runge & Simard 1990, Zakardjian et al. 1999, Sourisseau et al. 2006, Simard 2009, Maps et al. 2011, 2013). According to the mostly accepted view based on the similarity in zooplankton species composition among different regions of the SSLMP and local hydrodynamics, the head of the Laurentian Channel represents 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). Under this scheme, *Calanus* spp. would be 'non-resident' as they are advected into 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 a potential for local production and recruitment of large marine zooplankton (Rainville 1979).

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). Consequently, particulate organic carbon and plankton in these upstream regions show different stable isotope ratios ($\delta^{13}\text{C}$) from those in the more marine regions characterized by higher salinity with lower terrestrial carbon loads (Tan & Strain 1979a,b, 1983, Tremblay & Gagné 2009). The isotopic composition of copepods is likely to reflect these differences in carbon sources between regions, as they could acquire a region-specific isotopic signature when they feed during their active growth phase in the surface layer (0 to 100 m) in spring and summer (Fry 1981, Deegan & Garritt 1997, Perry et al. 1999). Afterward, they enter diapause as deep-dwelling (>100 m), non-feeding late stage copepodites in early (*C. hyperboreus*) and late summer (*C. finmarchicus*) until the following spring (Conover 1988, Plourde et al. 2001, 2003, Falk-Petersen et al. 2009, Johnson et al. 2008). These overwintering *Calanus* spp. are subjected to prevailing deep upstream circulation in the Laurentian channel toward the SSLMP, representing potential transport of several hundreds of kilometers (Sourisseau et al. 2006, Maps et al. 2011). In a previous study, we showed that diapausing (non-feeding) CVs of *C. finmarchicus* and *C. hyperboreus* captured in early fall conserved their $\delta^{13}\text{C}$ signature and percentage of body carbon (%C) in lipid extracted body structures over 4 months (Perrin et al. 2012). The present study confirms the utility of using stable isotopes and %C in determining the origins of *Calanus* spp. in an overwintering population in the SSLMP.

The aim of this study was to evaluate if *Calanus* spp. in the SSLMP originate from local or external sources, to help elucidate zooplankton connectivity in the Marine Park. We hypothesized that the $\delta^{13}\text{C}$ of *Calanus* spp. would differ among sub-regions of the St. Lawrence system (including the SSLMP) at the beginning of the overwintering period (summer) in response to variable environmental conditions. 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.

MATERIALS AND METHODS

Study site

The study area and distribution of sampling stations were selected to cover major potential source

regions of *Calanus* spp. into the SSLMP (Fig. 1). The study area included the SSLMP region comprising the Saguenay Fjord (SF) 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. 1). The 2-layer estuarine circulation typical of the Gulf of St. Lawrence is characterized by upstream transport of a cold intermediate layer (30 to 100 m; 0.10 m s^{-1}) and deep Atlantic waters (>100 m; $0.01\text{ to }0.02\text{ m s}^{-1}$) from the Gulf of St. Lawrence to the HLC in the LE (Koutitonsky & Bugden 1991, Saucier et al. 2003, 2009). General circulation in the St. Lawrence system is cyclonic. Upstream deep water currents are mostly restricted along the northern flank of the Laurentian Channel, and surface currents are more important along the southern coastline (Saucier et al. 2003, 2009). This circulation likely represents the predominant pathway for the transport and supply of deep-dwelling zooplankton (such as *Calanus* spp.) to the SSLMP from eastern downstream sources (Plourde et al. 2001, Sourisseau et al. 2006, Simard 2009, Maps et al. 2013). Stations were mainly distributed along the north side of the St. Lawrence, where the transport of deep-dwelling *Calanus* spp. should occur due to a predominant upstream current of deep water.

Sample collection

In 2009, sampling was carried out between 18 and 25 July at 9 stations in the SF from the RV 'Borealis' (Université du Québec à Chicoutimi), at the HLC from the RV 'Alliance' (Marine Park) and in the LE and NWG from the RV 'Frederick G. Creed' (Fisheries and Oceans Canada) (Fig. 1). The zooplankton sampling strategy aimed to separate actively growing *Calanus* spp., mainly occurring in the upper 100 m of the water column, from diapausing individuals at depths greater than 100 m (Plourde et al. 2001, 2003). Since we cannot presume the isotopic signature of actively growing animals, surface-dwelling (0 to 100 m) CVs were not considered in our analysis. All animals analysed in our study were captured in the deeper layer (>100 m) and were assumed to be in diapause. Sampling included a Seabird CTD profile from bottom to surface and a vertical tow initiated near the bottom to 100 m with a 75 cm diameter, 202 μm Nitex plankton net equipped with an opening-closing device. On 11 May 2010, deep-dwelling (>125 m), diapausing CV *Calanus* spp. were collected at 6 stations in the SF and at the HLC from the

RV 'Borealis' to identify *Calanus* spp. sources in deep waters of the Marine Park. All zooplankton samples were frozen in liquid nitrogen or on dry ice immediately after collection and subsequently transferred to a -80°C freezer until analysis.

Laboratory analysis

Frozen zooplankton samples were gently soaked in cold filtered sea water, allowing the sorting of individual CVs of *Calanus finmarchicus* and *C. hyperboreus* within a few minutes. Only *C. finmarchicus* CVs that were 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). CVs were analysed individually, and 12 replicates per species at each station were sorted. This number of replicates was sufficient to encompass within-station variability of CV carbon and nitrogen stable isotope signatures, and minimized the error term around the mean. For an unknown reason, no deep-dwelling *C. hyperboreus* CVs were collected at Stn Crx (Fig. 1), so this station was not analysed for this species.

CVs were individually rinsed in millipore water, lyophilised for 48 h in a pre-weighed 5×9 mm tin capsule and weighed using a microscale (Mettler MT5; ± 0.001 mg). Lyophilisation was used instead of oven drying to reduce the impact (i.e. alteration of the isotopic ratio) on both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (de Lecea et al. 2011). Lipids were subsequently extracted to minimize their influence on the isotopic signature associated with depleted $\delta^{13}\text{C}$ values in lipids (DeNiro & Epstein 1977, McConnaughey & McRoy 1979, Post et al. 2007) and their variable proportion of total weight 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. We used a lipid extraction technique (Bligh & Dyer 1959) adapted with a passive lipid extraction procedure (Ohman 1988) to avoid material loss during homogenisation. After 24 h, copepods were rinsed twice with the chloroform:methanol solution and then twice with millipore (ultrapure) water before being placed in a pre-weighed 5×9 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 (%Lipids) was estimated as the difference in dry weight before (DW) and after lipid extraction (DW_{LE}).

The isotopic composition of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) was expressed in parts per thousand (‰) relative to C and N standards as the relative proportion of ^{13}C to ^{12}C and ^{15}N to ^{14}N :

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

where R is the ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ of the sample and a standard (Peterson & Fry 1987). Deep-dwelling CVs were analysed individually (17.0 to 446.0 μg of 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). Due to small sample size (weight), the analyses were performed using a Costech zero blank autosampler (not open to the atmosphere but instead purged with helium) and the addition of a second set of resistors installed on collectors that amplified the signal 4 times over the typical setup. The standard reference material for $\delta^{13}\text{C}$ was Vienna Pee Dee Belemnite and atmospheric nitrogen for $\delta^{15}\text{N}$. For every 6 or 7 samples, 4 standards were run with C and N contents spanning the range found in our samples. Since the standard deviation of mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for standard reference materials was low ($<0.17\text{‰}$ for $\delta^{13}\text{C}$ and $<0.47\text{‰}$ for $\delta^{15}\text{N}$; data not shown), the accuracy of sample $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was considered statistically acceptable.

Data analysis

Temperature and salinity could reflect the contribution of terrestrial and marine $\delta^{13}\text{C}$ via the freshwater input—this difference being more pronounced at shallower depth due to different densities. Since CVs feed in surface waters, temperature and salinity were averaged at each station in the 0 to 10 m depth layer and plotted in a temperature–salinity diagram to visually identify regions with potentially distinct $\delta^{13}\text{C}$ signatures (Fig. 2) (Tan & Strain 1979a,b, 1983). Four sample regions were selected: (1) SF (1 station), (2) the LE (3 stations) (3) the HLC (2 stations) and (4) the NWG (3 stations). Even though the HLC is geographically a part of the LE, we considered the HLC a distinct region since it is situated within the boundary of the SSLMP (Fig. 1B). The HLC is characterized by strong mixing of many water sources, and therefore was expected to contain copepods with mixed $\delta^{13}\text{C}$ signatures. However, surface temperature and salinity data measured at these stations mostly resembled the conditions of the LE. 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.

To test differences among regions, 1-way ANOVAs were performed for each variable measured on deep-dwelling CVs in 2009, and used further for the quadratic discriminant function analyses (QDFA) ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C, DW_{LE} and %Lipids). Every analysis of variance was followed by *a posteriori* Tukey's HSD test. To evaluate if differences in $\delta^{13}\text{C}$ of deep-dwelling diapausing CVs (used further for the QDFA) were restricted to variations between regions and not among stations inside each region, a 2-way ANOVA was conducted for $\delta^{13}\text{C}$ with stations nested in regions. 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 the samples (<30 μg dry weight). Statistical analyses were carried out with JMP (version 7, SAS Institute).

QDFA with jackknifed validations were performed with SYSTAT (version 13) to classify *Calanus finmarchicus* and *C. hyperboreus* CVs captured in July 2009 from distinct regions based on individual characteristics ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C, DW_{LE} and %Lipids). We analysed data following 3 different steps, aiming to optimize the classification success of individuals and to assess if a QDFA conducted with a maximum number of variables was more effective than a QDFA using only variables observed to be constant over the overwintering period (Perrin et al. 2012). The initial QDFA considered all measured variables ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$,

%C, DW_{LE} and %Lipids), while the second QDFA employed only conservative variables ($\delta^{13}\text{C}$, %C). Because QDFAs are multivariate analyses, we included %C even if this variable did not show significant differences between regions (see 'Results'). Finally, the analysis with conservative variables was rerun after grouping regions that revealed low classification success due to reciprocal misclassifications. Since regions were unequal groups, and therefore CVs had a higher probability of being classified in the largest groups by chance alone (White & Ruttenberg 2007), we assigned equal prior probabilities to the groups. Pillai's trace was used to test the null hypothesis for QDFA. Data for each variable were tested for normality with Shapiro-Wilk's test, and for homogeneity of variances via visual evaluation of residual distributions. Grouped regions and assignment algorithms from the last QDFA were used to classify animals collected *in situ* from the SSLMP at the end of their overwintering period (i.e. May 2010; see next paragraph).

Differences in the $\delta^{13}\text{C}$ signatures in the SSLMP between 2009 and 2010 were tested for the entire Marine Park and its sub-regions (SF and HLC) with 1-way ANOVAs followed by *a posteriori* Tukey's HSD test. *Calanus 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 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.

RESULTS

Spatial characterization of the study area in July 2009

Region-specific averaged values and significance of comparisons among regions for each variable measured on *Calanus* spp. CVs are presented in Table 1. CVs showed significant differences in $\delta^{13}\text{C}$ among regions, but were similar within regions (Tables 1 & 2). Post-hoc Tukey's HSD test revealed that among regions, only the HLC and the LE had similar $\delta^{13}\text{C}$ for *C. finmarchicus* CVs. For *C. hyperboreus*, only CVs in the SF had $\delta^{13}\text{C}$ that were significantly different, whereas $\delta^{13}\text{C}$ was similar in the HLC, the LE and the NWG. The %C of lipid-

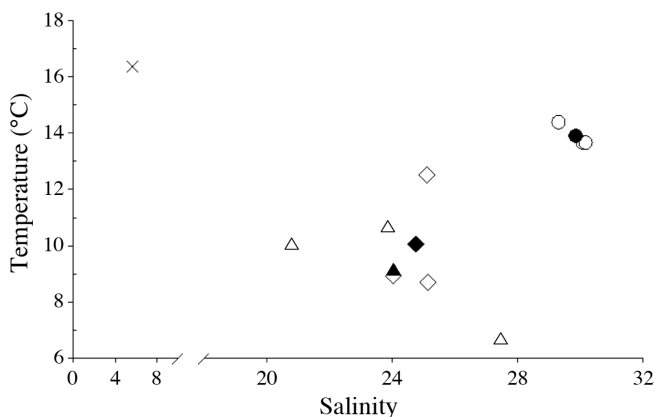


Fig. 2. Mean temperature and salinity of the 10 m top layer ($\text{SE} \leq 0.2$) of each station (open symbols) sampled in July 2009 including overall means (solid symbol) of different regions: Saguenay Fjord (x), Lower St. Lawrence Estuary (\diamond), Head of Laurentian channel (Δ) and Northwest Gulf of St. Lawrence (O) identified based on environmental data (temperature and salinity)

Table 1. *Calanus finmarchicus* and *C. hyperboreus*. Stable isotope signature of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), carbon percentage (%C), lipid extracted dry weight (DW_{LE}) and lipid percentage (%Lipids) of deep-dwelling (>100 m) (A) *C. finmarchicus* and (B) *C. hyperboreus* CVs in different regions of the study area in July 2009 (SF: Saguenay Fjord; HLC: Head of the Laurentian Channel; LE: Lower St. Lawrence Estuary; NWG: Northwest Gulf of St. Lawrence). Results of 1-way ANOVAs among regions in July 2009 are shown for each variable; values associated with different letters (columns) are significantly different (Tukey's HSD test, $p < 0.05$). Results are given as mean \pm standard error; NS: non-significant

	N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	%C	DW_{LE} (μg)	%Lipids
(A) <i>Calanus finmarchicus</i>						
SF	12	-23.1 ± 0.5 C	9.3 ± 0.2 A	47.9 ± 0.3 A	77.5 ± 3.6 A	72.7 ± 1.1 B
HLC	24	-19.1 ± 0.1 A	8.0 ± 0.2 B	46.1 ± 2.4 A	35.3 ± 2.2 B	75.9 ± 1.5 A
LE	36	-19.1 ± 0.1 A	9.1 ± 0.2 A	44.6 ± 0.4 A	67.0 ± 7.7 A	76.9 ± 0.8 A
NWG	36	-20.6 ± 0.1 B	9.3 ± 0.2 A	46.3 ± 0.3 A	80.0 ± 8.8 A	71.9 ± 1.1 B
		$F_{3,104} = 58.4$ $p < 0.001$	$F_{3,104} = 13.8$ $p < 0.001$	$F_{3,104} = 1.2$ NS	$F_{3,104} = 6.8$ $p < 0.001$	$F_{3,104} = 5.7$ $p = 0.001$
(B) <i>Calanus hyperboreus</i>						
SF	12	-20.8 ± 0.5 B	9.4 ± 0.2 AB	48.7 ± 0.2 A	119.6 ± 12.8 C	73.7 ± 2.1 A
HLC	12	-19.5 ± 0.2 A	9.0 ± 0.2 B	47.8 ± 0.5 A	159.8 ± 15.9 C	63.8 ± 2.3 B
LE	36	-19.2 ± 0.1 A	9.4 ± 0.1 A	48.3 ± 0.3 A	237.7 ± 14.9 B	77.6 ± 1.0 A
NWG	36	-19.3 ± 0.1 A	9.6 ± 0.1 A	48.3 ± 0.1 A	287.6 ± 13.7 A	76.5 ± 0.7 A
		$F_{3,92} = 12.1$ $p < 0.001$	$F_{3,92} = 3.2$ $p = 0.028$	$F_{3,92} = 0.7$ NS	$F_{3,92} = 15.5$ $p < 0.001$	$F_{3,92} = 13.8$ $p < 0.001$

Table 2. *Calanus finmarchicus* and *C. hyperboreus*. Two-way ANOVA conducted for $\delta^{13}\text{C}$ for deep-dwelling CVs of (A) *C. finmarchicus* and (B) *C. hyperboreus* in 2009 ($\alpha = 0.05$)

	df	SS	F	p
(A) <i>Calanus finmarchicus</i>				
Region	2	164.9	111.7	<0.001
Station(Region)	6	6.1	1.4	0.2
(B) <i>Calanus hyperboreus</i>				
Region	1	22.7	35.8	<0.001
Station(Region)	6	2.3	0.6	0.7

extracted animals was the only variable that was similar across the study area, reflecting the constant contribution of carbon to the structural weight (i.e. DW_{LE}). DW_{LE} of *C. finmarchicus* was lowest in the HLC, while that of *C. hyperboreus* was lowest in the SF (Table 1).

Origin discrimination of *Calanus* spp. in summer 2009

Calanus finmarchicus

The jackknifed validation of the classification matrix showed that the QDFA conducted with 5 variables ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C, DW_{LE} and %Lipids; Table 3A) resulted in an overall assignment success for *Calanus finmarchicus* of 70%, with maximum (91%) and minimum (47%) values obtained in the SF and the LE, respectively (Table 3A). Misclassified animals in the

Table 3. *Calanus finmarchicus*. Jackknifed classification matrices from the quadratic discriminant function analysis (QDFA) model conducted 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) with (A) 5 measured variables: $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C, lipid extracted dry weight (DW_{LE}) and %Lipids, (B) 2 conservative variables: $\delta^{13}\text{C}$ and %C, and (C) in newly grouped regions with 2 conservative variables ($\delta^{13}\text{C}$ and %C). Pillai's trace results are given below tables

Origin	N	Classified region				% correct
		SF	HLC	LE	NWG	
(A) 5 variables						
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
$F_{15,300} = 9.9, p < 0.001$						
(B) 2 conservative variables						
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
$F_{6,204} = 17.9, p < 0.001$						
Origin	N	Classified region			% correct	
		SF	HLC-LE	NWG		
(C) Grouped regions (2 variables)						
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	
$F_{4,206} = 26.6, p < 0.001$						

LE were distributed almost equally between HLC and NWG, but not in SF (Table 3A). When using only the 2 conservative variables, the classification success decreased by only 2% (Table 3B). The LE and HLC showed lower assignment success (58 and 52%, respectively) and a high proportion of reciprocal misclassification (Table 3B), suggesting a high degree of exchange or homogeneity of carbon signatures between these 2 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) (Figs. 1 & 2). We therefore combined these 2 regions (HLC-LE) and recalculated classification matrices, which improved our overall classification success from 70 to 84% and led to similar classification success among regions (Table 3C).

Calanus hyperboreus

The classification matrix with jackknifed validations conducted with the 5 variables resulted in an overall classification success of 67% for *Calanus hyperboreus* CVs (Table 4A). 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 4A). With only $\delta^{13}\text{C}$ and %C considered in the analysis, the overall classification success decreased by 15% (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 4B). The overall assignment accuracy was optimized at 81% after grouping the NWG, LE and HLC regions based on the reciprocal classification error (Table 4B), leading to 2 remaining regions: the SF and the St. Lawrence (SL) (Table 4C).

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

When comparing 2009 and 2010, the $\delta^{13}\text{C}$ was not significantly different in the SSLMP for both species (Table 5) and for *Calanus hyperboreus* in the SF and the HLC (Table 5B). However, the $\delta^{13}\text{C}$ signature of *C. finmarchicus* became similar in the SF and the HLC in 2010, whereas the difference between these 2 regions was 4‰ in 2009 (Table 5A).

Discriminant algorithms produced with $\delta^{13}\text{C}$ and %C and for grouped regions were used to assign individual *Calanus* spp. sampled in May 2010 to

Table 4. *Calanus hyperboreus*. Jackknifed classification matrices from the quadratic discriminant function analysis (QDFA) model conducted 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) with (A) 5 measured variables: $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C, lipid extracted dry weight (DW_{LE}) and %Lipids, (B) 2 conservative variables: $\delta^{13}\text{C}$ and %C, and (C) in newly grouped regions: SF and St. Lawrence (SL = HLC + LE + NWG) with 2 conservative variables ($\delta^{13}\text{C}$ and %C).

Pillai's trace results are given below tables

Origin	N	Classified region				% correct
		SF	HLC	LE	NWG	
(A) 5 variables						
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
$F_{15,264} = 11.4, p < 0.001$						
(B) 2 conservative variables						
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
$F_{6,180} = 6.1, p < 0.001$						
Origin	N	Classified region				% correct
(C) Grouped regions (2 variables)						
SF	12	9	3			75
SL	82	15	67			82
Total	94	24	70			81
$F_{2,91} = 20.4, p < 0.001$						

Table 5. *Calanus finmarchicus* and *C. hyperboreus*. ANOVAs comparing the $\delta^{13}\text{C}$ of deep-dwelling CVs of (A) *C. finmarchicus* and (B) *C. hyperboreus* in the Saguenay-St. Lawrence Marine Park (SSLMP) between 2009 and 2010 for the entire Marine Park, and for its sub-regions, the SF and the HLC. Values associated with different letters (columns) are significantly different (Tukey's HSD test, $p < 0.05$); NS: non-significant

	N	$\delta^{13}\text{C}$	
(A) <i>Calanus finmarchicus</i>			
2009	36	-20.4 ± 0.3	
SF	12	-23.1 ± 0.5	A
HLC	24	-19.1 ± 0.1	C
2010	72	-20.5 ± 0.1	
SF	36	-20.2 ± 0.2	B
HLC	36	-20.7 ± 0.2	B
SSLMP: $F_{1,106} = 0.1, \text{NS}$ Sub-regions: $F_{3,104} = 29.2, p < 0.001$			
(B) <i>Calanus hyperboreus</i>			
2009	24	-20.1 ± 0.3	
SF	12	-20.8 ± 0.5	A
HLC	12	-19.5 ± 0.2	B
2010	52	-19.9 ± 0.1	
SF	16	-20.6 ± 0.4	A
HLC	36	-19.6 ± 0.1	B
SSLMP: $F_{1,74} = 0.7, \text{NS}$ Sub-regions: $F_{3,72} = 6.4, p = 0.001$			

Table 6. *Calanus finmarchicus* and *C. hyperboreus*. Relative contribution of deep-dwelling CVs of (A) *C. finmarchicus* and (B) *C. hyperboreus* 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 Saguenay-St. Lawrence Marine Park (SSLMP) population in May 2010, including its 2 sub-regions (SF and HLC) and results for each sampled station (Cap, BM, Crx, BerN, BerS, EscN) (Fig. 1C)

(A) <i>Calanus finmarchicus</i>								
	Station	SF		HLC-LE		NWG		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) <i>Calanus hyperboreus</i>								
	Station	SF		SL			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	

likely regions of origin. Our results revealed that CVs sampled at the end of the overwintering period in the SSLMP (SF and HLC sub-areas) originated from both local and external sources (Table 6). Approximately 24% of *C. finmarchicus* sampled from 6 stations in the SSLMP (i.e. SF and HLC) were assigned to the SF group established with July 2009 data, suggesting that about 76% of the population sampled in May 2010 originated from the HLC-LE region (23.6%) and the NWG (52.8%) (Table 6A). The pattern for *C. hyperboreus* was similar (Table 6B). 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 6). Animals likely originating from the LE and NWG were observed in the SF, representing 56.3% (*C. hyperboreus*) and 80.5% (*C. finmarchicus*; 33.3% from the HLC-LE and 47.2% from the NWG) of the

population (Table 6). Likewise, *Calanus* spp. CVs ascribed to the SF were observed in the HLC region in spring 2010. The small sample size precluded any analysis at a smaller scale.

DISCUSSION

Potential origin of deep-dwelling CVs in the Marine Park

We found strong empirical evidence supporting a high connectivity among sub-areas of the LE-NWG region through the residual estuarine circulation. Our results support the hypothesis 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 *Calanus* spp. population of the SSLMP 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 copepodite stages upstream (mainly in the cold intermediate layer during summer, and deeper in autumn and winter), while surface-dwelling stages are flushed downstream in surface waters (Plourde & Runge 1993, Plourde et al. 2001, 2002, 2003, Maps et al. 2011, 2013).

In addition to the identification of distant source regions supporting the *Calanus* spp. populations in the SSLMP, our study provided the first evidence for local recruitment of these marine species in the SF. CVs ascribed to the SF due to their distinct $\delta^{13}\text{C}$ signature represented an important component of the *Calanus* spp. CV population in the SF, but also in the adjacent HLC (Fig. 1, Table 6). Previous studies suggested that *Calanus* spp. observed in the SF most likely originated 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 (Rainville 1979, De Ladurantaye et al. 1984, Schafer et al. 1990). However, these studies were not designed to detect local recruitment. Relatively high abundance of early copepodite stages of *C. finmarchicus* and overwintering stages of *C. hyperboreus* in the inner basin more than 170 km upstream of the fjord mouth have been observed (Rainville 1979, S. Plourde et al. unpubl. data). Our results do not prove that *Calanus* spp. could complete its whole life cycle inside the fjord. However,

the significant difference of the $\delta^{13}\text{C}$ signature in the SF compared to adjacent estuarine regions, combined with these observations of early development stages and accumulation of deep-dwelling *Calanus* spp. CVs in the upper part of the SF suggest local development and recruitment.

Discrimination of *Calanus* spp. CV origins was mostly attributable to differences in their $\delta^{13}\text{C}$ signatures among *a priori* regions determined using surface temperature and salinity (Table 1), while the other conservative variable (i.e. $\%C$) was similar across the sampling domain. Other variables differed somewhat among regions (Table 1), but they did not significantly contribute to spatial discrimination (Tables 3 & 4); their non-conservative and unpredictable nature during diapause (Perrin et al. 2012) likely made them poor indicators of *Calanus* spp. origin. Region-specific differences in DW_{LE} could be related to spatial differences in environmental growth conditions (i.e. temperature, food availability; Campbell et al. 2001) and in potential region-specific differences in the timing of arousal from, and entry into diapause (Johnson et al. 2008). Lipid content of deep-dwelling, overwintering *Calanus* spp. CVs sampled in July 2009 was lower than is typically observed at the beginning of the overwintering period, an artefact probably associated with damage observed to the normally large oil sacs (Plourde et al. 2003, Maps et al. 2010). Unfortunately, the reason for the oil sac damages is unknown. Animals collected in May 2010 had undamaged small oil sacs typical of the end of the overwintering period (Plourde et al. 2003, Maps et al. 2010) (Table 1). However, our results would not have been impacted by damage to the oil sacs because of the non-conservative nature of lipid storage during diapause (Perrin et al. 2012).

Results of the 2-way ANOVA conducted for $\delta^{13}\text{C}$ content of *Calanus finmarchicus* and *C. hyperboreus* CVs showed no significant variations among stations within each region, indicating that significant differences in $\delta^{13}\text{C}$ were restricted to variations among regions (Table 1; $p < 0.001$). These results strongly suggest that differences among regions were large enough to discriminate origins of *Calanus* spp. based on their $\delta^{13}\text{C}$ signature. Indeed, within-region standard error was less than 0.5‰ while significant differences among regions ranged between 1.3‰ (SF vs. HLC for *C. hyperboreus*) and 4.0‰ (SF vs. HLC-LE for *C. finmarchicus*) (Table 1).

Differences in the $\delta^{13}\text{C}$ of *Calanus* spp. CVs among regions could be related to biochemical and oceanographic processes across the study area. For both species in 2009, animals from the SF had the most

depleted $\delta^{13}\text{C}$ signature (Table 1). This low $\delta^{13}\text{C}$ signature might represent a greater proportion of terrestrial material via freshwater inputs (Fry & Sherr 1984, Michener & Kaufman 2007). In comparison, sources of carbon originating from the marine environment are typically enriched in ^{13}C compared to freshwater sources (Fry & Sherr 1984, Michener & Kaufman 2007). The St. Lawrence Estuary receives high amounts of fresh water from the Great Lakes, rivers and direct runoff from land. However, $\delta^{13}\text{C}$ signatures of *C. finmarchicus* in the HLC-LE had higher values than in the NWG (Table 1A), where the water had more marine characteristics (i.e. higher salinity and lower temperature; Fig. 2). In the St. Lawrence estuarine transition zone located upstream of the SSLMP, Martineau et al. (2004) estimated the $\delta^{13}\text{C}$ signature of phytoplankton (based on 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 estuarine transition zone. The enriched $\delta^{13}\text{C}$ of the phytoplankton is therefore likely to influence the stable isotopic signatures downstream in the HLC-LE. Another factor that could have influenced the $\delta^{13}\text{C}$ of *Calanus* spp. in the HLC-LE is high algal growth rates, which have been observed to increase the $\delta^{13}\text{C}$ 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), combined with enriched $\delta^{13}\text{C}$ signature of phytoplankton in the estuarine transition zone could help to explain why the $\delta^{13}\text{C}$ of *C. finmarchicus* was higher in the HLC-LE than in the NWG.

The study area encompasses high temporal variability in oceanographic conditions, but this variability could not significantly influence the discrimination of origins based on the $\delta^{13}\text{C}$ signature of deep-dwelling CVs. The stable isotope signature of CVs when they enter diapause (i.e. in non-lipid structures) is an integration of what they ate over the season. Short-term variability in the $\delta^{13}\text{C}$ signature of food during the period where CVs descend in depth (less than 1 mo; Plourde et al. 2001, 2002, 2003) would be reflected in tissues with high turnover rates

such as lipids (Tiezen et al. 1983, Graeve et al. 2005, Tamelander et al. 2006), which were removed in this study.

Based on abundance data of *Calanus finmarchicus* and *C. hyperboreus* collected at a fixed station near Rimouski (48°40'N, 68°35'W) in the LE between April and October in 2009 and 2010 (S. Plourde et al. unpubl.), we could confirm the diapausing status of our sampled *Calanus* spp. The July 2009 sampling period preceded the maximum abundance of deep dwelling *C. finmarchicus* CVs and occurred at the beginning of their entry into diapause in the central LE (Plourde et al. 2001, 2002, unpubl. data). In contrast, most of all *C. hyperboreus* CVs were in the deep layer, indicating that the population was in diapause at the time of sampling (Plourde et al. 2002, 2003, unpubl. data).

Species- and region-specific timing of diapause could have contributed to differences in classification assignments reported here for *Calanus* spp. (Tables 3 & 4). The $\delta^{13}\text{C}$ signatures reported for *C. hyperboreus* CVs sampled in the HLC-LE and NWG could not be discriminated as was done for *C. finmarchicus*. Since *C. hyperboreus* enters diapause earlier than *C. finmarchicus* (Plourde et al. 2001, 2002, 2003), diapausing *C. hyperboreus* CVs from the NWG may already have been transported to the estuary, thereby influencing mean $\delta^{13}\text{C}$ signatures of deep-dwelling populations in the HLC-LE. Data from S. Plourde et al. (unpubl.) show that during our sampling in July 2009, almost 100% of *C. hyperboreus* CVs in the LE were sampled in a depth of diapause (>100 m), about 2 wk after the end of entry into diapause. At the time of our sampling, the transport (supply) of deep-dwelling CVs from the NWG to the LE increased the abundance of diapausing CVs in the LE, and therefore caused the mixing of animals and their signatures from these 2 regions. The advection of deep-dwelling *C. hyperboreus* CVs from the NWG in HLC-LE was also confirmed by the QDFA conducted with separated regions (Table 4B). The assignment success in the NWG (71%) was relatively high compared to the LE (37%) and the HLC (25%), and a high proportion of misclassifications were attributed to the NWG (i.e. 11/35 in the LE and 5/11 in the HLC).

For both species, the arousal from diapause was well underway when our field sampling was conducted on 11 May 2010 (Plourde et al. 2001, 2002, 2003, unpubl. data). However, we are confident that our sampling strategy captured overwintering animals that had initiated diapause the year before. *Calanus hyperboreus* CVs typically increase their weight (and lipid content) in June, with an entry into

diapause in early July in central LE (Plourde et al. 2003). In *C. finmarchicus*, the production of the first cohort of young stages occurs in response to the phytoplankton bloom in late June and early July, with an entry into diapause as CVs in August (Plourde et al. 2001, Johnson et al. 2008). Therefore, the presence of newly diapausing individuals in depths below 125 m in May 2010 is unlikely.

The determination of potentially different regions of origin based on the 2009 summer sampling, and the proportions of animals from different origins in the SSLMP in May 2010 should not be interpreted as a complete assessment of the connectivity among *Calanus finmarchicus* and *C. hyperboreus* populations in the region. First, even if the sampling scheme covered the major part of the entry route, some animals could originate from other sources such as the east and south parts of the Gulf of St. Lawrence. Secondly, sampling in summer 2009 occurred over a short period, well before the main abundance peak and period of downward ontogenic migration of *C. finmarchicus* CVs in the region (Plourde et al. 2001, Johnson et al. 2008, Maps et al. 2010). Putting our sampling in the context of this annual time series indicates that only a fraction of the annual *C. finmarchicus* CV population was sampled. Moreover, examination of the time series clearly showed that we sampled the later part of the overwintering population of *C. finmarchicus* and *C. hyperboreus* in May 2010 (Fig. 3). Nevertheless, the proportion of animals in the SSLMP ascribed to different origins was consistent with what could be expected from the known residual circulation pattern and *Calanus* spp. life history; e.g. export of *Calanus* spp. in the HLC from the Saguenay Fjord and substantial import of *Calanus* spp. in the SSLMP originating from the adjacent LE and the more distant NWG (Table 6). Even though the $\delta^{13}\text{C}$ was not significantly different in the SSLMP for either species in 2009 or 2010 (Table 5), the $\delta^{13}\text{C}$ signature of *C. finmarchicus* became similar in the SF and the HLC in 2010, likely as a result of the import and export of animals from different origins. Inside the SF, the origin gradient reflected the import of CVs from outside regions. For both species, the proportion of 'local' CVs decreased downstream, while animals from the St. Lawrence regions were in general more abundant at the entrance of the fjord. For *C. finmarchicus*, the origin gradient in the SF also suggests that CVs from the HLC-LE entered the fjord earlier and further upstream than CVs originating from the NWG (Table 6A). In future studies, sample sizes should be increased, the sampling scheme should be extended further east and south in the Gulf

of St. Lawrence, and the number of stations inside the SF should be increased to enhance the characterisation of this area. Moreover, it would be preferable to test how the relative contribution changes seasonally and inter-annually with additional sampling sessions.

This study increases the comprehension of the population connectivity of *Calanus* spp. and the importance of the upstream advection of deep-dwelling CVs to sustain the high abundance of *Calanus* spp. in the SSLMP. Climate changes could have important impacts on the general water circulation (Saucier et al. 2009) and the distribution of the population of *Calanus* spp. in the Saguenay and St. Lawrence systems (Zakardjian et al. 2003). The biomass of zooplanktonic prey for higher trophic levels in the SSLMP could be affected by a reduction of the intensity of estuarine circulation and the upstream transport of deep waters caused by an increase in global temperature. Moreover, reduction of current intensity could lead to isolated zooplankton populations in the SF. The present study suggests local recruitment of *Calanus* spp., but this in itself is not evidence of sustainability of the local population without an input from external sources. An understanding of bio-physical processes of the SSLMP will help management efforts to protect the biodiversity and the ecosystems of this MPA. However, if changes occur in the distribution or abundance of the zooplankton community, it could become necessary to adapt the MPA to the situation (e.g. extend the range).

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

The stable isotopic marker could be an interesting tool in future studies to improve our understanding of the source-sink dynamics of zooplankton, which has important implications for the establishment and management of MPAs. The efficiency of $\delta^{13}\text{C}$ as a marker to estimate the origin of zooplankton was based on 3 main characteristics: (1) the presence of a non-feeding diapause stage in *Calanus* spp., (2) the stability of the $\delta^{13}\text{C}$ signature of lipid extracted tissues during diapause, and (3) the existence of spatial variability in $\delta^{13}\text{C}$ signatures of *Calanus* spp. in the study area. Although the laboratory experiments by Perrin et al. (2012) lasted 4 mo, a period much shorter than the estimated duration of the overwintering period of 200 to 300 d in the region (Johnson et al. 2008), we are confident that the experimental results showing a stability in $\delta^{13}\text{C}$ signatures over time were representative of the *in situ* dynamics. The shorter

duration of the laboratory incubations likely resulted in a higher metabolism due to changes in experimental conditions (e.g. weekly water changes, turbulence, light). However, these experiments were run until all CVs had used their lipid reserves and moulted to adult stages (*C. finmarchicus*) or lipid reserves were exhausted in females (*C. hyperboreus*) (Perrin et al. 2012), both characteristics being similar to the population dynamics at the end of the overwintering period in the region (Plourde et al. 2001, 2003, Johnson et al. 2008, Maps et al. 2010). Finally, the jackknifed classification success was high (overall >80%) and differences in the $\delta^{13}\text{C}$ signatures of CVs among regions of the study area were large enough to buffer a small variation in $\delta^{13}\text{C}$ signatures over the overwintering period.

The use of ANOVAs to compare $\delta^{13}\text{C}$ signatures between 2009 and 2010 was not as efficient as the QDFA to reveal the source-sink dynamics of *Calanus* spp. in the SSLMP. Both years had similar $\delta^{13}\text{C}$ signatures when comparing the entire SSLMP, and supply of *Calanus* spp. from external sources was detected only for *C. finmarchicus* (Table 5). For this species, the $\delta^{13}\text{C}$ signatures were similar among sub-areas of the SSLMP, increasing in the SF and decreasing in the HLC (Table 5A). Moreover, the ANOVAs could not show the retention of a part of the local *Calanus* spp. population in the SF and its export to the HLC. To detect the origin of *Calanus* spp. populations in the SSLMP, it was necessary to assess the origin of individuals—and the QDFA was useful for this purpose.

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 $\delta^{34}\text{S}$ (Peterson & Fry 1987, Michener & Lajtha 2007, Godbout et al. 2010, Couillard et al. 2011) could potentially be region-specific and discriminate the origin of *Calanus* spp., but their evolution during diapause would have to be assessed. However, $\delta^{34}\text{S}$ would be useful in studies using larger zooplankton species, since its analysis requires more material than the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. In the present study, the analysis of $\delta^{34}\text{S}$ could not be done in a single analysis with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Samples of *Calanus* spp. were already very small, and therefore could not be separated into 2 subsamples to get $\delta^{34}\text{S}$ values from the same individuals as for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

The viability of natural and anthropogenic variations of trace element composition also could be tested. This approach was used to delineate the ori-

gin and associated bay-ocean exchange dynamics of crustacean larvae (DiBacco & Levin 2000, DiBacco & Chadwick 2001). The elemental composition of the exoskeleton of brachyuran larvae reflected the trace elemental composition of ambient seawater where the exoskeleton was formed. Miller et al. (2013) showed that origin discrimination was also possible using trace elements in soft tissues. In the case of diapausing *Calanus* spp., the elemental fingerprint of its exoskeleton as well as soft tissues could serve as an additional and complementary variable to delineate the origins of diapausing zooplankton. Using trace elements could also increase the discrimination on a finer scale, since regions separated by only a few kilometers can be significantly distinct (Carson et al. 2008).

A study using our stable isotopes approach in combination with genetic techniques could be the next step to improve the understanding of the population connectivity in the SSLMP and along the St. Lawrence system. As stable isotopes and the other variables mentioned above would represent short term transport of *Calanus* spp. populations over the overwintering period, genetic markers would reflect the result of long term connectivity.

Acknowledgements. We thank the Ecosystem Research Initiatives (Department of Fisheries and Oceans Canada), the Canadian Coast Guard, the Université du Québec à Chicoutimi and the SSLMP (Parks Canada) for their assistance with sampling. Thanks to A. Bui for her help creating maps. We are grateful to Dr. P. Ouellet and J-P. Allard for their help in sampling and to P. Joly for his help in survey preparation, technical support and availability. This study was supported by the Canadian Healthy Ocean Network (CHONE), Québec-Océan and the Institut des Sciences de la Mer de Rimouski.

LITERATURE CITED

- Barnard C, Martineau C, Frenette JJ, Dodson JJ, Vincent WF (2006) Trophic position of zebra mussel veligers and their use of dissolved organic carbon. *Limnol Oceanogr* 51:1473–1484
- Bélanger C (2003) Observation and modeling of a renewal event in the Saguenay Fjord. PhD dissertation, Université du Québec à Rimouski
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37: 911–917
- Campbell RG, Wagner MM, Teegarden GJ, Boudreau CA, Durbin EG (2001) Growth and development rates of the copepod *Calanus finmarchicus* reared in the laboratory. *Mar Ecol Prog Ser* 221:161–183
- Carson HS, Morgan SG, Green PG (2008) Fine-scale chemical fingerprinting of an open coast crustacean for the assessment of population connectivity. *Mar Biol* 153: 327–335
- Conover RJ (1988) Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitudes of the northern hemisphere. *Hydrobiologia* 167–168:127–142
- Couillard MA, Cabana G, Dery JF, Daigle G, Dodson JJ (2011) Ontogenetic habitat shifts of the Atlantic tomcod (*Microgadus tomcod*) across an estuarine transition zone. *Estuar Coast* 34:1234–1245
- De Ladurantaye R, Therriault JC, Lacroix G, Côté R (1984) Advective processes and zooplankton distribution in a fjord. *Mar Biol* 82:21–29
- de Lecea AM, Smit AJ, Fennessy ST (2011) The effects of freeze/thaw periods and drying methods on isotopic and elemental carbon and nitrogen in marine organisms, raising questions on sample preparation. *Rapid Commun Mass Spectrom* 25:3640–3649
- Deegan LA, Garritt RH (1997) Evidence for spatial variability in estuarine food webs. *Mar Ecol Prog Ser* 147:31–47
- DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid-synthesis. *Science* 197:261–263
- DiBacco C, Chadwick DB (2001) Assessing the dispersal and exchange of brachyuran larvae between regions of San Diego Bay, California and nearshore coastal habitats using elemental fingerprinting. *J Mar Res* 59:53–78
- DiBacco C, Levin LA (2000) Development and application of elemental fingerprinting to track the dispersal of marine invertebrate larvae. *Limnol Oceanogr* 45:871–880
- Falk-Petersen S, Mayzaud P, Kattner G, Sargent J (2009) Lipids and life strategy of Arctic *Calanus*. *Mar Biol Res* 5: 18–39
- Fry B (1981) Natural stable carbon isotope tag traces Texas shrimp migrations. *Fish Bull* 79:337–345
- Fry B (1996) $^{13}\text{C}/^{12}\text{C}$ fractionation by marine diatoms. *Mar Ecol Prog Ser* 134:283–294
- Fry B, Sherr EB (1984) $\delta^{13}\text{C}$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib Mar Sci* 27:13–47
- Fry B, Wainright SC (1991) Diatom sources of ^{13}C -rich carbon in marine food webs. *Mar Ecol Prog Ser* 76:149–157
- Godbout L, Trudel M, Irvine JR, Wood CC, Grove MJ, Schmitt AK, McKeegan KD (2010) Sulfur isotopes in otoliths allow discrimination of anadromous and non-anadromous ecotypes of sockeye salmon (*Oncorhynchus nerka*). *Environ Biol Fishes* 89:521–532
- Graeve M, Albers C, Kattner G (2005) Assimilation and biosynthesis of lipids in Arctic *Calanus* species based on feeding experiments with a ^{13}C labelled diatom. *J Exp Mar Biol Ecol* 317:109–125
- Harvey M, Devine L (2009) Oceanographic conditions in the estuary and the Gulf of St. Lawrence during 2008: zooplankton. DFO Can Sci Advis Sec Res Doc 2009/083
- Harvey M, Starr M (2005) 2003 state of the ocean: chemical and biological oceanographic conditions in the Estuary and Gulf of St. Lawrence. DFO Can Sci Advis Sec Res Doc 2005/053
- Hélie JF, Hillaire-Marcel C, Rondeau B (2002) Seasonal changes in the sources and fluxes of dissolved inorganic carbon through the St. Lawrence River— isotopic and chemical constraint. *Chem Geol* 186:117–138
- Ingram RG, El-Sabh MI (1990) Fronts and mesoscale features in the St. Lawrence Estuary. In: El-Sabh MI, Silverberg N (eds) Coastal estuarine studies. Oceanography of a large-scale estuarine system: the St. Lawrence. Springer, New York, NY, p 71–93
- Johnson CL, Leising AW, Runge JA, Head EJH, Pepin P, Plourde S, Durbin EG (2008) Characteristics of *Calanus*

- finmarchicus* dormancy patterns in the Northwest Atlantic. ICES J Mar Sci 65:339–350
- Kattner G, Hirche HJ, Krause M (1989) Spatial variability in lipid composition of calanoid copepods from Fram Strait, the Arctic. Mar Biol 102:473–480
- Kattner G, Hagen W, Lee RF, Campbell R and others (2007) Perspectives on marine zooplankton lipids. Can J Fish Aquat Sci 64:1628–1639
- Koutitonsky VG, Bugden GL (1991) The physical oceanography of the Gulf of St. Lawrence: a review with emphasis on the synoptic variability of the motion. In: Therriault JC (ed) The Gulf of St. Lawrence: small ocean or big estuary? Can Spec Publ Fish Aquat Sci 113:57–90
- Laprise R, Dodson JJ (1994) Environmental variability as a factor controlling spatial patterns in distribution and species diversity of zooplankton in the St. Lawrence Estuary. Mar Ecol Prog Ser 107:67–81
- Lavoie D, Simard Y, Saucier FJ (2000) Aggregation and dispersion of krill at channel heads and shelf edges: the dynamics in the Saguenay-St. Lawrence Marine Park. Can J Fish Aquat Sci 57:1853–1869
- Laws EA, Popp BN, Bidigare RR, Kennicutt MC, Macko SA (1995) Dependence of phytoplankton carbon isotopic composition on growth rate and $[CO_2]_{aq}$: theoretical considerations and experimental results. Geochim Cosmochim Acta 59:1131–1138
- Mahalanobis PC (1936) On the generalised distance in statistics. Proc Natl Inst Sci India 2:49–55
- Maps F, Plourde S, Zakardjian B (2010) Control of dormancy by lipid metabolism in *Calanus finmarchicus*: a population model test. Mar Ecol Prog Ser 403:165–180
- Maps F, Zakardjian BA, Plourde S, Saucier FJ (2011) Modeling the interactions between the seasonal and diel migration behaviors of *Calanus finmarchicus* and the circulation in the Gulf of St. Lawrence (Canada). J Mar Syst 88:183–202
- Maps F, Plourde S, Lavoie D, McQuinn I, Chassé J (2013) Modelling the influence of daytime distribution on the transport of two sympatric krill species (*Thysanoessa raschii* and *Meganyctiphanes norvegica*) in the Gulf of St. Lawrence, eastern Canada. ICES J Mar Sci 71:282–292
- Martineau C, Vincent WF, Frenette JJ, Dodson JJ (2004) Primary consumers and particulate organic matter: isotopic evidence of strong selectivity in the estuarine transition zone. Limnol Oceanogr 49:1679–1686
- McConnaughey T, McRoy CP (1979) Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Mar Biol 53:257–262
- Michener RH, Kaufman L (2007) Stable isotope ratios as tracers in marine food webs: an update. In: Michener RH, Lajtha K (eds) Stable isotopes in ecology and environmental science: ecological methods and concepts, 2nd edn. Blackwell Publishing, Oxford, p 238–282
- Michener RH, Lajtha K (2007) Stable isotopes in ecology and environmental science: ecological methods and concepts, 2nd edn. Blackwell Publishing, Oxford
- Miller SH, Morgan SG, White JW, Green PG (2013) Trace element signatures in larval soft tissues reveal transport, but not population connectivity. Mar Ecol Prog Ser 481: 1–10
- Ohman MD (1988) Sources of variability in measurements of copepod lipids and gut fluorescence in the California Current coastal zone. Mar Ecol Prog Ser 42:143–153
- Parent GJ, Plourde S, Turgeon J (2011) Overlapping size ranges of *Calanus* spp. off the Canadian Arctic and Atlantic coasts: impact on species' abundances. J Plankton Res 33:1654–1665
- Perrin G, DiBacco C, Plourde S, Winkler G (2012) Assessing stable isotope dynamics of diapausing *Calanus finmarchicus* and *C. hyperboreus* during the overwintering period: a laboratory experiment. J Plankton Res 34: 685–699
- Perry RI, Thompson PA, Mackas DL, Harrison PJ, Yelland DR (1999) Stable carbon isotopes as pelagic food web tracers in adjacent shelf and slope regions off British Columbia, Canada. Can J Fish Aquat Sci 56:2477–2486
- Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320
- Petursdottir H, Falk-Petersen S, Hop H, Gislason A (2010) *Calanus finmarchicus* along the northern Mid-Atlantic Ridge: variation in fatty acid and alcohol profiles and stable isotope values, $\delta^{15}N$ and $\delta^{13}C$. J Plankton Res 32: 1067–1077
- Plourde S, Runge JA (1993) Reproduction of the planktonic copepod *Calanus finmarchicus* in the lower St. Lawrence Estuary: relation to the cycle of phytoplankton production and evidence for a *Calanus* pump. Mar Ecol Prog Ser 102:217–227
- Plourde S, Joly P, Runge JA, Zakardjian B, Dodson JJ (2001) Life cycle of *Calanus finmarchicus* in the lower St. Lawrence Estuary: the imprint of circulation and late timing of the spring phytoplankton bloom. Can J Fish Aquat Sci 58:647–658
- Plourde S, Dodson JJ, Runge JA, Therriault JC (2002) Spatial and temporal variations in copepod community structure in the Lower St. Lawrence Estuary, Canada. Mar Ecol Prog Ser 230:211–224
- Plourde S, Joly P, Runge JA, Dodson J, Zakardjian B (2003) Life cycle of *Calanus hyperboreus* in the lower St. Lawrence Estuary and its relationship to local environmental conditions. Mar Ecol Prog Ser 255:219–233
- Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montana CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152: 179–189
- Rainville L (1979) Étude comparative de la distribution verticale et de la composition des populations de zooplankton du fjord du Saguenay et de l'estuaire maritime du Saint-Laurent. PhD dissertation, Université Laval, Québec City
- Riera P, Richard P (1997) Temporal variation of $\delta^{13}C$ in particulate organic matter and oyster *Crassostrea gigas* in Marennes-Oléron Bay (France): effect of freshwater inflow. Mar Ecol Prog Ser 147:105–115
- Runge JA, Simard Y (1990) Zooplankton of the St. Lawrence Estuary: the imprint of physical processes on composition and distribution. In: El-Sabh MI, Silverberg N (eds) Coastal estuarine studies. Oceanography of a large-scale estuarine system: the St. Lawrence. Springer, New York, NY, p 296–320
- Saucier FJ, Chassé J (2000) Tidal circulation and buoyancy effects in the St. Lawrence Estuary. Atmos-Ocean 38: 505–556
- Saucier FJ, Roy F, Gilbert D, Pellerin P, Ritchie H (2003) Modeling the formation and circulation processes of water masses and sea ice in the Gulf of St. Lawrence, Canada. J Geophys Res 108:3269–3289
- Saucier FJ, Roy F, Senneville S, Smith G, Lefavre D, Zakardjian B, Dumais JF (2009) Modelling of the circula-

- tion in the Estuary and Gulf of St. Lawrence in response to variations in fresh water runoff and winds. *Rev Sci Eau* 22:159–176
- Schafer CT, Smith JN, Côté R (1990) The Saguenay Fjord: a major tributary to the St. Lawrence Estuary. In: El-Sabh MI, Silverberg N (eds) Coastal and estuarine studies. Oceanography of a large-scale estuarine system: the St. Lawrence. Springer, New York, NY, p 378–420
- Simard Y (2009) The Saguenay-St. Lawrence Marine Park: oceanographic processes at the basis of this unique forage site of northwest Atlantic whales. *Rev Sci Eau* 22: 177–197 (in French)
- Søreide JE, Falk-Petersen S, Hegseth EN, Hop H, Carroll ML, Hobson KA, Blachowiak-Samolyk K (2008) Seasonal feeding strategies of *Calanus* in the high-Arctic Svalbard region. *Deep-Sea Res II* 55:2225–2244
- Sourisseau M, Simard Y, Saucier FJ (2006) Krill aggregation in the St. Lawrence system, and supply of krill to the whale feeding grounds in the Estuary from the Gulf. *Mar Ecol Prog Ser* 314:257–270
- Tamelander T, Søreide JE, Hop H, Carroll ML (2006) Fractionation of stable isotopes in the Arctic marine copepod *Calanus glacialis*: effects on the isotopic composition of marine particulate organic matter. *J Exp Mar Biol Ecol* 333:231–240
- Tan FC, Strain PM (1979a) Organic carbon isotope ratios in recent sediments in the St. Lawrence Estuary and the Gulf of St. Lawrence. *Estuar Coast Mar Sci* 8:213–225
- Tan FC, Strain PM (1979b) Carbon isotope ratio of particulate organic matter in the Gulf of St. Lawrence. *J Fish Res Board Can* 36:678–682
- Tan FC, Strain PM (1983) Sources, sinks and distribution of organic carbon in the St. Lawrence Estuary, Canada. *Geochim Cosmochim Acta* 47:125–132
- Therriault JC, Legendre L, Demers S (1990) Oceanography and ecology of phytoplankton in the St. Lawrence Estuary. In: El-Sabh MI, Silverberg N (eds) Coastal and estuarine studies. Oceanography of a large-scale estuarine system: the St. Lawrence. Springer, New York, NY, p 269–295
- Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57: 32–37
- Tremblay L, Gagné JP (2009) Organic matter distribution and reactivity in the waters of a large estuarine system. *Mar Chem* 116:1–12
- White JW, Ruttenberg BI (2007) Discriminant function analysis in marine ecology: some oversights and their solutions. *Mar Ecol Prog Ser* 329:301–305
- Zakardjian BA, Runge JA, Plourde S, Gratton Y (1999) A biophysical model of the interaction between vertical migration of crustacean zooplankton and circulation in the lower St. Lawrence Estuary. *Can J Fish Aquat Sci* 56: 2420–2432
- Zakardjian BA, Sheng J, Runge JA, McLaren Y, Plourde S, Thompson KR, Gratton Y (2003) Effects of temperature and circulation on the population dynamics of *Calanus finmarchicus* in the Gulf of St. Lawrence and Scotian Shelf: study with a coupled, three-dimensional hydrodynamic, stage-based life history model. *J Geophys Res* 108:8016–8038

Editorial responsibility: Christine Paetzold,
Oldendorf/Luhe, Germany

Submitted: May 23, 2013; Accepted: November 15, 2013
Proofs received from author(s): February 8, 2014