Vol. 400: 143–154, 2010
doi: 10.3354/meps08413

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published February 11

Relating RNA:DNA ratio in *Eurytemora affinis* to seston fatty acids in a highly dynamic environment

Julien Pommier, Jean-Jacques Frenette*, Hélène Glémet

Groupe de Recherche sur les Écosystèmes Aquatiques (GREA), Université du Québec à Trois-Rivières (UQTR), Département de Chimie-Biologie, 3351, boul. des Forges, Trois-Rivières (QC) G9A 5H7, Canada

ABSTRACT: Nine stations were sampled across the St. Lawrence estuarine transition zone (ETZ) in summer 2006 in order to investigate the variability in *Eurytemora affinis* growth condition, as revealed by RNA:DNA ratios, and its relationship to the nutritional quality of seston, expressed in terms of fatty acid (FA) composition. The population of *E. affinis* in the St. Lawrence ETZ was under the influence of brackish waters, as well as fresh and saline waters landwards and seawards of the frontal mixing zone, respectively. The RNA:DNA ratio for *E. affinis* throughout the ETZ ranged from 2.14 to 23.01, with an average value of 10.30 ± 4.35. When compared with the previously reported RNA:DNA ratio for copepods under optimal growth conditions, our values suggest that the summertime population of *E. affinis* in the St. Lawrence ETZ was in good growth condition. The hydrodynamic imprint within the St. Lawrence ETZ translated into the distribution pattern of seston having various FA compositions. Freshwater seston was of better nutritional quality (i.e. richer in essential fatty acids) than that from brackish and more saline waters. However, this did not translate into a significantly different *E. affinis* RNA:DNA ratio between the 3 types of waters. Yet, the growth condition of *E. affinis* under the influence of fairly stable fresh waters was associated with the relative abundance of 20:5n3-rich seston advected from upstream despite the low contribution of chl *a* to total suspended particulate matter. Moreover, a comparison between seston and zooplankton FA composition revealed the potential trophic transfer of diatom-related FA from producers to consumers within fresh waters. No such correlations were evidenced within the most dynamic parts of the ETZ (brackish and more saline waters) where the relationship between *E. affinis* growth condition and seston quality was likely constrained by a spatial-temporal decoupling between seston and zooplankton.

KEY WORDS: *Eurytemora affinis* · RNA:DNA ratio · Fatty acids · Estuarine transition zone · St. Lawrence River

INTRODUCTION

The St. Lawrence River is one of the largest lotic ecosystems on Earth, connecting the Great Lakes with the North Atlantic Ocean. Understanding the ecological structure and function of such large riverine systems has been the goal of many investigations and has led to the formulation of several conceptual models in the past 30 yr, from the River Continuum Concept (Vannote et al. 1980) to the Riverine Ecosystem Synthesis (Thorp et al. 2006). While these models are useful tools for understanding the flow of matter and energy through the riverine part, sensu stricto, of lotic systems, little is known about how connectivity (Pringle 2003) operates at the downstream edge of rivers where fresh and saltwaters meet at the estuarine frontal zone.

The St. Lawrence estuarine transition zone (ETZ) is of paramount importance in terms of biogeochemical interactions between continental waters and the ocean. Particularly, the ETZ is recognized as a site of high biological production (Vincent & Dodson 1999) where maximal concentrations of chlorophyll *a* (chl *a*) (Frenette et al. 1995, Vincent et al. 1996) as well as high abundances of bacteria and protists (Frenette et al. 1995), zooplankton (Bousfield et al. 1975, Winkler et al. 2003) and anadromous fish larvae (Laprise & Dodson 1989) have been reported.

*Corresponding author. Email: jean-jacques.frenette@uqtr.ca

The zooplankton assemblage in the St. Lawrence ETZ is dominated by *Eurytemora affinis.* This calanoid copepod plays an important role in the trophic dynamics of the St. Lawrence upper estuary where it feeds on phytoplankton (Winkler et al. 2003), mainly freshwater species advected from upstream (Lapierre & Frenette 2008), as well as on aggregates and attached bacteria (Zimmermann-Timm 2002, Martineau et al. 2004). *E. affinis* is amongst the major prey items for anadromous fish larvae (Winkler et al. 2003, Barnard et al. 2006), such as *Microgadus tomcod* and *Osmerus mordax*, which may colonize downstream environments later in their ontogenic cycle. Given its key trophic position within the St. Lawrence ETZ, *E. affinis* may therefore be considered an important regulator of the biological connectivity between the St. Lawrence River and the ocean.

Within this connectivity context, it is still unclear whether the high abundance of *E. affinis* in the St. Lawrence ETZ is related to passive or active processes. On the one hand, the St. Lawrence ETZ has been described as a 'graveyard' of fresh and marine zooplankton passively advected with the 2-layer estuarine circulation, retained by the hydrodynamic entrapment, and killed by the adverse physical and chemical conditions (Bousfield et al. 1975). On the other hand, Simons et al. (2006) emphasize the importance of tidal vertical migrations (TVM), which may be used by *E. affinis* as a strategy to maintain its population in the St. Lawrence ETZ. By using TVM, *E. affinis* would optimize its utilization of the 2-layer estuarine circulation and avoid flushing by surface currents. *E. affinis* may also have developed growth strategies as trade-offs to maximize fitness (Devreker et al. 2008) and maintain actively growing populations in highly dynamic environments such as estuaries. Characterizing *E. affinis* growth within the St. Lawrence ETZ would shed light on the question of whether the high abundance of *E. affinis* in the St. Lawrence ETZ is related to passive or active processes.

The ratio of ribonucleic acid (RNA) to deoxyribonucleic acid (DNA) has proven to be a useful tool for the determination of short-term growth of various zooplankton organisms (Saiz et al. 1998, Wagner et al. 1998, Vrede et al. 2002, Chícharo & Chícharo 2008). This method offers the advantage of responding rapidly (e.g. within 5 h in freshwater daphniids; Vrede et al. 2002) to changes in environmental conditions. As the hydrodynamic variability in the St. Lawrence upper estuary is mostly related to the semidiurnal tidal cycle (Bourgault 2001), *E. affinis* in the ETZ can experience variations in their surrounding environment every ~6 h. Therefore, using the RNA:DNA ratio is particularly relevant to quantify relatively short-term growth processes of *E. affinis* at a local scale, since the temporal response of animals could match the frequency at which environmental variations in the St. Lawrence ETZ occur.

Zooplankton growth is influenced by various factors, among which food quality can be of more relevance than food quantity (Koski et al. 1998, Andersen et al. 2007). This important influence of food quality on zooplankton growth is likely to occur in the St. Lawrence ETZ where the bulk particulate organic matter (POM) is largely composed of detritus of poor nutritional quality and where primary consumers feed selectively on phytoplankton despite its low contribution to total POM (Martineau et al. 2004). Polyunsaturated fatty acids (PUFA), particularly eicosapentaenoic acid (20:5n3) and docosahexaenoic acid (22:6n3), are a key factor in food quality, affecting the growth and reproduction of zooplankton as well as trophic transfer efficiency in aquatic food webs (Müller-Navarra et al. 2000, Kainz et al. 2004, Kattner et al. 2007). Tide-driven changes in the St. Lawrence ETZ hydrodynamics may control the distribution of available seston rich in essential fatty acids (EFA) and its availability as high quality food for *E. affinis*.

Within this framework, the main objectives of our study were (1) to characterize the variability in *E. affinis* growth condition across the St. Lawrence ETZ, using the RNA:DNA ratio as a biochemical indicator of copepod growth, (2) to investigate the relationship between *E. affinis* growth condition and the nutritional quality of the seston, evaluated in terms of its fatty acid (FA) composition and (3) to assess the potential trophic transfer of EFA from producer to consumer, by relating the FA composition of *E. affinis* to that of the seston. It is hypothesized that tide-driven hydrodynamics in the St. Lawrence ETZ influences the distribution of EFA-rich seston, which translates into varying growth conditions of *E. affinis* across the ETZ.

MATERIALS AND METHODS

Sampling. Sampling was carried out from 12 to 15 August 2006 aboard the RV 'Lampsilis' from the Université du Québec à Trois-Rivières. Nine stations were sampled from Québec City to downstream of Île-aux-Coudres, Canada (Fig. 1, Table 1). On 14 August, additional sampling was carried out at Stns 46 and 48 during each phase of the tidal cycle, i.e. flood tide (FT), high waters (HW), ebb tide (ET), and low waters (LW).

Water samples were collected using an 8 l GO-FLO water sampler (Model 1080; General Oceanics) at 1 m below the surface and at a depth ranging from 3 to 37 m above the bottom depending on the station (Table 1). Turbidity, temperature, and salinity were measured at the same depths using profiles conducted with a

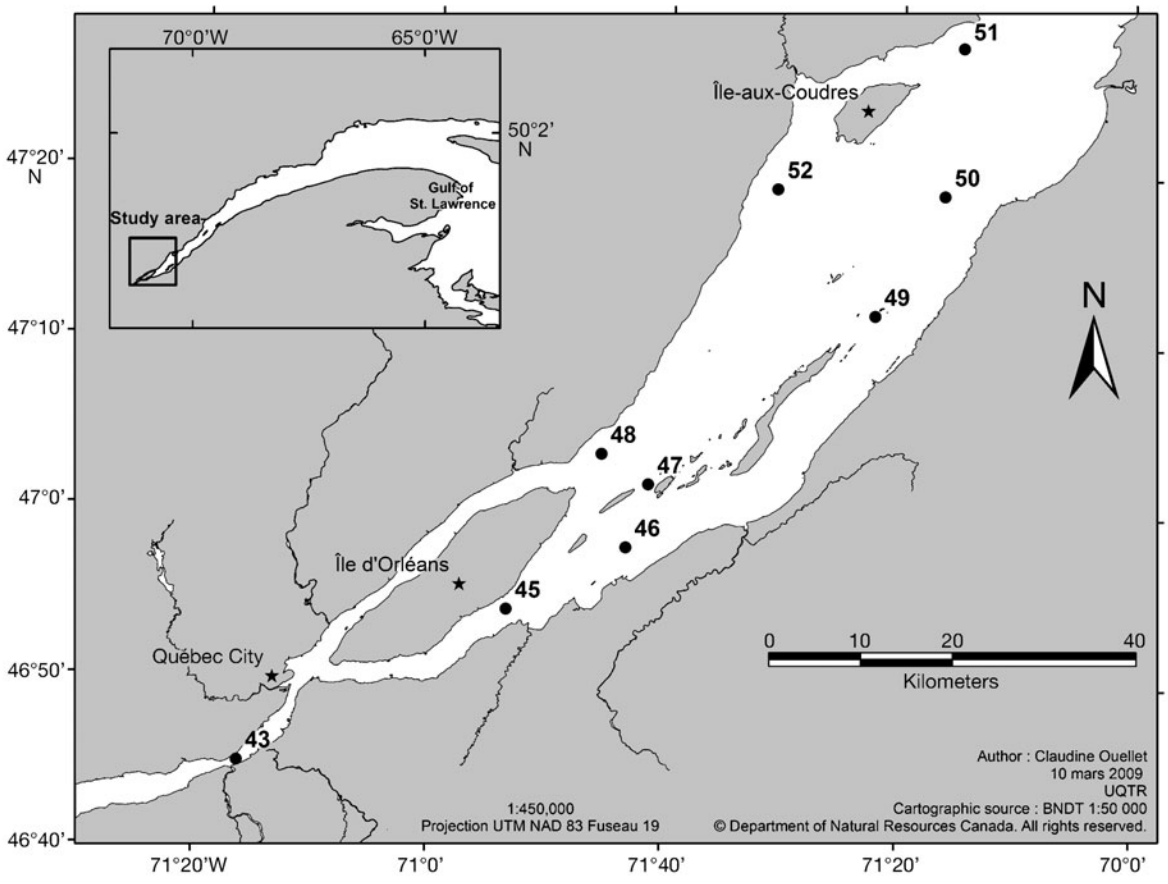

Fig. 1. Location of the sampling sites across the St. Lawrence River estuarine transition zone, i.e. from Île d'Orléans (Stn 45) to downstream of Île-aux-Coudres (Stn 51). In the vicinity of Québec City (Stn 43), an additional sampling was performed in order to obtain a freshwater reference point

YSI 6600 EDS-M sensor array (Yellow Spring Instruments). Subsamples for subsequent analyses were drawn from the GO-FLO bottles into acid-washed polyethylene bottles.

Subsamples were filtered (chl *a* measurements: 25 mm Millipore GF/F, nominal pore size 0.7 mm; seston FA determination: 45 mm Millipore GF/F precombusted for 4.5 h at 500°C) and filters were then kept at –80°C until analysis in the laboratory.

Table 1. Sampling site characteristics. 2 depths were sampled at each site. Dates given as d/mo/yr

Station	Date	Latitude (° N)	Longitude (° W)	Bottom depth (m)	Sampling depths (m)
43	12/08/06	46° 45′ 346	71° 16′ 205	10.9	1, 8
45	13/08/06	46° 54′ 802	70° 55′ 700	36	1, 15
46	13/08/06	46° 38′ 341	70° 41′ 428	13	1, 10
47	13/08/06	47° 03′ 587	70° 41′ 035	11.5	1, 10
48	13/08/06	49° 03′ 272	70° 41′ 428	17	1, 10
49	15/08/06	47° 10′ 901	70° 22′ 198	25	1, 10
50	15/08/06	47° 18′ 870	70° 14′ 067	7.9	1, 7
51	15/08/06	47° 24′ 687	70° 14′ 694	57.3	1, 20
52	15/08/06	47° 19′ 654	70° 30′ 590	15.9	1, 15

Zooplankton samples were collected using a conical net (1 m mouth opening, 150 µm mesh size) hauled horizontally at the same depths as those for water sample collection. Samples collected with the net were filtered onto a 500 µm mesh. The material retained on that mesh was poured into plastic jars, immediately frozen with liquid nitrogen, and stored onboard at –80°C until further biochemical analysis in the laboratory. Once in the lab, all manipulations where conducted in a cool room at 2 to 3°C where the frozen zooplankton samples were allowed to thaw gently and were then immediately placed into a Petri dish and sorted on ice under a binocular microscope. Copepodites Stage V and adults of *Eurytemora affinis* were sorted at all sampling sites except at the most downstream station, i.e. Stn 51, where *E. affinis* was replaced by *Eurytemora herdmanii*. The individual size of copepods collected in our study was relatively constant, with an average prosome length of 682 ± 93 µm. Duplicate sub-

samples of 7 individuals were placed into Eppendorf vials (acid washed and autoclaved) for RNA and DNA quantification. Another subsample of up to 100 individuals was sorted and poured into acid-washed and autoclaved Eppendorf vials for FA analyses. Upon collection of the appropriate number of individuals, the Eppendorf vials were immediately frozen with liquid nitrogen and stored at –80°C until analysis.

Analyses. Chl *a* and phaeopigment concentrations were measured with a Turner Designs 10-005R fluorometer, after sonication and 24 h extraction in 90% acetone at 4°C in the dark (Parsons et al. 1984).

RNA:DNA ratios were determined according to Kyle et al. (2003). Briefly, nucleic acids were extracted from duplicated pools of 7 copepods homogenized in a solution of N-lauroylsarcosine (0.1% final conc.; Sigma-Aldrich®) in Tris buffer (10 mM Tris, 1 mM Na EDTA, pH 7.5; Molecular Probes®). Aliquots of the extract were distributed into three 96-well microplates. Nuclease-free water was added to Plate 1 (control), whereas nucleases were added to Plates 2 (RNase) and 3 (RNase + DNase). Nucleic acids were stained using the cyanine base fluorescent dye RiboGreen™ (Molecular Probes), which binds non-specifically to nitrogenous bases of nucleic acids. RNA and DNA concentrations were determined from fluorescence readings (530 nm excitation, 590 nm emission filters) using a Varian™ Cary Eclipse Fluorescence spectrophotometer. Readings of Plates 1 (DNA + RNA conc.) and 2 (DNA conc.) were corrected for background fluorescence (Plate 3). RNA concentrations were then determined by subtracting the corrected fluorescence from Plate 2 from that of Plate 1.

Lipid extractions and FA analyses were performed on pools of 100 copepods following the protocol described by Hebert et al. (2009). Briefly, zooplankton samples were freeze-dried and weighted prior to FA analysis. Fatty acid methyl esters (FAME) were obtained by lipid extraction in chloroform:methanol (2:1 v/v), transesterification, and quantification using a capillary gas chromatograph (Agilent 6890N) coupled with a flame ionization detector. A 37-component FAME standard (Supelco no. 47885-U) was used to identify and quantify FAME in the samples by comparing their retention times to those of the FAME standard. Results are reported as concentrations (µg FAME mg^{-1} dry weight of tissue extracted) and as percentage values of identified total FA. The same protocol was applied for the seston samples.

Arachidonic acid (20:4n6), eicosapentaenoic acid (20:5n3), and docosahexaenoic acid (22:6n3) were designated as EFAs. Linoleic acid (18:2n6) and α-linolenic acid (18:3n3) were also considered as potential EFAs, as they can be converted to 20:4n6 and 20:5n3, respectively, when the 2 latter EFAs are in short supply (Kainz et al. 2004). Moreover, on the basis of the FA composition, a variety of ratios were determined as potential trophic markers to obtain information on the phytoplankton contribution to the seston and the feeding behaviour of copepods. The ratio of polyunsaturated FAs (PUFA) to saturated FAs (SAFA) was considered as a marker of the relative importance of phytoplankton versus detritus, the ratio of 16:1n7 to 16:0 was used to discriminate between diatom versus phytoflagellate, and the ratio of the n3 and n6 PUFA fractions was utilized as an indicator of the nutritive value of the seston (Biandolino et al. 2008 and references therein). Finally, the ratio of chl *a* to suspended particulate matter (SPM) was calculated after deriving the SPM concentration from turbidity measurements following the procedure of Pfannkuche & Schmidt (2003).

Data analyses. Data analyses were performed using the R graphical and statistical computing environment (www.r-project.org). Data were checked for normality and, when needed, transformation of the original data was applied using a power function. The index of power transformation was adjusted by computing normal Quantile/Quantile plots and Shapiro-Wilks normality tests. Results are presented as mean ± SD when applicable. The differences between the mean values of 2 sets of data were analysed using Student's *t*-test. A one-way analysis of variance (ANOVA) was used when 3 or more sets of data were compared and was followed by the Tukey's post-hoc test when a significant difference was detected. Correlation matrixes were calculated in order to relate the variability in RNA:DNA ratio to abiotic (temperature, salinity, turbidity) and biotic (chl *a*, phaeopigments, FA composition of seston and zooplankton) variables. A multiple linear regression analysis was used in order to relate the RNA:DNA ratio of *Eurytemora* spp. to several independent variables and to check for multicollinearity between them. Linear correlation analyses were also used to investigate the trophic transfer of FAs from seston to zooplankton. A clustering analysis (K-means method) was also performed, using the SAS JMP® statistical software, in order to discriminate the sampling stations into different groups, based on temperature and salinity data. These 2 abiotic variables were selected, as they are the imprint of the tide-driven hydrodynamics in the ETZ where cold and saline waters mix with fresh and relatively warmer waters.

RESULTS

The clustering analysis, based on temperature and salinity, allowed for the discrimination of the sampling stations into 3 groups (Fig. 2, Table 2) fairly well related to the hydrodynamics in the St. Lawrence ETZ

(Bourgault 2001, Simons et al. 2006). Group A (Stns 43, 45, 46 and 47) was characterized by the downstream flow of fresh waters with stable temperatures, low turbidity and peak concentrations in chl *a*. Group C (Stns 50, 51 and 52) was characteristic of the tidal upstream flow of saline and cold waters with low turbidity. Group B (Stns 48 and 49) corresponded to highly turbid brackish waters with stable temperatures slightly colder than those of Group A and located in the mixing zone of the 2 other water masses. Due to the Coriolis force, the mixing zone extended farther upstream on the north shore (Stn 48) and downstream on the south shore (Stn 49).

The pattern of spatial variability in the RNA:DNA ratio of *Eurytemora* spp. across the St. Lawrence ETZ is presented in Fig. 3. The RNA:DNA ratio ranged from 2.14 (Stn 45 at surface) to 23.01 (Stn 48 at depth during flood tide), with an average value throughout the ETZ of 10.30 ± 4.35. The RNA:DNA ratios at the surface were not significantly different from those at depth, except at Stns 45 and 49, and at Stn 46 during ebb tide and Stn 48 during high and low waters (Student's *t*-test, $p < 0.05$). The average RNA:DNA ratio was not significantly different (1-way ANOVA; $p < 0.01$) between fresh (Group A), brackish (Group B) and saline (Group C) waters (Tables 2 & 4).

The average FA composition of the seston for each group of stations is presented in Table 3. Within fresh waters (Group A), the most abundant FAs were 16:0, 16:1n7 and 20:5n3 (22, 15 and 12% of identified FA, respectively). The percent FA composition of seston from brackish waters (Group B) was dominated by 16:0, 18:0 and 16:1n7, whereas 16:0, 18:0 and 18:1n9 dominated the seston from saline waters (Group C). The seston from fresh waters was significantly richer in 16:1n7, 18:2n6, 18:3n3, 20:5n3 (1-way ANOVA; $p < 0.01$) and 22:6n3 (1-way ANOVA; $p < 0.05$) than seston from brackish and saline waters (Table 4). Moreover, the 16:1n7/16:0, ∑n3:∑n6, ∑PUFA:∑SAFA and chl *a*:SPM ratios were significantly higher in fresh waters (1-way ANOVA; $p < 0.01$) than in brackish and saline waters (Table 4).

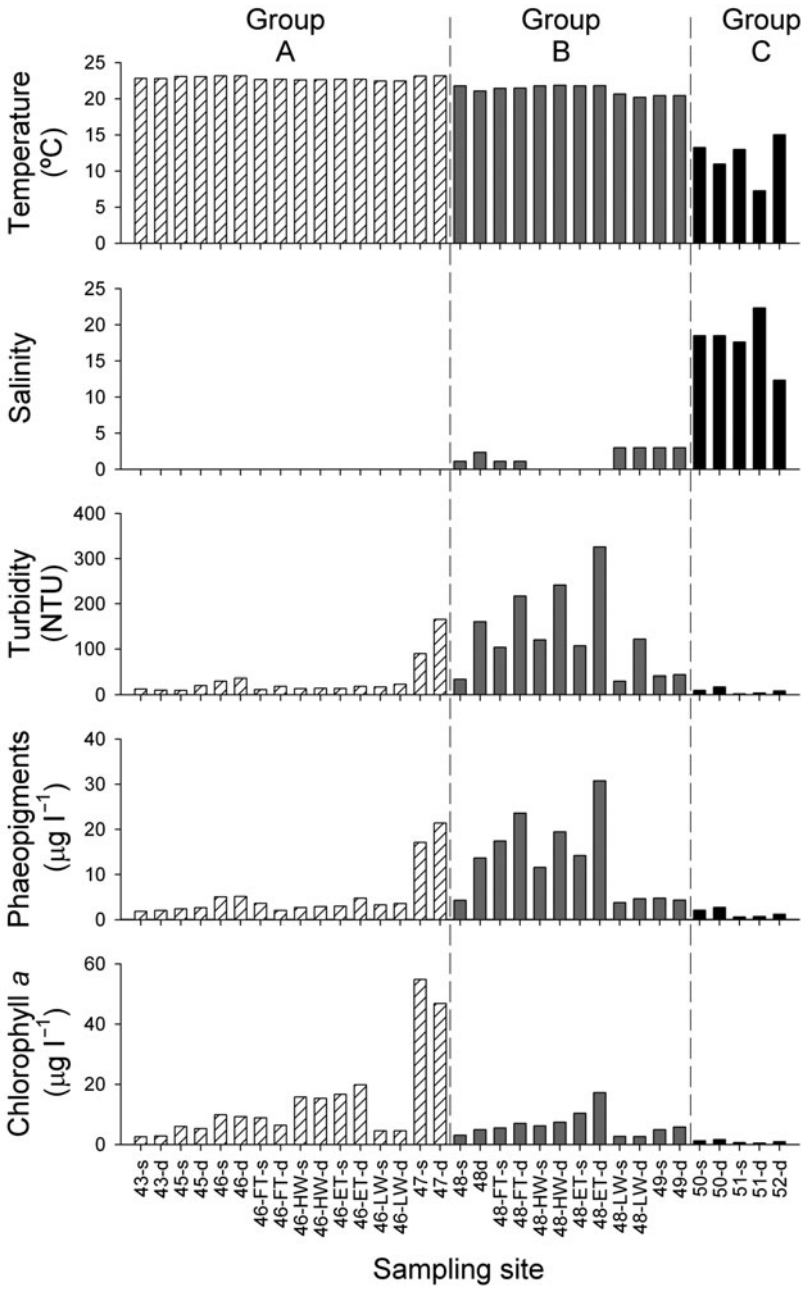

Fig. 2. Spatial changes in temperature (°C), salinity, turbidity (NTU; nepholometer turbidity units) and concentrations (µg l^{-1}) of phaeopigments and chl *a* within the St. Lawrence River estuarine transition zone. Sampling sites were discriminated into 3 groups (A, B and C) according to a clustering analysis based on temperature and salinity values. Surface and deep samples for each site are identified by letters following the station number (s and d, respectively). Additional sampling performed at Stns 46 and 48 during each phase of the tidal cycle are also identified by letters, i.e. flood tide (FT), high waters (HW), ebb tide (ET) and low waters (LW)

Throughout the St. Lawrence ETZ, the *Eurytemora* RNA:DNA ratio was not significantly correlated to any of the other abiotic and biotic variables measured (Pearson's product-moment correlations; $p > 0.05$). Within fresh waters (Group A), the RNA:DNA ratio was not sig-

Table 2. Average ± SD values of physical variables, concentrations of chl *a* and phaeopigment, ratio of chl *a* to suspended particulate matter (chl *a*:SPM) and RNA:DNA ratio within each of the 3 groups of stations discriminated using the clustering analysis

	Group A (Stns 43, 45–47)	Group B (Stns 48–49)	Group C (Stns 50–52)
Temperature (°C)	22.8 ± 0.2	21.2 ± 0.6	11.9 ± 2.9
Salinity	0.0 ± 0.0	1.5 ± 1.3	17.8 ± 3.6
Turbidity (NTU)[a]	31.3 ± 43.6	128.8 ± 93.2	8.2 ± 6.0
Chl *a* (µg l^{-1})	15.0 ± 16.2	6.5 ± 4.0	1.0 ± 0.5
Phaeopigments (µg l^{-1})	5.2 ± 6.0	12.7 ± 8.9	1.5 ± 0.9
Chl *a*:SPM (%)	36 ± 23	7 ± 3	5 ± 1
RNA:DNA	9.5 ± 3.3	10.5 ± 5.1	12.2 ± 5.3

[a]Nephelometer turbidity units

nificantly correlated to any abiotic variables nor to seston FA concentrations (Pearson's product-moment correlations; $p > 0.05$). However, the *Eurytemora* RNA:DNA ratio within Group A was significantly correlated to the concentration of chl *a* (Pearson's product-moment correlation; $r^2 = 0.63$, $p < 0.01$) and to the percent contributions of 20:5n3 ($r^2 = 0.63$, $p < 0.01$), 16:1n7 ($r^2 = 0.62$, $p < 0.01$) and n3 FAs ($r^2 = 0.58$, $p < 0.01$) to total FA composition (Fig. 4). A multiple linear regression analysis was performed in order to relate the *Eurytemora* RNA:DNA ratio within Group A to seston chl *a* concentration, %20:5n3 and %16:1n7 and to check for multicollinearity between independent variables. Although each of the 3 independent variables had a significant ($p < 0.01$) zero-order correlation with the copepod RNA:DNA ratio, only %20:5n3 had significant partial effects ($p < 0.05$) in the full model, which was able to account for 61% of the variance in the copepod RNA:DNA ratio within Group A ($F_9 = 10.77$, $p < 0.01$). Variance inflation factors for chl *a*, %20:5n3 and %16:1n7 within Group A were 2.77, 2.39 and 4.14, respectively, suggesting that there was no multicollinearity between these 3 independent variables within fresh waters. Within brackish (Group B) and saline (Group C) waters, the RNA:DNA ratio was correlated neither to abiotic variables nor to seston FA composition (Pearson's product-moment correlation; $p > 0.05$).

Eurytemora FA composition was not significantly correlated to that of the seston throughout the St. Lawrence ETZ (Pearson's product-moment correlations; $p > 0.05$). Within fresh waters (Group A), the concentrations of 20:5n3, 16:1n7 and total n3 FAs in *Eurytemora* were significantly correlated to those in the seston ($r^2 = 0.53$, 0.40

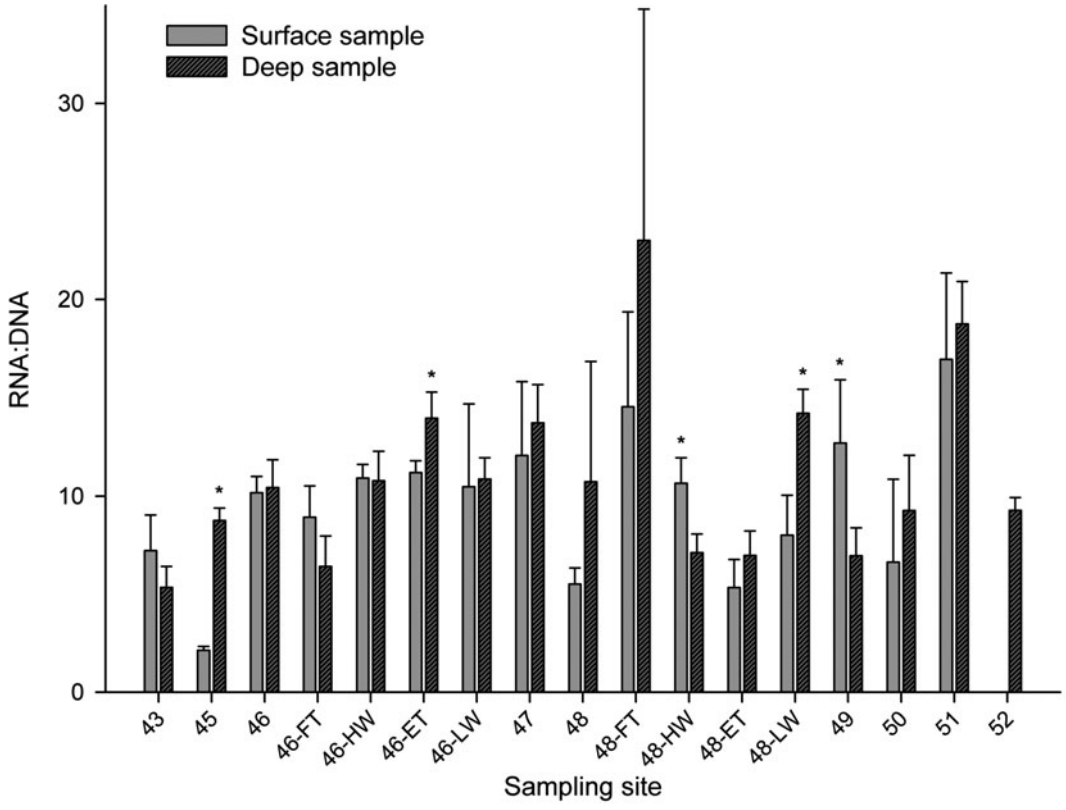

Fig. 3. *Eurytemora affinis* . Spatial changes in the RNA:DNA ratio of from surface (grey bar) and deep (shaded dark-grey bar) samples within the St. Lawrence River estuarine transition zone. Values are mean ± SD. Stations with significant differences ($p < 0.05$) in RNA:DNA ratio between surface and deep samples are indicated by an asterisk. Additional sampling performed at Stns 46 and 48 during each phase of the tidal cycle is identified by letters, i.e. flood tide (FT), high waters (HW), ebb tide (ET) and low waters (LW)

Table 3. Seston fatty acid composition within each of the 3 groups of stations discriminated by mean of the clustering analysis. Data are reported as average ± SD concentration and percentage value of reported total fatty acids. SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ∑n3, sum of omega-3 fatty acids; ∑n6, sum of omega-6 fatty acids. dry wt = dry weight

	Group A		Group B		Group C	
	(µg mg^{-1} dry wt)	(%)	(µg mg^{-1} dry wt)	(%)	(µg mg^{-1} dry wt)	(%)
SAFA						
14:0	0.06 ± 0.03	7	0.03 ± 0.03	6	0.04 ± 0.04	2
15ai	0.06 ± 0.04	7	0.02 ± 0.01	5	0.01 ± 0.01	1
15:0	0.05 ± 0.03	5	0.05 ± 0.04	5	0.04 ± 0.04	3
16:0	0.20 ± 0.15	22	0.11 ± 0.11	23	0.35 ± 0.29	28
18:0	0.08 ± 0.03	7	0.09 ± 0.08	13	0.34 ± 0.30	22
MUFA						
16:1n7	0.13 ± 0.05	15	0.02 ± 0.01	8	0.03 ± 0.02	5
18:1n7	0.02 ± 0.01	2	0.01 ± 0.00	2	0.02 ± 0.01	3
18:1n9	0.06 ± 0.04	6	0.02 ± 0.02	5	0.17 ± 0.15	9
PUFA						
18:2n6c	0.02 ± 0.01	2	0.00 ± 0.00	2	0.01 ± 0.01	2
18:3n3	0.02 ± 0.01	2	0.00 ± 0.00	1	0.00 ± 0.00	1
20:4n6	0.01 ± 0.01	2	0.01 ± 0.00	2	0.01 ± 0.01	2
20:5n3	0.11 ± 0.07	12	0.02 ± 0.01	6	0.02 ± 0.01	5
22:6n3	0.01 ± 0.01	2	0.01 ± 0.00	2	0.01 ± 0.01	1
∑n3	0.14 ± 0.07	16	0.03 ± 0.02	9	0.04 ± 0.02	7
∑n6	0.03 ± 0.01	4	0.01 ± 0.00	4	0.02 ± 0.01	4
∑SAFA	0.52 ± 0.40	55	0.32 ± 0.32	69	0.88 ± 0.72	68
∑MUFA	0.22 ± 0.10	25	0.07 ± 0.04	19	0.24 ± 0.23	19
∑PUFA	0.17 ± 0.09	20	0.04 ± 0.02	13	0.13 ± 0.12	13

and 0.32, respectively; $p < 0.05$) (Fig. 5). However, within Group A, the relative (%) FA content of *Eurytemora* was not significantly correlated to that in the seston ($p > 0.05$). No significant correlations were found between the FA composition of seston and that of zooplankton within brackish (Group B) and saline (Group C) waters (Pearson's product-moment correlation; $p > 0.05$).

Table 4. One-way ANOVA (and Tukey's post-hoc test) comparing the quality of seston (expressed as its concentration in selected fatty acid and its chl *a*:SPM ratio) and the RNA:DNA ratio of *Eurytemora affinis* between each of the 3 groups of stations (A, B and C) discriminated by means of the clustering analysis. ∑n3 and ∑n6, sum of omega-3 and omega-6 fatty acids, respectively; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids; SPM, suspended particulate matter. $^{**}p < 0.01$; $^{*}p < 0.05$

Variable	ANOVA		Tukey's post-hoc test		
			A-B	A-C	B-C
	F	p	p	p	p
16:1n7	26.81	<0.01**	<0.01**	<0.01**	9.56
18:2n6	9.66	<0.01**	<0.01**	0.29	0.19
18:3n3	14.14	<0.01**	<0.01**	0.02*	0.59
20:4n6	2.49	0.10	0.16	0.20	0.94
20:5n3	13.89	<0.01**	<0.01**	<0.01**	0.97
22:6n3	4.75	0.02*	<0.05*	0.54	0.45
16:1n7/16:0	10.25	<0.01**	<0.01**	<0.01**	0.95
∑n3:∑n6	11.02	<0.01**	<0.01**	<0.01**	0.56
∑PUFA:∑SAFA	6.99	<0.01**	<0.01**	0.03*	0.99
Chl *a*:SPM	14.43	<0.01**	<0.01**	0.01*	0.95
RNA:DNA	0.70	0.50	0.84	0.45	0.75

DISCUSSION

RNA:DNA ratio and growth condition of *Eurytemora affinis*

On an individual basis, DNA per somatic cell is assumed to be constant in sexually mature adults so that the RNA:DNA ratio can be related to the magnitude of RNA transcription, protein synthesis and hence growth. Yet, the RNA:DNA ratios reported in our study are relative to a pool of 7 individuals and consequently represent average values for the population of *E. affinis* in the St. Lawrence ETZ. In this context, the variability in RNA:DNA ratios may be related either to a population with variable DNA but constant RNA concentrations (i.e. population composed of individuals of different body sizes with fairly constant growth) or to a population with variable RNA but constant DNA concentrations (i.e. population of growing individuals of similar body sizes) or to a population with covariation in DNA and RNA concentrations. In our study, the RNA:DNA ratio was significantly correlated to RNA concentration ($r^2 = 0.61$, $p < 0.01$) but not to that of DNA ($p > 0.05$), and the size of the copepods collected in our study was relatively constant (see 'Materials and methods'). Together, these results suggest that the variability in RNA:DNA ratio was likely related to variable growth conditions of the population of *E. affinis* across the St. Lawrence ETZ.

The average RNA:DNA ratio of 10.30 ± 4.35 reported for *E. affinis* in our study is about 3 times higher than the maximal value of ~3.5 measured for the same species in the northern Baltic proper during the spring phytoplankton bloom (Gorokhova et al. 2007). Yet, our RNA:DNA average value is in fairly good agreement with a previous study reporting RNA:DNA ratio for other calanoid species under optimal nutritional and growth conditions. Speekman et al. (2007) reported RNA:DNA ratios for *Acartia tonsa* of up to 14, in laboratory experiments, when fed on the EFA-rich *Thalassiosira* sp. Similarly, RNA:DNA values of up to 8 were reported for females of *Calanus finmarchicus* under excess-food conditions (Wagner et al. 2001). Comparisons of our results with those found in studies of other species or populations should be used with caution, as our RNA:DNA values are relative to the population of *E. affinis* in the St. Lawrence ETZ, for which data have been collected only once, and should be calibrated with values from the same

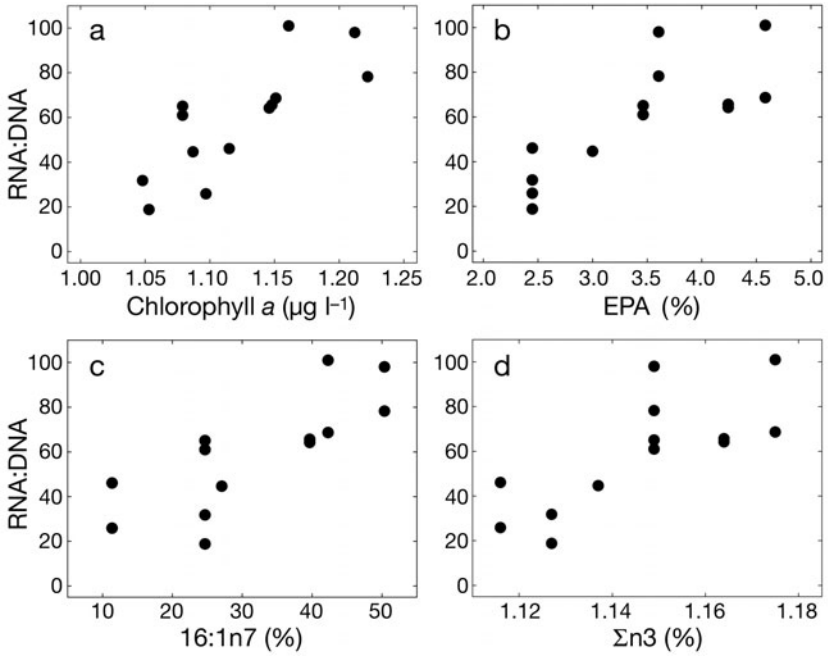

Fig. 4. *Eurytemora affinis*. Relationships between the RNA:DNA ratio and the seston composition in terms of (a) chl *a* (µg l^{-1}), (b) eicosapentaenoic acid (EPA) (20:5n3), (c) 16:1n7 fatty acid and (d) total n3 (Σn3) fatty acids within Group A. Fatty acids data are expressed as percentage values (%) of reported total fatty acids. For each graph, data are transformed values (see 'Materials and methods' for details)

population under low growth conditions. However, the presence of numerous ovigerous females carrying spermatophores, observed during the sorting of our samples, likely indicates that the summertime population of *E. affinis* within the ETZ was in growth condition good enough to allow for the allocation of energy towards reproduction.

Variability in the nutritional quality of seston and effect on copepod growth

The hydrodynamic imprint within the St. Lawrence ETZ translated into the distribution pattern of seston having various FA compositions. Indeed, apart from 16:0, which was the dominant FA in all groups, freshwater seston (Group A) was relatively rich in 20:5n3 and 16:1n7, i.e. 2 potential biomarkers of diatoms (Dalsgaard et al. 2003, Brett et al. 2009). Seston in saline waters (Group C) was relatively rich in 18:0 and 18:1n9, i.e. 2 dominant FAs different from those found in fresh waters and potential markers for detrital material (Søreide et al. 2008). Finally, the seston from brackish waters (Group B) integrated the signal of hydrodynamic mixing and was mainly composed of 16:1n7 and 18:0, dominant FAs in fresh and saline waters, respectively. Comparatively, Lapierre & Frenette (2008) reported the percent occurrences of the major phytoplankton genera sampled in summer 2006 in the St. Lawrence ETZ at Stns 46 (freshwaters), 48 (brackish waters) and 50 (saline waters). Diatoms and cyanophytes dominated the phytoplankton community structure at all sites, but no distinct longitudinal pattern was evidenced throughout the ETZ, as the abundance of diatoms and cyanophytes did not show any significant variations from Stns 46 to 50. The lack of a clear distinction in the dominant phytoplankton genera between the 3 groups of stations compared with the information provided with the seston FA composition may be attributed to the fact that chl *a* contributed a small fraction to total SPM throughout most of the St. Lawrence ETZ (Table 2) so that phytoplankton FA was probably a minor component of total seston FA.

The higher concentrations of EFAs and higher 16:1n7/16:0, ∑n3:∑n6, ∑PUFA:∑SAFA and chl *a*:SPM ratios in the seston from Group A compared with those within Groups B and C suggest that freshwater seston was of better nutritional quality than that from brackish and saline waters. Interestingly, on the basis of sulfur-stable isotopic ratios from planktonic algae, Lapierre & Frenette (2008) reported that high concentrations in chl *a* within the St. Lawrence ETZ are related to the advection and retention of freshwater algae (mainly diatoms and cyanophyceae) rather than to *in situ* primary production. In the context of hydrological connectivity within the St. Lawrence River, the ETZ may therefore be viewed as a receptacle in which seston of high nutritional quality, mostly associated with freshwater diatoms advected from upstream, mixes with seston of lower quality originating from the tidal flow of saline waters. Yet, the imprint of the variable quality of seston did not translate into differential growth conditions for *E. affinis* across the St. Lawrence ETZ, as no significant differences in the average RNA:DNA ratio were evidenced between fresh, brackish and saline waters.

Despite the lack of inter-group differences in *E. affinis* growth across the St. Lawrence ETZ, the variability in RNA:DNA ratio of *E. affinis* within Group A was related to that of seston quality. Previous studies have related copepod growth to temperature (Katona 1970,

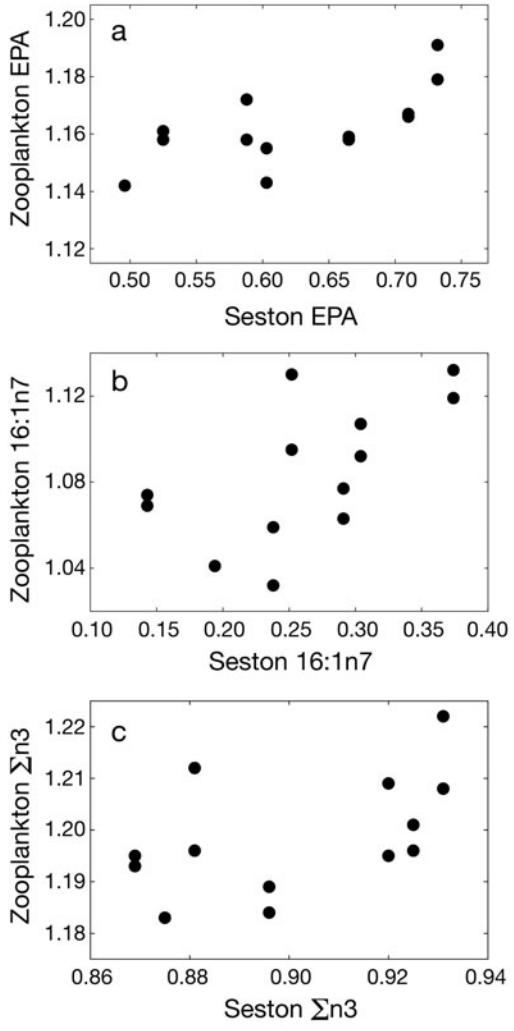

Fig. 5. Relationships between the concentrations (µg fatty acid methyl esters per mg dry weight of tissue extracted) of (a) eicosapentaenoic acid (EPA) (20:5n3), (b) 16:1n7 fatty acid and (c) total omega-3 fatty acids (∑n3) in seston and in zooplankton within Group A (freshwater samples). Data are transformed values (see 'Materials and methods' for details)

Lee et al. 2003) as well as food availability (Klein Breteler et al. 1982, Barthel 1983) and quality (Koski et al. 1998, Andersen et al. 2007). Temperature is recognized as the dominant factor influencing copepod growth under adequate food supplies; conversely, when the temperature range is narrow, food becomes the predominant determining factor for growth (Chícharo & Chícharo 2008 and references therein). Coherently, within Group A, temperature was fairly constant, and the zooplankton RNA:DNA ratio was significantly correlated to seston chl *a*, %20:5n3 and %16:1n7. The multiple regression analysis between the *Eurytemora* spp. RNA:DNA ratio and seston chl *a*, %20:5n3 and %16:1n7 revealed the lack of multicollinearity between chl *a* and seston FA and highlighted the importance of high quality food, particularly 20:5n3-rich seston, rather than food quantity (i.e. chl *a*) for *E. affinis* growth. This is also consistent with the fact that *E. affinis* growth was correlated to the relative FA content of seston but not to its FA concentration. *E. affinis* is an omnivorous suspension feeder, able to feed on a large particle spectra including algae, protozoa, bacteria, and detritus within the size range of 1 to 60 µm (Andersen & Nielsen 1997). Throughout the St. Lawrence ETZ, chl *a* contributed a small fraction to total SPM (Table 2), and turbidity was significantly correlated to the concentration of phaeopigments (r^2 = 0.88, $p < 0.01$) but not to that of chl *a,* emphasising the importance of detrital organic matter. However, zooplankton in the St. Lawrence ETZ is able to feed selectively on phytoplankton despite its low contribution to total POM (Martineau et al. 2004). Finally, the lack of relationship between %20:5n3 and %16:1n7 in the seston and that in zooplankton may be related to quasi-homeostatic FA composition responses of crustacean zooplankton to dietary FA availability (Brett et al. 2009). Yet, the significant relationships between 20:5n3 and 16:1n7 concentrations in the seston and those in zooplankton point toward the potential trophic transfer of these 2 FAs from diatoms to zooplankton. Our results are coherent with those of Müller-Navarra et al. (2000), which demonstrated that 20:5n3 is a good predictor of carbon transfer between planktonic primary producers and consumers in freshwater ecosystems. Our results also suggest that, even in systems with large amounts of detrital carbon from a variety of sources, nutritional factors associated with phytoplankton can be dominant in influencing zooplankton growth, as previously reported for *Daphnia* in the Sacramento River Tidal System (Müller-Solger et al. 2002).

Apparent decoupling between seston FA composition and zooplankton growth within the ETZ

In contrast with what has been shown within fresh waters, the lack of a relationship between the *E. affinis* RNA:DNA ratio and seston quality as well as between the FA composition of seston and that of the copepods within brackish and saline waters of the St. Lawrence ETZ suggests that *E. affinis* growth was not directly associated with seston of the locations inhabited by the copepods at discrete times. This uncoupling may be associated with various factors including TVMs, time-delay in zooplankton growth response to environmental variability, osmoregulatory processes and genetic diversity of *E. affinis* within the St. Lawrence ETZ.

Zooplankton can use TVM to take advantage of the 2-layer estuarine circulation and maintain their position in the estuary by swimming downwards to the bottom on ebb tide and by migrating (or being resuspended) upwards to the surface on flood tide (Devreker et al. 2008). Modelling studies (Simons et al. 2006) have demonstrated that TVM is an efficient mechanism for zooplankton retention in the St. Lawrence ETZ, although this has yet to be demonstrated *in situ*. TVM could have resulted in a spatial decoupling between zooplankton and seston so that the RNA:DNA ratio of zooplankton sampled at a given station could integrate the signal of feeding on some seston of different quality at a different location in the ETZ where they stood before migrating along with the tidal cycle.

We have considered the RNA:DNA ratio as an index of copepod growth, assuming a time-response of this ratio of 5 h, as reported for *Daphnia galatea* at 16.5°C (Vrede et al. 2002). However, the time-response of the RNA:DNA ratio may be slower in *Eurytemora* than in *Daphnia* owing to lower growth rates and longer generation times of copepods than those of cladocerans (Becker et al. 2004). This may have led to a temporal decoupling between zooplankton and seston, so that the RNA:DNA ratio of zooplankton sampled at a given station could instead integrate the signal of an earlier feeding episode on some other seston of different quality.

So far, the RNA:DNA ratio has been used as a proxy for copepod growth in relatively stable environments in terms of salinity. Yet, the St. Lawrence ETZ is characterized by a large salinity gradient where *Eurytemora* may experience osmoregulation (Gonzales & Bradley 1994). *E. affinis* can modify protein synthesis as an efficient short-term adaptation to salinity (Kimmel & Bradley 2001) and acclimate to salinity changes in less than 12 h (Roddie et al. 1984). This is particularly relevant given the semidiurnal pattern of tide-driven changes in salinity in the St. Lawrence middle estuary. Therefore, throughout the St. Lawrence ETZ, the variability in *Eurytemora* RNA:DNA ratio may not only express the signal of variable growth conditions but may also integrate that of protein synthesis in response to salinity stress. Finally, Winkler et al. (2008) revealed the coexistence of 2 morphologically identical but genetically distinct clades of *E. affinis* within the St. Lawrence ETZ. The non-invasive North Atlantic clade primarily occupies the variable brackish waters of the central portion of the St. Lawrence Middle Estuary (Group B), whereas the Atlantic clade invaded the more stable fresh waters of the upstream reaches of the estuary (Group A). These 2 clades of *E. affinis* are exposed to different salinity stresses and may have developed different physiological responses in terms of osmoregulation which may affect the RNA:DNA ratio signal, as mentioned above.

CONCLUSIONS

Understanding the factors responsible for the variability in copepod growth and production rates within estuaries is paramount in regard to both their crucial position in the trophic food web and their key role in supporting very productive fish nurseries. This study is the first to simultaneously examine the *in situ* growth condition of *Eurytemora affinis* and its relation to seston FA composition in an ETZ, using the RNA:DNA ratio as a proxy for copepod short-term growth condition. Our results demonstrated that the growth condition of *E. affinis* under the influence of fairly stable fresh waters was related to the relative abundance of 20:5n3-rich seston, likely associated with freshwater diatoms advected from upstream, despite the low contribution of chl *a* to total SPM. However, in the most dynamic part of the ETZ (i.e. the frontal mixing zone), evidence of the relationship between *E. affinis* growth and seston FA composition was impaired by a spatial-temporal decoupling likely associated with zooplankton TVMs and an unmatched time-response of the RNA:DNA ratio in *E. affinis* to tide-driven environmental variations. This study highlights the limits of using the RNA:DNA ratio as a proxy for copepod growth in a very dynamic and variable environment such as an ETZ.

Acknowledgements. This research was supported by grants from the Natural Sciences Research Council of Canada (NSERC shiptime program) to J.-J.F. (Principal Investigator), and from the NSERC discovery program and the Fonds québécois de la recherche sur la nature et les technologies (FQRNT) to J.-J.F. and H.G., and by a postdoctoral fellowship from the Groupe de recherche interuniversitaire en limnologie (GRIL) to J.P. We gratefully acknowledge Captain F. Harvey and the crew of the RV 'Lampsilis' for their invaluable support during the expedition. We are especially indebted to J. -F. Lapierre and P. Thibeault for technical assistance in the field. We also thank T. Bélanger, V. Cloutier and B. Kakou for nucleic acid analyses; M. T. Arts and M. Drebenstedt (National Water Research Institute/Canada Centre for Inland Waters; Burlington, Ontario, Canada) for FA analyses; G. Winkler and G. Méthot for assistance with zooplankton identification; P. Massicotte for assistance with data analysis and C. Ouellet for the map used in Fig. 1. This is a contribution to the GRIL research program.

LITERATURE CITED

Andersen CM, Nielsen TG (1997) Hatching rate of the egg-carrying estuarine copepod *Eurytemora affinis*. Mar Ecol Prog Ser 160:283–289

Andersen T, Fœrøvig PJ, Hessen DO (2007) Growth rate versus biomass accumulation: different roles of food quality and quantity for consumers. Limnol Oceanogr 52: 2128–2134

Barnard C, Martineau C, Frenette JJ, Dodson JJ, Vincent W (2006) Trophic position of zebra mussel veligers and their

use of dissolved organic carbon. Limnol Oceanogr 51: 1473–1484

Barthel KG (1983) Food uptake and growth efficiency of *Eurytemora affinis* (Copepoda: Calanoida). Mar Biol 74:269–274

Becker C, Feuchtmayr H, Brepohl D, Santer B, Boersma M (2004) Differential impacts of copepods and cladocerans on lake seston, and resulting effects on zooplankton growth. Hydrobiologia 526:197–207

Biandolino F, Prato E, Caroppo C (2008) Preliminary investigation on the phytoplankton contribution to the mussel diet on the basis of fatty acids analysis. J Mar Biol Assoc UK 88:1009–1017

Bourgault D (2001) Circulation and mixing in the St. Lawrence Estuary. PhD dissertation, McGill University, Montréal, QC

Bousfield EL, Filteau G, O'Neill M, Gentes P (1975) Population dynamics of zooplankton in the middle St. Lawrence Estuary. In: Cronin LE (ed) Estuarine research, Vol 1. Chemistry, biology, and the estuarine system. Academic Press, New York, p 325–351

Brett MT, Müller-Navarra DC, Persson J (2009) Crustacean zooplankton fatty acid composition. In: Arts MT, Brett MT, Kainz MJ (eds) Lipids in aquatic ecosystems. Springer-Verlag, New York, p 115–146

Chícharo MA, Chícharo L (2008) RNA:DNA ratio and other nucleic acid derived indices in marine ecology. Int J Mol Sci 9:1453–1471

Dalsgaard J, St. John M, Kattner G, Müller-Navara D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46:225–340

Devreker D, Souissi S, Molinero JC, Nkubito F (2008) Tradeoffs of the copepod *Eurytemora affinis* in mega-tidal estuaries: insights from high frequency sampling in the Seine estuary. J Plankton Res 30:1329–1342

Frenette JJ, Vincent WF, Dodson JJ, Lovejoy C (1995) Size-dependent variations in phytoplankton and protozoan community structure across the St. Lawrence River transition region. Mar Ecol Prog Ser 120:99–110

Gonzales CRM, Bradley BP (1994) Salinity stress proteins in *Eurytemora affinis*. Hydrobiologia 292/293:461–468

Gorokhova E, Edlund A, Hajdu S, Zhivotova EN (2007) Nucleic acid levels in copepods: dynamic response to phytoplankton blooms in the northern Baltic proper. Mar Ecol Prog Ser 349:213–225

Hebert CE, Weseloh DVC, Idrissi A, Arts MT and others (2008) Restoring piscivorous fish populations in the Laurentian Great Lakes causes seabird dietary change. Ecology 89:891–897

Kainz M, Arts MT, Mazumder A (2004) Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnol Oceanogr 49:1784–1793

Katona SK (1970) Growth characteristics of the copepods *Eurytemora affinis* and *E. herdmani* in laboratory cultures. Helgol Wiss Meeresunters 20:373–384

Kattner G, Hagen W, Lee RF, Campbell R and others (2007) Perspectives on marine zooplankton lipids. Can J Fish Aquat Sci 64:1628–1639

Kimmel DG, Bradley BP (2001) Specific protein responses in the calanoid copepod *Eurytemora affinis* (Poppe, 1880) to salinity and temperature variation. J Exp Mar Biol Ecol 266:135–149

Klein Breteler WCM, Fransz HG, Gonzalez SR (1982) Growth and development of four calanoid copepod species under experimental and natural conditions. Neth J Sea Res 16: 195–207

Koski M, Klein Breteler W, Schogt N (1998) Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). Mar Ecol Prog Ser 170:169–187

Kyle M, Watts T, Schade J, Elser JJ (2003) A microfluorometric method for quantifying RNA and DNA in terrestrial insects. J Insect Sci 3:1–7

Lapierre JF, Frenette JJ (2008) Advection of freshwater phytoplankton in the St. Lawrence River estuarine turbidity maximum as revealed by sulfur-stable isotopes. Mar Ecol Prog Ser 372:19–29

Laprise R, Dodson JJ (1989) Ontogeny and importance of tidal vertical migrations in the retention of larval smelt *Osmerus mordax* in a well-mixed estuary. Mar Ecol Prog Ser 55:101–111

Lee HW, Ban S, Ikeda T, Matsuishi T (2003) Effect of temperature on development, growth and reproduction in the marine copepod *Pseudocalanus newmani* at satiating food condition. J Plankton Res 25:261–271

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

Müller-Navarra DC, Brett MT, Liston AM, Goldman CR (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403:74–77

Müller-Solger AB, Jassby AD, Müller-Navarra DC (2002) Nutritional quality of food resources for zooplankton (*Daphnia*) in a tidal freshwater system (Sacramento–San Joaquin River Delta). Limnol Oceanogr 47:1468–1476

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Toronto

Pfannkuche J, Schmidt A (2003) Determination of suspended particulate matter concentration from turbidity measurements: particle size effects and calibration procedures. Hydrol Process 17:1951–1963

Pringle C (2003) What is hydrologic connectivity and why is it ecologically important? Hydrol Process 17:2685–2689

Roddie BD, Leakey RJG, Berry AJ (1984) Salinity-temperature tolerance and osmoregulation in *Eurytemora affinis* (Poppe) (Copepoda: Calanoida) in relation to its distribution in the zooplankton of the upper reaches of the Forth estuary. J Exp Mar Biol Ecol 79:191–211

Saiz E, Calbet A, Fara A, Berdalet E (1998) RNA content of copepods as a tool for determining adult growth rates in the field. Limnol Oceanogr 43:465–470

Simons RD, Monismith SG, Johnson LE, Winkler G, Saucier FJ (2006) Zooplankton retention in the estuarine transition zone of the St. Lawrence Estuary. Limnol Oceanogr 51: 2621–2631

Søreide JE, Falk-Petersen S, Hegseth NE, 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

Speekmann CL, Nunez BS, Buskey EJ (2007) Measuring RNA:DNA ratios in individual *Acartia tonsa* (Copepoda). Mar Biol 151:759–766

Thorp JH, Thoms MC, Delong MD (2006) The riverine ecosystem synthesis: biocomplexity in river networks across space and time. River Res Appl 22:123–147

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980) The river continuum concept. Can J Fish Aquat Sci 37:130–137

Vincent WF, Dodson JJ (1999) The need for an ecosystem-level understanding of large rivers: the Saint Lawrence River, Canada-USA. Jpn J Limnol 60:29–50

Vincent WF, Dodson JJ, Bertrand N, Frenette JJ (1996) Photo-

synthetic and bacterial production gradients in a larval fish nursery: the St. Lawrence River transition zone. Mar Ecol Prog Ser 139:227–238

Vrede T, Persson J, Aronsen G (2002) The influence of food quality (P:C ratio) on RNA:DNA ratio and somatic growth rate of *Daphnia*. Limnol Oceanogr 47:487–494

Wagner M, Durbin E, Buckley L (1998) RNA:DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. Mar Ecol Prog Ser 162:173–181

Wagner MM, Campbell RG, Boudreau CA, Durbin EG (2001) Nucleic acids and growth of *Calanus finmarchicus* in the laboratory under different food and temperature conditions. Mar Ecol Prog Ser 221:185–197

Winkler G, Dodson JJ, Bertrand N, Thivierge D, Vincent WF (2003) Trophic coupling across the St. Lawrence River estuarine transition zone. Mar Ecol Prog Ser 251:59–73

Winkler G, Dodson JJ, Lee CE (2008) Heterogeneity within the native range: population genetic analyses of sympatric invasive and noninvasive clades of the freshwater invading copepod *Eurytemora affinis*. Mol Ecol 17: 415–430

Zimmermann-Timm H (2002) Characteristics, dynamics and importance of aggregates in rivers–an invited review. Internat Rev Hydrobiol 87:197–240

Editorial responsibility: Hans Heinrich Janssen, Oldendorf/Luhe, Germany

Submitted: June 15, 2009; Accepted: November 11, 2009
Proofs received from author(s): February 4, 2010