# Secondary contact of two sibling species of *Eurytemora affinis*: distribution, diversity and trophic role in the St. Lawrence estuarine transition zone

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Abstract:

The St. Lawrence estuarine transition zone (ETZ), characterized by strong environmental gradients, is a secondary contact zone for two sibling species (clades) of the dominant estuarine copepod *Eurytemora affinis*. This study analysed pattern of distribution, genetic heterogeneity and the trophic role of the species complex of *E. affinis*. Sequencing of 652bp of the cytochrome oxidase I subunit (COI, mt-DNA) revealed a high degree of heterogeneity in genetic structure and habitat type along the salinity gradient. The two genetically-distinct clades showed pattern of niche partitioning within the St. Lawrence ETZ by separate geographic distribution. The North Atlantic clade occupied the central portion of the St. Lawrence Middle Estuary, whereas the Atlantic clade was more prevalent along the margins, in the upstream reaches of the estuary and downstream salt marshes. The genetic population structure observed at fine spatial scales may be responsible for differentiation of trophic roles between the two genetic clades when channelling carbon from primary producers up to higher trophic levels, due to longitudinally organized distribution of primary producers and predators such as mysids and larval fishes across the St. Lawrence ETZ. Furthermore this distribution pattern might result in corresponding spatial structure in secondary production patterns. Overall, this study illustrates the overriding importance to consider cryptic intra-specific diversity in studies on ecosystem functioning.

Key words: copepod sibling species complex, mt-DNA, COI, St. Lawrence estuary, trophic position, stable isotopes

#### Introduction

Eurytemora affinis is a calanoid copepod that dominates most of the estuarine transition zones in the Northern Hemisphere (Mauchline 1996). It is considered to be euryhaline, because it is found in freshwater, brackish and in highly fluctuating tidal ponds. It has a major role in the estuarine food web to channel carbon from primary producers to higher trophic levels such as mysids and larval fish ( Irvine et al. 1995, Fockedey & Mees 1999a, Sirois & Dodson 2000a, Viherluoto et al. 2000, North & Houde 2003, David et al. 2006, Winkler et al. 2003, 2007). Recently E. affinis was described to be a sibling species, composed of 6 genetically diverged major clades, but morphologically similar (Lee 1999, 2000, Lee & Frost 2002). Sibling species might exhibit distinct genetic structure, physiological characteristics, habitat preferences or life history. These distinct properties could strongly influence evolutionary trajectories and ecological dynamics of the sibling species (Knowlton 1993, 2000, Bilton et al. 2002). Relatively few studies have examined the functional and ecological consequences of genetic and demographic subdivision among sibling species (Lee and Gelembiuk 2008, Skelly et al. submitted). These intrinsic (genetic) factors together with extrinsic factors (environmental) influence patterns of spatial-temporal distribution and population dynamics that might affect ecosystem These factors have important consequences for biodiversity, functioning and productivity. biogeography, conservation, and fisheries management (Knowlton 1993, Lee 2000, 2002, Lecomte et al. 2000, Jolly et al. 2005, Gelembiuk et al. 2006).

Two genetically divergent clades of *E. affinis* were found in the estuarine transition zone (ETZ) of St. Lawrence estuary, one of the rare regions where sympatric distribution was found except at the west coast of North America (Lee 2000). This makes the ETZ an ideal study site to compare these two sympatric clades in terms of distribution, diversity and their ecological role. The ETZ, a 150 km long section between Ile d'Orléans and Tadoussac of the St. Lawrence estuary, is characterized by strong environmental gradients as a result of an effective estuarine circulation due to high freshwater discharge and tidal amplitudes up to 6m (Vincent & Dodson 1999). The plankton food web of the ETZ, including primary producers, herbivores, and planktivores, is longitudinally organized across the frontal gradient of the transition zone and shows strong spatial and trophic coupling (Winkler et al. 2003). Despite highly dynamic environmental conditions such as salinity and temperature in the ETZ, elevated primary production was observed at the head of the ETZ (Vincent et al. 1994, Frenette et al. 1995, Vincent et al. 1996), supporting a high zooplankton standing stock throughout the summer season which in turn is fed upon by invertebrate predators and larval fishes (Winkler et al. 2003). Therefore the ETZ is an important nursery zone for larval fishes (Laprise & Dodson 1989b, Dauvin & Dodson 1990, Sirois & Dodson 2000a). Stable isotope analysis revealed the estuarine food web to be composed of mostly omnivorous species (Winkler et al. 2007) with the bulk particulate organic matter (largely detrital) being un-coupled from the consumer food web (Martineau et al. 2004, Barnard et al. 2006). In addition, the inverse relationship between the abundance of zooplankton and phytoplankton provides further evidence that grazers selectively feed on phytoplankton despite its low contribution to total POM. First-order estimate of grazing by only one copepod species was about 20% of net algal production, suggesting a potential impact on phytoplankton biomass when including the entire grazer community (Winkler et al. 2003). Grazers, mostly zooplankton, are subject to predation by invertebrate predators and larval and juvenile fish. The dominant calanoid copepod *Eurytemora affinis* has a key role in channelling carbon from primary producers to higher trophic levels due to its dominant occurrence in gut contents of two mysid species (Winkler et al. 2003, 2007). As we know now two genetically divergent clades occur in the ETZ, which leads to the question: does each clade contribute in the same way to trophic pathways? To address this question the aim of this study is threefold (1) to compare the temporal-spatial distribution of two genetically divergent clades with predator distribution (here mysid and fish larvae), and (2) to identify potential consequences of the genetic structure on the estuarine food web.

#### Materials and Methods

### Study Site

The St. Lawrence Middle Estuary is located from 50 to 230 km downstream of the city of Québec and encompasses a salinity range of 0–25 psu (Fig. 1). Three channels (northern, middle, and southern) run through this part of the estuary. A combination of semidiurnal, lunar tides ranging from 3 to 5 m in amplitude, and freshwater discharge varying between 18 164 and 8863  $\text{m}^3 \cdot \text{s}^{-1}$  from May to August (Ministère de l'environnement Canada, Service météorologique) creates strong estuarine circulation. Because of the lateral variation in bathymetry (a deep northern channel and shallower middle and southern channels are separated by islands, sand banks, and shallow shoals), salt water intrudes farther upstream in the northern channel of the estuary, creating both along- and cross-estuary salinity gradients (Simons 2004). Mean current velocities exceed 0.6–3 m·s<sup>-1</sup>, and hydrodynamic trapping of inorganic and organic particles owing to estuarine circulation is responsible for high turbidity in this area (D'Anglejan 1981). This study concentrates on the southern part of the estuarine transition zone between Bethier sur mer and St. Roch des Aulnaies.

# Data

The data used in this study were obtained from several studies that differed in their major objectives, and thus, sampling protocols varied between years. To render the data comparable, I imposed the well-documented structure of zooplankton assemblages (Bousfield et al. 1973; Laprise and Dodson 1994; Winkler et al. 2003) on the data sets to form two putative zooplankton assemblages: tidal fresh water ( $\leq 0.5$  PSU), true estuarine (> 0.5 PSU). Three types of data 1) genetic data, 2) abundances and 3) stable isotope data are combined to analyse potential consequences of the sympatric occurrence of two genetically divergent clades of *Eurytemora affinis* on the food web of the ETZ.

#### Genetic identification of E. affinis

To demonstrate spatial and temporal distribution of the two genetic divergent clades we extended the sampling done in 2003-2004 (Winkler et al. 2008) along the south shore of the ETZ from May to July 2007. Eurytemora affinis were collected from 4 sites along the shore, Berthier sur Mer (B), Montmagny (M), St-Jean-Port-Joli (S), La Pocatière (L), Rivière du Loup (R) and Ile Verte (V) (Fig. 1). Furthermore 3 sites in the channel were sampled, one between Montmagny and St. Jean Port Joli, one across St. jean Port Joli and one off-shore of Rivière du Loup (Fig.1). These sites differ in salinity. Berthier sur Mer and Montmagny are characterized by tidal fresh water (< 0.5 PSU), Saint-Jean-Port-Joli and La Pocatière fall into the true estuarine category (0.5 - 15 PSU), Rivière du Loup shows salinities higher than 15 PSU and the tidal ponds at Ile Verte vary tremendously in salinity (5-35 PSU). Samples were taken at a depth of 1m with a plankton-net with a mesh size of 250um. At each site 24 to 60 individuals were amplified to recognize the clade. For each individual 652 bp of the mitochondrial COI gene were amplified. DNA was extracted from ethanol-preserved copepods using a cell-lysis buffer with Proteinase-K protocol modified from Hoelzel & Green (1992) in (Lee & Frost 2002). Primers COIH 2198 and COIL 1490 (Folmer et al. 1994) were used for polymerase chain reaction (PCR) amplification, using a PCR profile taken from Lee (2000). A second and third amplification with specific primers identifying the North-Atlantic clade and E. herdmani, respectively, were done. Individuals with a positive amplification with primers COIH 2198 and COIL 1490 and negative amplification results with the specific primers were evaluated as the Atlantic clade. 10 % of all individuals were sequenced to minimize the error (less than 2%) of clade determination by

amplification only. Sequencing was done according to Winkler et al. (2008). Specific primers were designed for the North Atlantic clade: COI-NA (5'-ACACCCGCTAAATGAAGCGAG-3') used in combination with COIL 1490 and for *E. herdmani*: COI-EH (5'-GGGGTTTGGGAATTGGCTC-3' in combination with COIH 2198.

# Abundance data of E. affinis and potential predators

Field surveys were conducted in 2001, we sampled one station at the head of the transition zone (freshwater <0.5 psu) and one station in the transition zone (0.5–6 psu) each week from 22 May to 31 July (Fig. 1). Stations were determined by real-time measures of surface salinity (i.e., a Lagrangian sampling design). For all sampling, salinity and temperature profiles were measured using a SeaBird Seacat profiler (Sea-logger SBE-19, Sea- Bird Electronics, Inc., Bellevue, Washington). For *Eurytemora affinis* abundance data zooplankton was collected by 5-m vertical tows in the surface layer with a 0.5-m standard net (63- $\mu$ m mesh size), for samples in the bottom layer an opening/closing device was used and a flow-meter was installed. A 1-m standard net (500- $\mu$ m mesh size) was used to sample potential predators such as mysids and larval fish. Samples were preserved in 95% ethanol and changed once in the first week after the first preservation to prevent the solution from becoming too acidic (Butler 1992). Abundance data was pooled from bottom and surface samples in this study. The following species were identified: calanoid copepods *E. affinis*, two mysid species *Neomysis americana* and *Mysis stenolepis* and fish larvae of two species *Osmerus mordax* and *Microgadus tomcod*.

# Stable isotope analyses

Zooplankton samples for isotopic analysis were obtained by horizontal trawls during 1 cruise from the  $29^{th}$  of Mai to  $4^{th}$  of June 2009 12 stations across the ETZ (fig.1; 6 stations in the tidal fresh-water zone < 0.5 PSU and 6 stations in the true-estuarine zone > 0.5 PSU) using 0.5 m standard net (250µm mesh size). Seston samples were prefiltered over a 63µm and 20µm net, respectively. The samples were preserved on ice until laboratory processing at latest 4hours after sampling. They were then sorted by hand, washed with deionised water, freeze-dried, ground with an agate mortar and pestle and kept in a desiccator until mass spectrometry analysis. All isotopic analyses were carried out with an gas chromatograph / elemental analyser, coupled to an isotopic ratio mass spectrometer (Trace GC Ultra/Delta Plus SP/ECS 4010, GE-EA-IRMS, ThermoElectron/COSTECH) at l'Institut des Sciences de la mer de Rimouski, UQAR, Quebec. Stable isotope ratios were expressed as parts per thousand according to the following equation:

 $\delta X = [(R \text{sample}/R \text{standard}) - 1] \times 1000 (1)$ 

where X is <sup>13</sup>C or <sup>15</sup>N and R is the corresponding ratio <sup>13</sup>C:<sup>12</sup>C or <sup>15</sup>N:<sup>14</sup>N. The reference standards for <sup>13</sup>C and <sup>15</sup>N were caffeine, chicken broth and atmospheric N2, respectively. Each samples of small zooplankton such as cladocerans (*Daphnia* sp. and *Bosmina* sp.), cyclopoid copepods and *Eurytemora affinis* contained at least 30 individuals; each mysid sample contained 1 to 5 individuals; single fish larvae were analysed. In the tidal freshwater zone a total of 3 replicates of *Eurytemora affinis*, 1 replicate of cladocerans, 1 replicate of rotifers, 1 replicate of amphipods, 3 replicates of larval fish, 5 replicates of *Eurytemora affinis*, 3 replicates of seston < 20µm and 5 replicates of cladocerans, 2 replicates of amphipods, 2 replicates of *Neomysis americana*, 3 replicates of *Mysis stenolepis*, 1 replicate of larval fish (*Microgadus tomcod*), 5 replicates of seston < 20µm and 5 replicates of Mysis stenolepis, 1 replicate of larval fish (*Microgadus tomcod*), 5 replicates of seston < 20µm and 5 replicates of Mysis stenolepis, 1 replicate of larval fish (Microgadus tomcod), 5 replicates of seston < 20µm and 5 replicates of Mysis Stenolepis, 1 replicate of larval fish (Microgadus tomcod), 5 replicates of seston < 20µm and 5 replicates of Seston < 63µm were analysed.

Results

To get a better understanding of the role of intra-specific diversity of *E. affinis* in the ETZ the spatialtemporal distribution of the *E. affinis* species complex along the salinity gradient is shown. The geographic pattern of the Atlantic and the North-Atlantic clade is compared to the spatial-temporal abundance patterns of *E. affinis* and its predators such as mysids and fish larvae in the two distinct salinity zones (tidal freshwater and true estuarine). In a third step the trophic pathways are characterized by a stable isotope analysis of the two clades, the Atlantic and North Atlantic clade (preliminary data).

# Eurytemora affinis, intra-specific diversity and geographic structure

Geographic structure was revealed by sequence analysis on the mt-DNA gene COI of *E. affinis* in the estuarine transition zone (ETZ) of the St. Lawrence estuary for pooled samples in 2003 and 2004 (Fig. 2A, Winkler et al. 2008). The ETZ is dominated by two genetically distinct clades, the Atlantic and the North-Atlantic clade, from the Eurytemora affinis complex. Along the south shore (150 km) the two divergent clades (12.4% sequence divergence) of E. affinis, two genetically distinct subclades (1% sequence divergence) within the Atlantic Clade and E. herdmani were geographically segregated along the salinity gradient in the shallow bays and tide pools of the south shore. To verify if habitat partitioning between the Atlantic and the North Atlantic clades is stable in time, E. affinis individuals from 6 sites along the south shore and 2 to 3 channel sites (Fig.1, 2) were identified by clade specific PCR in May, June and July 2007. Populations from the Atlantic clade occurred in the tidal freshwater zone at the head of the ETZ in shallow bays (sites B, M), in the salt marshes in the Baie de L'Ile Verte (sites V). The North Atlantic clade occurred predominantly in the central region (true estuarine zone) of the St. Lawrence ETZ (0.5-20 PSU; sites S, L, Fig. 2). In general this geographic segregation of the two divergent clades was similar to the one in 2003/2004 and varied only little throughout May to July in 2007. Variation and overlap of occurrence of the Atlantic and North-Atlantic clades were observed in the channel stations between Montmagny (site M) and Saint-Jean-Port-Joli (site S) and might be due to sampling at different tidal states and high current speeds. At La Pocatière (site L) the E. affinis population was dominated by the North Atlantic clade, 70%, 5% and 75% in May, June and July, respectively. At Rivière du Loup (site R), a station with high salinities around 20 to 25 PSU, the congener Eurytemora herdmani was dominant over the North-Atlantic clade, while the Atlantic clade was mostly absent (Fig. 2 B, C).

Spatial and temporal abundance distribution of *Eurytemora affinis* and its potential predators

Distribution and abundance of *E. affinis* and its potential prey was highly variable along the southern channel when all samples from May to July 2001 were pooled (Fig. 3 A-F). Significantly higher abundances of two mysid species, *Neomysis americana* (Mann-Whitney U test; z = -3.56, p<0.05, N=33) and *Mysis stenolepis* (Mann-Whitney U test; z = -2.02, p<0.05, N=33) were found in the true-estuarine zone (>0.5 PSU) than in the tidal freshwater zone (<0.5 PSU) (Fig. 3 C,D). No significant differences in the abundances of *E. affinis*, *Osmerus mordax* and *Microgadus tomcod* were found between the tidal freshwater and the true-estuarine zone (Fig. 3A, E, F).

Abundances also varied in time over the summer period from May to July in 2001 (Fig.4). The abundance of *E. affinis* in the tidal freshwater zone tended to increase from May to July, whereas a decrease was observed from May to July in the true estuarine zone (Fig. 4A). In contrast, no such tendency was found for *E. affinis* nauplii. Peak abundance of nauplii occurred in June in the true estuarine zone and was less pronounced in the tidal freshwater zone (Fig. 4B). *Neomysis americana* 

abundance in the true estuarine zone increased from May to the end of July up to a maximum of 200 ind. m<sup>-3</sup>, while abundances in the tidal freshwater zone were constantly very low (Fig. 4C). *Mysis stenolepis* showed peak abundance in June (7 ind. m<sup>-3</sup>) that was much lower than abundance of *N. americana*. Larval smelt (*Osmerus mordax*) showed opposite trends of abundance for the two salinity zones. In the true estuarine zone smelt abundances peaked in the beginning of June and decreased afterwards, whereas in the tidal freshwater zone peak abundance was found at the end of June (Fig. 4E). Larvae from the second important fish species the tomcod (*Microgadus tomcod*) were found only in the beginning of summer (end of May and beginning of June) showing higher abundances in the tidal freshwater zone.

# Trophic position of Eurytemora affinis in the food web

*Eurytemora affinis* showed significant differences in carbon and nitrogen isotopic ratios depending on the habitat type (Fig. 5). The Atlantic clade in the tidal freshwater zone had significantly lower carbon and nitrogen isotopic values than the N-Atlantic clade in the true estuarine zone (t-test,  $\delta^{13}$ C: df = 6, t = -4.9, p = 0.003;  $\delta^{15}$ N: df = 6, t = -6.21, p = 0.001) suggesting a different composition of food items in their diets. The seston pool, both size categories (<20µm and <63µm), of the tidal freshwater zone were significantly higher in nitrogen isotopic ratios than the seston pool in the true estuarine zone ( $\delta^{15}$ N: t-test: <20µm, df = 9, t = 3.49, p = 0.007; <63µm, df = 8, t = 3.97, p = 0.004). In contrast carbon isotopic ratios of the seston pool were similar in both habitats.

The nitrogen isotopic signatures of potential competitors in the same size range (cladocera, rotifera and amphipoda) revealed a much lower position in the food web than *E. affinis* (Fig. 5). In general, the potential predators such as fish larvae and mysids differed in carbon and nitrogen isotopic ratios from *E. affinis* (Fig. 5). In the freshwater zone the fish larvae had a carbon isotopic ratio similar to rotifers and amphipods but different from the Atlantic clade of *E. affinis*, suggesting that the Atlantic clade is not the major prey item for these larvae. In the true-estuarine zone the carbon isotopic ratios of larval tomcod is similar to that of the N-Atlantic clade of *E. affinis* and cladocerans, suggesting both as potential prey. However the nitrogen isotopic ratio is not an entire trophic level higher than *E. affinis*, but two higher than the cladocerans, so that a combination of these food items is likely. *Neomysis americana* and *Mysis stenolepis* showed similar carbon and nitrogen isotopic ratios (Fig. 5). Both mysids were situated at the same trophic level as the N-Atlantic clade of *E. affinis*, but their carbon isotopic ratios (Fig. 5).

#### Discussion

Within the St. Lawrence estuarine transition zone the zooplankton community contains 3 major zooplankton assemblages that are mainly characterized by differences in salinity (Laprise and Dodson 1994, Winkler et al. 2003), the tidal freshwater (<0.5 PSU), the estuarine (0.5 -10 PSU) and the estuarine-marine (>10 PSU) assemblage. The longitudinal distribution patterns are strongly dependent on freshwater discharge and tidal amplitudes.

Distribution of two Eurytemora affinis clades in the secondary contact zone

The segregated distribution of the two genetically divergent *E. affinis* clades, the Atlantic and the North-Atlantic found in 2003/2004 (Winkler et al. 2008) was confirmed in 2007. We could show that distribution patterns were stable over several months from May to July. At the sampling sites Berthier sur mer (B) and Montmagny (M) only the Atlantic clade was found, whereas further downstream from Montmagny around St.Jean Port Joli (S) the North Atlantic clade was dominant down to La Pocatière.

The absence of the North-Atlantic clade from the tidal freshwater zone, might be due to differences in salinity tolerance of both clades, which was confirmed by laboratory experiments (Skelly et al. submitted). In contrast salinity tolerance do not explain why the North Atlantic clade dominate in the true estuarine zone (<0.5 PSU) at St.Jean-Port Joli (S) and La Pocatière (L). Mechanism of potential competition between the Atlantic and North-Atlantic clade are still unknown. Further downstream where salinities ranged between 20-25 PSU, a congener of *E. affinis*, *Eurytemora herdmani* was the dominant species.

A different degree of mixing among *Eurytemora* spp. in the ETZ in time might be due to changes in freshwater discharge, normally decreasing from May to July and to the tidal state during sampling. For example the channel station between Montmagny (M) and St. Jean Port Joli (S) accommodated the Atlantic, or a mixture of both clades. This station illustrates nicely the dynamic of the estuarine transition zone. The variability in clade distribution might be due to the tidal cycle. In contrast to all other stations that were sampled at high tide, it was sampled at different tidal states each month, due to logistics with the research vessel.

In general the distribution patterns correspond to the division of the ETZ into three major zones characterized by salinity and zooplankton assemblages (Laprise and Dodson 1994, Winkler et al. 2003). The Atlantic clade occurs in the tidal freshwater zone/assemblage, the North-Atlantic clade is part of the true-estuarine zone/assemblage, whereas *E. herdmani* occurs in the estuarine-marine zone/assemblage. The habitat partitioning of the two *E. affinis* clades and *E. herdmani* probably has ecological consequences in terms of exploitation of resources as well as transfer of carbon to higher trophic level. The two clades of *E. affinis* and *E. herdmani* exploit different salinity zones of ETZ, which differ in quality and quantity of food resources (Lovejoy et al. 1993, Frenette et al. 1995, Vincent et al. 1996, Martineau et al. 2004, Barnard et al. 2006). High chl *a* values were found at the head of the transition zone diminishing further downstream due to grazing and dilution with marine water, while protists aggregate formation increased at higher salinities (>2).

Comparing abundances of *E. affinis* in the southern channel of the ETZ in 2001 for the tidal freshwater and the true estuarine zone with abundances of potential predators (mysids and fish larvae) revealed that Eurytemora affinis occurred in similar abundances in both zones. Assuming a stable genetic structure similar to that found in 2003/2004 and 2007, our data suggest that both clades were present in the ETZ, the Atlantic clade in the tidal freshwater zone and the North Atlantic clade in the true estuarine zone. The potential invertebrate predators, Neomysis americana and Mysis stenolepis showed significant higher abundances in the true-estuarine zone than in the tidal-freshwater zone, which confirmed data from earlier studies (Laprise and Dodson 1994, Winkler et al. 2003), suggesting a higher predation pressure on the North-Atlantic clade than on the Atlantic clade of E. affinis. Trophic relationships among these predators (mysids and fish larvae) and E. affinis has been shown for the St. Lawrence estuary (fish larvae: RobichaudLeBlanc et al. 1997, Sirois 2000, Sirois & Dodson 2000b), mysids: (Winkler et al. 2003, Winkler et al. 2007) and other estuarine transition zones in the northern Hemisphere (fish larvae: Meng & Orsi 1991, Thiel et al. 1996), mysids: (Viitasalo et al. 1998, Fockedey & Mees 1999, Viherluoto & Viitasalo 2001, Winkler & Greve 2004, David et al. 2006b). However, for the first time trophic relationships are related to genetic structure of copepod prey, here intra-specific clades of E. affinis.

Abundances of *E. affinis* and its potential predators varied also in time from May to July in 2001. Given the assumption that the two salinity zones represent the spatial division of the two genetic clades, differences in abundances of copepodites and nauplii of the Atlantic and North Atlantic clade of *E. affinis* in their respective habitat suggest differences in population dynamics and secondary production of the Atlantic and North-Atlantic clade. The Atlantic clade increased abundances during the summer season, whereas the North-Atlantic clade showed a decrease from May to July in 2001. This might have consequences in carbon transfer to higher trophic levels. Negative correlations

between the North-Atlantic clade abundance and the two mysids abundances may indicate a predatorprey relationship.

Larval *O. mordax* may feed on either genetic clade of *E. affinis* depending on their spatial-temporal distribution within the estuarine transition zone. Higher abundance of larval *O. mordax* were found at the end of May and in the beginning June in the true-estuarine zone, that may indicate potential predation on the North-Atlantic clade, whereas in the end of June more larval *O. mordax* were found in the tidal-freshwater zone, potentially feeding on the Atlantic clade of *E. affinis*. Similar distribution patterns were found by Laprise & Dodson (1989a); *O. mordax* associated with the true estuarine zooplankton assemblage (>0.5 PSU) in June showed an upstream migration as a result of selective tidal stream transport in July.

As the ETZ is a secondary contact zone for two genetically distinct races of *O. mordax*, the Arcadian and the Atlantic race (Bernatchez 1997, Pigeon et al. 1998) segregate into two populations the south shore and the north shore population with different frequencies of both races, so that distribution pattern and trophic relationships might be a lot more complicated than described above. Spawning grounds and larval nurseries of these two populations are separated longitudinally in the St. Lawrence estuary (Lecomte & Dodson 2004) which might have consequences on their alimentary regime. Further detailed studies are necessary to determine if feeding on different clades of *E. affinis* cause an impact on survival and recruitment success in the two races of *O. mordax*.

Larval *M. tomcod* occurred in May and June in the ETZ in 2001. Tomcod larvae might mostly feed on the Atlantic clade of *E. affinis* given the higher abundance of tomcod larvae in the tidal freshwater zone than in the true-estuarine zone. Laprise and Dodson (1989) proposed habitat partitioning among *O. mordax* and *M. tomcod* in the middle of the summer season, so that juvenile tomcod migrate downstream into colder more saline waters, whereas larger smelt larvae migrate upstream into tidal fresh water. This might explain why in 2001 no *M. tomcod* larvae were captured from the middle of June onwards.

# Trophic position of E. affinis

The Atlantic and the North-Atlantic clades differed significantly in their trophic position within the estuarine food web. Differences in carbon signature suggested that the food composition differs which in turn is translated also in different position in the nitrogen signature, showing the North-Atlantic clade  $2\%_0$  higher  $\delta$ 15N than the Atlantic clade. The seston pool might be one food source according to corresponding carbon signatures. This stands in contrast to earlier work in the ETZ of the St. Lawrence estuary, where overall POM was only weakly coupled to the metazoan food web (Martineau et al. 2004, Barnard et al. 2006).

However, great differences of 5 % $_{o}$  and 8 % $_{o}$  between seston and *E. affinis* in the tidal freshwater and true-estuarine zone, respectively, indicate that other food/prey is still lacking in this food web analysis. If *E. affinis* also feed on large algal cells and chains >63 µm, we would have missed these due to filtation over a 63µm sieve. As *E. affinis* is also known to be predacious, early life stages of other copepods (calanoid, cyclopoid, harpacticoid) might be a potential prey. Further analyses on that are on the way.

In the freshwater zone potential predators found were fish larvae that differed significantly in carbon signature from the Atlantic clade of *E. affinis*, but had a carbon isotopic ratio similar to rotifers and amphipods suggesting that the Atlantic clade was not the major prey item for these larvae. In the true-estuarine zone the North-Atlantic clade and cladocera were likely food items of larval *M. tomcod*, which confirms stomach content analysis indicating a majority of food items as *E. affinis* and *Bosmina* sp. (Yoneyama 2004, Winkler et al. 2003).

In contrast, mysids of the true-estuarine zone were on the same trophic level as the North-Atlantic clade and differed in significantly in carbon signature, so that in the beginning of June 2007 the mysids

as main predators of the North-Atlantic clade are unlikely. In contrast, high predation rates of *E. affinis* by *M. stenolepis* were documented and confirmed by stable isotope data (Winkler et al. 2007). One possible explanation for the discrepancy may be the time of sampling. In this study both mysid species were small sized (<6 mm) in the beginning of June, whereas in Winkler et al. (2007) used larger individuals of *M. stenolepis* (19-23 mm) later in the year. Verification and temporal changes in the estuarine food web will be done by an enlarged study on the food web with samples from July and August 2009.

# Conclusion

The estuarine transition zone of the ETZ acts as a secondary contact zone for two genetically divergent clades of the cryptic species complex *Eurytemora affinis*. Distribution pattern and genetic structure of the Atlantic and North-Atlantic clade correspond to zooplankton assemblage division into the tidal freshwater and the true estuarine assemblage, respectively, mostly characterized by salinity differences. Comparison of temporal-spatial abundance pattern of both clades and potential predators such as mysids and larval fish proposed (1) differentiation of population dynamics and secondary production of the Atlantic clade and both mysid species than between the Atlantic clade and mysids, (3) distribution of fish larvae was highly variable and differed between *O. mordax* and *M. tomcod*, so that trophic relation with either clade of *E. affinis* will depend on the actual longitudinal position of the larvae at a given time during the summer season. Trophic position revealed by stable isotope analysis of the two genetically divergent clades *E. affinis* was significantly different suggesting a different trophic role within the entire food web of the estuarine transition zone. Overall, this study illustrates the overriding importance to consider cryptic intra-specific diversity in studies on ecosystem functioning.

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Figure Legends:

Fig. 1: Location of stations sampled in the southern channel of the St. Lawrence estuarine transition zone in 2001 2007 and 2009. In 2001 symbols represent only average positions among field surveys, as the exact location of stations was determined on each occasion according to surface salinity.

Fig. 2: Spatial-temporal distribution of Eurytemora spp. along a salinity gradient in the estuarine transition zone of the St. Lawrence estuary. A) Data 2003-2004 combined (Winkler et al. 2008), B-D) May, June, July 2007, respectively. Color code shows the Atlantic clade (*E. affinis*) in red, the North Atlantic clade (*E. affinis*) in blue and *Eurytemora herdmani* in yellow.

Fig. 3: Spatial distribution of salinity and zooplankton abundance of *Eurytemora affinis* and its potential predators in the ETZ in 2001. A) salinity, B) *E. affinis*, C) *Neomysis americana*, D) *Mysis stenolepis*, E) larval *Osmerus mordax* and F) larval *Microgadus tomcod*. Data shown is pooled from Mai to July 2001. Light colours indicate the tidal fresh water zone with salinities < 0.5PSU, whereas darker colours indidate the true estuarine zone with salinities >0.5PSU. The sizes of the circles are relative to highest abundance found for a given species. *E. affinis* abundance max. = 8230 ind. m<sup>-3</sup>, *N. americana* abundance max. = 201 ind. m<sup>-3</sup>, *M. stenolepis* abundance max. = 7 ind. m<sup>-3</sup>, larval *O. mordax* abundance max. = 64 ind. 100m<sup>-3</sup>, larval *M. tomcod* abundance max. = 28 ind. 100m<sup>-3</sup>

Fig. 4 : Weekly distribution (abundance) of *Eurytemora affinis* and its potential predators (*Neomysis americana, Mysis stenolepis*, larval *Osmerus mordax* and larval *Microgadus tomcod*) in the tidal freshwater and true estuarine zone of the estuarine transition zone from May to July in 2001. Surface and bottom samples were pooled for each station.

Fig.5: Planktonic food web of the St. Lawrence estuarine transition zone at the beginning of June 2009, separated into the two salinity zones, tidal freshwater (red and orange colours) and the true estuarine (blue colours). Dual stable isotope plot for the 2 clades of *Eurytemora affinis* (Atlantic clade and N-Atlantic clade), their potential competitors: cladocera, rotifers; their potential predators: *Neomysis americana*, *Mysis stenolepis*, fish larvae, larval tomcod, and their potential prey: two size classes of seston < 20  $\mu$ m and <63  $\mu$ m. Replicates ranged between 1-6 and error bars indicate standard deviation for  $\delta^{13}$ C and  $\delta^{15}$ N.



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