Université du Québec
à Rimouski

Contribution des espèces cryptiques *Eurytemora affinis* et *E. carolleeae* au régime alimentaire des larves d'éperlan arc-en-ciel, *Osmerus mordax,* dans la zone de turbidité maximale de l'estuaire du Saint-Laurent

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PAR

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AVANT-PROPOS

Quitter son pays d'origine n'est pas toujours facile. En plus de cela, la pandémie de la COVID-19 a définitivement affecté toutes nos vies, y compris le monde universitaire. Frontières internationales fermées, procédures d'immigration retardées, vaccination massive, port obligatoire de masques, couvre-feux, cours virtuels, c'était certainement une période atypique pour démarrer un projet de maîtrise. Cependant, lorsque j'ai découvert ce projet pour la première fois, je savais que ce n'était pas seulement la possibilité de poursuivre mes études dans le pays dont je suis tombé amoureux de nombreuses années auparavant, mais aussi de continuer à étudier le monde fascinant de l'écologie trophique. Ayant une formation en écologie des élasmobranches, je savais que l'étude des niveaux inférieurs du réseau trophique me permettrait de mieux comprendre les processus qui façonnent la trophodynamique et définissent les écosystèmes. C'est ainsi que j'ai décidé de quitter la Colombie, commencer une nouvelle vie, et bien sûr comment ce projet a commencé à se concrétiser.

Ce travail nous a permis d'approfondir nos connaissances sur la dynamique trophique d'une espèce importante pour le Québec, l'éperlan-arc-en-ciel. Nous avons pu utiliser une nouvelle technique (PCR en temps réel) pour déterminer quels organismes contribuaient le plus au régime alimentaire, et malgré les limitations et contraintes, nous pensons que ce travail a ajouté des résultats importants pour élucider et comprendre la zone de turbidité maximale, une importante pouponnière dans le fleuve Saint-Laurent. Cette recherche permettra de continuer à évaluer les relations trophiques, afin de mieux comprendre l'écosystème et comment il pourrait réagir aux facteurs de stress environnementaux produits par l'activité humaine et bien sûr le changement climatique. Nous continuerons à étudier la dynamique trophique dans cette zone dans les années à venir et je ne peux qu'espérer que durant mon doctorat nous pourrons approfondir nos connaissances afin de mieux comprendre et protéger ce merveilleux écosystème.

RÉSUMÉ

Dans le fleuve et l'estuaire du Saint-Laurent, l'éperlan arc-en-ciel (*Osmerus mordax*) utilise la zone de turbidité maximale (MTZ) comme principale zone d'alevinage. Au stade larvaire, sa principale proie est le complexe de copépodes calanoïdes d'*Eurytemora affinis*. Représentant un lien essentiel entre la production primaire et les niveaux trophiques supérieurs, ce complexe d'espèces cryptiques sert de source de nourriture importante pour les jeunes stades de vie des poissons. Dans notre étude, nous avons cherché à évaluer la contribution d'*Eurytemora affinis* et d'*E. carolleeae* au régime alimentaire des larves de l'éperlan arc-en-ciel dans la mosaïque d'habitats hétérogènes de la MTZ, marquée par de forts gradients de salinité. Au cours de l'été 2021, nous avons réalisé quatre relevés (juin-août) échantillonnant une zone de 100 km s'étendant de Québec à l'Anse Sainte-Anne, révélant des distributions hétérogènes de proies d'éperlans. L'habitat oligohalin (0,5 – 5,0 PSU) présentait les abondances moyennes les plus élevées de larves d'éperlan arc-en-ciel, avec 20,41 ± 10,82 larves par 100 m³. Au fur et à mesure que les larves se développaient, nous avons observé des changements ontogénétiques de leur alimentation avec *Eurytemora* spp. représentant 76 % à 93 % du régime alimentaire en juin et juillet, et avec le mysidacé *Neomysis americana* représentant 80 % du régime alimentaire en août (habitat oligohalin). Grâce à un test de qPCR SYBR green nouvellement développé, nous avons révélé pour la première fois qu'*E. affinis* était la ressource la plus exploitée le long du gradient de salinité avec 72 % des estomacs ne présentant que cette espèce cryptique. Cependant, lorsqu'*E. carolleae* dominait dans l'environnement, une proportion élevée d'*E. carolleae était* également détectée dans le régime alimentaire, suggérant que les larves exploitent la ressource alimentaire disponible la plus abondante. Néanmoins, d'autres études sont nécessaires pour explorer la sélectivité au sein du complexe cryptique et son impact sur la croissance et la survie de l'éperlan arc-en-ciel. La compréhension de ces aspects fournira des informations précieuses sur la dynamique écologique complexe au sein de la MTZ et sur l'équilibre délicat qui soutient les premiers stades de vie de l'éperlan arc-en-ciel dans l'estuaire du Saint-Laurent.

Mots-clés : éperlan, copépodes, espèces cryptiques, trophique, qPCR.

ABSTRACT

In the St. Lawrence River, rainbow smelt (*Osmerus mordax*) relies on the maximum turbidity zone (MTZ) as its main nursery area, with its primary prey being the calanoid copepod complex of *Eurytemora affinis*. Representing a critical link between primary production and higher trophic levels, this cryptic species complex serves as a significant food source for fish larvae. In our study, we aimed to evaluate the contribution of *Eurytemora affinis* and *E. carolleeae* to the diet of early stages of rainbow smelt in the heterogeneous habitat mosaic of the MTZ, marked by strong salinity gradients. During the summer of 2021, we conducted four surveys (June-August), sampling an area of 100 km from Quebec City to Anse Sainte-Anne, revealing heterogeneous distributions of smelt and copepod prey. The oligohaline habitat (0.5–5.0 PSU) exhibited the highest mean abundances, with 20.41 ± 10.82 larvae per 100 m³. As the rainbow smelt larvae progressed in their development, we observed ontogenetic shifts in their diet with *Eurytemora* spp. comprising 76% to 93% in June and July, and with the mysid *Neomysis americana* accounting for 80% of the diet in August (oligohaline habitat). Through a newly developed SYBR green qPCR assay, we revealed for the first time that *E. affinis* was the most exploited resource along the salinity gradient with 72% of stomachs presenting only this cryptic species. However, when *E. carolleae* was dominant in the environment, a high proportion of *E. carolleeae* was also detected in larval diet (e.g., mid-June), suggesting that larvae exploit the most abundant food resource available. Nevertheless, further studies are needed to explore the selectivity within the cryptic complex and its impact on the growth and survival of rainbow smelt. Understanding these aspects will provide valuable insights into the intricate ecological dynamics within the MTZ and the delicate balance that supports the early life stages of rainbow smelt in the St-Lawrence Estuary.

Keywords: smelt, copepods, cryptic species, trophic, qPCR.

TABLE DES MATIÈRES

LISTE DES TABLEAUX

LISTE DES FIGURES

LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

MTZ	Maximum Turbidity Zone
NSP	North Shore Population
SSP	South Shore Population
PSU	Practical salinity units
SL	Standard length
Fst	Fixation index
mtDNA	Mitochondrial DNA
qPCR	Real time polymerase chain reaction
NADH	Nicotinamide adenine dinucleotide dehydrogenase
ND5	NADH-ubiquinone subunit 5 encoding gene
COI	Cytochrome c oxidase subunit 1 encoding gene
FI	Feeding incidence
CW	Carbon weight
PSIRI	Prey-specific index of relative importance
B	Levin's standardised niche breadth index
PERMANOVA	Permutational analysis of variance
SIMPER	Similarity percentages analysis
SD	Standard deviation
Df	Degrees of freedom

INTRODUCTION GÉNÉRALE

Zone de Turbidité Maximale de l'Estuaire du Saint-Laurent

Les estuaires sont des écosystèmes complexes et dynamiques où l'eau douce des rivières rencontre et se mélange avec l'eau salée de l'océan. Ces zones de transition sont des environnements hautement productifs et écologiquement riches qui abritent un large éventail d'organismes et jouent un rôle crucial dans la santé globale des zones côtières (Day *et al.,* 2012). Les zones de turbidité maximale (MTZ) se situent à l'intérieur des estuaires et se caractérisent par une augmentation de la turbidité en raison de la remise en suspension turbulente des sédiments et de la floculation des matières particulaires (Burchard *et al.,* 2018 ; Horemans *et al.,* 2020). La turbulence est entraînée par les forces de marée, les vagues et les courants induits par la densité de l'eau qui poussent une lame d'eau salée en amont sous le débit d'eau douce sortant (Simmons *et al.,* 2010 ; Wang *et al.,* 2022). Les grands estuaires comme baie de Chesapeake, baie de San Francisco ou l'estuaire du Saint-Laurent, pour ne nommer que certains des plus grands estuaires d'Amérique du Nord, sont caractérisés par des zones de turbidité maximale qui jouent un rôle important dans la distribution des espèces aquatiques, le cycle des nutriments et la dynamique globale de l'écosystème (Schubel, 1968 ; Baird & Ulanowicz, 1989 ; Peterson *et al.,* 1975 ; Vincent & Dodson, 1999 ; Laprise & Dodson, 1994).

Dans l'estuaire du Saint-Laurent, la zone de turbidité maximale (MTZ) est une zone à énergie marémotrice bien mélangée entre Québec et La Pocatière, où une circulation complexe axée sur la densité de l'eau, combinée à la remise en suspension des sédiments de fond, soutient une biomasse phytoplanctonique élevée et ainsi une productivité secondaire élevée (Simons *et al.,* 2006, 2010). Cette zone est un habitat d'alevinage important pour les larves et les juvéniles de poissons comme l'éperlan arc-en-ciel, le bar rayé, le baret, l'alose

savoureuse et le poulamon atlantique (Laprise & Dodson, 1989 ; Dauvin & Dodson, 1990; Vanalderweireldt *et al.,* 2020), offrant une protection contre les prédateurs et des proies abondantes le long de son gradient longitudinal. La communauté zooplanctonique de cette zone est composée de trois assemblages distincts : un assemblage tidal d'eau douce en amont dominé par des larves véligères du bivalve *Dreissena polymorpha* et des crustacés tels que *Bosmina* spp. et *Gammarus* spp., suivi d'un deuxième assemblage estuarien dominé par le complexe cryptique d'*Eurytemora affinis* ainsi que les mysidacés *Neomysis americana* et *Mysis stenolepsis*. Enfin, à des salinités plus élevées, un troisième assemblage d'espèces euryhalines et marines composé d'*Acartia* spp. *Eurytemora herdmani*, *Calanus* spp., *Mysis littoralis*, euphausiacés et chaetognathes (Laprise & Dodson, 1994 ; Vincent & Dodson, 1999 ; Winkler *et al.,* 2003, 2005, 2016). Ce gradient longitudinal de proies à travers la mosaïque d'habitats de la MTZ offre ainsi une nutrition adéquate aux larves de poissons au cours de leurs premiers stades de vie (Bousfield *et al*., 1975 ; Laprise & Dodson, 1989).

Écologie trophique des premiers stades de vie des poissons

Les larves de poissons sont parmi les plus petits vertébrés qui se développent extrêmement rapidement se métamorphosant en stades de vie très différents. Ces organismes subissent d'importants changements physiologiques et comportementaux lors de leur transition d'organisme planctonique (œuf, larve avec sac vitellin, larve) à celui de necton nageant activement (stade juvénile) pleinement indépendants (Marshall & Morgan, 2011). Au cours de ces stades, les individus traversent une période critique pour leur développement et leur survie pendant laquelle ils sont vulnérables aux facteurs environnementaux comme l'abondance de nourriture, la prédation et la dérive du courant (Hjort, 1914, 1926). Cette période englobe les premiers jours ou semaines de la vie et est caractérisée par des exigences métaboliques accrues et une croissance rapide, ce qui rend une nutrition adéquate essentielle à la survie (Figure 1 ; Houde, 2008 ; Cushing, 1990 ; Jobling, 1994). Ainsi, au cours de cette phase critique, les habitudes alimentaires et la disponibilité de la nourriture jouent un rôle crucial dans la détermination de leur croissance et survie (Robert *et al*., 2023).

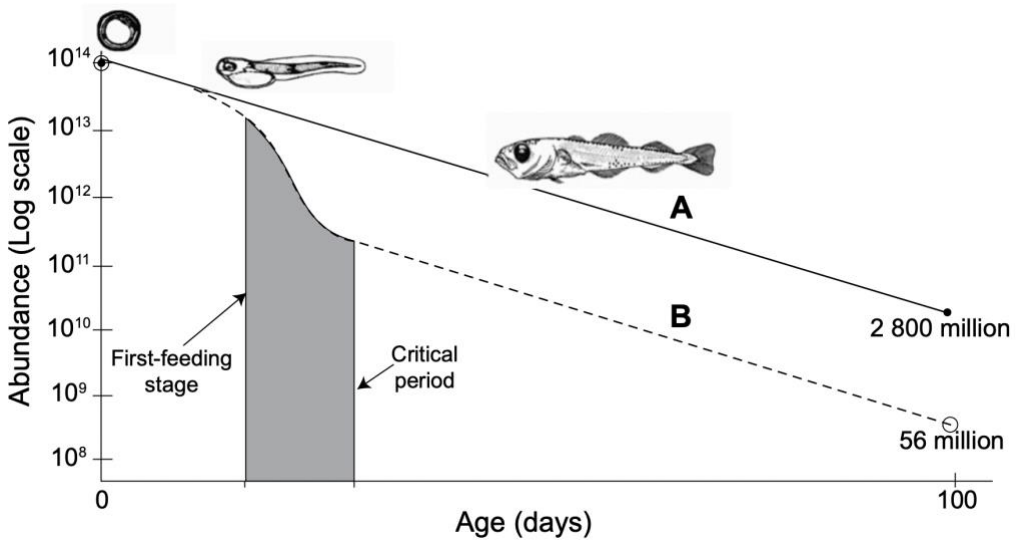

Figure 1. Illustration de la « période critique » selon l'hypothèse de Hjort (1914, 1926). À défaut de trouver des proies appropriées en quantité suffisante, une mortalité > 90 % survient peu de temps après que les larves aient absorbé leur vitellus et commencé à se nourrir (cohorte B). Dans cet exemple, le taux de mortalité quotidien, à l'exception de la période critique, est M = 0,1. Le recrutement à 100 jours post-éclosion diffère de 50 fois dans la cohorte B connaissant une période critique et dans la cohorte A ne connaissant pas de période critique. Tiré de Houde, 2008.

Les premiers stades de vie des poissons ont des besoins alimentaires uniques et présentent différentes stratégies d'alimentation pour faire face aux défis de trouver une nourriture appropriée dans leur environnement (Hauss *et al.,* 2023). La théorie de la stratégie optimale de recherche de nourriture (en anglais « optimal foraging theory ») est un cadre théorique utilisé pour comprendre les stratégies que les prédateurs comme les larves de poissons adoptent pour augmenter leur capacité à obtenir des proies, minimiser leur dépense énergétique et exploiter un éventail diversifié de proies potentielles (MacArthur & Pianka, 1966 ; Schoener, 1971 ; Carpenter *et al.,* 1983). La théorie suggère que les animaux ont évolué pour maximiser leur apport énergétique dépensé lors des activités de recherche de nourriture, en supposant que les organismes prendront des décisions de recherche de nourriture qui optimiseront leur condition physique globale. En effet, les larves de poisson sont confrontées à des compromis (trade-offs) ou à un équilibre entre des facteurs ou encore

des décisions contradictoires qui affectent leur comportement de recherche de nourriture et, en fin de compte, leur fitness (survie et reproduction). Ceci est d'autant plus important qu'ils traversent la période critique de leur développement pendant laquelle ils doivent trouver suffisamment de nourriture pour survivre et devenir adultes (Cushing, 1990 ; Houde, 2008).

Les stratégies alimentaires sont variées et il existe des espèces opportunistes qui captureront plusieurs proies de qualité nutritionnelle différente tandis que d'autres seront sélectives envers un certain type de proie. Ces stratégies peuvent varier selon les stades ontogéniques qui ont des capacités natatoires et visuelles très différentes mais aussi en fonction de la disponibilité de la nourriture (Elner & Hughes, 1978 ; Blaxter, 1986 ; Peck *et al.*, 2012 ; Moyano *et al.,* 2016). Au sein de l'éventail des proies, le zooplancton constitue une source de nourriture primaire pendant la période critique, fournissant les nutriments essentiels nécessaires à la croissance et au développement des larves. Ainsi, différentes espèces de poissons présentent des préférences alimentaires spécifiques pour certains types de zooplancton, de sorte que l'abondance des proies préférées peut déterminer le succès larvaire (Leclerc *et al.,* 2011 ; Demontigny *et al.,* 2012 ; Paradis *et al.,* 2012 ; Robert *et al.,* 2008, 2009, 2014). La composition et la disponibilité du zooplancton influencent donc la survie et le recrutement de la plupart des stocks de poissons (Cushing, 1990 ; Kiørbe, 1998).

Les populations d'éperlan arc-en-ciel dans l'estuaire du Saint-Laurent

L'éperlan arc-en-ciel (*Osmerus mordax*) est un poisson carnivore répandu et abondant natif du nord-est de l'Amérique du Nord qui occupe les lacs, les rivières, les estuaires et les eaux côtières. Il présente une grande diversité au niveau du cycle biologique des populations anadromes et enclavées ainsi que des écotypes nains et normaux d'eau douce (Nellbring, 1989 ; Taylor & Bentzen, 1993). Dans l'estuaire du Saint-Laurent, les larves d'éperlans anadromes sont transportées de leurs sites de fraie à la fois en amont ou à partir de petits affluents jusqu'à leur zone d'alevinage située dans la zone de turbidité maximale (MTZ). Étant donné que la nourriture y est abondante, que la prédation est minime et que la turbidité

(et/ou l'hydrodynamisme) est favorable à l'alimentation, ces larves de poisson restent tout l'été dans l'estuaire, ce milieu étant avantageux pour leur développement (Dodson *et al*., 1989 ; Laprise & Dodson, 1993 ; Sirois & Dodson, 2000a).

Des études moléculaires ont identifié deux lignées phylogénétiques distinctes d'éperlan distinguées par six sites apomorphiques sur le gène codant pour la sous-unité 5 de la NADH déshydrogénase, et séparées par une divergence moyenne de séquences par paires d'environ 0,8 %, une valeur intraspécifique relativement élevée chez les poissons tempérés (Figure 2 ; Bernatchez, 1997 ; Baby *et al.,* 1991 ; Bernatchez & Martin, 1996). Le nom de ces deux lignées mitochondriales (Acadienne ou A et Atlantique ou B) vient de leurs hypothétiques origines glaciaires (Baby *et al.,* 1991 ; Bernatchez & Martin, 1996 ; Bernatchez, 1997).

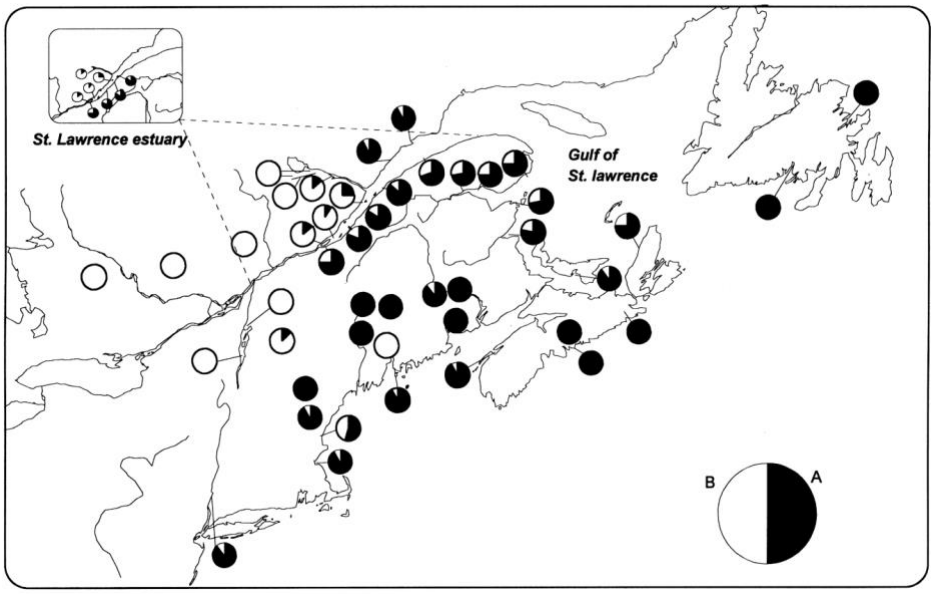

Figure 2. Distribution de fréquence des groupes d'ADNmt A et B parmi 49 populations indigènes d'éperlans arc-en-ciel. Tiré de Bernatchez, 1997.

Dans les eaux saumâtres de l'Estuaire Moyen du Saint-Laurent, deux populations sympatriques d'éperlan génétiquement distinctes (Fst = 0,178 ; Baby *et al.,* 1991 ; Bernatchez & Martin, 1996) ont alors été identifiées, étant associées aux lignées précitées. L'une se répartissait principalement dans les chenaux profonds de l'estuaire moyen (population de la rive nord) et la seconde se limitait aux hauts-fonds peu profonds de la rive sud de l'estuaire (population de la rive sud). Ces deux populations sont caractérisées par la présence des deux lignées d'ADN mitochondrial (ADNmt) mais à des fréquences significativement différentes. La population dite de la rive nord se caractérise par la prédominance de la lignée atlantique (85% des individus présentent une lignée ADNmt atlantique) et la population dite de la rive sud se caractérise par la prédominance de la lignée acadienne (82 % des individus présentent une lignée acadienne d'ADNmt) (Bernatchez & Martin, 1996). Cela suggère une période de mélange à la suite d'un contact secondaire des deux lignées fondatrices dans l'estuaire du Saint-Laurent qui ont ensuite produit deux populations sympatriques présentant des modèles distincts de cycle biologique, de morphologie et d'écologie (Lecomte & Dodson, 2004, 2005 ; Dodson *et al*., 2015).

Les larves d'éperlan arc-en-ciel anadrome des deux populations dominent la communauté ichtyoplanctonique dans la zone de transition estuarienne du Saint-Laurent (Dodson *et al.,* 1989 ; Sirois & Dodson, 2000b ; Vanalderweireldt *et al.,* 2020). Cette zone possède de forts gradients hydrographiques qui contrôlent les distributions du zooplancton et de l'ichtyoplancton, possiblement influençant leurs interactions trophiques. Considérant que les espèces fourragères comme l'éperlan arc-en-ciel jouent un rôle clé dans les écosystèmes marins et les économies du monde entier en soutenant de nombreux prédateurs et pêcheries à la fois directement et indirectement (Pikitch *et al.,* 2012), il est important d'étudier la dynamique trophique qui soutient les populations afin que nous puissions gérer correctement les espèces. En analysant le transfert d'énergie vers des prédateurs comme l'éperlan, nous pouvons comprendre les paramètres trophiques qui peuvent affecter la survie des larves et finalement prédire le recrutement du stock.

Eurytemora affinis : une espèce pivot dans l'estuaire du Saint Laurent

Les espèces cryptiques désignent des groupes d'organismes morphologiquement similaires, mais génétiquement distincts représentant des lignées évolutives différentes (Knowlton, 1993 ; Sáez & Lozano, 2005). Le terme « espèces cryptiques » met en évidence la difficulté d'identifier et de catégoriser ces espèces uniquement par les méthodes taxonomiques traditionnelles. Dans ce sens, les techniques de génétique moléculaire, telles que le séquençage de l'ADN, ont joué un rôle crucial dans le dévoilement de la présence d'espèces cryptiques en révélant des différences génétiques qui ne ressortent pas des caractéristiques phénotypiques (Hebert *et al.,* 2004 ; Bickford *et al.,* 2007 ; Behereharay & Caccone, 2007). Ces espèces sont largement distribuées et peuvent apparaître en raison de divers facteurs tels que l'isolement géographique, des différences de comportement ou des niches écologiques spécialisées (Pfenninger & Schwenk, 2007). Mais bien que ces espèces soient communes dans les taxons marins (Knowlton, 1993, 2000), certains groupes présentent des niveaux élevés de divergence génétique malgré leur conservatisme morphologique (Buckling *et al.,* 1996, 1998 ; Rocha-Olivares *et al.,* 2001 ; Lee & Frost, 2002). On trouve les copépodes parmi ces groupes d'organismes qui peuvent inclure de nombreux complexes cryptiques (Goetze, 2003).

En tant que groupe zooplanctonique dominant des écosystèmes aquatiques, les copépodes jouent un rôle essentiel dans les réseaux trophiques car ils permettent le transfert d'énergie des producteurs primaires vers les niveaux trophiques supérieurs (Longhurst, 1991; Stibor *et al.,* 2004 ; Teuber *et al.,* 2014). Au sein de ces organismes, on trouve *Eurytemora affinis* (Poppe, 1880), un complexe d'espèces cryptiques de copépodes important dans l'hémisphère nord qui habite une gamme d'habitats allant des eaux douces, saumâtres aux étangs hypersalins (Lee, 1999, 2000 ; Winkler *et al.,* 2003). Dans l'estuaire moyen, en particulier dans la zone de turbidité maximale (MTZ), deux clades génétiquement différents ont été signalés, le clade Nord-Atlantique et le clade Atlantique, probablement coexistant depuis la recolonisation de l'estuaire du Saint-Laurent après la dernière glaciation (Winkler

et al., 2008). Au sein de ce complexe, le clade Atlantique a été décrit comme une nouvelle espèce nommée *Eurytemora carolleeae* (Aleekseev & Soussi, 2011).

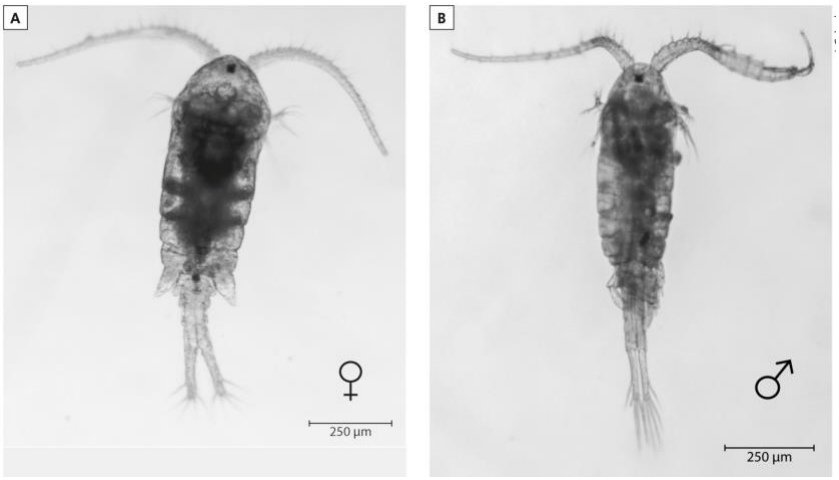

Figure 3. Femelle (A) et male (B) du complexe d'espèce *Eurytemora affinis*. Tiré de Winkler *et al.*, 2016.

Dominant dans la MTZ, ce complexe d'espèces cryptiques contribue grandement au recrutement de plusieurs espèces de poissons, dont plusieurs d'importance économique, qui utilisent la zone de turbidité maximale comme aire de reproduction et/ou d'alevinage. Parmi ces espèces on compte l'éperlan arc-en-ciel (*Osmerus mordax*), le poulamon atlantique (*Microgadus tomcod*), le bar rayé (*Morone saxatilis*), le baret (*Morone americana*) et l'alose savoureuse (*Alosa sapidissima*) (Sirois & Dodson, 2000a ; Martino & Houde, 2010 ; Vanalderweireldt *et al.,* 2019, 2020). Ce complexe d'espèces cryptiques constitue ainsi un lien trophique majeur au sein de l'estuaire en tant que proie importante des premiers stades de vie de nombreuses espèces de poissons. Néanmoins, malgré leur rôle important dans le réseau trophique estuarien, on ignore comment les deux espèces cryptiques *E. affinis* et *E. carolleeae,* affectent le flux d'énergie des producteurs primaires jusqu'aux espèces fourrages dans la zone de transition estuarienne du Saint-Laurent.

Les résultats préliminaires montrent que les deux espèces du complexe cryptique ont des caractéristiques morphométriques et reproductives différentes. *E. carolleeae* a une longueur corporelle totale légèrement plus petite que *E. affinis,* en revanche *E. affinis* montre une couvée plus petite contenant des œufs plus grands (G. Winkler, non publié). Ces différences de biovolume et de biomasse pourraient avoir des implications sur la façon dont les prédateurs les perçoivent comme proies potentielles. D'un point de vue énergétique, il pourrait être avantageux de consommer l'une des deux espèces en considérant que la qualité et la quantité de nourriture disponible influencent le développement et la croissance conditionnant ainsi les interactions trophiques (Schoo *et al*., 2012). Compte tenu de cet enjeu pour le réseau trophique, il est important d'étudier et d'évaluer le rôle écologique de ce complexe d'espèces. C'est en analysant le transfert d'énergie de chaque espèce vers les niveaux trophiques supérieurs dans la MTZ et en se concentrant sur l'ichtyoplancton, qu'il deviendra possible de mieux comprendre le fonctionnement de cet écosystème.

Objectifs de recherche et hypothèses

Compte tenu de l'importance du complexe d'espèces cryptiques d'*Eurytemora affinis* dans la zone de turbidité maximale (MTZ ; Winkler *et al.,* 2016) et du fait que l'éperlan arc-en-ciel est une espèce fourragère importante dans la région (Sirois & Dodson, 2000a, 2000b), l'objectif général de cette étude était : évaluer la contribution des deux espèces cryptiques, *E. affinis* et *E. carolleeae*, au régime alimentaire des premiers stades de vie de l'éperlan arc-en-ciel dans la mosaïque d'habitats de salinité de la MTZ durant les mois de juin, juillet et août 2021.

Le premier objectif de ce travail était d'analyser l'écologie trophique des premiers stades de vie de l'éperlan arc-en-ciel par l'analyse du contenu stomacal le long des différents habitats de salinité de la zone de turbidité maximale. Différentes études ont déjà analysé le

régime alimentaire des populations d'éperlans dans cette zone en utilisant l'analyse des contenus stomacaux (Sirois & Dodson, 2000a ; Lecomte & Dodson, 2004 ; Yoneyama, 2004), cependant c'est la première étude qui a effectué l'identification des proies à la résolution du stade de développement ce qui est utile pour mieux interpréter les interactions au sein des communautés d'ichtyoplancton (Demontigny *et al.,* 2012). En outre, ce travail est aussi le premier à effectuer ces comparaisons entre habitats de diverses salinités et lignées génétiquement distinctes.

Ensuite, des contenus stomacaux d'éperlans ont été utilisés pour atteindre notre deuxième objectif qui était d'estimer la contribution de chacune des espèces cryptiques d'*Eurytemora* à l'aide de techniques moléculaires, notamment la réaction en chaîne de la polymérase en temps réel. C'est la première fois qu'une nouvelle approche moléculaire est utilisée pour étudier le transfert d'énergie entre le complexe cryptique d'*Eurytemora affinis* et les niveaux trophiques supérieurs. Ceci est particulièrement important car ce complexe cryptique domine le mésozooplancton dans la zone de turbidité maximale et joue donc un rôle très important dans la survie et le recrutement des populations de poissons.

Des deux espèces cryptiques, *E. carolleeae* a une plus grande tolérance aux variations de salinité et se trouve dans la portion amont de la MTZ du Saint-Laurent (Winkler *et al.,* 2008 ; Favier & Winkler, 2014 ; Cabrol *et al.,* 2020), suggérant l'hypothèse que les larves de poissons qui préfèrent les eaux limnétiques se nourriront principalement de cette espèce. D'autre part, *E. affinis* se trouve principalement dans les eaux oligo- et mésohalines de la MTZ (Winkler *et al.,* 2008 ; Favier & Winkler, 2014 ; Cabrol *et al.,* 2020), de sorte que les larves des poissons présentes dans ces habitats se nourriraient davantage du clade nord-atlantique d'*E. affinis*. En revanche, dans la zone de chevauchement de la distribution de ces espèces, les larves de poissons pourraient potentiellement avoir des préférences envers l'une ou l'autre de ces deux espèces.

Ce travail a été financé par le programme individuel CRSNG (Conseil de Recherches en Sciences Naturelles et en Génie du Canada) de G. Winkler intitulé « zooplancton des écosystèmes estuariens hautement hétérogènes : les processus écologiques et évolutifs et connectivité des populations ». Les missions de terrain ont été financées par les fonds du temps de navire du programme Odyssée Saint-Laurent (OSL) du Réseau Québec Maritime (RQM) octroyés à G. Winkler, P. Sirois, C. Nozais et G. Dupéré ainsi que par la mission annuelle du Lampsilis du même programme. Un soutien financier et logistique a également été reçu de Québec Océan.

CHAPITRE 1

QUI CHOISIR ? CONTRIBUTION DE DEUX ESPÈCES CRYPTIQUES (*EURYTEMORA AFFINIS* ET *E. CAROLLEEAE*) AU RÉGIME ALIMENTAIRE DES PREMIERS STADES DE VIE DE L'ÉPERLAN ARC-EN-CIEL

1.1 RESUME EN FRANÇAIS DU PREMIER ARTICLE

L'éperlan arc-en-ciel (*Osmerus mordax*) utilise la zone de turbidité maximale (MTZ) de l'estuaire du Saint-Laurent comme zone d'alevinage et les proies zooplanctoniques sont fortement dominées par le complexe de copépodes calanoïdes *Eurytemora affinis* dans cette zone très productive. Ce complexe d'espèces cryptiques est considéré comme un lien important entre la production primaire et les niveaux trophiques supérieurs et représente une proie cruciale pour les larves de poissons. Nous avons cherché à évaluer la contribution d'*Eurytemora affinis* et d'*E. carolleeae* au régime alimentaire des premiers stades de l'éperlan arc-en-ciel tout au long des habitats hétérogènes de la MTZ. Quatre relevés ont été effectués au cours de l'été 2021. La répartition de l'éperlan et de ses proies copépodes étaient hétérogène et les abondances moyennes les plus élevées été trouvées dans l'habitat oligohalin (0,5 – 5,0 PSU ; 20,41 ± 10,82 larves 100 m^{-3}). Des changements ontogénétiques du régime alimentaire ont été observés, *Eurytemora* spp. étant la proie prédominante en juin et juillet (76 – 93 %), tandis que le mysidacé *Neomysis americana* a gagné en importance en août (80 %, habitat oligohalin). À l'aide d'un protocole qPCR nouvellement développé, nous avons révélé qu'*E. affinis* était la proie prédominante avec 72 % des estomacs ne présentant que cette espèce, et qu'*E. carolleeae* jouait un rôle mineur dans le transfert d'énergie. De même, *E. affinis* dominait dans l'environnement, ce qui signifie que les larves d'éperlan arc-en-ciel exploitent la ressource la plus abondante disponible. Néanmoins, d'autres études sont nécessaires pour examiner la sélectivité au sein du complexe cryptique et son influence sur la croissance et la survie des jeunes stades de vie des poissons.

Cet article, intitulé en anglais « Whom to chose? Contribution of two cryptic species (*Eurytemora affinis and E. carolleeae*) to the diet of early life stages of rainbow smelt », fut corédigé par moi-même ainsi que par les professeurs Gesche Winkler et Pascal Sirois, et la postdoctorante Maria Martinez-Silva. Il sera soumis dans la revue *Marine Ecology Progress Series*. En tant que premier auteur, j'ai contribué à l'essentiel de la recherche sur l'état de la question, au développement de la méthode, à l'exécution des analyses et à l'écriture de l'article. Les professeurs Gesche Winkler et Pascal Sirois ont fourni l'idée originale, ont aidé à la recherche sur l'état de la question, au développement de la méthode ainsi qu'à la révision de l'article. La postdoctorante Maria Martinez-Silva, a contribué au développement et l'exécution des protocoles génétiques, à la revue de la littérature, ainsi qu'à la révision de l'article. Une version abrégée de l'article a été présentée à la réunion scientifique annuelle de Québec Océan à l'hiver 2022 (virtuel) où j'ai reçu le prix des juges pour la meilleure affiche et à l'hiver 2023 à Rivière-du-Loup (Québec) où j'ai reçu le prix du public pour la meilleure présentation orale. À l'hiver 2023, une présentation orale a aussi été faite à la conférence de la *Société canadienne de science aquatiques* à Montréal (Québec). Finalement, les résultats les plus importants ont été présentés à la *Larval Fish Conference* à Lisbonne (Portugal) au printemps 2023.

Liste des communications :

Avila, L., Sirois, P. & Winkler, G. (2022, 31 janvier – 3 février). *L'hétérogénéité spatiale des jeunes stades d'éperlan arc-en-ciel et de poulamon atlantique dans la mosaïque des habitats de l'estuaire moyen du Saint-Laurent* [présentation par affiche]. Prix de la meilleure affiche par les juges. Réunion Scientifique Annuelle (RSA) de Québec Océan, Québec, QC, Canada.

Avila, L., Martinez-Silva, M., Sirois, P. & Winkler, G. (2023, 6 – 7 février). *Contribution of a cryptic copepod species complex to the early life stages of rainbow smelt in the zone of maximum turbidity of the St. Lawrence* [présentation orale]. Prix de la meilleure présentation par le public. Réunion Scientifique Annuelle (RSA) de Québec Océan, Rivière-du-Loup, QC, Canada.

Avila, L., Martinez-Silva, M., Sirois, P. & Winkler, G. (2023, 22 – 25 février). *Whom to choose? Feeding on a cryptic species complex by early life stages of rainbow smelt in the maximum turbidity zone of the St. Lawrence* [présentation orale]. Conférence de la société canadienne des sciences aquatiques (SCAS/SCSA), Montréal, QC, Canada.

Avila, L., Martinez-Silva, M., Sirois, P. & Winkler, G. (2023, 7 – 12 mai). *Ecological implications of a cryptic species complex on the diet of early life stages of rainbow smelt in the maximum turbidity zone of the St. Lawrence Estuary* [présentation orale]. Larval Fish Conference (LFC), Lisbonne, Portugal.

1.2 WHOM TO CHOOSE? CONTRIBUTION OF TWO CRYPTIC SPECIES (*EURYTEMORA AFFINIS* AND *E. CAROLLEEAE*) TO THE DIET OF EARLY LIFE STAGES OF RAINBOW SMELT

1.3 ABSTRACT

Rainbow smelt (*Osmerus mordax*) uses the maximum turbidity zone (MTZ) of the St. Lawrence Estuary as a nursery area and its prey field is strongly dominated by the calanoid copepod complex *Eurytemora affinis* in this highly productive area. This cryptic species complex is considered an important link between primary production and higher trophic levels and represents a crucial prey for fish larvae, so we aimed to evaluate for the first time the contribution of *Eurytemora affinis* and *E. carolleeae* to the diet of early stages of rainbow smelt throughout the heterogeneous habitats of the MTZ. Four surveys were carried out during the summer of 2021, showing a heterogeneous distribution of smelt and its copepod prey with highest mean abundances found in the oligohaline habitat (0.5–5.0 PSU; 20.41 ± 10.82 larvae 100 m^{-3}). Ontogenetic diet shifts were observed, *Eurytemora* spp. being the predominant prey in June and July (76–93%), while the mysid *Neomysis americana* increased in importance in August (80%, oligohaline habitat). Using a newly developed qPCR assay, we revealed that *E. affinis* was the predominant prey with 72% of stomachs presenting only this *Eurytemora* species, thus *E. carolleeae* played a minor role in energy transfer. Similarly, *E. affinis* dominated over *E. carolleeae* in the environment meaning the rainbow smelt larvae are exploiting the most abundant resource available. Further studies are required to examine selectivity within the cryptic complex and its influence on growth and survival.

1.4 INTRODUCTION

Zooplankton plays a crucial role in aquatic ecosystems, acting as an intermediary in the energy transfer, linking primary producers (phytoplankton) to upper trophic levels such as forage fish (Fenchel,1988; Bollens & Frost, 1989; Sommer & Stibor, 2002). The small size of zooplankton makes them suitable prey for early life stages of fish, which have limited feeding capabilities. During these stages, fish larvae undergo a critical period of high mortality when increased metabolic demands and rapid growth make proper nutrition essential to survival (Hjort,1914, 1926; Cushing, 1990; Jobling, 1994; Houde, 2008; Robert *et al.,* 2023). Zooplankton constitutes a primary food source during this critical period, providing essential nutrients required for larval growth and development. Moreover, different fish species exhibit specific feeding preferences for specific types of zooplankton, so that the abundance of preferred prey can determine larval survival success (Leclerc *et al.,* 2011; Demontigny *et al*., 2012; Paradis *et al.,* 2012; Robert *et al*., 2008, 2009, 2014). Zooplankton composition and availability thus influence the survival and recruitment of most fish stocks (Kiørboe,1998).

With its rapid growth and early sexual maturity, rainbow smelt *Osmerus mordax* can effectively transform zooplankton biomass and make it available to higher trophic levels. This small fish species native to the northern Atlantic Ocean is widely distributed along the coast of North America and it constitutes a vital prey species contributing to overall food web dynamics and energy transfer within aquatic ecosystems (Scott & Crossman, 1973; Nellbring, 1989; Dodson *et al.,* 1989; Lecomte & Dodson, 2005). In the St. Lawrence Estuary, anadromous rainbow smelt is probably one of the most abundant and important forage species from both an ecological and economic point of view as it supports recreational fisheries in the region (Sirois & Dodson, 2000a, 2000b). In this estuary two genetically distinct sympatric populations of rainbow smelt co-exist, namely the North Shore population (NSP) and the South Shore population (SSP) (Bernatchez & Martin, 1996; Bernatchez, 1997). These populations are associated with two phylogenetically distinct lineages (Atlantic and Acadian) separated by about 0.8% average pairwise sequence divergence in the NADH

dehydrogenase subunit 5 encoding gene (ND5) (Taylor & Dodson, 1994; Bernatchez, 1997; Dodson *et al*., 2015). They spawn in small tributaries along the Middle Estuary using the maximum turbidity zone (MTZ) as a nursery and feeding area (Lecomte & Dodson, 2004). Retention of rainbow smelt larvae in this zone would allow early life stages to exploit the abundant food sources (Dauvin & Dodson, 1990) and shelter from predation in the turbid waters (Bruton, 1985). High levels of suspended particle matter and nutrients support high levels of primary productivity that translate into high zooplankton abundance (Laprise & Dodson, 1994; Frenette *et al.,* 1995; Vincent *et al.,* 1996; Winkler *et al.,* 2003) providing an ideal feeding zone for smelt larvae during the critical period.

The zooplanktonic community of the highly productive MTZ is strongly dominated by the calanoid copepod *Eurytemora affini*s. This copepod represents an important link between primary producers and higher trophic levels such as early life stages of rainbow smelt (Laprise & Dodson, 1994; Winkler *et al.,* 2003; Martineau *et al.,* 2004; Barnard *et al.,* 2006). Nonetheless, *E. affinis* is recognized as a cryptic species complex distributed throughout the northern hemisphere (Lee, 1999, 2000). In the MTZ of the St. Lawrence estuary two clades, the North-Atlantic and the Atlantic clade co-exist (Winkler *et al.,* 2008). The latter was described in 2011 as a new species within the cryptic species complex of *E. affinis*, called *E. carolleeae* (Alekseev & Soussi, 2011). Different studies in the MTZ have shown that these two cryptic species differ not only genetically (Winkler *et al.,* 2008), but also in many different ecological aspects such as their distribution patterns (Favier & Winkler, 2014; Winkler *et al.,* 2016), their feeding behavior (Cabrol *et al.,* 2015) and their eco-physiological performance (Cabrol *et al.,* 2020). *E. carolleeae* shows greater tolerance to salinity, being present from freshwater and oligohaline habitats to polyhaline waters and salt marshes, while *E. affinis* seems to prefer oligo- and mesohaline waters (Winkler *et al*., 2016). Analyzes have also shown that even if the two cryptic species seem to ingest mainly phytoplankton, the two present different feeding behavior probably optimizing the accumulation of energy reserves according to their physiological needs (Cabrol *et al.,* 2015). These differences exhibited by the two cryptic species may have ecological implications

affecting not only their spatio-temporal distribution but also their ecological role within the food web and the entire ecosystem (Winkler *et al.*, 2016).

Preliminary results show that these two cryptic species have different morphometric and reproductive characteristics. *E. carolleeae* has a slightly smaller total body length but produces more eggs than *E. affinis*, on the other hand *E. affinis* has a smaller clutch size containing larger eggs (G. Winkler, unpublished). These differences in size and biomass could have implications for how predators like rainbow smelt perceive them as potential prey. From an energetic point of view, it could be advantageous to select one of the two species, according to the optimal feeding theory (MacArthur & Pianka, 1966; Schoener, 1971). The quality and quantity of food available to consumers influence development and growth, thus conditioning trophic interactions (Schoo *et al.,* 2012). Based on the general distribution pattern of the two cryptic species of *E. affinis* (Winkler *et al.,* 2008; Favier & Winkler, 2014; Winkler *et al.,* 2016), we hypothesize that smelt larvae in the tidal freshwater habitats would exploit *E. carolleaee* in a greater proportion while larvae downstream in the oligo- and mesohaline habitats would feed more on the North Atlantic clade of *E. affinis*. However, larvae in areas where distribution of the cryptic species overlap could potentially be choosing one species over the other. Given its importance to the estuarine food web of the MTZ, assessing the ecological role of this species complex is crucial for species and ecosystem management. By analyzing the energy transfer of each cryptic species to higher trophic levels, we can increase the understanding of the functioning of this ecosystem and how it might respond to external perturbations.

Taking this into consideration, the main goal of this study was to evaluate how the two cryptic species *E. affinis* North Atlantic clade and *E. carolleeae* are supporting early life stages of rainbow smelt in the maximum turbidity zone (MTZ). Therefore, we first aimed to evaluate the feeding ecology of larval smelt from June to August throughout the habitat mosaic of St. Lawrence Middle Estuary and second to estimate the contribution of the cryptic complex of *E. affinis*/*E. carolleeae* to the larval diet.

1.5 MATERIALS AND METHODS

1.5.1 Study site

The maximum turbidity zone (MTZ) of the St. Lawrence Middle Estuary is a well-mixed, tidally energetic area between Quebec City and La Pocatière, where the concentration of suspended material is much higher than in areas landward or seaward (Bousfield *et al.,* 1975; Dodson *et al.,* 1989). High turbidity levels maintained by a complex density-driven circulation in combination with the resuspension of bottom sediments near the head of the Middle Estuary (Simons *et al.,* 2006, 2010) support high phytoplanktonic biomass and also high secondary productivity. The zooplanktonic community of this zone is composed of three distinct assemblages: a tidal freshwater assemblage upstream dominated by veliger larvae of the bivalve *Dreissena polymorpha* and crustaceans such as *Bosmina* spp. and *Gammarus* spp., followed by a second estuarine assemblage dominated by the cryptic complex of *Eurytemora affinis* as well as the mysids *Neomysis americana* and *Mysis stenolepsis*. And finally, at higher salinities a third assemblage of euryhaline and marine species composed of *Acartia* spp. *Eurytemora herdmani*, *Calanus* spp., *Mysis littoralis*, euphausiids and chaetognaths (Laprise & Dodson, 1994; Winkler *et al.,* 2003, 2016). Although *Eurytemora affinis* dominates only the estuarine assemblage, this complex of cryptic species is also present, but in lower density, throughout the entire estuarine transition zone (Winkler *et al.,* 2008; Favier & Winkler, 2014). Estuarine smelt spawns from mid-April to early May with individuals from the north shore population spawning upstream in shallow shoals in the fluvial estuary and larvae then moving downstream to the MTZ where they remain for the rest of the season. South shore population smelt spawns downstream in small tributaries along the south shore, with larvae then descending passively to shallow bays that act as retention areas in the MTZ and further downstream, where they develop during the summer (Ouellet & Dodson, 1985; Lecomte & Dodson, 2004, 2005; Trencia *et al*., 2005).

1.5.2 Sampling

Sampling took place in the maximum turbidity zone (MTZ) between Quebec City and Anse Ste-Anne (La Pocatière) to cover known habitats of the copepod species complex *Eurytemora* spp. and fish larvae of rainbow smelt (*Osmerus mordax*) in the salinity range between 0 and 25 PSU. A total of 20 stations were sampled across the north, middle and south channels with asymmetrical distribution due to the salinity gradient (Fig. 1). Stations were classified according to the Venice salinity classification system (1958) into limnetic (<0.5 PSU), oligohaline (0.5–5.0 PSU), mesohaline (5.0–18.0 PSU) and polyhaline (18.0–30 PSU). Four different field surveys were carried out during late spring and summer of 2021 (June – August), one in mid-June (14th – 19th June), corresponding to the start of the growing season, followed by three surveys in late June (28th June – 1st July), mid-July (19th–23rd July) and early August (7th–13th August) considering the hatching period of rainbow smelt (Sirois & Dodson 2000b), as well as its first weeks of ontogeny. This period is characterized by a significant increase in zooplankton biomass (Laprise & Dodson, 1994).

To describe abiotic and biotic characteristics of the habitats, a CTD profile was carried out at each station, measuring physical-chemical parameters such as salinity, temperature and turbidity. These were accompanied by water samples to quantify chlorophyll *a* concentration at surface. Other variables like nutrients, seston (suspended particulate matter, SPM) and particulate organic matter (POM) were also quantified for complementary studies. Ichthyoplankton were collected using a 1 m diameter 500 µm mesh plankton net deployed obliquely throughout the water column. To assess the prey field of larval fish, concurrent zooplankton tows were carried out at each station using two zooplankton net types, a ring net with a 50 cm diameter, equipped with a 63 µm mesh deployed vertically and a ring net with a 1 m diameter and a 200 µm mesh deployed obliquely. All ichthyoplankton and zooplankton samples were immediately preserved in a 95% ethylic alcohol solution onboard the vessel.

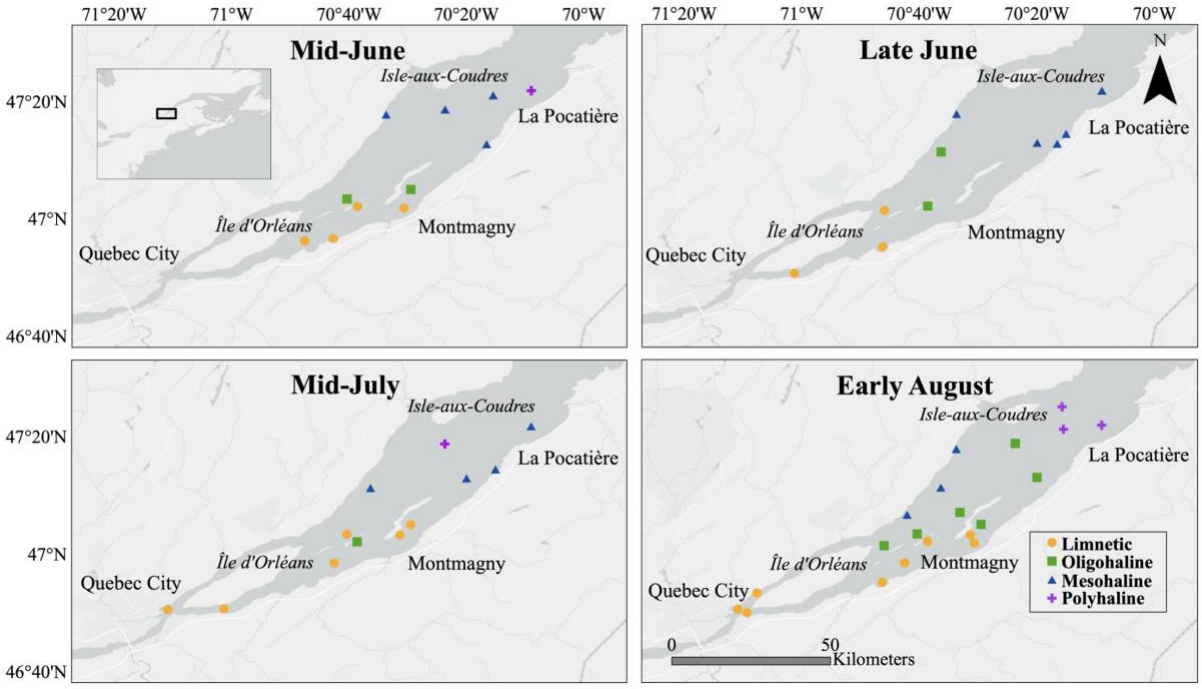

Figure 4. Map of 10–20 stations sampled along the salinity gradient of the maximum turbidity zone (MTZ) of the St. Lawrence Estuary where ichthyoplankton and zooplankton samples were collected in summer 2021.

1.5.3 Laboratory analyses

Following the field surveys, larval *O. mordax* were sorted, identified and stored at –20°C in falcon tubes using 95% ethylic alcohol. A subsample of 10–26 larvae from each salinity zone when available were chosen for molecular and trophic analyses. Then, a total of 197 larvae from the sampling periods were selected and measured for standard length (SL) (to the nearest 0.01 mm) under a stereoscopic microscope (Olympus SZX16, CellSens standard 3.2 software).

1.5.3.1 Larval molecular identification

Considering that the North Shore Population (NSP) of rainbow smelt is characterized by predominance of the Atlantic lineage (mtDNA lineage B, 87.1%, Lecomte & Dodson, 2004) and the South Shore Population (SSP) is mostly composed of the Acadian lineage (mtDNA lineage A, 80.9%, Lecompte & Dodson 2004), molecular identification of mtDNA lineage was carried out for each selected larva (n = 197). Muscular tissue between the adipose and caudal fins was dissected with flame sterilized dissecting tools for DNA extraction using QuickExtract™ Solution 1.0 (Biosearch Teachnologies, UK) following manufacturer's instructions with a modified volume of 50µL per sample. The NADH dehydrogenase subunit 5 encoding gene (ND5) was selected due to previous studies and available sequences of the mtDNA lineages (Pigeon *et al*., 1998). Specific primers for *O. mordax* (Table 1) were developed using the Primer-BLAST tool (National Center for Biotechnology Information) with public access ND5 sequences (AF034751.1 and AF034752.1, NCBI). Polymerase chain reaction was performed (Mastercycler EP Gradient S 5345, Eppendorf) with an initial denaturation of 95°C for 10 s followed by 30 cycles comprising a denaturation of 90°C for 30 s, annealing temperature of 59°C for 45 s and an elongation of 72°C for 60 s. A subsequent final elongation of 72°C for 5 min produced a 659 bp amplicon containing four identifiable apomorphies that allowed genetic differentiation between the two mtDNA lineages. All samples were sent for sequencing the two complementary DNA strains to the SANGER Sequencing Platform (CHU de Québec Research Center, Laval University, Quebec, Canada) and final sequences were analyzed using MEGA11 (Molecular Evolutionary Genetics Analysis version 11; Tamura *et al.,* 2021) so that each individual larva was allocated to mtDNA group A (Acadian) or B (Atlantic).

Table 1. Primers used for molecular identification of Atlantic lineage (mtDNA group B) and Acadian lineage (mtDNA group A) rainbow smelt larvae and their potential prey, the cryptic species complex of *E. affinis*/*E. carolleeae*, as well as congener species *E. herdmani*, through the newly developed SYBR green qPCR assay on stomach contents.

Species	Gene	Origin	Forward (5'-3')	Reverse (5'-3')
O. mordax	*ND5*	This study	ACCTCACCCCCTTCACTTCTT	CCTTGTTGAAGGTCTGTGGTGG
Universal	*COI*	Folmer *et al.*1994	GGTCAACAAATCATAAAGATATTGG	TAAACTTCAGGGTGACCAAAAAATCA
E. affinis	*COI*	This study	ACCTTAGGGAACTTGCGAGC	ACAAAATCGGATCTCCGCCC
E. carolleeae	*COI*	This study	CTCACGCAGGTAGGTCTGTC	CCCCTCCACTTGCGTCATAA
E. herdmani	*COI*	This study	TATTGCTCACGCTGGGAGTT	TAACACAGACCACGCGAACA

1.5.3.2 Gut content analyses

The digestive system of each larva (n = 197) was dissected (8–11.5x magnification) using flame sterilized dissecting tools for visual stomach content analysis before molecular tests. Each sample was transferred to a sterile 60x20 mm petri dish filled with a 97% ethylic alcohol solution. Individual preys were photographed, identified to the lowest taxonomic level, developmentally staged and measured for total length or prosome length (to the nearest 0.1 µm). Prey measurements were used to convert stomach contents to carbon weight (µgC) using previously determined equations from the literature (Table 2).

When more than 10 individuals from the same prey category and developmental stage were present or if they were too damaged to be accurately measured, the average length of the taxon and corresponding developmental stage was used. All prey and unidentified material, including stomach and intestinal walls, were then placed in a 1.5 mL tube with 100% ethylic alcohol and stored at -80°C. For molecular analysis, samples were centrifuged at 15000 rpm for 10 min at 4°C (5430 R, Eppendorf) and then the supernatant ethylic alcohol solution was carefully removed with a 1000 µL micropipette. Pellet was air dried for 20 minutes to assure the evaporation of residual ethylic alcohol followed by DNA extraction using QuickExtractTM Solution 1.0 (Biosearch Teachnologies, UK) according to manufacturer's instructions with a modified volume of 50µL per sample.

Table 2. Summary of number, size ranges and references on relationships between carbon content (C, in µg), prosome or total length (L, in µm), volume (V, in µL), ash-free dry weight (ADW in µg) and dry weight (DW, in µg) for prey found in larval rainbow smelt gut contents.

Prey item	n	Size range	Measure	Equation	Reference
Copepoda					
Eggs	6950	40–122	V	$4/3\ ((L/1000)/2)^3$	
			C	140V	Kiørboe *et al*., 1985
Nauplii	18	132–362	DW	$3.009\ (L/1000)^{1.706}$	Culver *et al.,* 1985
			C	$10^{(\log DW - 0.499)/0.991}$	Wiebe, 1988
***Eurytemora* spp. (N1–N6)**	232	112–404	DW	$3.009\ (L/1000)^{1.706}$	Culver *et al.,* 1985
			C	$10^{(\log DW - 0.499)/0.991}$	Wiebe, 1988
Copepodites	29	275–705	DW	$7.047\ (L/1000)^{2.399}$	Bottrell *et al.,* 1976
			C	44.7% DW	Mauchline, 1998
***Eurytemora* spp. (C1–C6)**	1970	289–1028	DW	$10^{2.088\ (L/1000) - 0.859}$	Burkill & Kendall, 1982
			C	44.7% DW	Mauchline, 1998
***Halicyclops* spp. (C1–C6)**	23	318–401	DW	$7.047\ (L/1000)^{2.399}$	Copepod conversion; Bottrell *et al.,* 1976
			C	44.7% DW	Mauchline, 1998
Cyclopoida	7	123–793	DW	$7.047\ (L/1000)^{2.399}$	Copepod conversion; Bottrell *et al.,* 1976
			C	44.7% DW	Mauchline, 1998
Acanthocyclops robustus	1	792	DW	$7.047\ (L/1000)^{2.399}$	Copepod conversion; Bottrell *et al.,* 1976
			C	44.7% DW	Mauchline, 1998
Diplostraca	1	180	C	$10^{4.15 \log L - 11.15}$	Uye, 1982
***Bosmina* spp.**	285	105–630	DW	$(10^{4.849 \log(L/1000) - 3.857} \times 10^5)/1000$	Rosen, 1981
			C	$10^{(\log DW - 0.499)/0.991}$	Wiebe, 1988
Mysidacea	6	476 –3053	DW	$6.605\ (L/1000)^{2.57}$	Chigbu & Sibley, 1996
			C	$10^{(\log DW - 0.499)/0.991}$	Wiebe 1988
Neomysis americana	19	1917–11121	DW	$6.605\ (L/1000)^{2.57}$	Chigbu & Sibley, 1996
			C	$10^{(\log DW - 0.499)/0.991}$	Wiebe, 1988
***Gammarus* spp.**	15	778–2380	DW	$9.616\ (L/1000)^{2.604}$	Pöckl, 1992
			C	$10^{(\log DW - 0.499)/0.991}$	Wiebe, 1988
Gammarus tigrinus	1	1609	DW	$9.616\ (L/1000)^{2.604}$	Pöckl, 1992
			C	$10^{(\log DW - 0.499)/0.991}$	Wiebe, 1988
Ostracoda	2	512–629	AFDW	$0.0228\ L^{2.3698}$	Mumm, 1991
			C	$10^{(\log AFDW - 0.410)/0.963}$	Wiebe, 1988
Crangon septemspinosa	1	2500	DW	$10^{0.039(L/100) + 0.51}$	Wilcox & Jeffries, 1973
			C	$10^{(\log DW - 0.499)/0.991}$	Wiebe, 1988
Gastropoda	1	306	DW	$6.07\ (L \times 10^{-6})^{2.59} \times 10^8$	Legendre & Michaud, 1998
			C	22.1% DW	Omori, 1969
Unidentified material			DW	$109.08\ V^{0.9591}$	Sirois & Dodson, 2000a
			C	$10^{(\log DW - 0.499)/0.991}$	Wiebe, 1988

1.5.3.3 Primer design for *Eurytemora* identification

First, cytochrome c oxidase subunit 1 (COI) gene was selected for primer design due to the absence of insertions and deletions and the combination of conserved and variable regions along it (Winkler *et al.,* 2008). Individuals from the cryptic species complex *E. affinis,* including the North Atlantic clade and *E. carolleae* were isolated and individually passed through two consecutive 5 min baths of TRIS (1M, pH 8.0, VWR) to remove ethylic alcohol before DNA extraction using QuickExtractTM Solution 1.0 (Biosearch Technologies, UK) following manufacturer's instructions with a modified volume of 30µL per sample. DNA of *Eurytemora herdmani* individuals were also extracted. This is a congener species that does not belong to the cryptic complex of *E. affinis* but inhabits the middle and lower St. Lawrence estuary (Winkler *et al.*, 2008) and therefore could potentially be present in sampled downstream stations. Universal primers HCOI 2198 and LCOI 1490 developed by Folmer *et al.* (1994) were used to perform polymerase chain reactions of the three species (Mastercycler EP Gradient S 5345, Eppendorf). Modified temperature profiles from Lee (2000) were used comprising an initial denaturation at 95°C for 10 s, followed by a first set of 5 cycles (denaturation at 90°C for 30 s, annealing at 45°C for 60 s and elongation at 72°C for 60 s), a second set of 28 cycles (denaturation at 90°C for 30 s, annealing at 55°C for 45 s and elongation at 72°C for 60 s) and a final elongation at 72°C for 5 min.

The resulting 652 bp amplicon was sent for sequencing to the SANGER Sequencing Platform (CHU de Québec Research Center, Laval University, Quebec, Canada). The resulting sequences (OP876784, OP876810 and OP876964, GenBank, NCBI) were processed and aligned to identify specific regions where mutations were present between the three species using MEGA11 (Molecular Evolutionary Genetics Analysis version 11; Tamura *et al.,* 2021). Specific primers (Table 1) were then developed on these regions with the Primer-BLAST tool (NCBI) for each species. The three pairs of specific primers were then tested to assure that they only amplified the target species. Amplification profiles

comprised an initial denaturation at 95°C for 10s followed by 30 cycles (denaturation at 90°C for 30 s, annealing at 57°C for 45 s and elongation at 72°C for 60 s) and a final elongation at 72°C for 5 min.

1.5.3.4 Real-time PCR assay

To diagnose and identify *Eurytemora* species in stomach content, a real-time polymerase chain reaction SYBR green assay (PowerUp™ SYBER™ Green Master Mix, applied biosystems, Thermo Fisher Scientific) was designed using the specific primers described above (Table 1). To calculate the detection limit of our assay (sensibility), qPCR was performed with ten-fold dilutions of mtDNA extracts of the three *Eurytemora* species in triplicate (standard curves). Reproducibility among replicas was attained at amounts of target DNA of 0.006 ng/L. Reliable detection of *Eurytemora* DNA was set at 29.6 Ct value for *E. affinis*, 27.9 Ct value for *E. carolleae* and 29.8 Ct value for *E. herdmani.* As expected, a ~3 Ct units increase corresponded to each 10-fold dilution (Albaina *et al.*, 2015). The assay was also tested against rainbow smelt tissue extracts (n = 5) to confirm there was no amplification with larval DNA. All stomach extracts (n = 197) were then diluted at 1/5 to assure adequate DNA concentration and tested through the assay with a total of three replicates per stomach per primer (*E. affinis* and *E. carolleae*). On stomachs from larvae captured on stations where salinity was higher than 5.0 PSU, three replicates were also tested with specific primers for *E. herdmani*. Three positive controls of pooled individuals of each species and three negative controls of distilled DNA-free water were also included in each plate to test cross-contamination on the plate.

1.5.3.5 Visual and molecular identification of the cryptic complex in the environment

To analyze the proportion of the cryptic complex in the environment and estimate the abundance of other important prey, zooplankton from the 63 µm and 200 µm nets was identified and counted through subsampling using a stereoscopic microscope (Olympus SZX16, CellSens standard 3.2 software). A representative subsample of 400 copepodites was counted or until one tenth of the sample was analyzed. *Eurytemora* spp. developmental stages (N1–N6, C1–C6) were determined, and distinction was made between adult males and females. The proportion of *E. affinis* and *E. carolleeae* in the environment was molecularly analyzed in each sampled station to compare it with stomach content results. A subsample of 30 randomly selected copepods per station were tested with the two specific primers developed for the cryptic complex (Table 1). To achieve that, DNA was extracted from each copepod and then a pool of DNA extracts of 10 individuals was amplified via PCR and verified with agarose gel electrophoresis. If positive results were obtained for the two species in a single pool, each individual copepod DNA extract was tested with PCR and agarose gel electrophoresis. Randomly selected individuals of the 2 species (n = 20) were sent for sequencing to the SANGER Sequencing Platform (CHU de Québec Research Center, Laval University, Quebec, Canada) to confirm correct species identification.

1.5.4 Trophic analysis

We analyzed the feeding ecology of larval rainbow smelt using feeding incidence, diet composition, trophic niche width and prey selectivity. Feeding incidence (*FI*) was calculated as the percentage of larvae having at least one prey item in their gut content. Diet composition was studied using the carbon weight percent contribution ($\%CW_i$) of each visually identified prey item and the more integrative Prey-Specific Index of Relative Importance (*%PSIRI*; Brown *et al.,* 2012). The carbon weight percent contribution ($\%CW_i$) was calculated as follows:

$$\%CW_i = \frac{\sum_{j=1}^{n} \%CW_{ij}}{n};$$

where $\%CW_{ij}$ is the estimated carbon weight percentage of prey category I in a stomach sample j, and n is the total number of stomachs.

In order to calculate the PSIRI, prey-specific abundance values (in carbon weight $\%PCW_i$ and in number $\%PN_i$) were also calculated following Brown *et al.* (2012):

$$\%PA_i = \frac{\sum_{j=1}^{n} \%A_{ij}}{n_i};$$

where $\%A_{ij}$ is the percent abundance (by carbon weight or number) of prey category i in a stomach sample j, and n_i is the number of stomachs containing prey i. The Prey-Specific Index of Relative Importance ($\%PSIRI$) was then used to incorporate prey-specific abundance in carbon weight ($\%PCW_i$) and number ($\%PN_i$) with frequency of occurrence ($\%FO_i$) (Brown *et al.,* 2012):

$$\%PSIRI = \frac{\%FO_i \times (\%PCW_i + \%PN_i)}{2};$$

where $\%FO_i$ = percent frequency of occurrence, $\%PCW_i$ = percent prey-specific carbon weight, and $\%PN_i$ = percent prey-specific number.

We estimated niche width (B_i) using Levin's standardized index (Krebs, 1999) to determine diet specialization of rainbow smelt larvae along the salinity gradient following:

$$B_i = \frac{(1/\sum P_{ij}^2)-1}{n-1};$$

where B_i is Levin's standardized index for predator i, P_{ij} is the proportion of the diet of predator i that is made up of prey j, and n is the number of prey categories. Proportions were calculated using the estimated carbon weight contribution of each prey. This index ranges from 0 to 1, with values close to 0 indicating a specialized diet and values close to 1 indicating a generalist diet (Krebs, 1999).

Prey selectivity along the salinity gradient was also calculated for each larva using Chesson's α-selectivity (Chesson, 1978):

$$\alpha_j = \frac{d_j/p_j}{\sum \frac{d_i}{p_i}};$$

Where d_j is the proportion of prey taxon j by occurrence in the larval diet and p_j is the proportion of prey taxon j in the prey field. Given the technical limitations to quantify proportions with stomach content qPCR presence/absence results, selectivity between *E. affinis* and *E. carolleeae* could not be determined. Due to the low number of larvae with only *E. carolleeae* DNA in gut content, selectivity was then only calculated for larvae that had exclusively consumed *E. affinis* according to our SYBR-green qPCR assay results.

Prey taxa that represented in average more than 2% of the larval diet by number were used in this equation (Govoni *et al.,* 1986): *E. affinis*, *N. americana*, *Bosmina* sp. and *Gammarus* sp.. *E. affinis* was subdivided by stage of nauplius and copepodite development in six categories (N1–N3 , N4–N6, C1–C3, C4–C5, C6 female, C6 male) across all salinity

zones for a total of nine taxa for mid-June, late June and mid-July. In mid-June, only one larva had exclusively *E. affinis* DNA in limnetic stations, therefore Chesson's alpha for this category was not calculated. For early August, nauplius stage data from the environment were not available so given that larvae did not consume nauplii during this period, these prey taxa were not included in the analysis resulting in a total of seven taxa for this month.

Neutral selectivity was calculated as an α value of $1/n$, where n is the number of prey taxa used un Chesson's equation. An α value higher than $1/n$ indicates positive selection for any given prey taxon as it was ingested in a higher proportion than its relative frequency in the surrounding environment, while an α lower than $1/n$ indicates selection against the prey. The threshold for neutral selectivity was calculated at 0.11 for mid-June, late June and mid-July, and 0.14 for early August. Following Burns *et al*. (2020), we defined positive selection as 'strong' when Chesson's α was $>2/n$, meaning that the proportion of the taxon in the larval diet was at least double that of its proportion in the potential prey field. Similarly, negative selection was defined as strong when α was $<1/2n$, meaning that the proportion of the taxon in larval diet was less than half of its proportion in the environment. The threshold for strong positive and negative selection were determined as 0.22 and 0.06 for mid-June, late June and mid-July, and 0.28 and 0.07 for early August, respectively. Chesson's α was averaged among larvae within the same salinity zone in the same period to determine differences in preference throughout larval development across the salinity gradient.

1.5.5 Statistical analysis

To assess whether the rainbow smelt lineage (Atlantic or Acadian), salinity zone (limnetic, oligohaline and mesohaline) or time of year (mid-June, late June, mid-July and early August) influenced rainbow smelt diet, we used permutational analysis of variance (i.e., three-way PERMANOVA) for data from visual gut content analysis. PERMANOVA analyses were based on Bray-Curtis dissimilarities (Bray & Curtis, 1957) and were performed using 9999 permutations. The homogeneity of dispersion was verified prior to

each PERMANOVA. Individual larvae were tested using the carbon weight (μgC) of each prey consumed divided by the standard length (mm) of the larva. Preliminary tests revealed no statistical influence of larval lineage in diet differences ($p < 0.05$), so this factor was removed from the analysis and two-way PERMANOVA were employed for diet composition comparison. Pairwise multiple comparisons tests were used if significant PERMANOVA results were found to identify differences according to each factor. To analyze the contrasts of diet composition, similarity percentage analyses (SIMPER) were performed to identify the taxa responsible for the dissimilarities (Clarke, 1993).

To assess if the salinity zone or time of the year influenced *Eurytemora* composition in the diet, two-way PERMANOVAs were also used for data from molecular gut content analysis. Diet composition in terms of the complex *E. affinis/E. carolleeae* based on percentage of qPCR results per station was determined. Stations within salinity zones were considered as replicates. All analyzes were carried out using PRIMER v7 PERMANOVA+ software (Anderson *et al.,* 2008) and R software v4.2.3 (R Core Team, 2018) running the vegan package (Oksanen *et al.,* 2019). All throughout the manuscript variation among the mean is noted as standard error (SE).

1.6 RESULTS

1.6.1 Distribution and density of rainbow smelt populations

A total of 1461 larvae and juveniles of rainbow melt ranging from 12–54 mm were captured during the summer of 2021 in the maximum turbidity zone (MTZ) of the St. Lawrence Estuary. Distribution was heterogeneous among habitats (Fig. 2) with higher densities found in oligohaline stations throughout the sampling period (20.41 $\pm$ 10.82 larvae 100 m^{-3}, 20 stations), followed by mesohaline (11.46 $\pm$ 5.70 larvae 100 m^{-3}, 32 stations) and limnetic stations (5.02 $\pm$ 2.91 larvae 100 m^{-3}, 34 stations). Densities also fluctuated as the season progressed with mesohaline stations showing the greatest variation in general abundances with an average of 20.45 $\pm$ 6.97 larvae 100 m^{-3} (18 stations) in mid and late June followed by a decrease in average abundance for mid-July and early August with 1.75 $\pm$ 0.87 larvae 100 m^{-3} (14 stations). In oligohaline stations, densities for mid and late June were on average 25.87 $\pm$ 6.43 larvae 100 m^{-3} (8 stations) and for mid-July and early August 15.51 $\pm$ 8.29 larvae 100 m^{-3} (12 stations). Finally, in limnetic stations, larval densities were on average 7.38 $\pm$ 3.34 larvae 100 m^{-3} (11 stations) for mid and late June and 3.82 $\pm$ 2.02 larvae 100 m^{-3} (23 stations) for mid-July and early August.

Molecular identification of the 197 larvae chosen for genetic lineage identification and subsequent trophic analysis showed predominance of individuals from the Atlantic lineage (mtDNA group B) with 80.03% of genotyped larvae belonging to this lineage (Fig. 2). Lineage distribution remained constant among salinity zones and throughout the season with all stations being dominated by the Atlantic lineage except for Anse Ste-Anne close to La Pocatière during late June (Fig. 2), where most genotyped individuals (75%) belonged to the Acadian lineage (mtDNA group A).

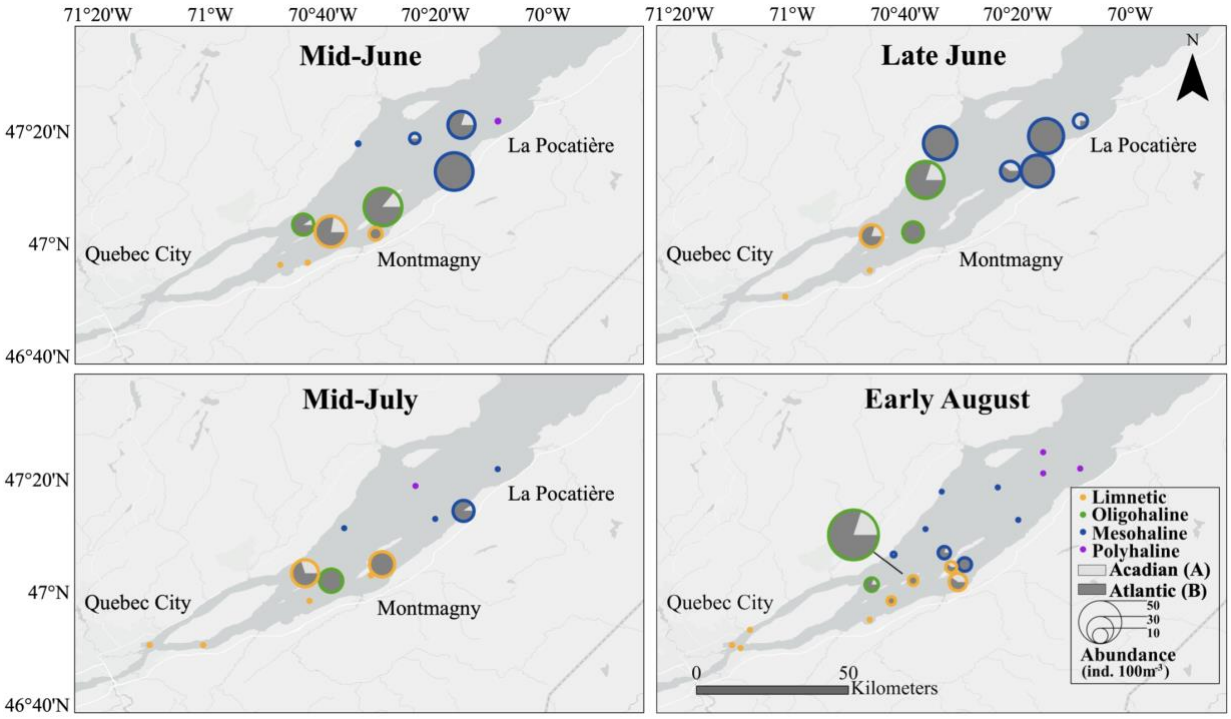

Figure 5. Distribution and relative abundance of sympatric lineages (Atlantic or Acadian) of estuarine rainbow smelt larvae along the salinity gradient of the MTZ of the St. Lawrence Estuary in summer 2021.

1.6.2 Visual characterization of rainbow smelt larval diet along the salinity gradient

In total 14 food items corresponding to 5 classes, 6 orders, 6 families and 8 species were identified through visual analysis of larval gut content (Table 3). Feeding incidence was high across the sampling period with limnetic stations having the highest percentage through the season (*FI* > 95% Table 3,). Larvae from oligohaline and mesohaline stations showed lower feeding incidences in mid and late June (FI = 65–80% Table 3) that increased in mid-July (*FI* = 91.67–100% Table 3). By early August, all larvae exhibited feeding incidences of 100% across all salinity zones.

Table 3. Diet composition expressed as percent carbon contribution of the prey categories identified in rainbow smelt larval diet in each salinity zone sampled throughout summer 2021 in the MTZ of the St. Lawrence Estuary. Feeding statistics and physical-chemical variables of each salinity zone are included as references.

					Limnetic				Oligohaline				Mesohaline			
Class	**Order**	**Family**	**Item**	**Stage**	**Mid-June**	**Late June**	**Mid-July**	**Early Aug.**	**Mid-June**	**Late June**	**Mid-July**	**Ear. Aug.**	**Mid-June**	**Late June**	**Mid-July**	**Early Aug.**
Branchiopoda	Diplostraca	Bosminidae	*Bosmina sp.*		1.39	0.33	0.65	0.57	3.38	0.07	0.02					
			Unidentified Cladocera						0.03							
Malacostraca	Amphipoda	Gammaridae	*Gammarus tigrinus.*					4.66								
			Gammarus sp.		6.15		0.31	11.6		4.90						
	Decapoda	Crangonidae	*Crangon septemspinosa*	Zoea										2.02		
	Mysida	Mysidae	*Neomysis americana*								9.83	56.20		25.01		
			Unidentified Mysida							6.25		1.16		3.86		
Maxillopoda (Copepoda)	Calanoida	Temoridae	*Eurytemora sp.*	N1		0.14			0.23							
				N2		0.75			0.37							
				N3		0.58			3.21						0.16	
				N4		1.39			5.08						0.19	
				N5		2.76			5.79						1.38	
				N6		2.48			8.49						0.32	
				C1		1.73			3.76				4.58		2.56	
				C2		0.96			0.94			0.00			5.04	0.84
				C3								0.10	5.95		0.28	1.87
				C4		0.41		0.09				1.97	2.31	0.16	5.11	8.59
				C5	1.45	2.10	1.67	0.86	12.33	0.43	4.70	6.09		25.67	15.62	42.34
				C6 (fem.)	40.03	20.01	45.76	58.66	28.91	25.37	32.34	9.00	13.40	13.51	25.41	7.89
				C6 (male)	16.16	65.10	32.18	21.67	4.02	50.69	50.65	24.47	37.08	14.38	33.67	22.19
				Eggs	7.32	0.68	2.93	1.59	2.13	1.14	2.46	0.65		0.31	0.89	0.18
	Cyclopoida	Cyclopidae	*Acanthocyclops robustus*	C1–C6	6.02											
			Halicyclops sp.	C1–C6		0.51		0.13								
				Eggs				0.01								
			Unidentified Cyclopidae	C1–C6			0.01		0.04			0.04			0.10	

Table 3. *Continued.*

Class	Order	Family	Item	Stage	Limnetic				Oligohaline				Mesohaline			
					Mid-June	Late June	Mid-July	Early Aug.	Mid-June	Late June	Mid-July	Early Aug.	Mid-June	Late June	Mid-July	Early Aug.
			Unidentified Copepoda	C1–C6	0.05	0.07			20.99				0.69			
				N1–N6				0.09	0.29	6.42		0.04		0.85	0.17	16.10
				Eggs					0.02				0.82			
Ostracoda			Unidentified Ostracoda							0.00					0.00	
Gastropoda			Unidentified Gastropoda											0.15		
Unidentified material					21.43		16.49			4.72		0.28	35.18	14.09	9.09	
Feeding statistics		Number of fish examined			16	10	20	20	20	20	10	20	20	26	12	3
		Mean SL of fish (mm)			19.69	21.00	27.93	33.32	17.76	22.03	28.76	37.86	16.72	18.80	24.33	36.51
		SE SL of fish (mm)			0.36	0.67	0.64	0.83	0.32	0.83	0.69	1.48	0.41	0.29	0.60	4.53
		Feeding incidence (FI, %)			100.0	100.0	95.00	100.0	65.00	80.00	100.0	100.0	80.00	73.08	91.67	100.0
		Levin's standardised index			0.48	0.21	0.36	0.19	0.21	0.26	0.32	0.02	0.45	0.21	0.16	0.11
Physical-chemical variables		Mean temperature (°C)			19.32	20.86	22.27	21.96	17.93	19.58	22.10	20.84	14.92	15.90	19.39	18.33
		SE temperature (°C)			0.66	-	0.29	0.18	0.67	1.07	-	0.14	0.61	1.30	0.77	-
		Mean salinity (PSU)			0.13	0.34	0.19	0.18	1.44	2.46	0.52	1.27	8.04	10.49	6.09	7.05
		SE salinity (PSU)			0.00	-	0.07	0.02	0.93	1.85	-	0.35	1.08	2.49	0.49	-
		Mean turbidity (FTU)			81.47	104.2	159.9	95.00	101.2	62.47	91.01	80.74	77.22	57.22	33.04	19.40
		SE turbidity (FTU)			47.87	-	55.53	26.20	49.00	10.72	-	23.74	49.16	12.62	15.00	-
		Mean surface chl a (mgChla.m^{-3})			25.13	16.49	32.85	14.56	4.95	4.56	10.09	12.20	2.59	3.88	9.56	1.81
		SE surface chl a (mgChla.m^{-3})			11.33	-	23.58	0.77	2.32	1.29	-	2.80	1.43	0.77	6.65	-
		Number of stations			2	1	2	4	2	2	1	4	3	5	2	1

Table 4. Results of permutational analyses of variance (PERMANOVA) performed on carbon weight data from visual analysis of larval gut content and data from molecular *Eurytemora* spp. identification in larval gut content via qPCR.

Factor	df	Visual		Molecular	
		Pseudo-F	p-value	Pseudo-F	p-value
Salinity habitat	2	4.331	**0.001**	6.258	**0.013**
Survey	3	2.163	**0.003**	1.411	0.261
Salinity habitat×survey	6	3.262	**0.001**	0.928	0.507

Diet composition in larval rainbow smelt was dominated by *Eurytemora* spp. through the sampling area and period with an average of carbon weight contribution of 75.56 $\pm$ 4.77% (Fig. 7). Nonetheless, temporal and spatial variations in diet composition were present (Table 4, Fig. 6).

In Mid-June, in the oligohaline habitat larval diet differed significantly from diets in other salinity habitats, with *Eurytemora* spp. adults and nauplii contributing the most to these differences (SIMPER Fig. 6a). Larvae in the oligohaline habitat fed in a higher proportion on nauplii (23.17% *CW* Fig. 7) compared to larval diets in limnetic and mesohaline habitats that were dominated by adults (56.19% *CW* and 50.04% *CW*, respectively, Fig. 7). Other prey categories contributing to diets in low salinity habitats included *Gammarus* sp. and eggs of *Eurytemora* spp. in the limnetic habitat (7.32% and 6.15% *CW,* respectively, Fig. 7) and *Bosmina* sp. in the oligohaline habitat (3.38% *CW* Fig. 7).

In late June, larval diet in the mesohaline habitat differed significantly from the diet of low salinity habitats (Fig. 6a). A relatively high contribution of the mysid *Neomysis americana* was noted for mesohaline habitats (25.01% *CW* Fig. 7, SIMPER Fig. 6a) while *Eurytemora* spp. adults dominated in limnetic and oligohaline larval diet (85.11% and 76.06% *CW*, respectively, Fig. 7) explaining most of the dissimilarity (SIMPER Fig. 6a).

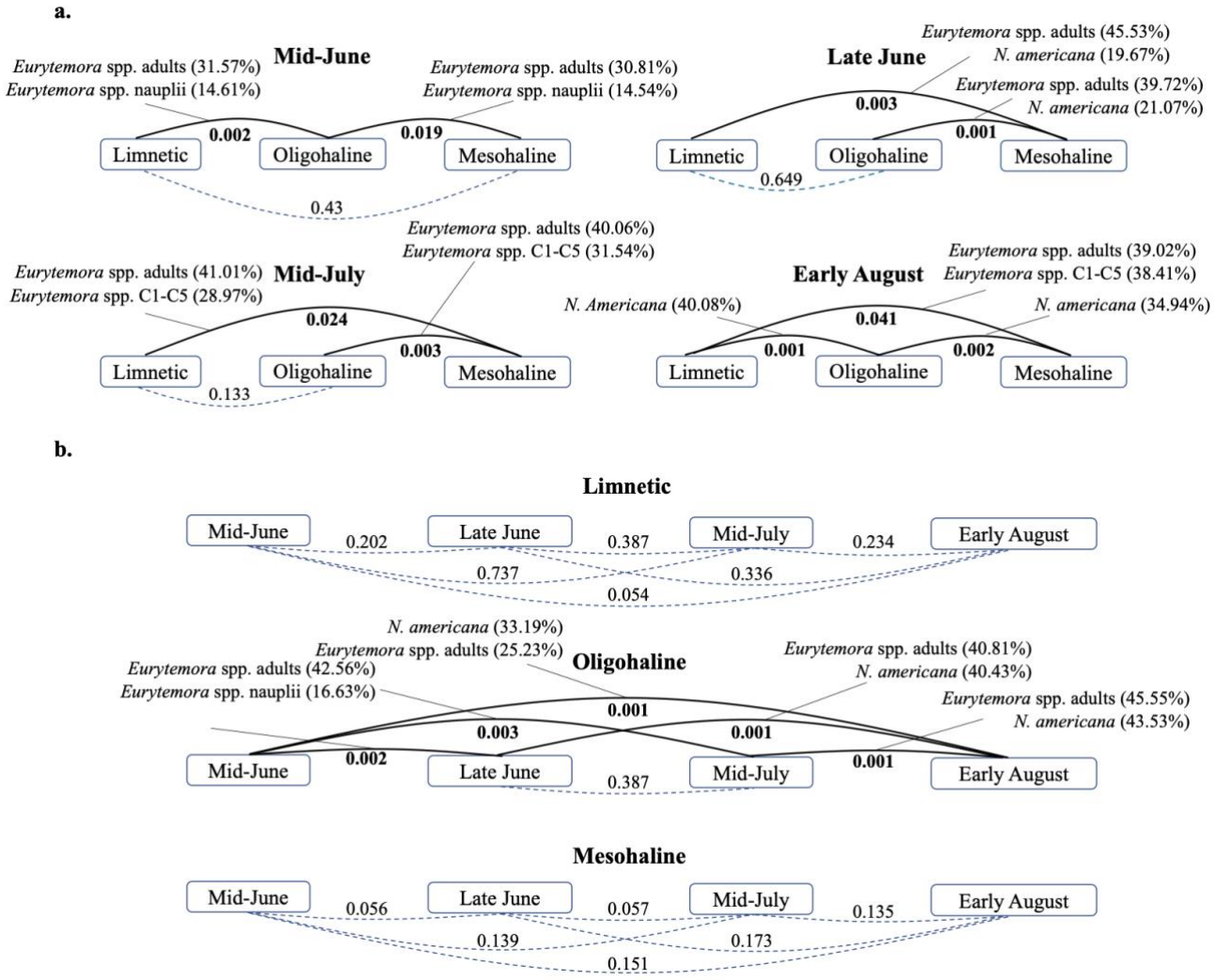

Figure 6. Spatial-temporal comparisons of larval diet through salinity zones and time, based on visual gut contents. Summary of pair-wise comparisons from the permutational analysis of variance (PERMANOVA). Spatial comparisons between salinity habitats in each survey (a) and temporal comparisons of each salinity habitat through the sampling period (b) are showed with significant interactions and p–values in bold. Prey with highest percent contribution to the dissimilarity (SIMPER) are showed for interactions with significant p-values.

In Mid-July, *Eurytemora* spp. adults were the prey that contributed the most to larval diet in all salinity habitats (77.93% *CW* limnetic, 82.99% *CW* oligohaline and 59.04% *CW* mesohaline, Fig. 7). Larvae of the mesohaline habitat, however, exhibited statistically different diet to those of lower salinity habitats (Fig. 6a) with a lower contribution of *Eurytemora* spp. adults and a higher contribution of copepodites C1-C5 (28.61% *CW* Fig. 7, SIMPER Fig. 6a). Other significant prey in this period were *Neomysis americana* in the oligohaline habitat (9.83% *CW* Fig. 7) and eggs of *Eurytemora* in the limnetic habitat (2.93% *CW* Fig. 7).

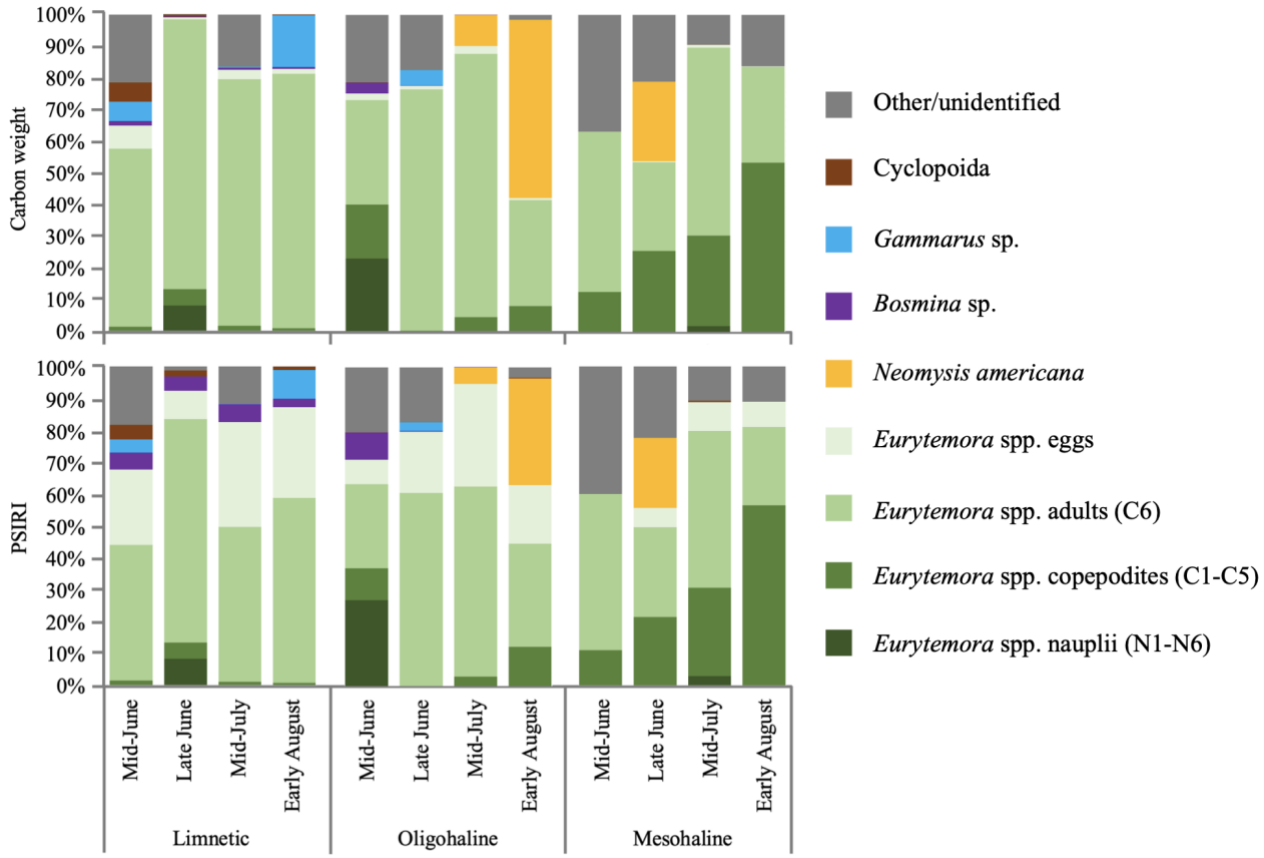

Figure 7. Carbon weight percent contribution (top) and prey specific index of relative importance (PSIRI, bottom) for the most frequently consumed prey taxa in rainbow smelt larval diet in each salinity zone sampled throughout summer 2021 in the MTZ of the St. Lawrence Estuary.

Finally in early August, all salinity zones exhibited differences in diet (Fig. 6a) with consumption of *N. americana* dominating in the oligohaline habitat (56.20% *CW,* Fig. 7) and accounting for most of the differences between this habitat and the limnetic and mesohaline habitat (SIMPER, Fig. 6a). Larvae of the limnetic and the mesohaline habitat differed in their consumption of *Eurytemora* spp. with a higher proportion of adults in the limnetic habitat (80.27% *CW* Fig. 7*,* SIMPER Fig. 6a) compared to a higher consumption of copepodites C1-C5 in the mesohaline habitat (53.64% *CW* Fig. 7, SIMPER Fig. 6a), however a low number of larvae was analyzed in the latter (n = 3). Other important taxa included *Gammarus* spp. (16.22% *CW* Fig. 7) for the limnetic habitat.

Regarding temporal differences in the diet within salinity zones, the oligohaline habitat exhibited the highest variation with early August differing significantly from all the other sampled periods (Fig. 6b). Most of the variation was explained by higher consumption of adult stages of *Eurytemora* spp. through the months of June and July compared to a higher consumption of the mysid *Neomysis americana* in early August (SIMPER Fig. 6b). Oligohaline habitat diet also differed between mid-June and late June (Fig. 6b) and mid-June and mid-July (Fig. 6b) mostly explained by higher consumption of *Eurytemora* spp. adults in late June and mid-July in contrast to nauplii consumption in mid-June (SIMPER Fig. 6b). No temporal differences in diet were found in the limnetic and the mesohaline habitat through the sampling periods.

The Prey-Specific Index of Relative Importance (PSIRI) also revealed that the cryptic complex of *Eurytemora affinis* was indeed the most important prey through the sampling period with an average of 82.44 $\pm$ 5.32% for the limnetic habitat, 77.21 $\pm$ 6.77% for the oligohaline habitat and 73.13 $\pm$ 9.14% for the mesohaline habitat (Fig. 7, Fig. S2). Similar general patterns observed with percent contribution of carbon weight were present with this index with a few exceptions. Numerous but low carbon contributing prey taxa like *Eurytemora* spp. eggs and *Bosmina* sp. exhibited higher PSIRI than carbon weight percentages. Conversely, less abundant but high carbon contributing prey taxa like *N. americana* and *Gammarus* sp. showed lower relative importance values. For example, in

early August, in terms of carbon weight contribution *N. americana* dominated the oligohaline habitat diet (56.20% CW, Fig. 7) followed by *Eurytemora* spp. (42.28% CW, Fig. 7) while in terms of relative importance it became the second most important prey item (33.26% PSIRI, Fig. 7) after *Eurytemora* spp. (63.16% PSIRI, Fig. 7).

Trophic niche width of the larvae showed relatively higher values of Levin's standardized index (Table 4) at the beginning of the season in mid-June ($B > 0.21$) with limnetic and mesohaline stations having the highest values ($B = 0.48$ and 0.45, respectively). This would represent a slightly more generalist feeding behaviour of smaller larvae (19.69 $\pm$ 0.36 mm SL limnetic and 16.72 $\pm$ 0.41 mm SL mesohaline) in these salinity habitats. Lower values were registered for early August ($B < 0.19$) with the lowest value reported in oligohaline stations ($B = 0.01$) revealing more specialist feeding habits of larger larvae (37.86 $\pm$ 1.48 mm SL).

1.6.3 Contribution of the cryptic complex to rainbow smelt diet

From the 197 DNA extracts of visually identified larval gut contents that were used in our newly developed SYBER green real-time PCR assay, a total of 164 samples (83.25%) reported a positive signal for *Eurytemora* spp.. More than half of the larvae (115 larvae, 58.38%) showed ingestion of only *E. affinis*, fourteen larvae (7.11%) fed exclusively on *E. carolleeae,* and thirty-four larvae (17.26%) consumed both *E. affinis* and *E. carolleeae* (Table 5). One larva from a mesohaline station close to La Pocatière showed positive signal for both *E. affinis* and *E. herdmani* in mid-July.

Table 5. Results of the newly developed SYBR green q-PCR assay to identify presence of *Eurytemora* spp. in rainbow smelt larval diet expressed as number of individual gut content samples for each salinity habitat sampled in the MTZ of the St. Lawrence Estuary during summer 2021.

	Limnetic				Oligohaline				Mesohaline			
Species	**Mid-June**	**Late June**	**Mid-July**	**Early Aug.**	**Mid-June**	**Late June**	**Mid-July**	**Early Aug.**	**Mid-June**	**Late June**	**Mid-July**	**Early Aug.**
E. affinis	1	8	12	12	13	10	8	15	16	8	9	3
E. carolleeae	7	0	1	0	4	1	0	1	0	0	0	0
E. affinis & *E. carolleeae*	8	2	6	8	3	3	2	2	0	0	0	0
E. affinis & *E. herdmani*	0	0	0	0	0	0	0	0	0	0	1	0
Negative signal	0	0	1	0	0	6	0	2	4	18	2	0
Total qPCR positive signals	16	10	19	20	20	14	10	18	16	8	10	3
Total of qPCR samples	16	10	20	20	20	20	10	20	20	26	12	3
Total identif. with visual analysis	13	10	17	20	11	14	10	20	12	14	10	3

Among all positive samples, 141 larvae corresponded to individuals where visual identification of *Eurytemora* spp. was reported. The remaining 23 samples corresponded to larval guts that were visually classified as empty or with no copepods identified but where *Eurytemora* spp. DNA was detected. From the remaining 33 gut content DNA extracts that showed no positive signal for *Eurytemora* spp., 14 samples corresponded to larval guts that were visually classified as empty or without copepods while 16 samples corresponded to larvae with 1-3 highly digested copepods visually identified through exoskeleton structures. Remaining samples corresponded to 3 larvae with 6-8 copepods in various stages of digestion. Most of negative signals (18) were reported for mesohaline stations in late June (Table 5), potentially due to conservation issues of the larvae.

When comparing qPCR results across the salinity gradient through the sampling period, significant differences were only observed between salinity zones (Table 4). Larval gut contents in the mesohaline habitats were dominated by *Eurytemora affinis* while lower salinity habitats exhibited the presence of larvae consuming both cryptic species in different proportions (mesohaline - limnetic Pair-wise test: $t = 3.78$, $p = 0.001$; mesohaline – oligohaline $t = 2.44$, $p = 0.02$). Larvae consumed the two species in the limnetic and oligohaline habitat ($p > 0.05$), however lower salinity stations (limnetic) exhibited higher consumption of *E. carolleeae* (Table 5).

In general, the distribution of the two cryptic species in the environment showed similar patterns across the salinity gradient. *E. affinis* were predominant in mesohaline habitats and both cryptic species were present in lower salinity limnetic and oligohaline habitats (Fig. 8). This pattern repeated itself through the sampling period except for late June and mid-July, where the oligohaline habitat was exclusively dominated by *E. affinis*. However, during these two periods qPCR results of stomach content confirmed the presence of *E. carolleeae* DNA in larvae captured in these stations.

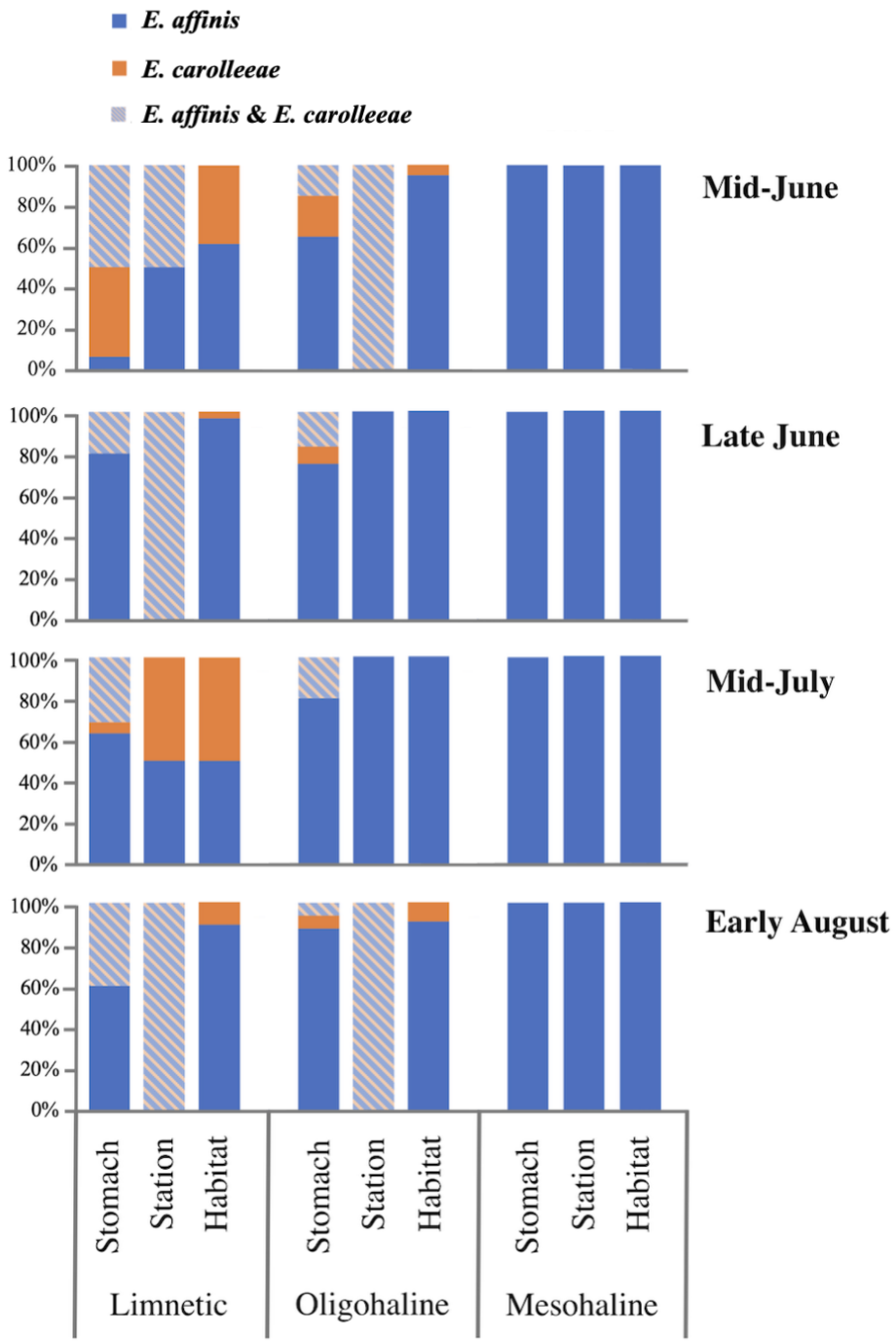

Figure 8. Proportion (%) of positive results for *E. affinis*, *E. carolleeae* or both in stomach content qPCR analysis (left bars) versus presence of *E. affinis*, *E. carolleeae* or both in sampled stations (center bars) and general proportion of *E. affinis* and *E. carolleeae* in the habitat (right bars) as obtained through individual genotyping in the environment for each salinity zone sampled throughout the summer of 2021 in the MTZ of the St. Lawrence Estuary.

1.6.4 Preference and selectivity between different stages of *E. affinis*

In general, rainbow smelt larvae exhibited strong positive selection for *E. affinis* adults, however variability between sexes and across salinity zones was present (Fig. 9). In Mid-June, larvae of the mesohaline habitat showed strong positive selection for both males and females of *E. affinis* while strong negative selection was noted for C4–C5 copepodites. Larvae of the oligohaline habitat on the other hand showed strong, although variable positive selection for N4–N6 nauplii and strong negative selection for smaller N1–N3 nauplii. In late June, larvae selected strongly for male *E. affinis*, showing highest selectivity indices of Chesson's alpha for all salinity zones, secondly positive selection was found for female *E. affinis* in the oligohaline habitat, for *Bosmina* sp. in the two low salinity habitats and for *N. americana* in the mesohaline habitat. Strong negative selection values were reported for N1-N3 nauplii and C1–C5 copepodites in the limnetic habitat. In mid-July, *E. affinis* females and males were strongly selected by larvae across all salinity zones, while nauplii, copepodites, *N. americana, Bosmina* sp. and *Gammarus* sp. were in general strongly negatively selected. In early August, females were strongly selected in the mesohaline habitat and to a lesser extent in the oligohaline habitat. In the latter habitat, rainbow smelt larvae strongly selected *N. americana* and moderately selected *Bosmina* sp.. Smaller C1–C3 copepodites showed strong negative selection in oligohaline and mesohaline habitats while C4–C5 copepodites were negatively selected in the limnetic habitat. Prey taxa that did not contribute at least 2% to the larval diet by prey number but represented >2% of the potential prey field can also be considered strongly selected against by larval rainbow smelt. Those potential prey taxa avoided by larval rainbow smelt were bivalves (all sampling period) and harpacticoid and cyclopoid copepods (mid-July) in the limnetic habitat, and bivalves (all sampling period), harpacticoid copepods (late June, mid-July and early August), and Tintinnina (late June) for the oligohaline habitat. For mesohaline stations avoided prey included harpacticoid copepods (all sampling period), Tintinnina, bivalves (late June and mid-July), *Acartia* spp. (late June), and Cirripedia nauplii (late June). Rotifera were also abundant in the environment through

all the sampling period, however they were not considered in this list as they are often missed in stomach content visual analysis due to rapid digestion.

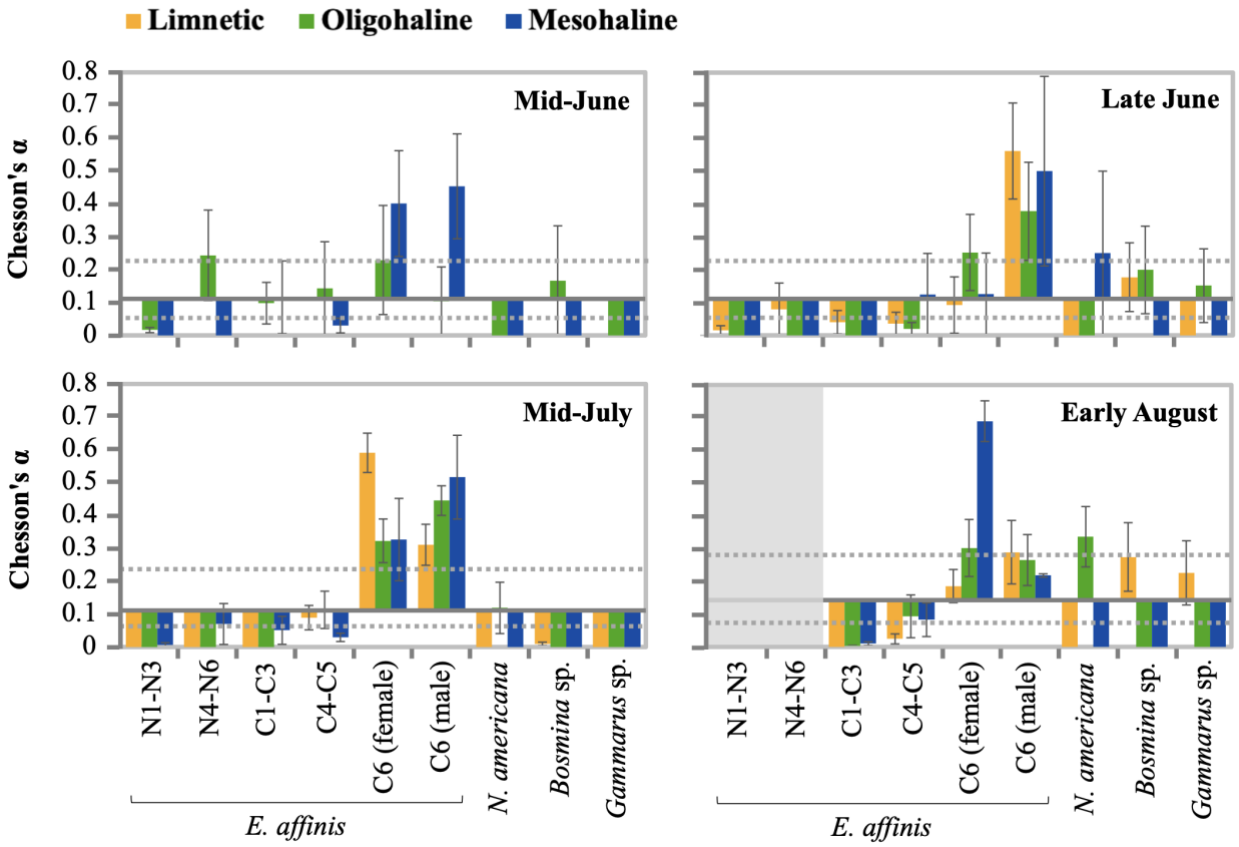

Figure 9. Chesson's alpha-selectivity indices (±SE) for the four most frequently consumed prey taxa in the diet of rainbow smelt larvae that contained only *E. affinis* DNA across the three salinity zones. *E. affinis* nauplius stage were not available for early August (grey area). Neutral selectivity corresponded to 0.11 for mid-June, late June and mid-July, and 0.14 for early August. Dotted lines on the *y*-axis at 0.22 and 0.06 for mid-June, late June and mid-July, and 0.28 and 0.07 for early August indicate strong positive and strong negative selection thresholds, respectively. In mid-June, only one larva had exclusively *E. affinis* DNA in limnetic stations, therefore Chesson's alpha for this category was not calculated.

1.7 DISCUSSION

Feeding ecology of rainbow smelt in the maximum turbidity zone

The most important prey of the early life stages the anadromous rainbow smelt of is the estuarine cryptic species *Eurytemora affinis* throughout the three different salinity habitats of the maximum turbidity zone (MTZ), confirming previous studies. The predominance of *E. affinis* in larval diet has been reported for different fish species in the area, such as rainbow smelt, Atlantic tomcod and striped bass (Dauvin & Dodson, 1990; Sirois & Dodson, 2000a; Winkler *et al.,* 2003; Yoneyama, 2004; Vanderweireldt *et al.,* 2019). Sirois and Dodson (2000a) examined 905 larval rainbow smelt gut content, finding, as with the present study, high feeding incidence, but a low diversity diet based primarily on *E. affinis* and the diplostracan *B. longirostris*. Throughout the MTZ the cryptic species complex of *E. affinis* is the predominant mesozooplankton species (Bousfield *et al.,* 1975; Runge & Simard, 1990; Winkler *et al.,* 2003), representing the most available prey for fish larvae.

Although consumption of the *Eurytemora affinis* complex was prevalent from June to August in diet composition, we found significative spatiotemporal variation. Different proportions in the consumption of the various stages of development of the *Eurytemora* complex accounted for most of the variation among habitats. Consumption of advanced copepodite stages and adults was constant through all salinity habitats and throughout the sampling period while nauplii consumption was variable and mainly present in the months of June and July when larvae were smaller compared to August (Fig. S1). Selectivity results suggest that smelt larvae are not only exploiting the most abundant resources, but also positively selecting high carbon contributing taxa. Many fish species exhibit strong positive prey preference during larval stages which would provide faster growth and increased survival (Robert *et al.,* 2014). In the case of rainbow smelt, adult female and male stages of the cryptic *E. affinis* complex are the preferred prey in the area for all salinity habitats. This has been previously reported for rainbow smelt and other species inhabiting the MTZ such

as the Atlantic tomcod (Yoneyama, 2004), and in other temperate estuaries like Chesapeake Bay for species such as white perch and striped bass (Campfield & Houde, 2011), confirming that advanced stages of the *E. affinis* complex play an important role in larval trophic ecology.

Bigger species such as the mysid *Neomysis americana* appears in the diet in late June and becomes the second most preferred prey by early August in the oligohaline habitat. Although in a smaller proportion, the amphipod *Gammarus* spp. gains importance in the diet in August and are positively selected together with diplostracan *Bosmina longirostris* within the limnetic habitat. Other studies have concordantly found that mysids and gammarids compose the diet of advanced larval stages, juveniles and adult rainbow smelt (Sirois & Dodson, 2000a, Lecomte & Dodson, 2004). Theses ontogenetic shifts in diet are common, bigger larvae consuming larger prey that would satisfy their physiological demands (Sogard, 1997; Scharf *et al.,* 2000). *N. americana* is an abundant resource in the area (Winkler *et al.,* 2003, 2007; Lecomte & Dodson, 2004) of good quality, as it contains a high proportion of essential fatty acids (J. J. García-Gonzalez, unpublished) that could fulfill nutritional requirements of larvae during advanced early life stages. Gammarids are commonly used in aquaculture as they are rich in protein and other essential biomolecules (Harlıoğlu & Farhadi, 2018) so they could also represent an important prey for larger smelt larvae and juveniles in freshwater habitats upstream. Differences in trophic niche width suggest that bigger larvae possess slightly more specialist feeding habits, which could correspond to active selection towards high carbon contribution prey or stages like adult *Eurytemora* spp., *N. americana* or *Gammarus* spp. in most cases, or towards small but numerous preys like *B. longirostris* when available in the environment. These shifts in types and sizes of selected prey are common as successful larval foraging improves with ontogeny, which is mostly related to mouth gape increase and enhanced swimming and prey detection capabilities (Shirota, 1970; Pepin & Penney, 1997).

Contribution of the cryptic *Eurytemora* complex to rainbow smelt diet

For the first time we were able to show through the molecular approach that one cryptic species of the *Eurytemora affinis* complex, the *E. affinis* North Atlantic clade was the predominant prey with 75.64% of occurrence, whereas the occurrence of *E. carolleeae* was low. Rainbow smelt larvae fed on *E. carolleeae* only in the low salinity limnetic and oligohaline habitats and on the *E. affinis* North-Atlantic clade in all zones. This only partially confirmed our hypothesis, as we did not expect to find *E. affinis* in larval diet in the limnetic habitat.

Distribution patterns of the two cryptic species throughout the salinity habitats most likely explain the contribution of these in the diets. *E. carolleeae* was only present in low salinity habitats: in limnetic waters through all the season and in oligohaline waters only in mid-June and early August, whereas *E. affinis* occurred and dominated in all zones including the limnetic habitat. This has rarely been observed and is contradictory to generally found distribution patterns in the MTZ (Winkler *et al.,* 2008; Favier & Winkler, 2014; Winkler *et al*., 2016). The North Atlantic clade of *E. affinis* is mostly present in the oligo- and mesohaline habitats of the MTZ and it does not colonise freshwater habitats upstream of the MTZ (Winkler *et al.,* 2008). *E. carolleeae* has been reported in dominant proportion in limnetic habitats from the Great Lakes to the upstream portion of the MTZ, and in lower abundances in the oligohaline and mesohaline stretches of the estuary (Lee, 1999, 2000; Winkler *et al.,* 2016). Some studies have suggested that each cryptic species might be well adapted to the environmental conditions of their specific preferred habitat but that *E. carolleeae* is capable of rapidly acclimating to higher salinity conditions and possesses the physiological ability of exploiting the oligo- and mesohaline habitats (Cabrol *et al.,* 2013, 2015), where *E. affinis* is mostly predominant. However, it has been shown that these distribution patterns are more dynamic in the MTZ and can vary through the season and among years (Winkler *et al.,* 2016). The presence of *E. affinis* in estuarine limnetic waters was observed early in the growing season in early June (Favier & Winkler, 2014). Furthermore, in laboratory experiments a certain tolerance to freshwater was observed under

certain temperature (<17 °C) and food conditions (high chl. *a* concentrations) (Lee *et al.,* 2011, 2013). This suggested that *E. affinis* could effectively tolerate low salinity habitats but that abiotic parameters such as water temperature during summer might reduce this capacity. During our sampling period in summer 2021, presence of the two cryptic species was reported in low salinity habitats but was dominated by *E. affinis* along the salinity gradient with no discernible temporal pattern. Thus, environmental conditions may then play an important role in modulating the presence of the two cryptic species within the MTZ (Winkler *et al.,* 2016). The specific reasons as to why *E. affinis* continued to dominate all throughout the season or why *E. carolleeae* did not thrive if it is in fact physiologically equipped to do so, as shown in experimental studies (Lee & Petersen, 2002; Lee *et al.,* 2011, 2013; Devreker *et al.,* 2012; Cabrol *et al.,* 2015, 2020) are still unclear. Competition or prey availability could possibly play a role (Cabrol *et al.,* 2015; Winkler *et al.,* 2016), but further studies are necessary to fully understand the variability in these distribution patterns.

From the perspective of the predator and taking into consideration the partial segregation between *E. affinis* and *E. carolleeae* within the MTZ, larval distribution of rainbow smelt could also condition which cryptic species contributes more to the larval diet. Spatial distribution of early stages of rainbow smelt in the Middle Estuary of the St. Lawrence has been studied and associated to several factors including suspended particle matter distribution and the trophic dynamics of this area (Ouellet & Dodson, 1985; Dodson *et al.,* 1989). In the present study, distribution was heterogeneous with larvae present all along the salinity gradient at the beginning of the season in mid-June, however as the season progressed larvae concentrated upstream in the limnetic and oligohaline habitats in the months of July and August. Laprise and Dodson (1989) reported that the longitudinal distribution of rainbow smelt larvae in the MTZ is influenced by both tidal circulation within the estuary and active vertical migration. Larvae would use selective tidal stream transport to migrate upstream, placing themselves near the surface during flood tides and descending to the bottom layers during ebb tides. This would be an adaptation to maximize the longitudinal retention of high densities of larvae in a zone of high prey biomass (Dauvin & Dodson, 1990), specifically the oligohaline habitat, as found in our study. In this sense, trophic patterns could change over

time and an ontogenetic shift in the consumption of the cryptic species could occur in migrating larvae. As our molecular results suggested, smelt in mesohaline habitats would feed exclusively on *E. affinis* while larvae that would actively migrate upstream to freshwater limnetic habitats would exploit *E. carolleeae* in a higher proportion.

Overall, it seems that the reported trophic patterns are probably a result of a complex interaction between intrinsic (i.e., physiology) and extrinsic (i.e., salinity, temperature, prey availability) factors influencing *Eurytemora* distribution, as well as factors influencing rainbow smelt larval distribution within the MTZ. Although selectivity analysis between the two cryptic species was not possible, studying preference between *Eurytemora* species in the field and/or through experimental approaches would give an idea if early life stages were truly exploiting the most abundant resource or if they could be actively selecting to a certain extent one of the species when present in the environment.

Ecological implications of the cryptic *Eurytemora* complex

Predominance of the Atlantic rainbow smelt lineage over the Acadian lineage (80.03% mtDNA lineage B versus 19.07% mtDNA lineage A) was found, with no evidence of diet differences between the two groups. Nonetheless, the two studied lineages clearly use the maximum turbidity zone as a nursery area (Lecomte & Dodson, 2004; Trencia *et al.,* 2005; Legault & Lecomte, 2012). These two ancestral lineages appear to have come into secondary contact in the St. Lawrence after the last glaciation over 8000 years ago (Dodson *et al.*, 2015). Since then, they have produced two sympatric populations (north shore/south shore) that have stayed in the Middle Estuary and that profit of its resources and habitats despite exhibiting different patterns of life history, morphology and ecology (Lecomte & Dodson, 2004, 2005). In this sense, the cryptic species complex *Eurytemora affinis* must be an advantageous prey that fulfills the nutritional needs of estuarine populations of smelt, supporting early life stages through the critical period. Given the importance of feeding in early life stages (Hjort 1914, 1926; Cushing, 1990; Houde, 2008), this species complex is

crucial to larval survival and recruitment in the Middle Estuary. Understanding the role of *Eurytemora* during the critical period is then vital for fisheries monitoring, conservation efforts and predicting the impacts of environmental changes on populations.

According to our results, the North Atlantic (NA) clade of *E. affinis* seems to be predominantly the species that contributes to the energy transfer. However, these trophic patterns seem to be variable, and each species might contribute to diet at different times through larval development, during the seasons and among years. An interannual and possibly seasonal fluctuation in the distribution of the two cryptic species of the *E. affinis* complex (Winkler *et al*., 2016) could then potentially play a role in supporting smelt populations through time. *E. affinis* NA clade would certainly play a critical role in the maintenance of estuarine populations of smelt as found with our molecular results, but *E. carolleeae* may also be important for the interannual regulation of the stock as it is present just outside of the traditional distribution center of smelt larvae. Indeed, trophic interactions between the cryptic complex and higher trophic levels are much more complex than previously thought and a thorough analysis of trophic dynamics through time is required to correctly assess energy transfer in the system.

Moreover, differential feeding between the cryptic species could be advantageous to predators like smelt giving them enhanced chances of surviving during the critical period. Although both cryptic species are omnivorous and prefer phytoplankton, their nutritional characteristics such as fatty acid composition differ. Cabrol *et al.* (2015) found evidence that fatty acid composition differed significantly between the two cryptic species with *E. carolleeae* accumulating higher proportions of FA, marking for terrestrial organic matter and bacteria whereas *E. affinis* (North Atlantic) stored more FA derived from diatoms, dinoflagellates and ciliates. Essential fatty acid content is higher in *E. affinis* compared to *E. carolleae*, indicating potentially better quality (Cabrol *et al.,* 2015, 2020). In addition, preliminary results suggest that both cryptic species exhibit different biovolume, with *E. carolleeae* having a slightly smaller total body length but producing more eggs than *E. affinis*, and *E. affinis* having a smaller clutch size containing larger eggs (G. Winkler, unpublished).

These differences could represent advantages to predators in terms of mass per prey and prey quality and thus influence growth, mortality and overall recruitment of stocks. Based on our results, we could hypothesize that feeding on the North Atlantic clade of *E. affinis* would be advantageous. Nonetheless, analyzing preference between the two cryptic species by the rainbow smelt larvae would allow to better understand the relationship between prey availability and larval feeding success, growth and survival (Robert *et al.,* 2014).

In the context of climate change where ecosystems in general are facing pressures that may influence their vulnerability and ultimately affect their ecological resilience (Malhi *et al.,* 2020) changes in prey availability and composition might have consequences on larval fish recruitment (Pörtner & Peck, 2010; Kristiansen *et al.,* 2014; Asch *et al.*, 2018). The St. Lawrence system is subject to an increasing number of drivers of environmental change (Beauchene *et al*., 2020) and thus environmental conditions that control primary productivity and consequently secondary productivity are rapidly changing. Rising water temperatures and intrusion of marine water and associated organisms threaten estuarine habitats like the MTZ (Kennedy, 1990; Gillanders *et al.,* 2022). This could have differential consequences on dominant zooplankton species like *E. affinis* and *E. carolleeae* that differ in their ecophysiology (Cabrol *et al.,* 2020). Effects of climate change (i.e., ocean warming) on these species could include altered phenology, changes in community structure, increase or decline of abundances and biomass (i.e. depending on temperature limits), as well as distribution changes. Spatial, temporal, bioenergetic and/or evolutionary mismatches between zooplankton and fish larvae could result in altered larval fish growth and survival (Dam & Baumann, 2018). Bottom-up processes involving the cryptic species complex may then be essential for the survival of many ecologically and economically important species like rainbow smelt. Furthermore, abundance of rainbow smelt stocks has been linked to air temperature and water level, with the influence of these hydroclimatic variables differing significantly between sympatric populations in the MTZ (Mingelbier *et al.,* 2001). Consequently, changes in environmental conditions coupled with other stressors like spawning habitat loss, agricultural pollution and geomorphological changes in coastal areas

could also be detrimental for smelt populations, ultimately affecting larval survival and recruitment.

Unraveling the intricacies of the trophic interactions involving *E. affinis* and *E. carolleeae* is a key step towards assessing the ecological significance of the MTZ as a nursery area under climate change. A deeper understanding of the trophic relationships and energy transfer within the cryptic complex is pivotal in evaluating the carrying capacity of this ecosystem. Given that food limitation is of major influence in determining the carrying capacity of nursery habitats (Le Pape & Bonhommeau, 2013), analyzing trophic patterns with further dominant species in the ecosystem like Atlantic tomcod and mysid shrimps could help elucidate the role of this cryptic species complex. Future studies examining the spatiotemporal interannual tendencies in energy transfer and its consequences on species growth and survival would allow to better comprehend the dynamics within the ecosystem. This would shed light on the factors influencing the recruitment success and survival of rainbow smelt larvae, ultimately contributing to the sustainable management of this ecologically important species.

CONCLUSION GÉNÉRALE

Le complexe cryptique d'*E. affinis* et d'*E. carolleeae* est l'espèce zooplanctonique dominante dans la zone de turbidité maximale (MTZ) de l'estuaire du Saint-Laurent (Winkler *et al*., 2016). Ce complexe d'espèces soutient activement les populations des espèces dominantes comme l'éperlan arc-en-ciel, une espèce écologiquement et économiquement importante dans la MTZ, qui dépendent de cette proie au cours des premiers stades de leur développement (Sirois & Dodson, 2000a, Lecomte & Dodson, 2004). Compte tenu de cette importance, à travers la présente recherche nous avons cherché non seulement à élucider le rôle du complexe dans le régime alimentaire des premiers stades de vie de l'éperlan arc-en-ciel, mais aussi à analyser la contribution de chacune des espèces durant leur ontogenèse et au sein de la mosaïque hétérogène des habitats présents le long du gradient de salinité de cette importante zone d'alevinage.

Dans le but de réaliser cette recherche, notre premier objectif d'étude consistait à analyser l'écologie alimentaire des premiers stades de vie de l'éperlan arc-en-ciel dans la MTZ. Grâce à l'analyse du contenu stomacal, nous avons pu confirmer que le complexe cryptique d'*E. affinis* était en effet la proie prédominante dans le régime alimentaire de larves d'éperlan arc-en-ciel estuarien à travers la mosaïque d'habitats de salinité de la zone de turbidité maximale. La prédominance de la lignée atlantique de l'éperlan a été constatée, mais aucune différence significative dans le régime alimentaire n'a été constatée entre les lignées d'éperlans. Cependant, il est clair que les deux lignées utilisent la MTZ comme zone d'alevinage (Ouellet & Dodson, 1985 ; Dodson *et al.,* 1989 ; Laprise & Dodson, 1989 ; Pigeon *et al.,* 1998 ; Sirois & Dodson, 2000b ; Lecomte & Dodson, 2004), depuis probablement la dernière glaciation. Ainsi le complexe d'espèces cryptiques semble être une proie avantageuse qui occupe cette zone.

Également, en plus de confirmer le rôle important que joue le complexe cryptique dans le régime alimentaire de l'éperlan arc-en-ciel estuarien, les résultats de cette étude ont permis de mettre en évidence le fait que les larves d'éperlan sélectionnent positivement des proies riches en carbone et aussi un changement vers des proies plus grosses au cours du développement ontogénétique. Ce changement de préférence alimentaire s'est traduit par une consommation accrue de *N. americana* pour l'habitat oligo- et mésohalin, et d'amphipodes gammaridés pour l'habitat limnétique. Ces changements développementaux du régime alimentaire sont courants, les larves d'une taille croissante consommant des proies de plus en plus grosses, pour satisfaire leurs exigences physiologiques accrues (Sogard, 1997 ; Scharf *et al*., 2000).

Conformément à notre deuxième objectif de recherche qui consistait à estimer la contribution de chaque espèce cryptique du complexe d'*E. affinis/E. carolleeae*, il a été possible de constater, grâce à notre essai de PCR en temps réel nouvellement développé, qu'au sein du complexe, c'est en fait le clade Nord-Atlantique (NA) d'*E. affinis,* l'espèce qui contribuait principalement au transfert d'énergie au cours de l'été 2021. Ce résultat n'a que partiellement confirmé notre hypothèse car nous ne nous attendions pas à trouver *E. affinis* dans l'habitat limnétique. Grâce à ce travail, nous avons pu déterminer que cette situation s'expliquait très probablement par la distribution de l'espèce dans l'environnement, avec une dominance d'*E. affinis* dans toutes les zones de salinité alors qu'*E. carolleeae* n'était présent que dans l'habitat limnétique et en juin et août dans l'habitat oligohalin. Ce constat était certainement inattendu et contradictoire avec les modèles de distribution généralement documentés dans la MTZ (Winkler *et al.,* 2008 ; Favier & Winkler, 2014 ; Winkler *et al.,* 2016). Bien que les conditions environnementales joueraient certainement un rôle dans la modulation de la distribution spatio-temporelle du complexe d'espèces (Favier & Winkler, 2014), les facteurs précis expliquant la dominance continue d'*E. affinis* tout au long de la saison, ou le manque apparent de succès d'*E. carolleeae* malgré ses capacités physiologiques démontrées (Lee & Petersen, 2002 ; Lee *et al.,* 2011, 2013 ; Devreker *et al.,* 2012 ; Cabrol *et al.,* 2015, 2020), restent incertains. Bien que la compétition ou la disponibilité des proies puissent potentiellement exercer une influence (Cabrol *et al.,* 2015 ; Winkler *et al.,* 2016),

une compréhension globale des variations de ces schémas de distribution nécessite des travaux de recherche ultérieurs.

Par ailleurs, les résultats de cette étude ont permis d'émettre l'hypothèse que le clade NA d'*E. affinis* pourrait représenter des avantages alimentaires pour les prédateurs en termes de masse par proie et de qualité nutritionnelle. La teneur en acides gras essentiels est plus élevée chez *E. affinis* que chez *E. carolleae*, indiquant une qualité nutritionnelle potentiellement meilleure (Cabrol *et al.,* 2015, 2020). De plus, les résultats préliminaires suggèrent que les deux espèces cryptiques présentent un biovolume différent, *E. carolleeae* ayant une longueur corporelle totale légèrement inférieure mais produisant plus d'œufs qu'*E. affinis*, et *E. affinis* ayant une couvée plus petite contenant des œufs plus gros (G. Winkler, inédit). L'alimentation différentielle entre les espèces cryptiques pourrait donc être avantageuse pour les prédateurs comme l'éperlan arc-en-ciel, en leur donnant de meilleures chances de survie pendant la période critique et ainsi influençant la croissance, la mortalité et le recrutement global des stocks.

La présente recherche est particulièrement importante pour l'habitat de pouponnière de la MTZ car elle nous a facilité d'établir une base sur les schémas de transfert d'énergie dans la zone. Les résultats recueillis grâce à notre analyse nous ont permis de conclure que les schémas trophiques rapportés sont probablement le résultat d'une interaction complexe entre des facteurs intrinsèques (c'est-à-dire la physiologie) et extrinsèques (par ex. la salinité, la température, la disponibilité des proies) influençant la distribution d'*Eurytemora*, ainsi que des facteurs influençant répartition des larves d'éperlan arc-en-ciel dans la MTZ et sa sélectivité des proies. Chaque espèce dans ce complexe pourrait contribuer à différents moments du développement des larves de poissons, à différents moments de la saison et aussi au fil des années. Cependant le clade NA d'*E. affinis* serait crucial pour soutenir les populations estuariennes d'éperlan arc-en-ciel au cours des premiers stades de leur vie, en particulier pendant la période critique. Parallèlement, *E. carolleeae* pourrait jouer un rôle pour la régulation interannuelle des populations d'éperlans arc-en-ciel de l'estuaire car elle est habituellement distribuée en marge du centre de distribution traditionnel de l'espèce. Ces

résultats sont essentiels pour mieux comprendre l'écologie trophique des populations locales et la survie des premiers stades de vie des poissons.

Comme pour toutes les études scientifiques, il est important de reconnaître les limitations associées à la collecte d'échantillons et aux approches méthodologiques. En termes d'échantillonnage larvaire, nous n'avons pas pu échantillonner la transition de l'alimentation endogène vers l'alimentation exogène, les larves de poissons ayant déjà dépassé ce stade étant donné le début précoce de la saison en 2021. Des températures plus chaudes que la moyenne, couplées à un faible débit d'eau au printemps (Pêches et Océans Canada, 2023), auraient certainement influencé la saison de frai. Un échantillonnage additionnel d'éperlan arc-en-ciel plus tôt dans son développement permettrait de capturer une image complète de l'ontogenèse larvaire et de confirmer les interactions trophiques durant cette phase critique.

En termes de méthodes, les techniques moléculaires sont en effet importantes pour l'étude des espèces cryptiques (Hebert *et al.,* 2004 ; Bickford *et al.,* 2007 ; Behereharay & Caccone, 2007). La PCR en temps réel est une nouvelle technique qui permet d'identifier rapidement l'ADN cible dans l'analyse du contenu stomacal. Cependant, il ne quantifie pas l'ADN dans des échantillons individuels, de sorte qu'une analyse de sélectivité entre les espèces du complexe était logistiquement impossible à réaliser. L'analyse de la préférence entre les deux espèces cryptiques par les larves d'éperlan arc-en-ciel permettrait de mieux comprendre la relation entre la disponibilité des proies et le succès alimentaire, la croissance et la survie des larves (Robert *et al.,* 2014). En ce sens, la prochaine étape serait d'identifier ou de développer une technique qui permettrait d'effectuer une analyse de sélectivité afin de déterminer si les premiers stades de vie des prédateurs exploitent la ressource la plus disponible dans l'environnement, ou encore s'ils possèdent la capacité de sélectionner l'une des espèces cryptiques. Cela pourrait être accompli à l'aide d'expériences de sélectivité *in vitro* ou *in situ*, ou par des futures techniques plus raffinées pour quantifier l'ADN du contenu de stomacal. Par la suite, il s'agirait d'établir si l'ingestion de l'une des deux espèces

cryptiques influence la croissance, et éventuellement, le recrutement de l'éperlan arc-en-ciel et d'autres espèces importantes dans la région.

De plus, nos résultats mettent en évidence le rôle potentiel des espèces cryptiques dans la modulation des populations de prédateurs, influençant ainsi des aspects tels que leur croissance, leur mortalité et, en fin de compte, la dynamique de leur recrutement. Les échantillons utilisés dans cette étude seront utiles pour poursuivre et approfondir nos connaissances sur la contribution du complexe cryptique d'*Eurytemora* dans le réseau trophique de la MTZ. Plus précisément, des analyses des isotopes stables permettront de mieux comprendre la position trophique du complexe et ses interactions avec les autres composants du réseau. De plus, la lecture des otolithes des larves permettra d'effectuer des analyses de croissance et de calculer les taux de mortalité, ce qui pourrait nous donner une idée de la manière dont la consommation de ce complexe d'espèces cryptiques pourrait conditionner le cycle de vie de l'éperlan dans l'estuaire. Cette démarche serait cruciale pour comprendre la dynamique des populations et la façon dont cette espèce fourrage importante pourrait réagir aux changements trophiques et environnementaux comme par exemple, ceux liés au changement climatique. La compréhension de ces aspects fournirait des informations précieuses sur la dynamique écologique au sein de la MTZ et sur l'équilibre délicat qui soutient les premiers stades de vie de l'éperlan.

Dans l'ensemble, en abordant nos objectifs de recherche, nous avons non seulement fait progresser la compréhension des interactions trophiques au sein de cet habitat crucial pour le développement des jeunes stades de poissons, mais également révélé des schémas de distribution inattendus des espèces cryptiques. La dominance d'*E. affinis* tout au long de la saison et les difficultés apparentes rencontrées par *E. carolleeae*, malgré ses capacités physiologiques démontrées, restent des sujets intrigants donnant lieu à une exploration plus approfondie. Bien que des facteurs tels que la compétition et la disponibilité des proies puissent jouer un rôle, une compréhension complète de ces dynamiques spatiales nécessite des études ultérieures.

Les implications de cette recherche vont au-delà de la compréhension écologique, touchant l'équilibre complexe de l'écosystème aquatique et la distribution des espèces. Aussi, elle offre un cadre fondamental pour de futures investigations, où le raffinement des techniques et l'approfondissement des connaissances sur l'alimentation sélective pourraient améliorer notre compréhension de ces relations trophiques complexes. Alors que nous naviguons dans un monde en constant changement, les résultats de cette recherche contribuent non seulement à la préservation des espèces locales, mais également à notre compréhension globale de la façon dont des relations écologiques délicates façonnent la survie et la durabilité de la vie aquatique.

ANNEXES

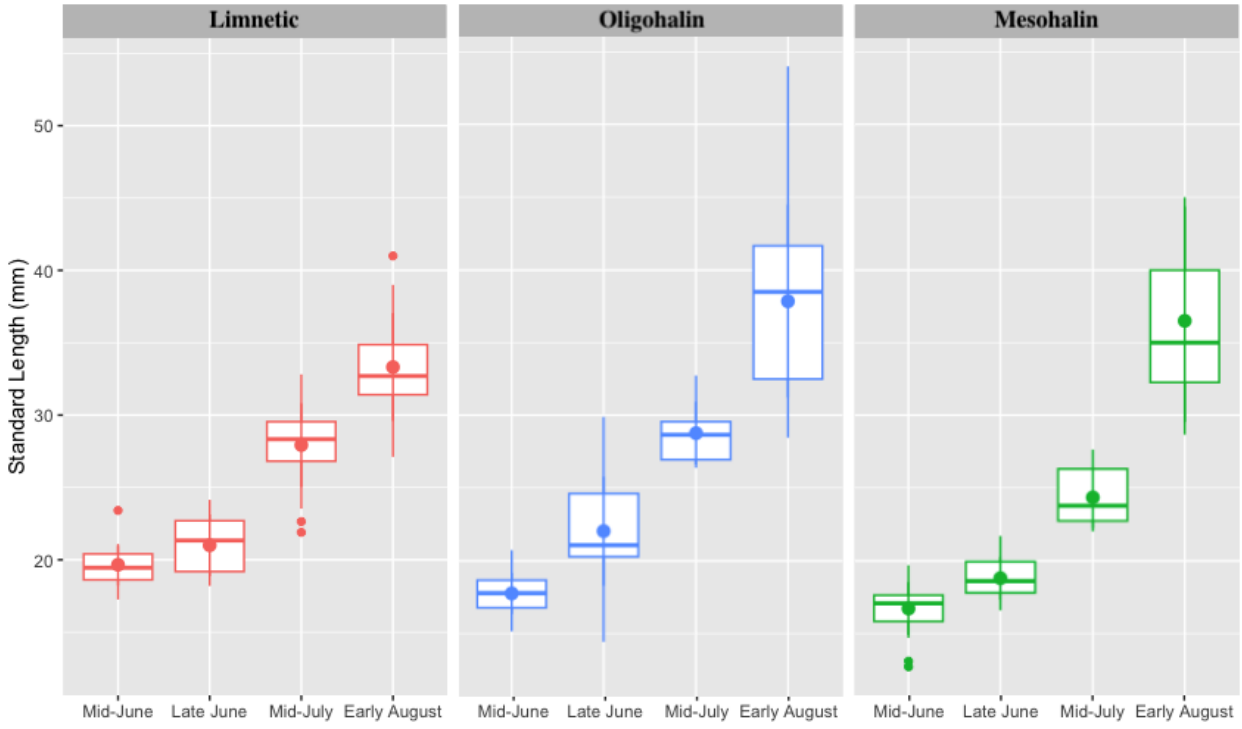

Figure S1. Mean standard length (mm) of rainbow smelt larvae in each salinity habitat in summer 2021. Mean values and standard deviation are showed for each.

Table S1. Diet composition expressed as percentage of prey-specific index of relative importance (%PSIRI) for the prey categories identified in rainbow smelt larval diet in each salinity zone sampled throughout summer 2021 in the MTZ of the St. Lawrence Estuary. Feeding statistics and physical-chemical variables of each salinity zone are included as references.

					Limnetic				Oligohaline				Mesohaline			
Class	**Order**	**Family**	**Item**	**Stage**	**Mid-June**	**Late June**	**Mid-July**	**Early Aug.**	**Mid-June**	**Late June**	**Mid-July**	**Ear. Aug.**	**Mid-June**	**Late June**	**Mid-July**	**Early Aug.**
Branchiopoda	Diplostraca	Bosminidae	*Bosmina sp.*		5.32	4.39	5.52	2.68	8.51	0.29	0.06					
			Unidentified Cladocera						0.16							
Malacostraca	Amphipoda	Gammaridae	*Gammarus tigrinus.*					2.41								
			Gammarus sp.		4.12		0.16	6.61		2.68						
	Decapoda	Crangonidae	*Crangon septemspinosa*	Zoea										2.32		
	Mysida	Mysidae	*Neomysis americana*								5.15	33.25		21.50		
			Unidentified Mysida							6.25		1.13		3.30		
Maxillopoda (Copepoda)	Calanoida	Temoridae	*Eurytemora sp.*	N1		0.30			0.23							
				N2		1.28			0.92							
				N3		0.74			5.97						0.35	
				N4		1.60			6.92						0.36	
				N5		2.52			6.34						2.03	
				N6		1.92			6.80						0.43	
				C1		1.09			2.49				3.27		2.84	
				C2		1.97			0.58			0.13			3.95	1.82
				C3								0.18	4.38		0.60	2.77
				C4		0.33		0.07				3.85	3.54	0.96	6.82	10.40
				C5	1.59	1.73	1.17	0.78	6.95	0.26	3.33	8.44		20.24	13.40	41.55
				C6 (fem.)	27.17	13.09	26.15	38.93	21.31	16.24	21.06	7.59	12.51	11.49	17.46	5.31
				C6 (male)	15.27	56.91	22.33	18.96	4.89	44.19	38.25	24.66	36.40	16.09	31.55	19.15
				Eggs	23.63	8.93	32.91	28.40	7.63	19.13	32.14	18.34		5.95	9.02	7.92
	Cyclopoida	Cyclopidae	*Acanthocyclops robustus*	C1–C6	4.57											
			Halicyclops sp.	C1–C6		1.92		0.27								
				Eggs				0.73								
			Unidentified Cyclopidae	C1–C6			0.01		0.16			0.26			0.51	

Table S1. *Continued.*

Class	Order	Family	Item	Stage	Limnetic				Oligohaline				Mesohaline			
					Mid-June	Late June	Mid-July	Early Aug.	Mid-June	Late June	Mid-July	Early Aug.	Mid-June	Late June	Mid-July	Early Aug.
			Unidentified Copepoda	C1–C6	1.07	1.28			19.75				0.84			
				N1–N6				0.16	0.25	7.37		0.13		2.62	0.84	11.08
				Eggs					0.13				3.84			
Ostracoda			Unidentified Ostracoda							0.05					0.76	
Gastropoda			Unidentified Gastropoda											1.39		
Unidentified material					17.27		11.74			3.53		2.03	35.24	14.14	9.09	

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