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Structure génétique et physiologie évolutive chez le mysidacé *Boreomysis nobilis*

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PAR

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À ma mère et mon père, pour
m'avoir offert la curiosité et le courage.

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AVANT-PROPOS

Ce mémoire traite du potentiel d'acclimatation à la température du mysidacé *Boreomysis nobilis*, dont une probable population relique glaciaire vit dans le fjord du Saguenay (QC, Canada). Il s'agit d'une étude fondamentale et exploratoire, dont j'ai rédigé la proposition de projet lors de mes études de premier cycle universitaire. Ce mémoire présente les premiers travaux portant sur la structure génétique et le phénotype métabolique de l'espèce à notre connaissance. Aussi, *B. nobilis* n'a que très peu été étudié par le passé, ce qui a apporté son lot de défis à la réalisation du projet. Les principaux défis auxquels j'ai dû faire face étaient liés à la manipulation des individus et à leur préservation. En effet, considérant le manque d'informations quant aux conditions d'élevage ainsi qu'aux limites de tolérance thermique de l'espèce, le potentiel d'acclimatation à la température de l'organisme a dû être évalué à l'aide d'un proxy *in vitro*, soit la mesure de l'activité enzymatique, de manière à induire le moins de biais possible qui auraient pu nuire à l'interprétation des données de nature métaboliques.

L'échantillonnage s'est déroulé de manière opportuniste lors de diverses missions océanographiques, notamment dans le Saguenay lors de la mission Sillex 2018 à bord du N/R Coriolis II, ainsi que dans l'archipel arctique canadien à l'occasion de la mission ArcticNet à l'été 2019, puis lors des essais en mer à l'automne 2020, à bord du NGCC Amundsen.

Ma proposition de recherche et mes résultats ont été présentés à l'occasion de la rencontre annuelle de Québec-Océan, à l'automne 2018, ainsi qu'au Forum des Sciences de la Mer, à l'automne 2019. L'expérience que j'ai acquise à bord des navires de recherche a été un point tournant de ma vie professionnelle et personnelle, me permettant entre autre d'enseigner aux étudiantes et étudiants du cours d'Écologie marine, offert au baccalauréat à l'Université du Québec à Rimouski à l'automne 2018 et 2019.

RÉSUMÉ

Le changement du niveau marin et les changements climatiques associés à la fin du Pléistocène ont participé à limiter la dispersion de certaines populations d'espèces marines, ce qui a pu entraîner leur isolement. Les populations isolées ont pu diverger au niveau de leur structure génétique et de leur phénotype métabolique d'origine et devenir des reliques glaciaires. Nous avons relevé des indices suggérant que la population du mysidacé arctique *Boreomysis nobilis* G. O. Sars, 1879 retrouvée dans le fjord du Saguenay (Québec, Canada) serait une relique glaciaire. La diversité et la structure génétique ainsi que le phénotype métabolique de la population du Saguenay ont été comparés avec des individus issus de différentes régions arctiques du point de vue de la diversité et de la structure de deux gènes mitochondriaux, soit la sous-unité I du cytochrome *c* oxydase et la sous-unité 5 de la nicotinamide déshydrogénase. La réponse métabolique à la température a également été évaluée par la mesure de l'activité d'enzymes clés, soit la citrate synthase (CS) et la lactate déshydrogénase (LDH), à la suite de l'exposition de tissus préservés à une gamme de températures. Une signature génétique différente que celle retrouvée dans les régions arctiques a d'ailleurs été décelée dans la population du Saguenay, ce qui soutient l'hypothèse selon laquelle la population du Saguenay serait une relique glaciaire. Les données génétiques ont révélé que les individus retrouvés dans les régions analysées ont probablement traversé le dernier maximum glaciaire au sein de refuges distincts. La distance génétique et la diversité étaient plus élevées et les fréquences haplotypiques étaient différentes au sein de la population du Saguenay comparativement aux régions arctiques, mais aucun indice d'expansion récente des populations n'a été observé. Les individus du Saguenay avaient également un taux moyen d'activité enzymatique généralement plus élevé, ainsi qu'un rapport CS : LDH ayant une tendance négative significativement plus élevée. Aucune différence n'a toutefois été observée au niveau des points de rupture (*breakpoints*) pour les deux enzymes analysées. Nous présentons ici les premières données empiriques, à notre connaissance, qui soutiennent que la population de *B. nobilis* du Saguenay serait une relique glaciaire.

Mots clés : Relique glaciaire, structure génétique, métabolisme énergétique, arctique canadien, Saguenay, mysidacea, *Boreomysis nobilis*.

ABSTRACT

Global sea level and climatic changes associated with the end of the Pleistocene have represented ecological barriers to population dispersal and thus resulted in their isolation. Populations isolated by sea level changes and the resulting geophysical impacts on the marine environment can diverge from their original genetic and phenotypical state and become glacial relicts. Here we investigated insights of local acclimatization to temperature in a putative relict population of the Saguenay Fjord (Quebec, Canada) of the Arctic opossum shrimp *Boreomysis nobilis* G. O. Sars, 1879. We determined the level of differentiation among the genotypes and the metabolic phenotypes of *B. nobilis* from the Saguenay Fjord and the Canadian Arctic. This was assessed by mtDNA diversity and structure analyses, along with metabolic response to temperature, as inferred by the activity of key enzymes following the exposure of preserved tissues to a range of temperature. Genetic data revealed that the specimens originating from analyzed regions probably survived the last glacial maximum within distinct refugia. Integrating genetic and metabolic proxies, we showed that the Saguenay Fjord population displayed slight but significant differences when compared to analyzed Arctic *B. nobilis*. Furthermore, a unique genetic signature was found in the putative relict population. This evidence is consistent with the hypothesis that the Saguenay Fjord population is indeed a glacial relict. Genetic distance, diversity and haplotype frequencies were greater within the Saguenay Fjord population when compared to Arctic regions, but no indication of population expansion was observed. The distinctiveness of the Saguenay Fjord population was also underlined at the metabolic level, as individuals of this region had a generally higher mean enzyme activity rate, which represent an indication of local metabolic acclimatization to warmer temperature, along with a CS:LDH ratio showing a steeper negative slope. No difference was observed in thermal response of metabolic activity by breakpoint analysis for any analyzed enzyme. However, mean enzyme activity ratios displayed the significantly steepest negative slope in individuals from the Saguenay Fjord when compared to the slopes obtained from Arctic *B. nobilis*. Here we present the first empiric evidence that the Saguenay Fjord population of *B. nobilis* is a glacial relict.

Keywords: Glacial relict, genetic structure, metabolism, phenotype, Canadian Arctic, Saguenay, mysidacea, *Boreomysis nobilis*

TABLE DES MATIÈRES

LISTE DES TABLEAUX

LISTE DES FIGURES

LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

FS	Fjord du Saguenay, QC, Canada
EMSL	Estuaire maritime du Saint-Laurent
PMSSL	Parc marin du Saguenay-Saint-Laurent
CIF	Couche intermédiaire froide
ADNmt	Acide désoxyribonucléique mitochondrial
COI	Cytochrome *c* oxydase, sous-unité I (*Cytochrome c oxydase, subunit I*)
ND5	Nicotinamide déshydrogénase, sous-unité 5 (*Nicotinamide dehydrogenase, subunit 5*)
HS	Détroit d'Hudson (*Hudson Strait*), Canada
QK	Qikiqtarjuaq, Nunavut, Canada
ES	Tasiujaq (lately Eclipse Sound), Nunavut, Canada
LGM	Last Glacial Maximum
SF	Saguenay Fjord, Quebec, Canada
SLE	St. Lawrence Estuary
mtDNA	Mitochondrial deoxyribonucleic acid
CS	Citrate synthase
LDH	Lactate déshydrogénase (*Lactate dehydrogenase*)

NADH	Nicotinamide Adenine Dinucleotide
DTNB	5,5’ – dithiobis (2 – nitrobenzoic acid)
AMOVA	Analysis of Molecular Variance
GLM	Generalized Linear Model
EMM	Estimated Marginal Means (Least-Squares Means)
ANCOVA	Analysis of covariance
CI	Confidence interval

INTRODUCTION GÉNÉRALE

1. GLACIATIONS ET RELIQUES GLACIAIRES

La glaciation du Wisconsinien a pris fin il y a environ 18 000 ans (Dyke & Prest, 1987). Elle a engendré d'importants changements du niveau marin relatif et du régime de température, ce qui a modifié la géomorphologie du territoire ainsi que la biogéographie de nombreuses espèces (Dyke & Prest, 1987; Hillaire-Marcel & De Vernal, 1987 ; De Vernal et al., 2011). L'écoulement des glaciers alors présents sur le territoire boréal et arctique a résulté en l'érosion du roc, ce qui a grandement altéré le paysage et permis la formation de certains écosystèmes, comme les fjords (Locat & Levesque, 2009). La formation du fjord de la rivière Saguenay (FS) origine d'un événement d'affaissement tectonique du bouclier canadien, datant du Précambrien, il y a environ 500 millions d'années (Locat & Levesque, 2009). Ce type d'effondrement induit l'apparition de failles, appelées graben, qui sont propices à l'érosion, notamment glaciaire. Les glaciations du Pléistocène ont donc permis l'établissement d'un glacier dans le graben du Saguenay, soit l'effondrement d'une section de plaque tectonique délimité des failles. Celui-ci a façonné la bathymétrie du FS en y creusant trois bassins, dont le plus profond, en amont, atteint aujourd'hui plus de 270 m de profondeur. De plus, l'action du glacier a laissé une série de trois dépôts morainiques, qui séparent les trois bassins (Fig. 1). Trois seuils prennent actuellement place, de l'amont vers l'aval, à 115, 60 et 20 m de la surface. Le FS draine aujourd'hui un bassin versant de plus de 78 000 km^2 (Smith & Walton, 1980). Il prend naissance en deux points, soit le delta de la rivière Saguenay et la ville de La Baie. Il s'étend sur plus de 110 km, jusqu'à son embouchure au contact de l'estuaire maritime du Saint-Laurent (EMSL) (Fig. 1).

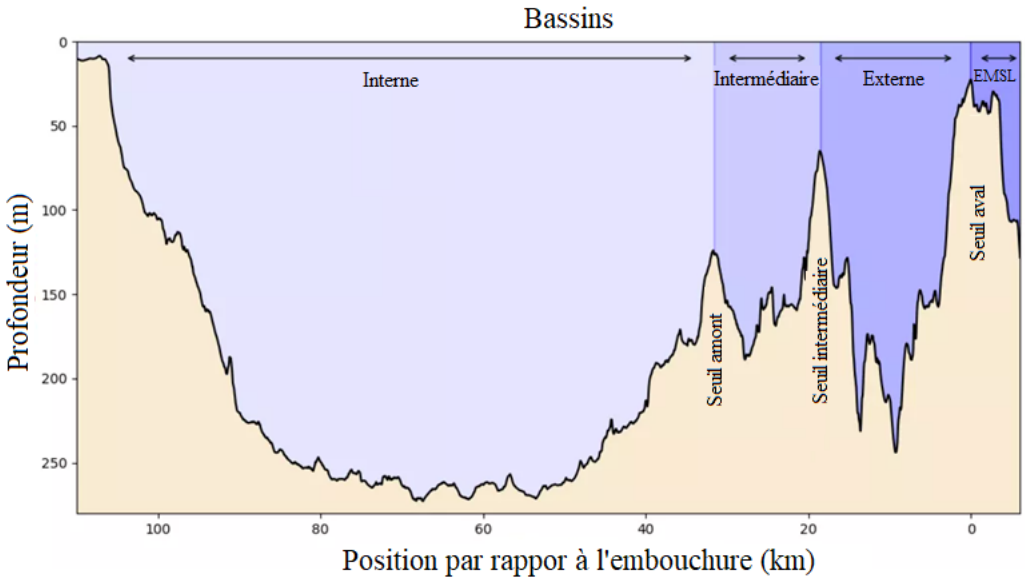

Figure 1. Bathymétrie du fjord du Saguenay (QC, Canada) représentant les trois bassins (interne, intermédiaire et externe), les trois seuils (amont, intermédiaire et aval) ainsi que l'embouchure du fjord sur l'estuaire maritime du Saint-Laurent (EMSL). © Guay, non-publié, modifié avec permission.

Le fjord du Saguenay représente un écosystème unique dont l'abondance de zooplancton soutien une importante biodiversité (De Ladurantaye et al., 1984). Un important apport d'eau froide s'insère dans le FS en provenance de la couche intermédiaire froide (CIF) retrouvée dans l'EMSL à des profondeurs variant de 40 à 150 m. La CIF est composée d'eau avoisinant le point de congélation (< 1 °C) et est composée d'eaux ayant été refroidies en hiver (Galbraith, 2006). Une partie de cette masse d'eau est advecté dans le FS sous l'effet de la marée montante ainsi que de probables ondes internes, des vagues sous-marines induites par le cisaillement entre les masses d'eau (De Ladurantaye et al., 1984 ; Belzile et al., 2016). Le FS est une étendue d'eau subarctique qui abrite des espèces propres aux climats arctiques (Butler et al., 1996). Le régime de température retrouvé dans la FS semble d'ailleurs rencontrer les besoins de la niche thermique de ces espèces arctiques, soit la gamme de température au travers de laquelle un organisme performe suffisamment bien pour se maintenir (Drainville, 1970 ; Judkins & Wright 1974; Magnuson et al., 1979). En effet, Bossé

et al. (1996) dénombraient plus de 410 espèces d'invertébrés marins dans le FS, dont 11% était d'affiliation arctique. De plus, 20% des espèces retrouvées dans le FS n'étaient pas présentes dans l'EMSL adjacent, ce qui signifie que ces espèces seraient contraintes aux eaux du fjord (Bossé et al., 1996). Cette ségrégation est également retrouvée chez certains *Calanus* spp., des espèces copépodes dominantes dans l'écosystème du PMSSL, dont près du quart (23 %) de leurs effectifs retrouvés dans l'ensemble du PMSSL était originaire du FS (Perrin et al., 2014). En effet, la présence de seuil dans les fjords est associée à une diminution des échanges fauniques de part et d'autre du seuil (Allen & Simpson, 1998). Le FS a d'ailleurs été considéré par le passé comme une enclave glaciaire, soit un refuge au sein duquel les espèces arctiques sont contraintes, notamment parce qu'elles y retrouvent des conditions favorables à leurs besoins. Cette hypothèse a toutefois été réfutée par certains auteurs, puisque des échanges fauniques récents entre le FS et l'EMSL adjacent y prennent place chez certaines espèces de poissons de fond et de crustacés d'eaux froides (Sévigny et al. 2009). Sévigny et al. (2009) avancent d'ailleurs que le renouvellement rapide des masses d'eau, qui se réalise en tout aux plus six mois pour le bassin supérieur, favoriserait la dispersion de ces organismes, notamment au niveau larvaire (Sévigny et al., 2009 ; Belzile et al., 2016; Galbraith & Bourgault, 2018). Cette particularité propre au FS le distingue de la majorité des fjords, où le renouvellement des masses d'eau est épisodique et très lent, ce qui favorise souvent le phénomène d'hypoxie des eaux profondes (Farmer & Freeland, 1983). Les travaux de Sévigny et al. (2009) ont toutefois porté uniquement sur des espèces de necton présentant une bonne capacité de dispersion, telles que le flétan du Groenland *Reinhardtius hippoglossoides matsuurae* Jordan & Snider, 1901 et la crevette nordique *Pandalus borealis* Krøyer, 1838. Ces espèces présentent toutefois des phases larvaires planctoniques qui peuvent être transportées par les courants. Certaines espèces associées aux climats arctiques sont cependant toujours présentes dans le FS et pourraient être considérées comme des populations reliques de la dernière période glaciaire (Judkins & Wright, 1974 ; Hampe & Jump, 2011). Ces populations, dites reliques glaciaires, étaient retrouvées au sud de leur distribution actuelle lors de la dernière glaciation. Elles auraient été laissées derrière à la suite du réchauffement du climat ayant mené au retrait des inlandsis vers la fin de la

glaciation (Hampe & Jump, 2011). Ces reliques glaciaires ont la particularité de présenter une diversité et des fréquences haplotypiques grandement différentes de celles retrouvées dans le reste de l'aire de répartition, en plus de distances génétiques importantes. L'étude des reliques glaciaires permet de reconstituer en partie les répartitions géographiques passées, en plus d'apporter davantage de connaissances sur des populations ayant persisté malgré des changements climatiques importants (Audzijonyte & Väinölä, 2006; Hampe & Jump, 2011). Elles sont retrouvées à la limite sud de leur distribution, ce qui les prédisposent à une pression sélective accrue (Hampe & Petit, 2005). Les reliques glaciaires sont donc des modèles intéressants pour étudier le potentiel adaptatif face à des changements des conditions environnementales.

2. IMPACTS DE L'ISOLEMENT SUR LES RELIQUES GLACIAIRES

L'isolement des populations reliques favorise leur divergence, tant génotypique que physiologique. En effet, cet isolement entre la population relique et le reste de la distribution de l'espèce peut notamment induire la divergence et/ou une diversité génétique réduite accompagnée d'une prépondérance de certains allèles dans une population issue d'un nombre réduit d'individus, ce qui est connu en tant qu'effet de goulot d'étranglement (*bottleneck effect*) (Templeton, 1980). D'autres processus évolutifs, notamment la dérive génétique, peuvent également favoriser une divergence génétique marquée de la population relique comparativement à la population générale. Ce phénomène serait beaucoup plus fréquent chez les populations près des limites de la distribution d'une espèce (*rear-edge population*) que chez les individus au centre de cette distribution, comme chez les reliques glaciaires (Vucetich & Waite, 2003). Des indices de ces procédés évolutifs peuvent être mise au jour par l'analyse de la structure de l'ADN mitochondrial (ADNmt), qui permet notamment de décrire le niveau de divergence génétique entre les populations au sein d'une même espèce (Brown et al., 1979). Plus précisément, la sous-unité 1 du cytochrome *c* oxydase (COI) est utilisé dans les études qui portent sur des espèces intimement reliées, voir même au niveau

intraspécifique (Hebert et al., 2003). L'utilisation du COI a d'ailleurs permis d'identifier une différenciation génétique rapide et importante entre certaines lignées de mysidacés considérées comme des reliques glaciaires (Audzijonyte & Väinölä, 2006). Également, le gène mitochondrial codant pour la sous-unité 5 de la nicotinamide déshydrogénase (ND5) a été utilisé afin de discriminer des groupes de puces d'eau du complexe d'espèces *Daphnia pulex* (Vergilino et al. 2009). Ces gènes permettent donc de décrire l'évolution de l'isolement entre les populations de crustacés au niveau intra- spécifique.

L'isolement des reliques glaciaires peut également induire des adaptations locales au niveau physiologique. Certaines espèces de mysidacés considérées comme des reliques glaciaires ont d'ailleurs développé d'importantes adaptations afin de faire face à différentes conditions environnementales. En effet, certaines populations du groupe *Mysis relicta, sensu lato* ont persisté malgré des changements de la salinité de leur environnement, passant de l'eau douce à l'eau marine à répétition sous l'effet des glaciations du Pléistocène, et ce, jusqu'à récemment, il y a moins de 10 000 ans (Eronen et al., 2001). En plus de devoir ajuster leur tolérance à ces changements de salinité, ces populations ont également adapté localement la sensibilité spectrale de leurs yeux à la lumière (Donner et al., 2016). De plus, les reliques glaciaires peuvent développer des adaptations du métabolisme, notamment en ce qui a trait à la tolérance et l'optimum thermique. La température a un effet majeur sur les processus métaboliques (Pörtner, 2002; Somero, 2002 ; 2004 ; Hofmann, 2005; Pörtner et al., 2017). Cet effet est d'autant plus important chez les organismes ectothermes, qui sont incapables de réguler leur température interne pour faire face à un changement de température du milieu. Ainsi, une variation de la température suffisante pour perturber l'activité d'un organisme et favoriser son adaptation représente l'effet limite (*threshold effect*), tel que décrit par Hochachka et Somero (2002). De plus, les organismes possèdent une gamme de températures optimales, soit celles auxquelles leur activité métabolique est maximale (Pörtner, 2002; Somero, 2002 ; 2004 ; Pörtner et al., 2017). Des conditions au-dessous ou au-delà de cet optimum se traduisent par une réduction des performances physiologiques, une diminution du fitness individuel, des effectifs de la population et, ultimement, en une modification de la distribution géographique (Brown et al., 1996; Gaston et al., 1997 ; Gaston, 2009).

L’ensemble des processus physiologiques d’une population donnée se font sur une gamme de température retrouvée dans l’environnement de cette population, appelée l’enveloppe thermique (Addo-Bediako et al., 2000; Pörtner, 2002). La tolérance et l’optimum thermiques permettent d’estimer la niche thermique d’un organisme (Gaston, 1996 ; Gaston & Spicer, 2001 ; Gaston, 2009). En effet, l'activité des enzymes d'un individu peut être un indicateur de son niveau d'adaptation à son environnement thermique (Somero & Hochachka, 1971; Stillman & Somero, 1996). Ceci peut être testé dans des conditions de laboratoire en analysant l’activité d’enzymes clés du métabolisme énergétique à la suite d’une exposition à une plage de températures. Ceci permet d’établir la thermosensibilité de l’enzyme en comparant la température où l'activité enzymatique diminue de manière significative. Cette thermosensibilité devrait être plus grande chez les individus et les populations adaptés à un climat plus chaud par rapport aux individus adaptés aux températures froides. Ceci se traduirait par une diminution drastique de l’activité enzymatique à des températures plus élevées, puisque l'énergie nécessaire pour briser les liens biochimiques entre les protéines est plus élevée (Stillman & Somero, 1996 ; Bennett et al., 2021). Cependant, les informations disponibles sur l'adaptation métabolique des populations reliques sont limitées, car peu d'études ont été entreprises à leur sujet, notamment en raison des problèmes d'échantillonnage et de transport, ainsi que de la difficulté de maintenir les individus dans des conditions de laboratoire (Calosi et al., 2010; Thibault et al. 2020 et références incluses). Ainsi, le fait de pouvoir évaluer les phénotypes métaboliques à l’aide de méthodes *in vitro* est très utile afin d’étudier les populations reliques, puisque le manque de connaissance relatif à leur physiologie ne permet pas de les maintenir en conditions de laboratoire. Afin de palier à cette limitation, il est possible d’utiliser des outils *in vitro*, tels que l’analyse de l’activité enzymatique afin de décrire les différences au niveau des phénotypes de tolérance thermique retrouvées au sein de différentes populations.

Les populations d’espèces zooplanctoniques arctiques toujours présentes en région boréale offrent la possibilité d’étudier l’histoire biogéographique post-glaciaire de ces espèces ainsi que leur potentiel adaptatif face au réchauffement climatique post-glaciaire. Notamment, les mysidacés (Crustacea; Malacostraca) ont la particularité de porter leurs œufs

dans un marsupium, ce qui diminue leur capacité de dispersion et favorise par conséquent la rétention de populations au sein d'environnements changeants en termes notamment de régime thermique (Mauchline, 1980 ; Audzijonyte et al., 2006, 2015).

3. BIOLOGIE, ECOLOGIE ET DISTRIBUTION DE *BOREOMYSIS NOBILIS*

Une espèce représentant un modèle idéal pour une étude portant sur le potentiel d'acclimatation à la température des espèces de crustacés arctiques est *Boreomysis nobilis* G. O. Sars, 1879 (Malacostraca; Pericarida). Il s'agit d'une espèce de l'ordre *Mysida* et de la famille *Mysidae* qui a été proposée à titre de relique glaciaire par Drainville (1970), mais dont le statut n'a jamais été confirmer par des travaux d'ordre génétique ou physiologique à ce jour. Sa morphologie se distingue de celles des autres *Boreomysinae* par son telson aux abords plutôt droits et dont la partie postérieure présente une ouverture de forme obtuse (Fig. 2B). Aussi, ses écailles antennaires présentent les traits caractéristiques de cette sous-famille, soit une marge assez large sur toute la longueur de l'écaille, qui se termine par une pointe latérale dans l'axe de cette dernière (Fig 2C). L'espèce semble se reproduire tout au long de l'année, avec une augmentation du nombre d'individus juvéniles vers le début de l'été, ce qui pourrait indiquer une activité reproductive plus importante à cette période de l'année (Clark & Threlfall, 1993). Tels les autres mysidacés, *B. nobilis* porte ses embryons au sein d'une chambre incubatrice, le marsupium, formée de sclérites modifiées (Fig. 2A), jusqu'à l'émergence des juvéniles. L'espèce présente donc un mode de développement direct. Autrement, les études traitant spécifiquement de *B. nobilis* sont rares, ce qui fait en sorte que les connaissances relatives, notamment, à son histoire de vie, ses paramètres physiologiques optimaux et son alimentation, ne sont pas disponibles à ce jour.

L'espèce, qui présente une distribution circumpolaire arctique, a notamment été récoltée dans la mer de Beaufort, la baie de Baffin, dans certains fjords de Terre-Neuve ainsi

que dans le fjord de la rivière Saguenay, au Québec (Judkins & Wright, 1974; Petryashov, 2009 ; Fig. 3). Cette région est la plus méridionale ou l'espèce a été documentée et pourrait abriter une population relique glaciaire (Drainville, 1970). Cette hypothèse a toutefois fait l'objet de critiques de la part de la communauté scientifique, qui proposait que les échanges fauniques entre l'EMSL et le FS étaient trop importants pour que cette région soit considérée comme une enclave glaciaire (Sévigny et al., 2009).

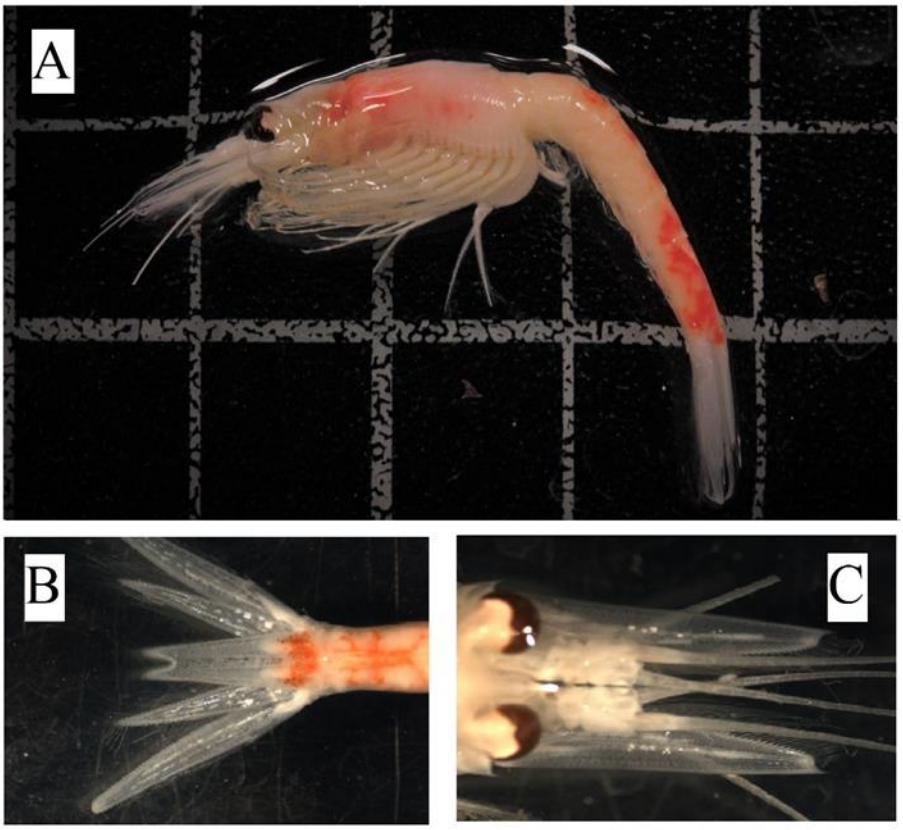

Figure 2. Morphologie générale (A), vue rapprochée du telson (B) et de la tête, présentant les écailles antennaires (C) d'un individu femelle du mysidacé *Boreomysis nobilis* prélevé dans le fjord du Saguenay en 2018 et préservé dans l'éthanol 95 %.

B. nobilis n'a cependant jamais été récoltée à l'extérieur du FS en territoire québécois. En effet, sa distribution semble discontinue dans sa partie méridionale. Les individus présents au sud du cercle polaire ne le sont que dans des environnements présentant une bathymétrie

combinant des sections à grande profondeur et des seuils, typiques des fjords (Clark & Threlfall, 1993; Petryashov, 2009). L'espèce est définie comme étant hyperbenthique et bathypélagique, les densités les plus importantes étant retrouvées dans les 150 m au-dessus du fond et à des profondeurs allant à plus de 1 000 m (Judkins & Wright, 1974 ; Clark & Threlfall, 1993). Elle n'effectuerait pas de migrations nycthémérales, ce qui réduirait davantage son potentiel de dispersion (Clark & Threlfall, 1993). De plus, le fait que l'espèce porte ses embryons dans un marsupium limite davantage sa capacité de dispersion. Aussi, sa présence vraisemblablement exclusive en eau profonde et l'absence d'indice de migration verticale importante abondent dans le sens de l'hypothèse de Drainville (1970), selon laquelle les individus du FS formeraient une population relique glaciaire.

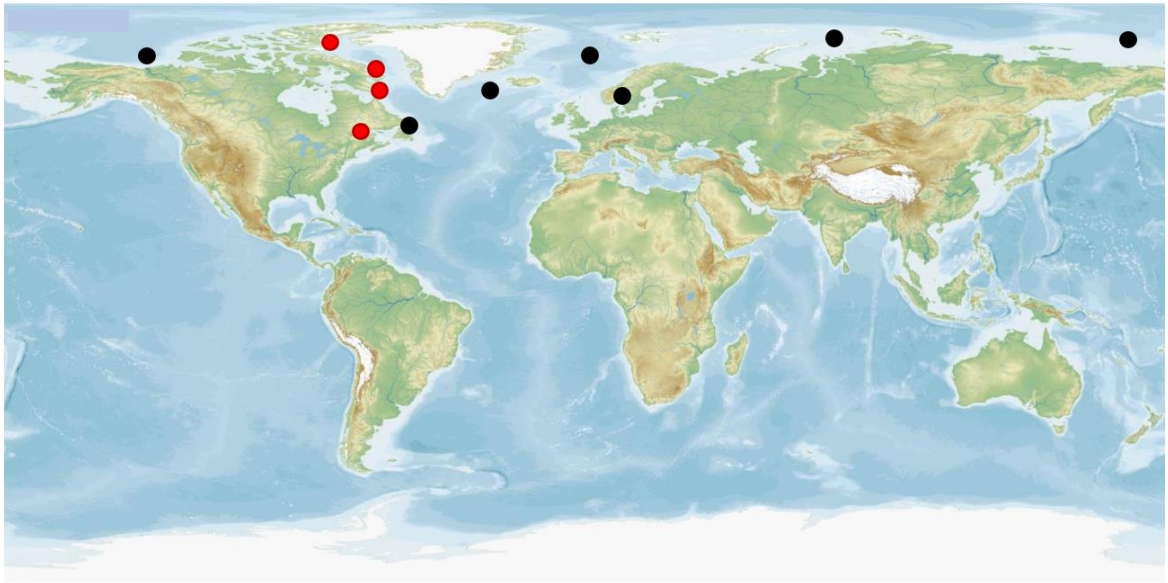

Figure 3. Distribution globale de *Boreomysis nobilis* (à partir de Petryashov, 2009). Les cercles rouges indiquent les régions échantillonnées dans le cadre de la présente étude. Les cercles noirs représentent les mentions historiques de l'espèce.

4. OBJECTIFS

Ce projet vise à déterminer si les individus de *B. nobilis* présents dans le FS forment effectivement une population relique glaciaire. Pour ce faire, la structure de l'ADNmt et la réponse métabolique à la température des individus provenant de trois régions de l'Arctique canadien, soit du détroit d'Hudson (HS), de la région de Qikiqtarjuaq (QK) et de Tasuijaq, anciennement Eclipse Sound (ES), ont été comparées avec le FS. Le niveau de différenciation génétique, a été déterminé à l'aide d'analyses de la diversité et de la structure de l'ADNmt. Puis, la réponse métabolique à la température a été déduite par l'activité d'enzymes clés suite à l'exposition de tissus préservés à une gamme de températures.

Sur la base de l'écologie des reliques glaciaires, notamment les travaux de Hewitt (1996, 2000, 2004) et de Väinölä (1992) et Väinölä et al. (1994), nous émettons l'hypothèse que la distance génétique de deux gènes mitochondriaux, soit le COI et le ND5, est plus grande entre les individus du FS et ceux provenant des régions arctiques qu'au sein des régions arctiques en elles-mêmes. Nous émettons également l'hypothèse que *B. nobilis* provenant de la région du FS présentera une faible diversité génétique, puisqu'elle a probablement subi un effet fondateur ou un effet de goulot d'étranglement.

Les différences au niveau du phénotype métabolique des individus provenant des régions du FS et de l'Arctique ont été déterminées en évaluant l'activité d'enzymes clés du métabolisme énergétique, soit la citrate synthase (CS) en tant que proxy de la thermosensibilité du métabolisme aérobie, la lactate déshydrogénase (LDH) en tant que proxy de la thermosensibilité du métabolisme anaérobie. Le ratio de ces deux enzymes (CS:LDH) a également été calculé afin de déterminer le phénotype métabolique sur une plage de températures atteignant la limite supérieure de leur tolérance thermique, ce qui permet d'évaluer la thermosensibilité des enzymes analysées. Nous émettons alors l'hypothèse que les individus provenant de la région la plus au sud, soit celle du FS, présente une thermosensibilité enzymatique plus faible, c'est-à-dire que les enzymes maintiennent leur

intégrité à des températures plus élevées comparativement aux individus des régions arctiques.

Le présent projet permettra d'apporter davantage de connaissances sur l'espèce, dont l'histoire de vie est peu connue, malgré son importance probable en tant qu'espèce fourragère. De plus, il permettra de discuter du potentiel d'acclimatation à la température des espèces de crustacés marins ayant une capacité de dispersion limitée dans le contexte des changements climatiques globaux.

CHAPITRE 1
STRUCTURE GENETIQUE ET PHYSIOLOGIE EVOLUTIVE CHEZ LE MYSIDACE *BOREOMYSIS NOBILIS*

1.1 INTRODUCTION

1.1.1 Relict species and populations

Global sea level and climatic changes resulting from the transition between the Pleistocene's Last Glacial Maximum (LGM) and the Holocene have represented ecological barriers to populations dispersal and thus resulted in isolated populations (Dyke & Prest, 1987; Hewitt, 2004; Hampe & Jump, 2011). These populations, known as glacial relicts, are remnants of the distribution of the species before the end of LGM that were left behind following a range shift event induced by a change in climate (Hampe & Jump, 2011). Populations isolated by sea level changes and the resulting geophysical impacts on the marine environment can diverge from their original genetic and phenotypical state and become glacial relicts. Indeed, the genetic structure of an organism is often dictated by past climate-driven distribution changes (Hewitt, 2000; 2004; Audzijonyte et al., 2006; Jiménez-Alfaro et al., 2016). Rear-edge glacial relict populations, living at the edge of a species' range, often show a higher level of genetic variation and phenotypic differentiation when compared to other populations of the same species, which can be induced by reproductive isolation from all other populations of the species, bottleneck effects, genetic drift and selection processes (Hampe & Jump, 2011). In addition, as a result of geographical isolation and post-glacial modification of environmental conditions, such as temperatures, local adaptation may occur, notably in rear-edge populations (Hampe & Petit, 2005). Moreover, rear-edge populations are even more susceptible to extinction when compared to "central" populations, since their environment are often close to their niche's limit, thus less favorable (Gaston, 2003; Hampe

& Petit, 2005; Jiménez-Alfaro et al., 2016). Glacial relict populations live in a way "beyond" the limit of the species' current distribution, thermal tolerance window and niche's limits.

1.1.2 Isolation of populations in a fjord environment

Glacial relict populations can notably be found in environments with restricted connectivity, such as fjords (Väinölä et al., 1994; Sköld et al., 2003). These particular environments are characterized by their glacial origin, having formed *via* erosion of the bedrock by a glaciers' flow (Butler et al., 1996). With their shallow sills, deep basins, and water masses circulation patterns, they can limit dispersal capacity of some species and act as a modern refuge (Sköld et al., 2003; Jørstad et al., 2004; Perrin et al., 2014). Indeed, it is suggested that genetic structure of fjord populations, as described by allozymes, microsatellites and mitochondrial DNA (mtDNA) analyses of the Norwegian American lobster populations, is dictated by the particular morphology of these fjords and the resulting limitation they impose to dispersal capacity (Jørstad et al., 2004). Thus, fjords can act as glacial refuges in boreal climate regions. The Saguenay Fjord (SF; QC, Canada) has been considered as a glacial refuge (Drainville, 1970), but this assumption could not be confirmed, since it exhibits very dynamic water circulation, which promotes migration of marine organisms between the fjord and the adjacent St. Lawrence Estuary (SLE) (De Ladurantaye et al., 1984; Saucier et al., 2003; Harvey et al., 2009; Sévigny et al., 2009; Galbraith & Bourgault, 2018). However, 11 % of the 410 benthic invertebrates' species found in the Saguenay Fjord are Arctic ones, and 14 of these species have never been collected in adjacent SLE system (Bossé et al., 1996). Thus, some of these species could potentially represent glacial relicts. In comparison, the Arctic region of Baffin Bay has two major currents that make up a cyclonic circulation in the region: the slightly warmer and saltier West Greenland Current flowing northwest, and the colder and less salty Baffin Current flowing south (Gratton et al. 2006). The latter flow into Baffin Bay from Lancaster Sound. Few studies have established temperature time series in the region, but available data generally place

temperatures below 0 °C (Gratton et al. 2006). In contrast, temperatures in the SF vary seasonally between 1 and 3.5 °C (Belzile et al., 2016; Galbraith & Bourgault, 2018).

1.1.3 Biology and ecology of *Boreomysis nobilis*

One of these putative relicts is a bathypelagic and hyperbenthic species, the opossum shrimp *Boreomysis nobilis* G. O. Sars, 1879, which occurs in the SF, but has never been found in the Estuary nor the Gulf of St. Lawrence (Drainville, 1970). Otherwise, *B. nobilis* is found in circumpolar Arctic waters, notably across the Canadian Arctic archipelago, Baffin Bay, Hudson Bay, in some Newfoundland fjords (Banner, 1954; Judkins & Wright, 1974; Astthorsson, 1985; Clark & Threlfall, 1993; Sirenko et al., 1996; Petryashov, 2009). It appears to be an important forage species, found in the stomach contents of many fish species of commercial interest, such as cod and halibut (Astthorsson, 1985). In the southernmost regions of its geographic distribution, *B. nobilis* is exclusively found in fjords with deep basins and shallow sills (Judkins & Wright, 1974; Clark & Threlfall, 1993). Some evidence suggests highest densities are found in the 50 m above sea floor, and that no diel vertical migration occurs in *B. nobilis* in Newfoundland's fjords (Clark & Threlfall, 1993), which could result in a limited dispersal capacity. In the Arctic regions, this species is found in midwater down to the seafloor, at water depth greater than 200 m and temperature is below 2 °C. Given the hyperbenthic and bathypelagic position of *B. nobilis* from the southernmost region, their isolated presence in boreal fjords could be due to sea level changes that occurred after the LGM. The Saguenay Fjord underwent a fast post-glacial isostatic adjustment. Since this isostatic adjustment that took place around 7 000 y ago, it induced a significant sea-level decrease (Dyke & Prest, 1987; Dionne & Occhietti, 1996), that probably strongly limited connectivity between the SF and Arctic regions. Thus, *B. nobilis* from the SF region have probably been highly isolated; this being likely enhanced by the fact that the dispersal of this species might be very restricted. Indeed, *B. nobilis*, like other mysids, keeps its eggs and embryos inside a brood pouch (*marsupium*) until the emergence of the juveniles.

Furthermore, *B. nobilis* does not undergo nycthemeral migration, at least in the Newfoundland fjords, which can further decrease their dispersal capacity on one hand but increase opportunities for local adaptation on the other (Clark & Threlfall, 1993). Consequently, *B. nobilis* from the SF region was suggested as a potential glacial relict population (Drainville, 1970; Judkins & Wright, 1974; Clark & Threlfall, 1993; Petryashov, 2009).

1.1.4 Impacts of isolation of *B. nobilis* on its genetic diversity and structure and metabolic phenotype

Greater genetic distance and a different haplotypic distribution can be expected between *B. nobilis* putative relict populations and *B. nobilis* populations from the Arctic than among different Arctic regions. This should be reflected in mitochondrial genetic structure (Audzijonyte et al., 2005; 2006). Insights of these selective processes can be obtained by analyzing the structure of mtDNA, which makes it possible to describe the level of genetic divergence among populations from different regions within the same species (Brown et al., 1979). In addition, organisms that have evolved through a change in thermal regime may show signs of local metabolic adaptations. Indeed, enzymatic kinetic is temperature-dependent and a set of enzymes in a population can long-term acclimatize/adapt to its thermal environment (Somero & Hochachka, 1971; Stillman & Somero, 1996). Such signs can be tested under laboratory conditions by analyzing the enzymatic activity following exposure to a range of temperatures. This establishes the thermosensitivity of the enzyme by comparing the temperature at which the enzyme activity significantly decreases. This thermosensitivity is expected to be greater in individuals acclimated to a warmer climate compared to individuals acclimated to cold temperatures (Stillman & Somero, 1996; Calosi et al. 2008, 2010; Sunday et al. 2019; Bennett et al., 2021). However, information on metabolic phenotypes of relict populations is scarce, since few studies have been undertaken, notably due to sampling and transport challenge, along with the difficulty to maintain the individuals under laboratory conditions (Calosi et al., 2008; 2019; Thibault et al. 2020). Assessing their

physiological performance can thus be difficult, since many environmental parameters allowing to maintain the organisms in laboratory conditions are unknown and can induce significant bias in the interpretation of the results. The assessment of metabolic enzymes *in vitro* is an alternative solution to compare the performance of relict populations like *B. nobilis*, about which life history data are very scarce (Drainville, 1970; Judkins & Wright, 1974; Clark & Threlfall, 1993).

1.1.5 Objectives and hypotheses

This project aims to determine if *B. nobilis* from the SF region is a glacial relict. In order to do this, we determined the level of genetic differentiation and the metabolic phenotypes in individuals collected from the SF and different Canadian Arctic regions. This was assessed by mtDNA diversity and structure analyses, along with metabolic response to a range of temperature, as inferred by the activity of key enzymes, namely citrate synthase (CS) and lactate dehydrogenase (LDH), following the exposure of preserved tissues to a range of temperatures. Based on relict ecological and evolutionary theories, we hypothesize that the genetic distance of two mitochondrial genes (Cytochrome *c* oxidase, subunit I; COI and nicotinamide dehydrogenase, subunit 5; ND5) is greater among *B. nobilis* from the SF and the Arctic regions investigated, than among the Arctic regions themselves. We hypothesize that SF will display low genetic diversity if it has effectively gone through a recent bottleneck. Differences in phenotypic plasticity of *B. nobilis* for physiological traits from the SF and the Arctic regions investigated have been determined by evaluating the activity of key enzymes on a temperatures range reaching the temperature at which enzymatic activity collapses. We hypothesize that *B. nobilis* from the southernmost region, SF, keeps CS and LDH functional at higher temperatures when compared to *B. nobilis* from the Arctic regions investigated.

1.2 METHODS

1.2.1 Sampling locations

Adult specimens of the opossum shrimp *B. nobilis* were collected from three regions in the Canadian Arctic Archipelago: Hudson Strait (HS), Qikiqtarjuaq (QK) and Tasiujaq (lately Eclipse Sound; ES) and from the putative glacial relict of the SF in boreal Quebec (Fig. 4). In ES, specimens were sampled in one location (n = 53), five locations in QK (n = 158), one location in HS (n = 2) and 11 in SF (n = 213). Sampling in SF took place onboard the R/V Coriolis II in October 2017 and July and October 2018. Sampling in the Canadian Arctic was undertaken onboard CCGS Amundsen between August and September 2019.

Figure 4. Sampling locations for each region where individuals of the opossum shrimp *Boreomysis nobilis* were collected during this study. A total of 10 locations (F15, F13, F11, 90, 82, 72, 62, 52, S1-A, S2-A and S3; see Table 1 for more details) were sampled in Saguenay Fjord (SF), one (354) in Hudson strait (HS), five (E5, E2, E1, D5, D1-177) around Qikiqtarjuaq (QK), and one (2.4) in Tasiujaq (Eclipse Sound; ES).

1.2.2 Specimen collection and storage

We used the most appropriate gear to maximize specimen collection in different regions across a latitudinal gradient (Table 1). Vertical and horizontal tows were performed using Jacknet, Tucker net, Isaacs-Kidd midwater trawl (IKMT) and Agassiz trawl (Table 1) to obtain alive specimens, which were sorted immediately after collection and preserved individually in cryovials. Most individuals were stored in cryovials at - 80 °C for either genetic or physiological analyses. However, some individuals were used for a combination of genetic and physiological analyses, and some from SF were preserved in 95% v/v ethanol and frozen at - 20 °C until processing (Table 1). These individuals were only used for genetic analyses.

1.2.3 DNA extraction, amplification, and sequencing

We amplified and sequenced 619 bp of mtDNA COI from 41 individuals for each region, except HS, where only two individuals were sampled (Table 1). Of these individuals, 10 from each region were also used in mtDNA ND5 analysis, where 255 bp were amplified, for 30 individuals in total. No specimen from HS were sequenced for ND5. Considering the low number of individuals sampled in HS, results regarding COI in this region should be taken with care in further analyses. We used LCOI1490 and HCOI2198 primers (Folmer et al., 1994) for COI amplification, and sets of primers for ND5 were designed using *Boreomysis arctica* Krøyer, 1861 mitochondrial genome (Tempestini et al., unpublished; Table 2). All 125 extracted DNA sequences of COI and 30 sequences of ND5 were proofread and aligned using MEGA X software (Kumar et al., 2018).

Table 1. Date, coordinates, sampling depth, gear (J = Jacknet 1 m diam. 202 μm mesh, TM = Tucker multinet 1 m² opening 202 μm mesh, IKMT = Isaacs-Kidd Midwater Trawl 2 mm mesh, A = Agassiz net 10 mm mesh), tow type (VO = Vertical-Oblique, O = oblique, H = horizontal, B = benthic), analysis and storage techniques) for each region and location where specimens of *Boreomysis nobilis* were collected.

Region	Location	Date	Lat. (°N)	Long. (°W)	Max net depth (m)	Bottom depth (m)	Sampling gear[1]	Tow type[2]	Molecular (n)		Enzymatic (n)		Storage
									COI	**ND5**	**CS**	**LDH**	
Saguenay Fjord	F11	2017-10-03	48.32990	-70.31350	252	267	J	O	3	-	-	-	EtOH 95 %
	F13	2017-10-3	48.36822	-70.59732	245	260	J	O	9	-	-	-	EtOH 95 %
	F15	2017-10-03	48.41322	-70.75360	125	208	J	O	2	-	-	-	EtOH 95 %
	52	2018-07-07	48.17702	-70.14239	193	264	TM	VO	12	2*	-	-	- 80 °C
	62	2018-07-07	48.20970	-70.21176	194	264	TM	VO	5	2*	4	-	- 80 °C
	72	2018-07-07	48.21512	-70.28729	205	257	TM	VO	4	1*	-	1	- 80 °C
	82	2018-07-07	48.22071	-70.37057	199	246	TM	VO	3	1*	-	-	- 80 °C
	90	2018-07-07	48.21532	-70.43097	199	219	TM	VO	3	-	2	-	- 80 °C
	S1-A	2020-09-18	48.24938	-70.10496	208	223	J	VO	-	1**	-	2	- 80 °C
	S2-A	2020-09-19	48.32501	-70.29272	236	259	J	VO	-	2**	-	2	- 80 °C
	S3	2020-09-21	48.32554	-70.29409	246	260	J	VO	-	1**	-	1	- 80 °C
Hudson Strait	354	2019-09-03	60.97898	-64.75070	400	557	IKMT	H	2	-	-	-	- 80 °C
Qikiqtarjuaq⸸	E5	2019-08-22	69.31459	-63.13343	450	1931	IKMT	H	9	-	1*	-	- 80 °C
	E2	2019-08-23	68.53774	-64.67042	516	516	A	B	9	6**	1*	3	- 80 °C
	E1	2019-08-23	68.27970	-6514856	450	450	A	B	3	-	-	-	- 80 °C
	D1-177	2019-08-24	67.47443	-63.68728	375	678	IKMT	H	10	4*	4	-	- 80 °C
	D5	2019-08-26	68.99704	-61.40536	461	1837	IKMT	H	10	-	-	3	- 80 °C
Eclipse Sound⸸	2.4	2019-08-18	72.64637	-79.33670	410	685	IKMT	H	41	10*	6*	6	- 80 °C
								Total	125	30	18	18	

⸸ Notes of collection: Individuals from Qikiqtarjuaq and Tasiujaq (Eclipse Sound) were sampled in Baffin Bay's open waters, at a few hundred meters from the bottom. They did not seem to be restricted to a particular position in the water column, as they were in Saguenay Fjord.
* Individuals were also used for COI structure analysis
** Individuals were also used for LDH activity analysis

Table 2. Information on primers used for *Boreomysis nobilis* COI (n = 125) and ND5 (n = 30) amplification.

Gene	Name	Pairing Temp. (°C)	Sequence
COI	HCOI-r	55.3	5’ – TAAACTTCAGGGTGACCAAAAAATCA – 3’
COI	LCOI	55.3	5’ – GGTCAACAAATCATAAAGATATTGG – 3'
ND5	ND5-150F	62.0	5’ – AGTTAAAGTTCCGGCGAGTATT – 3’
ND5	ND5-394R	62.1	5’ – GTGTTAGCTCAAAGATCCTTTGTTAG – 3’
ND5	ND5-32F	61.9	5’ – AAATCTGGTAAGCCTAAACCTACT – 3’
ND5	ND5-272R	61.8	5’ – TTTAGTGCCCTAGTTCACTCTTC – 3’

DNA was extracted using a DNeasy Blood & Tissue Kits (QIAGEN) and according to the manufacturer’s protocol. In brief, the thoracopods or abdominal muscle tissue of each individuals were triturated with a razor blade and placed in 180 µL lysis buffer. Samples were digested in 20 µL of proteinase K and incubated overnight at 37 °C. Extraction was finalized on QIAGEN MiniSpin columns. COI amplification was realized by Polymerase Chain Reaction (PCR) using, for each sample, 1 µL of DNA extract, 1X buffer (IBI RP010), 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.32 µM of each primer, nanopure sterile water and 1 unit of Taq polymerase (IBI RP002 #18T01001), for a final PCR volume of 25 µL. PCR protocol (adapted from Lee, 2000) included initial denaturation (95 °C, 90 s), followed by six cycles of denaturation (90 °C, 30 s), annealing (45 °C, 30 s) and elongation (72 °C, 90 s), 28 cycles of denaturation (90 °C, 30 s), annealing (55 °C, 45 s) and elongation (72 °C, 60 s), and a final elongation (72 °C, 5 min). ND5 amplification of each sample included 1 µL of DNA extract, 1X buffer (IBI RP010), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.32 µM of each primer, nanopure sterile water and 1 unit of taq polymerase (IBI RP002 #18T01001), for a final PCR volume of 50 µL. PCR protocol included initial denaturation (94 °C, 60 s), followed by five cycles of denaturation (94 °C, 60s), annealing (45 °C, 90 s) and elongation (72 °C, 90 s), 28 cycles of denaturation (94 °C, 60 s), annealing (53.5 °C, 90 s) and elongation (72 °C, 90 s), and a final elongation (72 °C, 5 min). Migration on 2 % agarose gel using SybrSafe stain (Invitrogen, SybrSafe DNA gel stain) was realized to confirm PCR success. We sequenced both strands for each individual, to ensure accuracy of the DNA sequence. Amplified samples were sent to Plateforme de Séquençage et de Génotypage (CRCHUL,

Quebec, QC, Canada) for sequencing. All COI sequences were deposited in the GenBank/BOLD database (GenBank accession numbers MW680165 to MW680288) which can be found on the following web page:

https://www.ncbi.nlm.nih.gov/nuccore/?term=MW680165:MW680288%5baccn%5d.

1.2.4 Characterization of metabolic phenotypes

We characterized the activity levels of two enzymes: citrate synthase (CS), lactate dehydrogenase (LDH) and their ratio (CS:LDH), in order to define the variation in mean metabolic phenotypes and provide evidence for potential local acclimatization to temperature. We measured mean CS activity as a proxy of aerobic metabolism. Indeed, CS is known as the limiting enzyme of the Tricarboxylic Acid cycle (TCA), since it catalyzes the first reaction of the cycle (Suissa et al., 1984). As for LDH, it was used as a proxy of anaerobic metabolism. CS:LDH ratio was used as a proxy to discriminate different metabolic phenotypes. Measurements were obtained by analyzing individuals originating from different regions exposed in vitro to a gradient of temperatures. In more detail, the abdominal muscle tissues of six individuals from the analyzed regions (ES, QK and SF) and for each enzyme, for a total of 36 individuals, were homogenized on ice in PBS buffer following a 1/10 dilution. Each sample was fractionated in 1.5 mL microtubes (subsamples of 30 µL) and kept frozen at -80 °C until analyses were carried out. Enzymes' activity was measured by mean of spectrophotometry-based approach at different temperature, selected along a temperature gradient by firstly measure CS and LDH activity using a thermal bath (Alpha RA12, Lauda, Lauda-Königshofen, Germany). The upper limit of the gradient was defined as the temperature at which no activity was measured. Treatment temperatures were subsequently selected from the range obtained to ensure a sufficient level of detail during subsequent analyses to identify the maximal temperature at which *B. nobilis'* analyzed enzymes are functional. The temperature treatment range spanned from 0 to 40 °C with 5 °C increments for CS, and from 0 to 70 °C with 10 °C increments for LDH. Tissue homogenates were

exposed to treatment temperature for 30 min before their enzymatic activity were estimated. Following exposure, measurements were undertaken in duplicates of the same individual, at 25 °C using a spectrophotometer (Cary 100, Aglient Tech., Santa Clara, CA, USA).

For CS's activity measurement, reaction medium comprised 20 µL of diluted and homogenized tissue, 0.16 mM nicotinamide adenine dinucleotide (NADH) and 0.4 mM pyruvate. Measurements at 340 nm wavelength were undertaken with extinction coefficient of NADH (Ɛ340) of 6.22 mL cm^{-1} μmol^{-1} (Thibault et al., 1997). Reaction medium for LDH's activity measurement comprised 20 µL of diluted and homogenized tissue, 0.1 mM of 5,5' – dithiobis (2 – nitrobenzoic acid) (DTNB), 0.113 mM of acetylated coenzyme A and 0.15 mM oxaloacetic acid. Measurements at 412 nm wavelength were undertaken with extinction coefficient of DTNB (Ɛ412) of 13.6 mL cm^{-1} μmol^{-1} (Thibault et al., 1997). LDH's activity were measured as proxy of anaerobic metabolism's activity. Furthermore, we assessed the extend of energy metabolism pathways through CS:LDH ratio. Protein content, which enables to normalize enzyme activity, was estimated using the bicinchoninic acid method (Smith et al., 1985), to normalize enzymatic activity. Protein content was measured by spectrophotometry using 25 µL of 1/100 diluted homogenized tissue in PBS buffer. Samples were incubated for 30 min at 37 °C and measurements were compared with a standard curve obtained with bovine albumin solution with a known protein content.

1.2.5 Data analysis

1.2.5.1 Genetic diversity and structure

The genetic diversity, defined as number of haplotypes, number of private haplotypes, haplotype diversity (Hd; probability to sample randomly two different haplotypes in a sample (Nei, 1987)), and nucleotide diversity (π; average nucleotide number of differences for each site, (Nei, 1987)), as estimated using DNASP version 6.10.4 (Rozas et al., 2017). The genetic structure of *B. nobilis* according to regions was assessed by the construction of statistical

parsimony haplotype network using TCS package of PopART software version 1.7.2 (Clement et al., 2002).

Pairwise p-distances, which represent the number of amino acid differences per site from averaging over all sequence pairs between groups, were obtained using MEGA X software (Kumar et al., 2018). Analysis of Molecular Variation (AMOVA) was computed to assess for genetic differentiation within and among regions. Pairwise fixation indices (Φ_{ST}; the variance among regions and within regions divided by total variance) among individuals from all regions were also computed by treating mtDNA haplotypes as allelic data at a single locus. Significance was assessed by bootstrapping using 1000 permutations (Excoffier et al., 2005). Deviations from neutrality of Wright-Fisher model was assessed based on COI sequences only with Fu's Fs, Fu and Li's F*, Fu and Li's D*, and Tajimas's D statistic, estimated using DNASP 4.10.4 (Tajima 1989; Rozas et al., 2017). Due to a lack of sufficient individuals available for this analysis, the ND5 sequences have not been tested for a population expansion event.

1.2.5.2 Metabolic phenotype

To assess differences in mean metabolic phenotypes (i.e. enzymatic activity) from each tested regions, we used a Generalized Linear Model (GLM) tests with "Temperature" treatments and "Region" as fix factors, and their interaction, with the term "Individual" as a random factor, to account for repeated measures. No significant effect of "Individual" was detected for any of the analyses performed, and it was therefore removed from the analyses. GLM tests, for which residual normality was assessed, were accompanied by Estimated Marginal Means (Least-Squares Means, EMM) post-hoc contrast analyses. Post-hoc tests were carried out using R Studio emmeans package (Lenth, 2020). Mean activity ratio (CS:LDH) trends in tested temperature levels were assessed with a least-squared regression using a linear model. Slopes of the linear regressions were then compared using an analysis of covariance (ANCOVA). In addition, we estimated breakpoints where mean enzymatic

activity and their ratios were altered by temperature treatments, outlining at which temperature the activity or the ratio begins to decrease, using the Segmented R Studio package (Muggeo, 2017). The breakpoints were compared between regions using the 95 % confidence interval (CI) test, where the 95 % CI were calculated as follows:

[Eq. 1] $$95\%\ CI = z\ {}^{\sigma}/_{\sqrt{n}}$$

Here, *z* represents the z-score (i.e., the number of standard deviations separating a result from the mean), σ the standard deviation, and *n* the number of individuals. All statistical analyses related to metabolic measurements were carried out using R Studio (R Studio Team, 2020).

1.3 RESULTS

1.3.1 Genetic polymorphism and mtDNA diversity

The highest number of COI haplotypes (20) of 41 *B. nobilis* analyzed was found in the SF, of which 17 haplotypes are private to the region (Table 3, Fig. 5). In HS two haplotypes were present, of which one was exclusive, however, only 2 individuals were analyzed. In QK and ES, 41 individuals were analyzed for each region. Eight COI haplotypes were found, and four of them were exclusive to those regions. One haplotype was shared among all regions, and one was shared among SF, QK and ES (respectively shown in red and green, Fig. 5). Two highly dominant COI haplotypes were shared between QK and ES (shown in light and deep blue, Fig. 5), one of which was dominant in both regions. Similar pattern emerged from the ND5 analyses, where the highest number of haplotypes (6) were found in *B. nobilis* of the SF, of which five were exclusive to the region. In QK, three shared ND5 haplotypes were detected and in ES, three of five ND5 haplotypes were exclusive. One haplotype was shared between SF and QK but was not predominant in the region (shown in red, Fig. 5). QK showed three ND5 haplotypes and none were exclusive. Nucleotide diversity was similar for COI in

QK and ES regions. Highest ND5 haplotype diversity (Hd) was also observed in SF, but highest ND5 nucleotide diversity (π) was observed in ES. In QK haplotype and nucleotide diversity for ND5 was lowest (Table 3). Parsimony haplotype network (TCS) was inferred from haplotype frequencies of *B. nobilis* from each region (Fig. 6). For COI, four mutations separated the four main haplotypes observed in QK and ES. Three of these were also observed in SF. The private haplotypes of SF were all one mutation apart. A similar network structure was observed for ND5. QK and ES shared three of their four main haplotypes, but more mutations separated these. The parsimony haplotype network of COI showed limited structure and haplotypes were closely related. Limited structure was also observed in the ND5 haplotype network, and potentially due to low number of mutational steps among haplotypes, likely due to small number of individuals analyzed for this gene.

Table 3. Mitochondrial DNA polymorphism of two mtDNA genes, COI and ND5 of *Boreomysis nobilis* in four different regions (Saguenay Fjord, Hudson Strait, Qikiqtarjuaq and Tasiujaq (Eclipse Sound) of two origins (Boreal and Arctic).

Gene	Origin	Region	n	No. haplotypes	No. private haplotypes	Haplotype diversity (Hd) ± SE	Nucleotide diversity (π) ± SE
COI	**Boreal**	**Saguenay, QC**	41	20	17	0.927 ± 0.022	0.00291
	Arctic	**Hudson Strait**	2	2	1	---	---
		Qikiqtarjuaq	41	8	4	0.699 ± 0.047	0.00155
		Tasiujaq (Eclipse Sound)	41	8	4	0.680 ± 0.055	0.00147
	Overall		**125**	**30**		0.850 ± 0.002	0.00263
ND5	**Boreal**	**Saguenay**	10	6	5	0.889 ± 0.075	0.01124
	Arctic	**Qikiqtarjuaq**	10	3	0	0.378 ± 0.181	0.00688
		Tasiujaq (Eclipse Sound)	10	5	3	0.844 ± 0.080	0.01429
	Overall		**30**	**11**		0.846 ± 0.54	0.01547

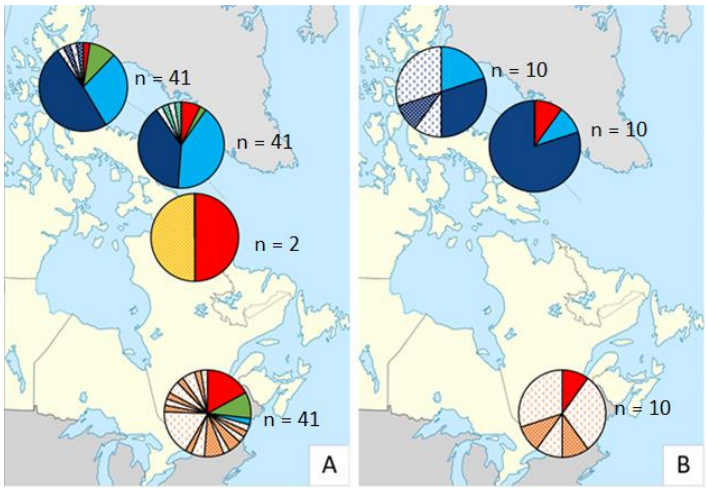

Figure 5. Geographic distribution of haplotype frequencies with 125 sequences of 619 bp of mtDNA COI (A) and 30 sequences of 255 bp of mtDNA ND5 (B) in *Boreomysis nobilis*. Textured colors represent private haplotypes, solid colors show shared haplotypes.

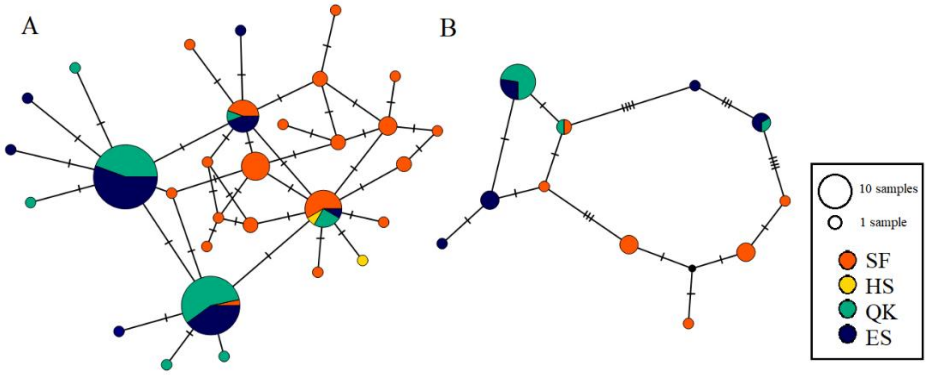

Figure 6. Parsimony mtDNA haplotype networks (TCS) built from 619 bp fragment of COI (A) and 255 bp fragment of ND5 (B) in *Boreomysis nobilis* from each region investigated (SF: Saguenay Fjord, HS: Hudson Strait, QK: Qikiqtarjuaq, ES: Tasiujaq (Eclipse Sound)). Black lines show unobserved haplotypes.

In general, genetic distances among regions were low for both mtDNA COI and ND5 genes (*p* distance < 0.01). However, SF showed higher genetic distance (COI) when compared to individuals from Arctic regions. Highest COI p-distance of 0.00614 ± 0.00439 was observed

between SF and QK, and 0.00593 ± 0.00420 was found between SF and ES. In contrast, lowest distance was observed between the two Arctic regions (Table 4). Genetic distances for ND5 were similar among all regions ($p > 0.05$; Table 4).

The AMOVA analyses revealed that a substantial covariance of COI and ND5 among regions of 32.75% and 41.06%, suggesting some level of genetic separation among them (Table 5). This was also supported by pairwise and similar Φ_{ST} for COI and ND5, yet slightly higher in the latter. Differentiation revealed by Φ_{ST} between SF and QK or SF and ES, ranged between 0.39 and 0.58 (Table 6). Furthermore, no history of population expansion events were found based on Wright-Fisher standard neutral model, as no significant deviation from the standard neutral model was present in each region (Table 7).

Table 4. Pairwise p-distances for 125 sequences of 619 bp of mtDNA COI and 30 sequences of 255 bp of mtDNA ND5 in *Boreomysis nobilis*. Below the diagonal distances are shown and standard deviation are above the diagonal.

	COI			**ND5**		
	SF	**QK**	**ES**	**SF**	**QK**	**ES**
SF		0.00439	0.00420		0.00497	0.00567
QK	**0.00614**		0.00104	0.00512		0.00497
ES	**0.00593**	**0.00153**		0.00585	0.00497	

Table 5. Analysis of Molecular Variance (AMOVA) and fixation indices (Φ_{ST}) based on 125 sequences of 619 bp of mtDNA COI and 30 sequences of 255 bp of mtDNA ND5 in *Boreomysis nobilis*. Asterisks (***) indicates level of significance, $p < 0.0001$.

	Sum of squares		**Variance components (σ^2)**		**Covariation (%)**	
Source of variation	**COI**	**ND5**	**COI**	**ND5**	**COI**	**ND5**
Among regions	25.304	22.193	0.297	0.944	32.75	41.06
Within regions	72.376	37.936	0.609	1.355	67.25	58.94
Total	97.680	60.129	0.905	2.300		
Fixation indices (Φ_{ST})	0.328***	0.411***				

Table 6. Pairwise Φ_{ST} indices analysis based on 125 sequences of 619 bp of mtDNA COI and 30 sequences of 255 bp of mtDNA ND5 in *Boreomysis nobilis*. Pairwise Φ_{ST} values are shown below and *p* values above the diagonal.

	COI			ND5		
	SF	**QK**	**ES**	**SF**	**QK**	**ES**
SF		< 0.0001	< 0.0001		< 0.0001	< 0.0001
QK	**0.3926**		0.1171	**0.5811**		0.1802
ES	**0.3893**	0.0253		**0.4043**	0.0753	

Table 7. Statistical tests of deviation from the Wright-Fisher standard neutral model for COI in *Boreomysis nobilis*, used to test for population growth. Marginally significant values (*p* between 0.05 and 0.10) are identified by a single asterisk (*), as significant values ($p < 0.05$) are identified by two asterisks (**).

Region	Fu's FS	Fu and Li's F*	Fu and Li's D*	Tajima's D
SF	-17.868	-1.95947	-1.88012*	-1.21477
QK	-3.873	-2.06765*	-2.12155*	-0.97845
ES	-3.741	-2.04926*	-2.12155*	-0.92767

1.3.2 Metabolic phenotypes

CS activity ranged between 0.003 (QK, 40 °C) and 0.032 μmol mg protein^{-1} min^{-1} (SF, 10 °C; Fig. 7). Mean CS response to temperature differed among the three regions tested, as indicated by the presence of a significant interaction between the terms "Region" and "Temperature" (Fig. 7, Table 8). In more detail, mean CS activity was stable when tested between 0 and 25 °C for all analyzed regions and decreased as treatment temperature continued to increase (Fig. 7). Individuals from SF displayed significantly higher mean CS activity when compared to individuals from QK and ES between 0 and 20 °C, but not at 5 °C where no difference was observed among these three regions (Fig. 7). CS activity significantly decreased between 25 and 30 °C and between 30 and 35 °C in all regions. Significant breakpoints were obtained for each region, but no significant difference between regions could be found among them (Table 9).

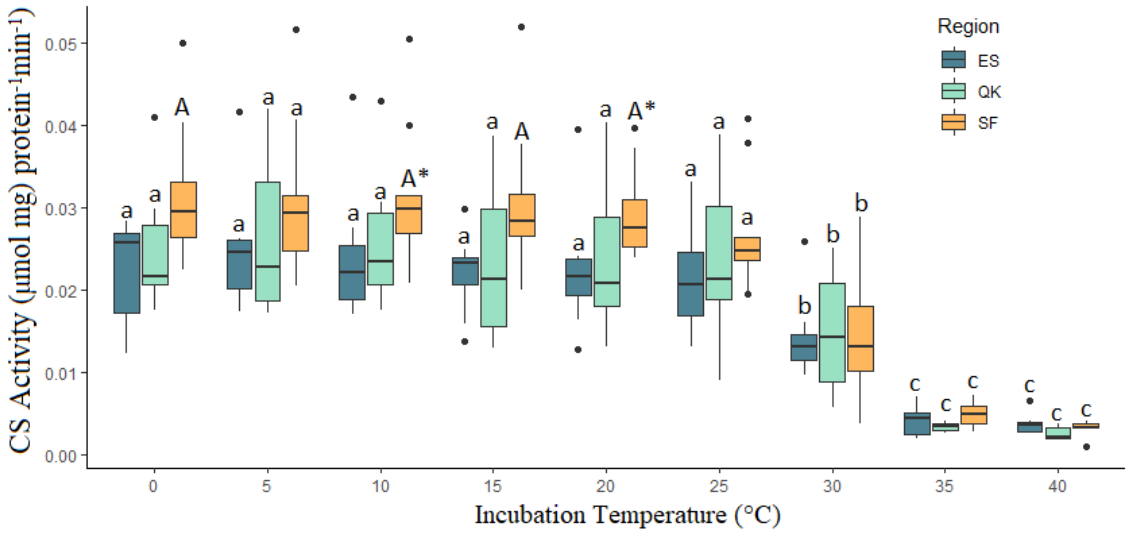

Figure 7. Effect of temperature exposure on mean Citrate Synthase (CS) activity in *Boreomysis nobilis* from Tasiujaq (Eclipse Sound; ES), Qikiqtarjuaq (QK) and Saguenay Fjord (SF). Colored box plots indicate 2nd (lower) and 3rd (upper) quartiles with horizontal lines indicate the median (central). Vertical bars indicate the smallest/highest observation greater than or equal to lower hinge - 1.5 * inter-quartile range and dots show highest/lowest values. Different low case letters indicate significant differences among incubation temperatures within regions, while capital letters show significant differences among regions, within temperatures. Asterisks show marginally significant differences between regions, within temperatures (*p* value between 0.05 and 0.10).

Table 8. Results for the GLM tests performed to investigate the effect of temperature on mean citrate synthase (CS) activity, mean lactate dehydrogenase (LDH) activity and mean CS:LDH ratio in *Boreomysis nobilis* from different regions.

Source	d.f.	M.S.	F	p
CS activity (μmol mg protein^{-1} min^{-1}) [a]				
Region	2	0.0002647	1.6274	0.2203
Temperature	8	0.00227784	140.0663	< 0.0001
Region:Temperature	**16**	**0.00003641**	**2.2386**	**0.0058**
LDH activity (μmol mg protein^{-1} min^{-1}) [b]				
Region	2	82.113	16.202	0.0002
Temperature	7	140.322	27.688	< 0.0001
Region:Temperature	**14**	**67.315**	**13.282**	**< 0.0001**
CS:LDH activity ratio [c]				
Region	2	0.00049135	47.934	< 0.0001
Temperature	1	0.00017061	33.288	0.0003
Region:Temperature	**2**	**0.00010979**	**10.711**	**0.0042**
Residuals	9	0.00004613		

[a] Among nine temperature treatments.
[b] Among eight temperature treatments.
[c] Among five temperature treatments.

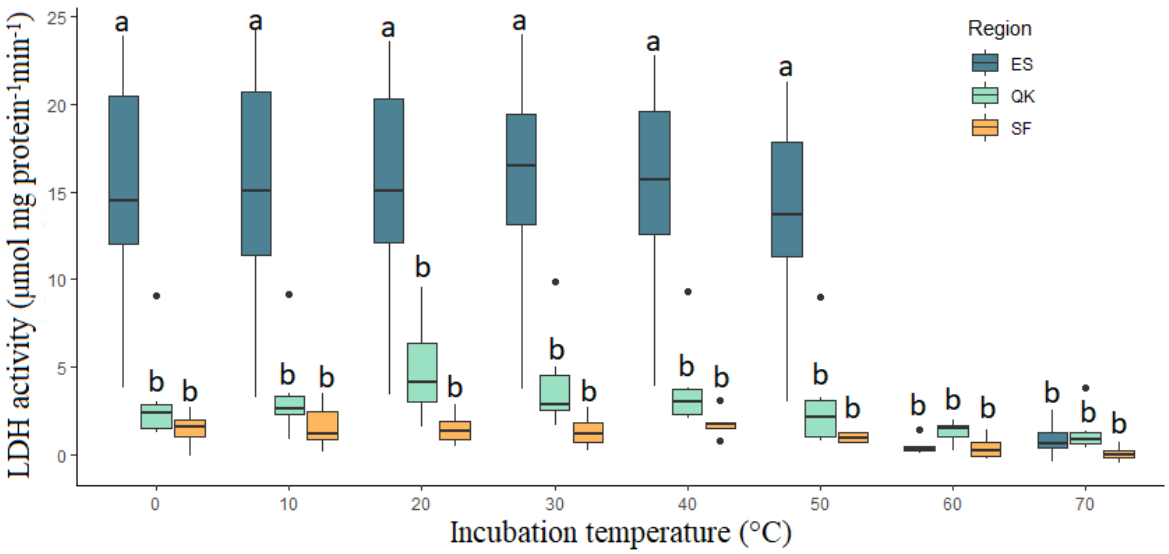

Figure 8. Effect of temperature exposure on mean LDH activity in *Boreomysis nobilis* from Tasiujaq (Eclipse Sound; ES), Qikiqtarjuaq (QK) and Saguenay Fjord (SF). Colored box plots indicate 2nd (lower) and 3rd (upper) quartiles with horizontal lines indicate the median (central). Vertical bars indicate the smallest/highest observation greater than or equal to lower hinge - 1.5 * inter-quartile range and dots show highest/lowest values. Different letters indicate significant differences among treatments.

Table 9. Results for the inferred temperature breakpoint analysis (with standard error; SE) and confidence intervals (CI) for CS, LDH and CS:LDH in *Boreomysis nobilis* from (ES, QK and SF.

	CS			**LDH**			**CS:LDH**		
	ES	**QK**	**SF**	**ES**	**QK**	**SF**	**ES**	**QK**	**SF**
Breakpoint (SE)	23.81 (2.36)	24.23 (2.37)	21.43 (2.44)	43.78 (6.71)	23.16 (4.17)	40.00 (9.77)	23.43 (2.19)	24.23 (2.37)	20 (2.97)
CI	4.63	4.65	4.78	13.15	8.17	19.15	4.29	4.64	5.83
Lower 95% CI limit	19.18	19.58	16.65	30.63	14.99	20.85	19.14	19.59	14.17
Upper 95% CI limit	28.44	28.88	26.21	56.33	31.33	59.15	27.72	28.87	25.83

Mean LDH activity ranged between 0.381 (SF, 60 °C) and 15.532 μmol mg protein^{-1} min^{-1} (ES, 30 °C; Fig. 8). ES displayed the highest mean LDH activity throughout temperature treatments, while SF and QK showed a similar and low mean activity level. This trait responded to temperature differently depending on the region, as indicated by the presence of a significant interaction between the terms "Region" and "Temperature" (Table 8). Mean LDH activity in ES was stable when tested between 0 and 50 °C and decreased between the 50 and 60 °C treatments, whereas SF and QK showed similar responses to the increasing temperature, i.e. a low and stable mean activity rate over the entire temperature range tested (Fig. 8; Table 8). In contrast, mean LDH activity in QK and SF was similar throughout treatments. One significant breakpoint was estimated for each region (Table 9), but no significant differences among break points could be found.

Temperature had a significant effect on mean CS:LDH ratio of individuals from the SF (t = 5.214, p = 0.0008), as indicated by the presence of a significant interaction between the term "Region" and "Temperature", while for individuals from the Arctic regions no significant effect of the exposure to increasing temperature was observed (Table 8). Furthermore, CS:LDH ratios were significantly different among regions, with this trait being significantly higher in SF when compared to those measured in the Arctic regions (Table 11). Finally, SF displayed a significantly negative trend of CS:LDH activity ratio in relation to an increase of incubation temperature, but no significant relationship was found for QK and ES (Fig. 9).

Table 10. Results for the ANCOVA test on the effect of incubation temperature (°C) and CS:LDH activity ratio of *Boreomysis nobilis* for each analyzed region. Asterisks indicates level of significance.

	DF	**Sum. Sq.**	**Mean Sq.**	***F***	***p***
Temperature	4	0.00019184	4.7959e-05	2.8485	0.0970
Region	2	0.00049135	2.4567e-04	14.5916	0.0021 **
Residuals	8	0.00013469	1.6837e-05		

Table 11. Results for the post-hoc Estimated Marginal Means (EMM) on the effect of temperature on contrasts between analyzed regions for mean CS:LDH activity ratios in *Boreomysis nobilis*. Asterisks indicates level of significance (*$p < 0.05$, ** $p < 0.01$).

Contrast	Estimate	S.E.	DF	*t* ratio	*p*
ES - QK	-0.00359	0.0026	8	-1.383	0.3934
ES - SF	-0.01353	0.0026	8	-5.214	0.0021 **
QK - SF	-0.00994	0.0026	8	-3.831	0.0124 *

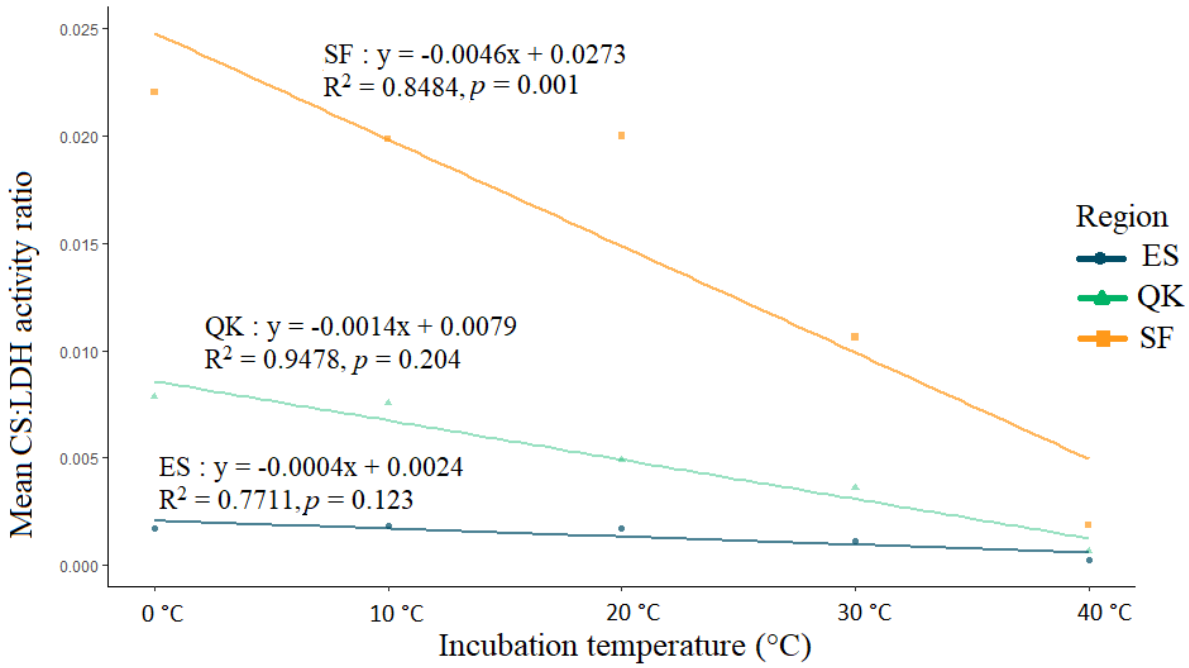

Figure 9. Relationship between incubation temperature (°C) and mean CS:LDH activity ratio of *Boreomysis nobilis* in the different regions. Points represent mean CS:LDH ratio and continued lines represent linear regressions fitted for each region, with the corresponding equation and R^2 above each regression line.

1.4 DISCUSSION

Here we present the first empiric evidence supporting the idea that the opossum shrimp *Boremysis nobilis* from the SF region could be considered a glacial relict population. Our study shows that *B. nobilis* from the SF region present characteristics of a glacial relict in terms of differentiation of its genetic structure and diversity, as well as signs of long-term acclimatization/adaptation of their metabolic phenotypes to temperature when compared to *B. nobilis* from the Arctic.

1.4.1 Genetic structure

In the present study, the level of genetic distance shown by individuals of *B. nobilis* from different regions is generally low. The two Arctic regions, QK and ES, do not show a significant differentiation, so that they can be considered a panmictic population. However, genetic distances among the SF region and Arctic regions are four times higher than the ones observed between the two Arctic regions. Moderately high genetic distance has already been observed between two species of opossum shrimps (*Mysis oculata* Fabricius, 1780 and *M. litoralis* Banner, 1948) and was attributed to ancient population bottleneck effects of species with a low dispersal capacity (Väinölä, 1992). Thus, one could argue that the observed genetic structure in *B. nobilis* could have resulted from a founder effect or a bottleneck effect in the SF and/or the Arctic regions after the Last Glacial Maximum (LGM). These would have reduced the genetic diversity in these regions. A recent founder effect, which is described as the weakening of genetic structure induced by the establishment of a new population by a very limited number of individuals (Mayr, 1978), and a subsequent expansion event, which could explain their genetic diversity is however unlikely in the present study. *B. nobilis* from the SF region displays higher nucleotide and haplotype diversity then what is observed in the two Arctic regions. However, no population expansion event isfound in the recent history, so that a founder effect (due to establishment of the

population after the LGM) or a bottleneck effect becomes unlikely to explain the differentiation between SF and the Arctic regions. It is possible that bottleneck effects occurred long enough ago for *B. nobilis* to stabilize. The two Arctic regions show very similar haplotype frequencies, and no significant differentiation is found in terms of genetic distance nor diversity, suggesting a panmictic population.

Differences in genetic diversity might be related to genetic drift in the three regions. Genetic drift can result in the marked genetic divergence observed in SF region compared to the Arctic regions, since this phenomenon would be much more frequent in rear-edge populations than in individuals at the centre of its distribution, such as in glacial relicts (Vucetich & Waite, 2003). Thus, the marked difference between haplotype frequencies in *B. nobilis* from the SF region compared to QK and ES regions could indicate that genetic drift occurred in SF, or that the individuals underwent a selective pressure (Hochachka & Somero, 2002). Since the conditions at the margins of a species distribution are often less favourable, rear-edge populations are generally known to be genetically less diverse (Lawton, 1993; Vucetich & Waite, 2003). However, this is not the case for *B. nobilis* from the SF region, which shows significantly higher nucleotide and haplotype diversity for both analysed genes.

Post-glacial marine water submersion of the SF took place around 10 000 yr BP (De Vernal et al., 2011; Dionne & Occhietti, 1996; Dyke & Prest, 1987; Dyke et al., 2002). Colonization of the SF by *B. nobilis* have occurred subsequently and could therefore only be undertaken since that time at the earliest. Similar results were also observed in the coastal populations of other opossum shrimps, namely *Neomysis americana* S. I. Smith, 1873 in the SLE and the American East coast, and *N. integer* Leach, 1824 in Northeast-Atlantic (Remerie et al., 2009, Cortial et al., 2019). *N. americana* was determined as a cryptic species in the SLE and presented a genetic structure which could result from the species being segregated in different glacial refugia throughout the LGM (Cortial et al. 2019). This phenomenon is known to have shaped the phylogeography of many opossum shrimps around the globe, particularly in the Pleistocene era (Audzijonyte and Väinölä, 2006; Audzijonyte et al., 2006;

Remerie et al., 2009). Remerie et al. (2009) attributed the occurrence of this pattern to the probable presence of a glacial refuge where the Southern populations of *N. integer* could have survived the LGM. Pflaumann et al. (2003) also suggested that at least one glacial refuge was present east of the Laurentian channel during the LGM. Thus, specimens from the SF region could have originated from a southerly glacial refuge and settle in their actual environment when the St. Lawrence drainage basin, and eventually the SF, would spare their ice coverage. *B. nobilis* from the Arctic regions could therefore have survived the LGM in the ice-free waters of Baffin Bay and then colonized the regions investigated in this study. Indeed, the waters of Baffin Bay in southeast Greenland were suggested as a glacial refuge, enabling species of seaweeds to develop high genetic diversity through contact-isolation episodes (Bringloe et al., 2020). Moreover, the haplotype pattern consisting in the predominance of shared COI haplotypes in the northernmost regions, as opposed to the elevated number of private haplotypes in the south is consistent with pre-LGM panmixia followed by post-glacial geographic isolation (Maggs et al., 2008).

Furthermore, *B. nobilis* appears to have a highly limited dispersal capacity, notably attributed to their distribution in the water column, their reproduction mode and the lack of nycthemeral migration (Mauchline, 1980; Clark & Threlfall, 1993). Species with low dispersal capacities are expected to show marked signs of the impacts of the LGM within their genetic structure (Hewitt, 2000). On the other hand, some Arctic water flea species belonging to the *Daphnia pulex* complex, which possess high dispersal ability, have high mtDNA diversity, in addition to a distinct phylogeographic subdivision (Weider et al., 1999). Despite their limited dispersal capacity, specimens of *B. nobilis* from the Arctic regions do not appear to be restricted to a specific stratum of water, nor to occur in an environment restricting dispersion capacities due to the presence of physical barriers to dispersal such as the fjords' sills, as found in the SF and some Newfoundland's fjords (Clark & Threlfall, 1993). Indeed, individuals were collected in the open waters of Baffin Bay, at a few hundred meters from the bottom, as reported in Table 1. Connectivity between analysed Arctic regions is thus probably higher as compared to the SF region. This can be attributed to the Labrador current, which creates favourable environmental conditions and to

a bathymetry which does not present any major physical barrier to be overcome by individuals, that can therefore disperse freely. Increased connectivity between individuals in the Arctic regions could result in greater homogeneity, which could explain the observed lower haplotypic diversity and dominance of shared haplotypes.

Some authors estimated the divergence time of other mysid species. Populations with a low dispersal capacity, such as the glacial relict *Mysis salemaai* (Audzijonyte & Väinölä, 2005) showed small population size and fast post-glacial COI divergence rate of 0.27 % per 10 000 yr. (Audzijonyte & Väinölä, 2006). If *B. nobilis* from the different regions analyzed in the present study had survived the LGM within the same glacial refuge, the observed divergence (0.59 % over 10 000 yr.) would have been about twice as high as that estimated in Audzijonyte & Väinölä's study, which is highly unlikely, when compared to other crustacean species (Knowlton & Weigt 1998, Winkler et al. 2011, Cortial et al. 2019). Thus, a slower divergence rates of 0.14 % *per* 10 000 yr, calibrated from the divergence of sister species of the snapping shrimp *Alpheus*, (Knowlton & Weigt 1998), would place the moment where divergence of the SF and Arctic regions occurred even further back in time.

1.4.2 Metabolic phenotype

In addition to show the highest genetic distances and diversity, specimens from the SF region exhibit a mean citrate synthase (CS) activity that is different than its Arctic counterparts, as indicated by the singular patterns of CS activity in relation to a temperature increase. However, SF and QK show a similar LDH metabolic phenotype, while ES shows higher mean LDH activity. In addition, a significantly higher CS:LDH ratio in SF as compared to the Arctic regions is also observed, which suggests local metabolic phenotypes and reflects the trends observed in genetic structure.

The effects of temperature on enzyme activity is well documented (Arrhenius, 1915; Haldane, 1931; Kavanau, 1950; Hochachka & Somero, 1973, 2002). For a species to persist in a changing environment, it is critical for all life stages to cope with and thrive under the

local physical and chemical conditions. Within this context, an organisms' tolerance range can be described as the thermal tolerance polygon, according to which the size of the polygon corresponds to the organisms' thermal window of tolerance (Brett 1952, 1956; Brett & Groves, 1979; Eme & Bennett, 2009). Within this window, an organism has a surplus of energy to allocate to somatic and reproductive growth. However, when the organism gets closer to its tolerance extremes, its growth performance decreases and it must adjust through plasticity (for example by adjusting physiological processes; Somero & Hochachka, 1971; Somero, 2004; Byrne, 2012) or move to a more favorable environment (*fight or flight;* Cannon, 1915). For example, even a slight change in temperature can alter enzymatic activity by inducing a change in an enzyme's activity rate, or by inducing a modification of the molecular bonds ensuring the integrity of the enzyme's tridimensional structure (Hochachka & Somero, 1973). Thus, cold-acclimated individuals must display higher level of thermosensitivity than warm-acclimated ones under increasing temperature. Indeed, in order for a cold-acclimated protein to maintain its three-dimensional structure and function, a lower number of hydrogen or disulfuric bounds is needed to stabilize the secondary, tertiary and quaternary structure of proteins, while ensuring its functionality (Hochachka & Somero, 1973; Dougherty, 1998). On the contrary, warm-acclimated proteins display higher number of chemical bounds between the proteins' subunits. This allows a lower level of fluidity for protein structure, to remain stable at higher temperature. Enzymes' thermosensitivity should thus be lower (i.e., enzymes maintain integrity at higher temperature) in warm-acclimated individuals when compared to cold-acclimated ones, since the energy required to break the biochemical bounds is higher (Stillman & Somero, 1996; Bennett et al., 2021). Our results suggest that individuals from the FS region demonstrate temperature acclimation of their aerobic metabolism, as evidenced by the fact that they show a higher mean CS activity rate. On the other hand, individuals from the ES region seem to demonstrate a certain level of temperature acclimation of their anaerobic metabolism, as highlighted by the fact that they possess a significantly higher mean LDH activity rate. Individuals from the QK region share similarities with those from the FS region in terms of aerobic metabolic response to temperature, measured as mean CS activity rate, whereas these similarities are higher

between individuals from the QK and ES regions in terms of mean anaerobic metabolic activity, measured as mean LDH activity rate. Furthermore, individuals from the SF region demonstrate a significantly different metabolic phenotype from its Arctic counterparts, as suggested by their higher CS:LDH ratio when compared to the latter.

CS is generally known as the limiting enzyme of the Tricarboxylic Acid cycle (TCA), and thus, aerobic energy metabolism, since it catalyses the first reaction of the cycle (Suissa et al., 1984). Changes in the catalytic capacity of CS can thus have tremendous impacts on the performance and survival of an organism. Our results suggest that the metabolic phenotype of specimens from the SF region may be long-term acclimatized (or adapted) to warmer waters. However, a different trend is observed for mean LDH activity, which is consistently higher in ES when compared to QK and SF. LDH activity is an indicator of anaerobic energy metabolism, since it is a glycolytic enzyme that catalyses the interconversion of pyruvate and lactate (Dunn et al., 1991; Hochachka & Somero, 2002). It has been historically studied for the characterisation of thermal adaptation (Hochachka, 1965; Dunn et al., 1991; Hochachka & Somero, 2002 and references therein). For example, when tested at the identical temperature, LDH extracted from brain tissues of cold-adapted ectothermic notothenioid fishes displayed an activity rate three times higher than that of warm-adapted tropical fishes (Kawall et al., 2002). In addition, a clear latitudinal segregation of LDH allozymes along the Atlantic coast in the minnow *Fundulus heteroclitus* Linnaeus, 1766 was documented, indicating that temperature must be a driver of local adaptation of this enzyme (Place & Powers, 1984). Hence, LDH activity trend in specimens from the ES region show evidence for cold-acclimated metabolism. However, this is not observed in individuals from the QK region, which instead display mean LDH activity comparable to those observed in SF. Specimens from the QK region thus seem to display an intermediate metabolic phenotype, since their mean CS activity is comparable to that of specimens from the northernmost region (i.e., ES), whilst LDH activity is comparable to that of specimens from the southernmost region (i.e., SF).

We assessed the metabolic pathways' phenotypes (i.e., aerobic v/s anaerobic) by comparing the enzymatic ratios between mean CS and LDH activity rate among regions. These results can be explained by the fact that CS is bound to the inner mitochondria membrane, whilst mitochondrial LDH is located in the matrix (Eventoff et al., 1977; Wiegand & Remingtonl, 1986). The ratio between CS and LDH is used as a proxy for mitochondria density: i.e., a higher CS:LDH ratio means denser mitochondria, thus higher aerobic capacities. The highest mean CS:LDH ratio we report is that for specimens from the SF region throughout the gradient of temperatures tested here. Individuals from this region should thus have a higher aerobic metabolism capacity. However, it has been shown in past studies that LDH is not only found in mitochondria matrix, but also in the cytosol of a variety of cells (Rasmussen et al., 2002). It is thus not possible to assess the aerobic metabolism capacity of the analysed regions using the CS:LDH ratio exclusively.

In general, organisms use a large range of other metabolic adaptation strategies to respond and buffer changes in temperature to maintain a stable homeostasis. This can be achieved on the short term by increasing its metabolic rates (measured as oxygen uptake - Schulte, 2015), or adjusting the abundance of an enzyme to increase its overall catalytic capacity. On the other hand, long-term metabolic plasticity can include a shift among different isozymes (i.e., enzymes with identical function but different structure) to ensure a more efficient catalytic activity under new environmental conditions (Hochachka & Somero, 1973). Furthermore, adjustments in the cholesterol and saturated lipid levels in the mitochondrion's membrane can enable an organism to maintain an appropriate membrane fluidity in response to a change in environmental temperature (Hazel, 1995). The use of such proxies should be evaluated in subsequent work to provide more nuance to the results of this study.

To further discuss the findings of our study, other proxies, namely adjustment of cellular membrane lipid composition, catalytic capacity and abundance of certain enzymes should be investigated in future works. Also, a characterisation of the metabolic phenotypic response of multiple life stages should be investigated, since different ontogenic stages

display different sensitivities to these conditions. A bottleneck effect can thus be observed at certain critical stages of development in species with complex life cycles (Small et al., 2015; Noisette et al., 2021). Future work should also investigate this further, using respirometry on multiple life-stages following common garden experiments over multiple generations. However, with an estimated generation time of 2 yr. (Clark & Threlfall, 1993), such work would be challenging.

In conclusion, we present genetic and metabolic evidence supporting the hypothesis that *B. nobilis* from the SF region is a glacial relict and more precisely a rear-edge relict population. Genetic diversity is higher in the SF region as compared to the Arctic regions investigated, along with significant differences in pairwise genetic differentiation. Individuals from the SF region also showed different haplotype frequencies when compared with the Arctic regions, suggesting that populations found in these regions would have passed through the LGM in separate glacial refugia, and then colonized the waters in which they are currently found. This hypothesis appears to be partially supported in physiological terms, as individuals from the SF and ES regions exhibited different metabolic phenotypes when compared to individuals from the QK region. Individuals from this region could thus be considered as intermediate between SF and ES. Our study confirms the relevance of integrating the investigation of genetic relationship with the characterisation of physiological long-term acclimatization/adaptation through *in vitro* proxies to describe the thermal responses of poorly documented species. Understanding the metabolic effects of temperature throughout life history will be of capital importance in future studies predicting the impacts of global climate change on their adaptation capacity.

CONCLUSION GÉNÉRALE

La présente étude a permis de relever les premières données empiriques proposant les individus de *B. nobilis* présents dans le FS en tant que population relique glaciaire. De plus, les travaux entrepris ont permis de séquencer un fragment de deux gènes mitochondriaux, ce qui n'avait jamais été entrepris auparavant. Cette étude a également permis de mettre en valeur l'importance de l'utilisation d'outils d'analyse *in vitro* afin de mettre en lumière le potentiel d'acclimatation du métabolisme à la température. Ceci a pu être réalisé à l'aide de mesures comparatives de l'activité de deux enzymes clés dans le cadre d'études portant sur des espèces ayant fait l'objet de peu ou pas d'études, sur lesquelles aucune donnée génétique ou physiologique n'est disponible. L'utilisation de ces outils permet donc de développer un portrait global de la structure génétique et de la physiologie adaptative d'une espèce méconnue.

STRUCTURE ET DIVERSITE GENETIQUE

L'analyse de la structure et de la diversité génétique de deux gènes mitochondriaux a permis de révéler que *B. nobilis* de la région du FS présentait une différenciation génétique supérieure comparativement à ceux retrouvés au sein des régions arctiques analysées, ainsi qu'une composition génétique significativement différente à ces dernières. L'analyse de la structure et de la diversité génétique des individus de *B. nobilis* provenant des différentes régions analysées permet également d'énoncer des hypothèses par rapport à la colonisation post-glaciaire ayant pris place à partir de la fin du Pléistocène. En effet, la structure génétique observée tend à démontrer que *B. nobilis* retrouvés dans les régions arctiques ainsi que dans le FS auraient été en contact avant le LGM. Ils auraient toutefois potentiellement traversé cette période dans des refuges glaciaires séparés, soit au large du chenal Laurentien pour les

individus de la région du FS, et dans la baie de Baffin pour *B. nobilis* des régions arctiques analysées, puisque celle-ci est demeurée libre de glace tout au long du LGM.

PHENOTYPE METABOLIQUE

Les individus de la région du FS étaient caractérisés par une activité enzymatique relative de la CS plus élevée, ainsi qu'une courbe de ratio CS : LDH en fonction de la température plus grande que celle observée chez les individus provenant des régions arctiques analysées. Cette tendance n'a toutefois pas été observée avec la LDH, pour laquelle les individus de la région d'ES présentaient une activité moyenne significativement plus élevée que les individus des régions du FS et de QK. De plus, les individus provenant de la région du FS semblaient démontrer un phénotype métabolique associé à une acclimatation à des températures plus élevées que ses contreparties arctiques. Les individus de la région la plus nordique, ES, a d'ailleurs démontré une activité de la LDH plus élevée, probablement associée à la production des isozymes plus adaptés aux températures froides. En effet, la baie de Baffin comporte deux courants majeurs qui composent une circulation cyclonique dans la région, soit les eaux du courant de l'ouest du Groenland, légèrement plus chaudes et salées qui circulent en direction nord-ouest, et celles du courant de Baffin, froides et moins salées, en direction sud (Gratton et al. 2006). Ces dernières affluent vers la baie de Baffin en provenance notamment de Lancaster Sound, où se trouve la région de Tasiujaq (ES). Peu d'études ont permis d'établir des séries temporelles de température dans la région, mais les données disponibles situent généralement ces températures sous la barre de 0 °C (Gratton et al. 2006). Dans le FS, ces températures varient selon les saisons entre 1 et 3,5°C (Belzile et al., 2016 ; Galbraith & Bourgault, 2018). Ces résultats mettent en lumière le potentiel des populations isolées, comme les reliques glaciaires, d'ajuster ou adapter leur métabolisme à l'aide de diverses stratégies afin de se maintenir et de prospérer dans un nouvel environnement thermique.

LIMITES DE L'ETUDE ET PERSPECTIVES

Cette étude a permis de caractériser davantage une espèce peu connue, soit *B. nobilis*, et ce, sur une grande étendue latitudinale. Les analyses entreprises comportaient de nombreuses limitations, notamment liées à l'échantillonnage et à l'induction de biais attribuables par les méthodes d'analyses. En effet, l'espèce, dont aucune donnée génétique ou physiologique n'était disponible jusqu'à présent, ne pouvait pas être maintenue en conditions de laboratoire afin de procéder à des analyses physiologiques poussées, telles les analyses en jardin commun (*common garden*) et de respirométrie, sans induire un biais attribué à un stress physiologique impossible à quantifier. Ces études auraient permis de préciser si les différences génétiques et physiologiques observées étaient attribuables à des adaptations à la température, ou encore s'il s'agissait d'acclimatation. Également, l'absence de données génétiques sur l'espèce nous a amenés à devoir élaborer des amorces en suivant le principe d'essai/erreur afin de pouvoir mener nos analyses sur la structure et la diversité génétique de l'espèce. Des travaux complémentaires soutenus devraient également inclure l'analyse de l'expression des gènes liés, entre autres, aux isozymes propres à chaque région, ainsi qu'une caractérisation du phénotype métabolique de plusieurs stades de vie, puisque les différents stades ontogéniques présentent souvent des sensibilités différentes aux conditions de température. De plus, l'élargissement de l'aire d'étude afin d'englober l'ensemble des régions où l'espèce est présente, notamment dans les fjords de Terre-Neuve et les autres régions arctiques où l'espèce a été historiquement répertoriée, permettrait d'avoir un portrait plus juste de la structure génétique de l'espèce, en plus d'apporter davantage de robustesse dans les analyses du phénotype métabolique. Le fait de souligner les effets de la température sur le métabolisme énergétique d'une espèce tout au long de son cycle de vie est d'une importance capitale pour prédire les impacts du changement climatique global sur sa capacité d'acclimatation à la température. De manière plus générale, ce projet a permis d'apporter davantage de connaissance sur l'écologie des mysidacés, d'émettre des hypothèses quant à leur histoire pré- et post-glaciaire ainsi que de détailler les possibilités en termes d'acclimatation métabolique à la température pour une espèce peu étudiée.

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