UNIVERSITÉ DU QUÉBEC À RIMOUSKI

DÉTERMINANTS PHYSIOLOGIQUES DE LA TOLÉRANCE AUX EXTRÊMES DE TEMPÉRATURE CHEZ LE MICROCRUSTACÉ *DAPHNIA PULEX*

MÉMOIRE PRÉSENTÉ

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Avertissement

« La connaissance scientifique, telle qu'élaborée par la modernité, réduit, quantifie, convertit en faits et en lois, ce qui était l'instant d'avant un être plein de vie et de mystère ».

- Estelle Zhong Mengual

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RÉSUMÉ

Chaque organisme vivant est caractérisé par des seuils de température au-delà desquels les fonctions vitales sont perturbées puis réduites à néant. D'un côté, ces seuils sont modelés par l'environnement à travers les processus d'acclimatation et d'adaptation. De l'autre, ils déterminent la niche écologique des espèces et la distribution des populations. Les seuils inférieurs et supérieurs de tolérance thermique sont ultimement liés à la sensibilité de certains processus physiologiques clés face aux changements de température. La compréhension de ces mécanismes permettra de mieux comprendre comment la température affecte la distribution des êtres-vivants et leur capacité d'adaptation dans des environnements changeants. Ce mémoire explore les fondements physiologiques des seuils de tolérance thermique inférieurs et supérieurs chez les ectothermes et comment ceux-ci peuvent affecter la distribution des individus. Le modèle d'étude utilisé est le complexe nord-américain *Daphnia pulex* qui est composé de clones d'origines et de ploïdies variées.

Le premier article a pour but de vérifier si la prépondérance des polyploïdes en hautes latitudes et la distribution des clones est expliquée par la tolérance au froid, mesurée par la méthode du CTmin. Il explore le lien entre la tolérance au froid et deux traits affectés par la ploïdie ayant une influence majeure sur la physiologie des organismes : la taille corporelle et la taille cellulaire. Les résultats ont montré que la polyploïdie permet une meilleure résistance au froid. Les tailles corporelles et cellulaires n'étaient pas liées au CTmin. Les résultats suggèrent que la présence des triploïdes en régions nordiques est en partie associée à une meilleure capacité à résister au froid mais que des traits autres que la taille des cellules expliquent cela. Des différences dans l'expression de certains gènes ou dans la composition lipidique pourraient en être la cause.

Le second article a pour but d'explorer le rôle de la composition en acides gras des membranes cellulaires sur la tolérance à la chaleur, mesurée par la méthode du CTmax. Les résultats ont montré que les propriétés membranaires générales telles que les indices d'insaturation et de peroxydation n'étaient pas liées à tolérance à la chaleur. En revanche, la proportion en acide eicosapentaénoïque était corrélée négativement au CTmax. Nous avons montré pour la première fois que les daphnies polyploïdes élevées à haute température suraccumulaient les acides gras omega-6.

Mots clés : CTmax, CTmin, polyploïdie, tolérance thermique, acides gras, taille cellulaire.

ABSTRACT

Every living organism is characterized by temperature thresholds beyond which its vital functions are disrupted and then reduced to nothing. On the one hand, these thresholds are shaped by the environment through the processes of acclimatization and adaptation. On the other, they determine the ecological niche of species and the distribution of populations. Lower and upper thermal tolerance thresholds are ultimately linked to the thermal sensitivity of certain key physiological processes. Understanding these mechanisms will provide a better understanding of how temperature affects the distribution of living organisms and their ability to adapt to changing environments. This thesis explores the physiological bases of lower and upper thermal tolerance thresholds in ectotherms and how these may affect the distribution of individuals. The study model used is the North American *Daphnia pulex* complex, which is composed of clones of varying origins and ploidy.

The first article aims to verify whether the preponderance of polyploids at high latitudes and the distribution of clones is explained by cold tolerance, measured by the CTmin method. It explores the link between cold tolerance and two traits affected by ploidy that have a major influence on the physiology of organisms : body size and cell size. The results showed that polyploidy improves cold tolerance. Body and cell size were not related to CTmin. The results suggest that the presence of triploids in northern regions could be partly due to a better ability to withstand cold temperatures, but that traits other than cell size explain that. Differences in the expression of certain genes or in lipid composition could be the cause.

The second article aims to explore the role of the fatty acid composition of cell membranes on heat tolerance, as measured by the CTmax method. The results showed that general membrane properties such as unsaturation and peroxidation indices were not related to heat tolerance. However, the proportion of eicosapentaenoic acid was negatively correlated with CTmax. We showed for the first time that polyploid *Daphnia* grown at high temperature over-accumulated omega-6 fatty acids.

Keywords : CTmax, CTmin, polyploidy, thermal tolerance, fatty acids, cell size.

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INTRODUCTION GÉNÉRALE

Comment les extrêmes thermiques affectent les populations d'ectothermes

Les extrêmes climatiques ont des effets à tous les niveaux d'organisation du monde vivant (Bellard et al., 2012; Parmesan, 2006). Les organismes ectothermes représentent la majorité de la biomasse terrestre et environ 99% des espèces connues (Ohlberger, 2013). Par définition, ce sont des organismes qui ne régulent pas leur température corporelle. Celle-ci suit donc celle de leur environnement, notamment chez les ectothermes aquatiques. Ils sont donc particulièrement sensibles aux changements de température. Au vu de leur importance, ce mémoire s'attachera à l'étude des organismes ectothermes et à leur sensibilité face aux extrêmes thermiques.

Les extrêmes thermiques ont des impacts subléthaux et léthaux chez les populations d'ectothermes du monde entier. Une analyse des causes des événements de mortalité de masse dans plusieurs milliers de populations animales indique que le nombre de mortalités dues à des températures froides a diminué au cours des dernières décennies alors que le nombre de mortalités dues à des températures élevées a augmenté (Fey et al., 2015). L'effet des chocs thermiques (canicules et gels) est bien documenté chez les espèces végétales de grandes cultures chez lesquelles ils réduisent les rendements, fragilisant les sécurités alimentaires humaines (Vautard et al., 2023; Vogel et al., 2019; Barlow et al., 2015; Zheng et al., 2012). Les vagues de chaleur extrêmes, parfois en combinaison avec d'autres facteurs comme la sécheresse (milieu terrestre) ou l'hypoxie (milieu aquatique), causent des mortalités de masse chez de nombreux groupes animaux (coraux : Leggat et al. (2019) ; spongiaires : Ereskovsky et al. (2019) ; poissons : Cheung and Frölicher (2020)). Les vagues de froid entraînent parfois des mortalités à grande échelle comme chez les poissons et notamment chez les espèces sténothermes des milieux tropicaux (Szekeres et al., 2016). Les principaux effets subléthaux des extrêmes thermiques sont une réduction de la reproduction et/ou de la

croissance à cause de changements d'allocation du budget énergétique vers les mécanismes physiologiques permettant la survie pendant le stress thermique. Ainsi, bien qu'un extrême thermique puisse ne pas causer de diminution de l'abondance des populations de manière directe, il peut en engendrer de manière indirecte à travers les effets sur la reproduction et la croissance (Breedveld et al., 2023; Siegle et al., 2022; Carey et al., 2021; Adamczuk, 2020).

Appréhender le futur des ectothermes grâce à la physiologie

Plusieurs questions fondamentales ont émergé du constat de la sensibilité des ectothermes face aux extrêmes thermiques. Les populations d'ectothermes persisteront-elles avec une occurrence accrue d'extrêmes thermiques ? Comment leur distribution et dynamiques de populations seront affectées ? Une riche littérature sur le rôle de la plasticité dans la tolérance à de nouvelles conditions environnementales et sur le potentiel évolutif des limites de tolérance thermiques des organismes est née. D'abord confinées à l'étude des effets d'une augmentation graduelle des températures, les études suivantes ont commencé à intégrer la variabilité climatique et les extrêmes de température dans leurs analyses (Buckley and Kingsolver, 2021; Vasseur et al., 2014; Bellard et al., 2012).

La plasticité est la capacité d'un génotype à produire plusieurs phénotypes en réponse à des changements dans les conditions environnementales. Elle peut inclure des changements morphologiques, physiologiques ou comportementaux (Donelson et al., 2019; Pigliucci, 2001). L'adaptation inclut un changement dans les fréquences alléliques au sein des populations. L'adaptation peut être rapide et survenir en quelques générations si le trait sélectionné est suffisamment variable dans la population, qu'il est héritable et que la sélection sur ce trait est suffisamment forte (Catullo et al., 2019; Yousey et al., 2018; Geerts et al., 2015; Barrett et al., 2011). La plasticité et l'adaptation sont vues comme des mécanismes importants pour aider les espèces à faire face aux changements environnementaux mais des limites physiologiques semblent exister (Bennett et al., 2021; Gunderson and Stillman, 2015; Hoffmann et al.,

2013). Une meilleure compréhension des traits qui influencent la performance des organismes face aux températures extrêmes aidera à évaluer quels traits sont soumis à la sélection, dans quelles conditions environnementales ils le sont, s'ils sont corrélés à d'autres traits et dans quelle mesure ils sont plastiques et/ou héritables.

À travers son influence sur la vitesse des réactions chimiques, la température du milieu influence par effet de cascade la capacité de respiration, de locomotion, d'alimentation ou de croissance des ectothermes (Angilletta and Angilletta Jr, 2009; Hochachka and Somero, 2002). Face aux changements de température, ceux-ci ajustent leur comportement ou leurs paramètres physiologiques afin de maintenir leur homéostasie (Soyano and Mushirobira, 2018; Abram et al., 2017). Malgré cette capacité d'ajustement, le dépassement de seuils critiques de température peut mener à de graves dysfonctions physiologiques et à la mort de l'individu. Ce travail s'intéresse à certains aspects clés de la tolérance aux extrêmes de température pour comprendre les mécanismes qui la contraignent.

Taille et tolérance thermique

Liens entre taille et température du milieu chez les ectothermes

Des liens entre la taille corporelle des ectothermes et la température de leur milieu ont été notées depuis longtemps et plusieurs règles clé en ont été déduites : la règle de Bergmann, la règle de James et la règle température-taille. La règle de Bergmann décrit comment chez des espèces ectothermes phylogénétiquement proches, les espèces de petite taille sont associées à des environnements chauds alors que les espèces de grande taille sont associées aux environnements froids (Bergmann, 1847; Vinarskiĭ, 2013). La règle de James est similaire mais s'applique aux différentes populations des mêmes espèces (James, 1970). La règle température taille (temperature size rule) décrit comment un développement à température élevée entraîne une vitesse de croissance plus rapide et une taille à maturité plus petite, tandis qu'à plus basse température la croissance est plus lente, plus longue et engendre des indivi-

dus de plus grande taille (Angilletta et al., 2004; Sibly and Atkinson, 1994). Malgré plusieurs exceptions (Atkinson, 1995), cette règle prévaut chez une majorité d'espèces ectothermes eucaryotes ou procaryotes de milieux terrestres ou aquatiques (Angilletta et al., 2004). La réduction de la taille des ectothermes aquatiques a été nommée comme l'une des réponses universelles aux changements climatiques, en plus des changements de distribution des espèces et des changements phénologiques (Daufresne et al., 2009).

La taille corporelle est une fonction du nombre et de la taille des cellules d'un organisme, si bien que chez les espèces dont les individus grandissent par l'accroissement du volume cellulaire, on observe des relations positives entre la taille des cellules et la taille corporelle (Beaudreau et al., 2021). La taille d'une cellule est en partie dictée par la taille du noyau, lui-même influencé par la taille du génome (Gregory, 2001; Cavalier-Smith, 1978; Szarski, 1970). Les règles qui lient température et taille décrites ci-dessus trouvent un parallèle avec la taille des génomes : chez plusieurs taxons végétaux ou animaux, on retrouve les espèces à large génome dans des environnements froids de hautes latitudes (Lorch et al., 2016; Hessen et al., 2013; Dufresne and Jeffery, 2011; Arendt, 2007).

Taille et seuils de tolérance thermique

À la lumière des patrons entre taille corporelle, taille cellulaire et température du milieu, peut-on établir des liens entre la taille et les seuils de tolérance thermique ? Leiva et al. (2019) ont comparé les seuils de tolérance thermique minimaux et maximaux de 500 espèces d'ectothermes et les ont liés à la taille corporelle, à la taille du génome (utilisé comme indicateur de la taille des cellules) et aux modes de respiration (terrestre et aquatique) tout en prenant en compte les relations phylogénétiques entre les différentes espèces. Ils montrent que les limites de tolérance thermique sont bel et bien liées à la taille corporelle et cellulaire mais que les relations diffèrent selon les espèces et les modes de respiration. La durée du stress thermique a une influence importante sur les relations entre la taille et les seuils de tolérance thermique (Peralta-Maraver and Rezende, 2020; Leiva et al., 2019).

De manière générale, l'augmentation de la taille corporelle chez les ectothermes s'accompagne d'une moins grande tolérance aux températures élevées et ce de manière prononcée chez les ectothermes aquatiques (Peralta-Maraver and Rezende, 2020; Leiva et al., 2019). Ces résultats sont cohérents avec les effets de la règle température-taille. L'effet de la taille corporelle sur la tolérance au froid peut être très variable selon les espèces ou individus d'une même espèce avec des effets positifs, négatifs ou nuls répertoriés (Gonzalez et al., 2022; Monsimet et al., 2021; Baudier and O'Donnell, 2018; Di Santo and Lobel, 2017; Oyen et al., 2016).

Comme pour la taille corporelle, la taille des cellules semble corrélée négativement à la tolérance aux températures élevées chez les ectothermes aquatiques (Verspagen et al., 2020; Bernier, 2020; Leiva et al., 2019). Peu d'études se sont penchées sur les liens entre la tolérance au froid et la taille des cellules chez les ectothermes. Leiva et al. (2019) ont démontré que la tolérance au froid augmentait avec la taille du génome chez les ectothermes terrestres. La relation était plus marquée lors de stress thermiques de longue durée. Chez les ectothermes aquatiques, cette relation était moins apparente et les auteurs suggèrent que cela est dû au lien entre la tolérance au froid et le gel de l'eau. Les données sont encore trop peu nombreuses pour établir avec certitude les liens entre les seuils de tolérance thermiques minimaux des ectothermes aquatiques et la taille de leurs cellules. Le premier article de ce mémoire cherchera à combler ce manque de connaissances.

Par quels mécanismes la taille corporelle et cellulaire peuvent influencer la tolérance thermique ?

L'oxygène, molécule clé du métabolisme aérobie, peut-il être la pierre angulaire pour comprendre les liens entre la taille des organismes, la température du milieu et les seuils de tolérance thermique ? L'augmentation de la température entraîne chez les ectothermes une augmentation du taux métabolique et de la consommation d'oxygène. Cependant la disponibilité de l'oxygène reste stable en milieu terrestre voire décroît en milieu aquatique du fait de

la diminution de sa solubilité à haute température (Woods, 1999; Dejours, 1981). Il en résulte un déséquilibre entre une demande accrue en oxygène et un approvisionnement insuffisant. Les mécanismes d'adaptation et/ou d'acclimatation à des températures chaudes comprennent la mise en place d'un phénotype qui améliore la diffusion et le transport de l'oxygène. Les traits concernés sont par exemple la composition des membranes et leur capacité de diffusion de l'oxygène (Möller et al., 2016; Hazel and Williams, 1990), l'expression des différentes sous-unités de l'hémoglobine (Zeis et al., 2013; Seidl et al., 2005), la morphologie des branchies (McBryan et al., 2016), la taille corporelle ou la taille des cellules (Verspagen et al., 2020; Verberk et al., 2020; Czarnoleski et al., 2013).

Les contraintes sur l'approvisionnement et la consommation d'oxygène forment la base de l'hypothèse de la limitation de la tolérance thermique par l'oxygène (hypothèse OCLTT, Pörtner (2010); Pörtner and Knust (2007); Frederich and Pörtner (2000)). Elle propose que les seuils de tolérance thermique des ectothermes sont causés par le déséquilibre entre l'approvisionnement et la demande en oxygène qui survient lors des changements de température (Pörtner, 2010; Pörtner and Knust, 2007; Frederich and Pörtner, 2000). Cette hypothèse est activement débattue dû à l'invalidation de plusieurs de ses prédictions (Lefevre, 2016; Schulte, 2015; Wang et al., 2014) et à la difficulté de tester ses postulats (Jutfelt et al., 2018, 2014; Clark et al., 2013). Des recherches supplémentaires seront nécessaires pour mieux appréhender les liens entre les seuils de tolérance thermique et l'oxygène.

La règle température-taille décrite précédemment peut en partie s'expliquer à travers le rôle de l'oxygène. Les individus de grande taille ont des besoins en oxygène élevés et il pourrait être difficile pour eux de les satisfaire à haute température. L'évolution aurait donc favorisé des individus de plus petite taille en conditions chaudes pour limiter les risques liés à l'hypoxie (Verberk et al., 2020). Chez les espèces aquatiques, chez qui l'approvisionnement en oxygène est plus contraint, l'effet de la température sur la taille est plus fort que chez les espèces terrestres. Cette observation appuie l'hypothèse d'une limitation de la taille corporelle par l'oxygène (Forster et al., 2012).

La taille des cellules d'un organisme est un déterminant majeur de son taux métabolique et cette relation serait due aux effets du rapport surface-volume ou des distances de transport intracellulaires (Glazier, 2022). En règle générale, le rapport surface-volume d'une cellule diminue avec sa taille. Cela signifie que la surface d'échange entre les milieux intra et extracellulaires par unité de volume d'une grande cellule est plus petite que celle d'une petite cellule. Il en résulte que le coût énergétique du maintien des gradients ioniques entre les milieux intra et extracellulaires de grandes cellules est plus faible que pour les petites cellules (Kozłowski et al., 2020; Szarski, 1983). Malgré une augmentation de leur coût énergétique, les échanges d'ions et de molécules sont facilités pour les petites cellules ce qui permet un meilleur approvisionnement en oxygène et autres ressources ainsi qu'une meilleure élimination des déchets métaboliques (Glazier, 2022). Un tissu composé de petites cellules contient proportionnellement plus de membranes de phospholipides qui sont une voie de diffusion privilégiée pour l'oxygène (Pias, 2021; Subczynski et al., 1989). Les distances de transport et de diffusion sont également plus faibles dans les petites cellules. Il a donc été proposé que des tissus composés de petites cellules facilitent l'approvisionnement en oxygène (Verberk et al., 2022; Glazier, 2022; Shestopaloff, 2016).

Le rapport avantage/coût lié à la taille des cellules serait maximisé selon la température et la durée du stress thermique. Chez les drosophiles, les individus possédant de petites cellules résistent mieux à une augmentation rapide de température, tandis que ceux qui possèdent de grandes cellules tolèrent mieux un stress thermique modéré de plus longue durée (Verspagen et al., 2020). Chez les poissons, l'effet de la taille des cellules sur l'approvisionnement et la consommation d'oxygène dépend de la température (Verberk et al., 2022). L'approvisionnement et la consommation d'oxygène sont plus élevés à basse température pour les poissons possédant des grandes cellules. L'inverse est observé chez les poissons possédant de petites cellules, avec un approvisionnement et une consommation d'oxygène plus élevés à haute température (Verberk et al., 2022). Chez les espèces comprenant des individus diploïdes et polyploïdes, les polyploïdes ont tendance à se développer plus vite à basse température et cet avantage pourrait être lié à la taille supérieure de leurs cellules (Hermaniuk et al., 2021,

2016; Dufresne and Hebert, 1998). De manière générale, il apparaît donc que les petites cellules sont avantageuses à haute température alors que les grosses cellules sont avantageuses à basse température et que les contraintes sur l'oxygène y jouent un rôle prépondérant.

Cette section a mis en lumière les diverses relations qui existent entre la taille des ectothermes et la température du milieu. Elle montre que les seuils de tolérance thermique sont liés à la taille corporelle et à la taille cellulaire mais que le signe et la force des relations dépend de nombreux facteurs. Les contraintes sur la consommation et l'approvisionnement en oxygène sont une clé de compréhension du lien taille-température. Toutefois, elles peinent à expliquer quels mécanismes physiologiques précis limitent les seuils de tolérance thermique. L'oxygène est l'une des molécules clé du métabolisme aérobie et intervient comme accepteur final d'électrons de la système de respiration des mitochondries. D'autres processus liés au métabolisme aérobie pourraient limiter les seuils de tolérance thermique. La partie suivante explique le rôle possible du stress oxydant mitochondrial dans la tolérance thermique.

Mitochondries et tolérance thermique

Les mitochondries sont de plus en plus ciblées pour leur rôle dans la tolérance aux hautes températures chez les ectothermes (Chung and Schulte, 2020). Ces organites sont indispensables pour le fonctionnement du métabolisme aérobie qui fournit la majorité de l'énergie nécessaire au fonctionnement des processus vitaux. L'adénosine triphosphate (ATP) intervient au niveau du système de transport des électrons des mitochondries. Le système de transport des électrons désigne une suite d'enzymes située sur la membrane interne des mitochondries dont le rôle est de créer un gradient de protons entre l'espace interne et l'espace intermembranaire des mitochondries. L'ATP est synthétisée grâce à l'énergie fournie par le passage des protons depuis l'espace intermembranaire vers l'espace interne à travers l'enzyme ATP-synthase. Le fonctionnement du système de transport des électrons produit des dérivés réactifs de l'oxygène (DRO), particulièrement au niveau des complexes enzymatiques

I et III (Figure 0.1). Les DRO sont des atomes ou molécules composés d'oxygène et qui sont particulièrement réactifs dû aux électrons non-appariés qu'ils contiennent. Le DRO produit par les complexes I et III est l'ion superoxyde. Cet ion est transformé en peroxyde d'hydrogène par l'enzyme superoxyde dismutase. Le peroxyde d'hydrogène, qui est lui-même un DRO, est ensuite transformé en eau par l'enzyme catalase. À concentration modérée, les DRO ont un rôle en tant que messagers cellulaires (Klumpen et al., 2017; D'Autréaux and Toledano, 2007). Leur concentration est régulée par le système antioxydant de la cellule.

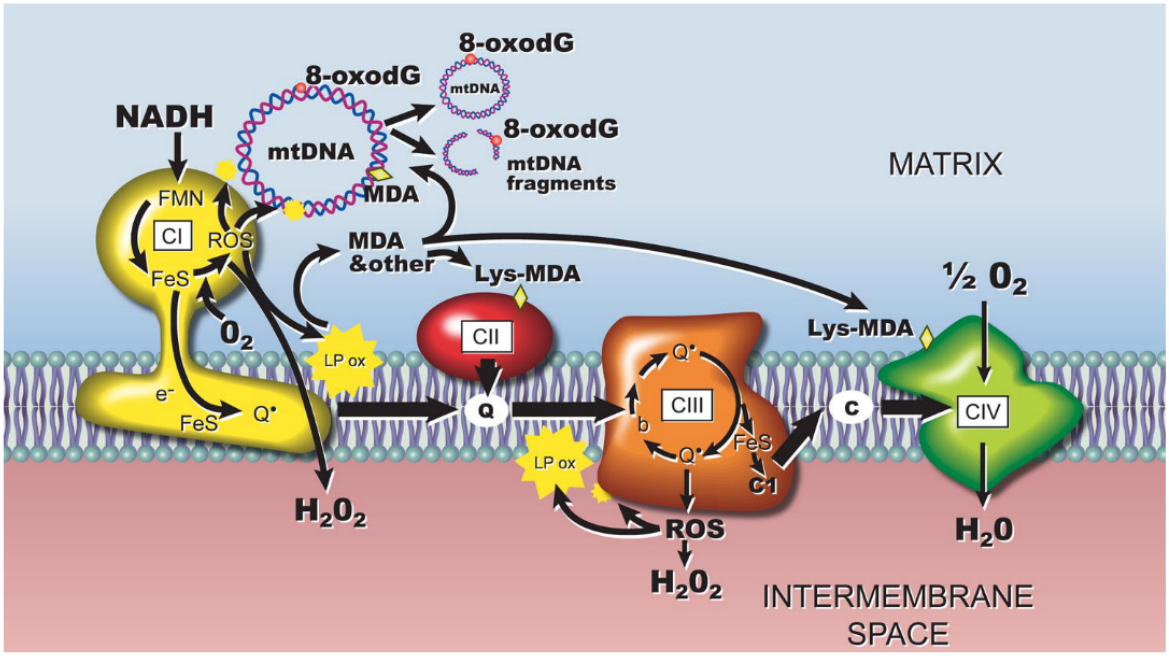

Figure 0.1: Représentation schématique du système de transport des électrons des mitochondries montrant les principaux lieux de production des dérivés réactifs de l'oxygène et la péroxidation des lipides (LP ox). D'après Hulbert et al. (2007).

Stress oxydant et peroxydation des lipides

Dû à leur réactivité, les DRO peuvent endommager les composantes cellulaires (acides gras, acides nucléiques, protéines) si l'activité du système antioxydant est insuffisante pour en plafonner la concentration (Hulbert et al., 2007). Ce processus est connu sous le nom de stress oxydant. S'il est trop important, le stress oxydant peut conduire à des lésions cellulaires et à

leur mort. Le stress oxydant est lié au vieillissement et à de nombreuses pathologies comme des maladies cardiaques ou dégénératives (Peoples et al., 2019; Marchio et al., 2019). La réaction au cours de laquelle les DRO réagissent avec les atomes de carbone des chaînes d'acides gras est la peroxydation des lipides. Les DRO réagissent tout particulièrement avec les atomes de carbone liés par une liaison unique et situés entre des doubles liaisons. Les acides gras polyinsaturés sont donc des cibles privilégiées de la peroxydation là où les acides gras saturés et monoinsaturés y sont peu sensibles (Hulbert et al., 2007). La peroxydation des lipides produit des radicaux de lipides qui peuvent eux-mêmes oxyder les chaînes d'acides gras par propagation.

Effets du stress thermique sur la peroxydation des lipides

Les changements de température affectent le fonctionnement des enzymes du système de transport des électrons avec pour conséquence une surproduction des DRO (Blier et al., 2014). La surproduction de DRO et le stress oxydant généré ont été proposés comme l'un des mécanismes physiologiques qui limite la tolérance à la chaleur des organismes ectothermes (Chung and Schulte, 2020). Chez l'omble chevalier, Christen et al. (2018) ont mis en évidence qu'à l'approche du seuil critique de tolérance à la chaleur, la consommation d'oxygène des mitochondries cardiaques plafonnait alors que la production de DRO augmentait de manière disproportionnée. Christen et al. (2020) ont ensuite mis en évidence que la tolérance à la chaleur d'ombles augmente avec la résistance des membranes des cellules cardiaques au stress oxydant. Plus précisément, les membranes cellulaires des individus résistants à la chaleur avaient des indices de peroxydation plus faibles et contenaient moins d'acide arachidonique et d'acide eicosapentaénoïque. L'indice de peroxydation est un indicateur de la sensibilité d'une membrane au stress oxydant (Hulbert et al., 2007) tandis que l'acide arachidonique et l'acide eicosapentaénoïque sont des acides gras polyinsaturés sensibles au stress oxydant. La tolérance à la chaleur était également meilleure chez les individus avec une capacité antioxydante élevée. Chez les daphnies, l'acclimatation à basse température induit une insaturation

des membranes qui engendre une peroxydation accrue des lipides membranaires (Zeis et al., 2019). Ces indices montrent un lien clair entre le dysfonctionnement des mitochondries à haute température, le stress oxydant et la tolérance à la chaleur.

La diminution de la température peut aussi entraîner du stress oxydant. Il a été suggéré que la surproduction de DRO est dans ce cas liée à la limitation du flux d'électrons au niveau des complexes III et IV (Blier et al., 2014). Des stress thermiques chroniques ou aigus à basse température peuvent engendrer du stress oxydant. Chez la grenouille *Nanorana pleskei*, l'acclimatation à basse température a augmenté le stress oxydant et la peroxydation des lipides tout en réduisant la capacité antioxydante totale, avec des effets différents selon les organes (Zhang et al., 2021). Chez le coléoptère *Alphitobius diaperinus*, un stress thermique aigu à basse température a engendré une augmentation de l'activité de la superoxyde dismutase, suggérant un besoin de régulation du stress oxydant à basse température (Lalouette et al., 2011). Chez les poissons également, les chocs thermiques à basse température sont souvent associés au stress oxydant et à la peroxydation des lipides et la réponse du système antioxydant varie en fonction de l'intensité de la baisse de température. Dans certains cas, le système antioxydant peut faillir à contrer l'augmentation des DRO (Reid et al., 2022). De manière générale, il apparaît donc que le stress thermique à basse température engendre du stress oxydant. Sa sévérité et la mitigation par les antioxydants dépendent de nombreux facteurs comme l'espèce, l'organe, l'intensité et la vitesse du changement de température ou l'acclimatation préalable de l'individu.

Lu et al. (2019) ont mis en évidence un lien fort entre la tolérance au froid – mesurée comme la survie après une baisse de température – et le stress oxydant induit par le froid chez le poisson zèbre. Leur expérience a montré que le froid causait une augmentation des dégâts oxydatifs sur les lipides et les protéines et que la supplémentation des poissons avec des antioxydants diminuait le stress oxydant et améliorait la survie des individus. Le stress oxydant pourrait donc être un mécanisme déterminant du seuil critique minimal de tolérance thermique mais le nombre d'études sur le sujet chez les animaux est encore faible.

Contrairement à l'acclimatation à haute température qui induit une diminution de l'insaturation des membranes donc une diminution de leur sensibilité au stress oxydant, l'acclimatation à basse température se fait à travers l'augmentation de l'insaturation des membranes cellulaires pour garantir une fluidité membranaire adéquate (Hazel and Williams, 1990; Sinensky, 1974). Les membranes insaturées étant plus sujettes à la peroxydation (Hulbert et al., 2007), une question soulevée est de savoir si la restructuration des lipides membranaires induite par le froid se traduit par une augmentation du stress oxydant lors du choc au froid, et si cela impacte la tolérance. Crockett (2008) a proposé qu'à température physiologique, c'est-à-dire la température à laquelle les individus sont acclimatés, les taux de peroxydation des lipides sont similaires quelle que soit la température d'acclimatation. La stabilité du taux de peroxydation des lipides à travers les températures d'acclimatation serait dans ce cas garantie par un équilibre entre production de DRO et sensibilité des membranes face au stress oxydant. Dans le cas d'un choc thermique à basse température, le stress oxydant pourrait toutefois dépasser les seuils d'équilibre et désavantager les individus possédant des membranes sensibles au stress oxydant.

Les seuils de tolérance thermique inférieurs et supérieurs sont-ils liés ?

La perspective d'une augmentation de la variabilité climatique et de l'occurrence de changements brutaux de température amène la question des liens entre les seuils de tolérance thermique inférieurs et supérieurs. Au sein d'une espèce ou d'une population, les individus les plus tolérants à la chaleur sont-ils par défaut moins tolérants au froid ? L'acclimatation ou l'évolution à des conditions chaudes rendent-elles plus vulnérable à un choc thermique froid ?

Entre les individus des mêmes espèces ou d'espèces apparentées, et pour des conditions d'acclimatation similaires, les seuils de tolérance thermique ne sont pas nécessairement liés. Chez les insectes terrestres, les études actuelles montrent une absence de corrélation entre CTmin et CTmax aussi bien au niveau intra qu'interspécifique (Gonzalez et al., 2022; Baudier

and O'Donnell, 2018). L'effet de la température d'acclimatation chez les ectothermes est plus systématique : une augmentation de la température s'accompagne dans de nombreux cas d'une augmentation simultanée des CTmin et des CTmax chez de nombreuses espèces (Reyes-Avalos et al., 2023; Dash et al., 2021; Sørensen et al., 2020; Safi et al., 2019; Di Santo and Lobel, 2017; Dülger et al., 2012). D'autres études montrent une modification de l'un des seuils de tolérance thermique avec l'acclimatation tandis que l'autre reste constant (Kieffer and Bard, 2022). Les processus évolutifs peuvent donc entraîner la modification d'un seuil de tolérance thermique sans modifier l'autre. Cela a par exemple été décrit chez une population de lézards ayant évolué une meilleure tolérance au froid tandis que la tolérance à la chaleur restait inchangée (Leal and Gunderson, 2012). L'évolution vers une plus grande plage de tolérance thermique peut toutefois se solder par une diminution de la performance de certains traits, un effet connu sous le nom de « compromis spécialiste-généraliste » (Buckley and Kingsolver, 2021).

Selon Leiva et al. (2019), les mécanismes qui sous-tendent les seuils de tolérance au froid sont différents de ceux qui sous-tendent les seuils de tolérance à la chaleur. Les seuils de tolérance thermique à basse température sont généralement associés à la dépolarisation des membranes (Overgaard et al., 2021; Williams et al., 2018; Hayward et al., 2014) tandis que les seuils à haute température sont associés aux contraintes sur le métabolisme aérobie et aux dysfonctions mitochondriales liées (Chung and Schulte, 2020; Pörtner and Knust, 2007). Ces différences de mécanismes pourraient expliquer pourquoi le CTmin et le CTmax peuvent évoluer indépendamment l'un de l'autre. L'acclimatation thermique cause généralement un changement conjoint des seuils de tolérance thermique qui pourrait être dû aux effets opposés de la température sur certains traits. Par exemple, l'acclimatation à des hautes températures chez les ectothermes s'accompagne d'une augmentation de la saturation des membranes tandis que l'acclimatation à basse température diminue leur saturation.

Le présent travail explorera les liens entre les seuils de tolérance thermique chez plusieurs populations d'une espèce d'ectotherme aquatique acclimatées à des conditions ther-

miques différentes.

Le modèle d'étude : le complexe *Daphnia pulex* nord-américain

Les daphnies (genre *Daphnia*) sont des crustacés cladocères de la famille des Daphniidae. Il en existe environ 150 espèces à travers le monde (Schön et al., 2009). Les daphnies sont en grande majorité des espèces dulçaquicoles qui ont une grande importance dans les réseaux trophiques de leur écosystème en servant de source alimentaire pour les consommateurs de zooplancton. Ce genre constitue un modèle d'étude intéressant car il est facile de l'élever en laboratoire et son cycle de vie est court. Le genre *Daphnia* se reproduit par parthénogenèse cyclique ou obligatoire (Figure 0.2). Ce type de reproduction permet le maintien d'un génotype constant à travers les lignées. La reproduction par parthénogénèse cyclique combine les avantages de la reproduction asexuée et de la reproduction sexuée. L'asexualité permet aux individus de se reproduire rapidement sans avoir besoin de trouver de partenaire sexuel. Des changements dans les conditions environnementales comme la température, la qualité et/ou la quantité de la nourriture déclenchent la reproduction sexuée qui permet un brassage génétique et la production d'oeufs de dormance appelés éphippies (Koch et al., 2009).

Le complexe d'espèce *Daphnia pulex* a une large répartition en Amérique du Nord, allant des zones tempérées aux zones arctiques. Le niveau de ploïdie des représentants de *D. pulex* est variable : il existe des clones diploïdes et des clones triploïdes. L'apparition de la triploïdie serait due à l'hybridation entre des espèces phylogénétiquement proches (Dufresne and Hebert, 1994). Les clones triploïdes sont retrouvés en haute latitude, dans les zones subarctiques à arctiques, alors que les diploïdes sont retrouvés en zones tempérées à subarctiques. Ce patron de distribution différentié n'est pas unique : chez les espèces animales et végétales composées de populations de ploïdies variables on retrouve souvent les diploïdes et polyploïdes dans des habitats différents sans que les raisons aient pu être clairement identifiées (Ramsey and Ramsey, 2014; Ehrendorfer, 1979). La compréhension de ce phénomène est

complexifiée par le fait que l'apparition de la polyploïdie est accompagnée d'un passage à la reproduction asexuée qui pourrait aussi avoir pour effet la différentiation de la distribution (Tilquin and Kokko, 2016).

L'une des hypothèses émises pour expliquer la répartition différentiée de la ploïdie chez *D. pulex* est celle de la tolérance thermique, à savoir que les différences de distribution des clones s'expliquent par des différences dans les seuils critiques de tolérance thermique. Bernier (2020) a montré que les clones triploïdes sont moins résistants aux choc thermiques à haute température. Parallèlement, les clones triploïdes et/ou les clones de hautes latitudes pourraient être plus tolérants au froid, ce qui leur permettrait de commencer leur saison de reproduction plus tôt ou de survivre à des températures inférieures. L'hypothèse de la tolérance au froid sera explorée dans le premier article de ce mémoire. Le complexe *D. pulex* est un modèle de choix pour comprendre les causes des seuils de tolérance thermique et des patrons de distribution observés. L'existence de clones d'origines climatiques et de ploïdies variées ainsi que la possibilité de les soumettre à différentes conditions environnementales en laboratoire permet d'étudier les effets du génotype, du phénotype et les effets de l'acclimatation à la température sur les seuils de tolérance thermique des clones.

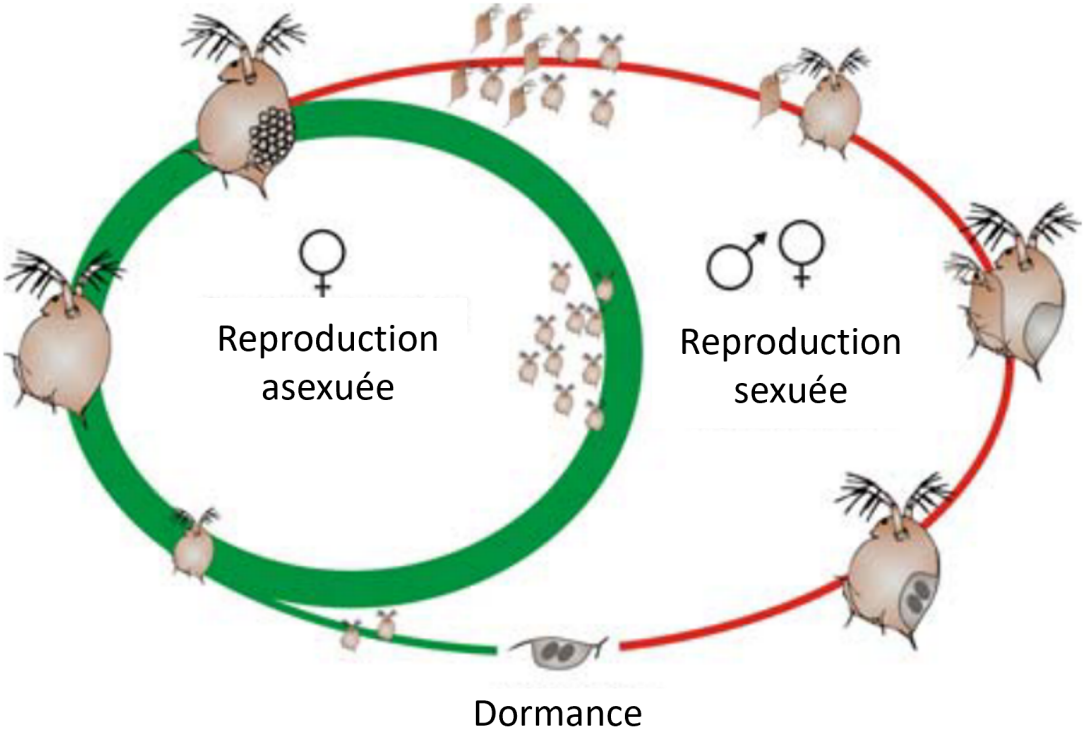

Figure 0.2: Cycle de vie parthénogénétique du genre *Daphnia*. En conditions favorables, la reproduction parthénogénétique prend place sur une ou plusieurs générations (vert). La reproduction sexuée (rouge) produit des œufs de dormance qui peuvent éclore quand les conditions environnementales deviennent à nouveau favorables. Certains groupes ont supprimé les mâles du cycle et produisent des œufs dormants de manière de manière asexuelle. Modifié d'après Schön et al. (2009).

Problématique

Les changements climatiques engendrent une augmentation de la température moyenne globale et une augmentation de la fréquence et de l'intensité d'extrêmes de température. Les ectothermes sont particulièrement touchés par ces événements dû à leur incapacité à réguler leur température corporelle. Les communautés de zooplancton ont une importance cruciale dans les chaînes alimentaires. Elles sont et seront impactées par les changements de régimes thermiques. Il est donc d'intérêt de connaître leur vulnérabilité face aux extrêmes de température. Il existe, selon les espèces, les populations d'une même espèce ou les individus, une

variabilité dans la tolérance aux extrêmes de température. Cette variabilité permet de comprendre par quels moyens les organismes tolèrent ou non les extrêmes de température. En connaissant les traits qui déterminent les seuils de tolérance thermique, il deviendra plus aisé de comprendre le potentiel d'acclimatation et d'adaptation des espèces dans un environnement changeant.

Objectifs, hypothèses et prédictions

Les objectifs principaux de ce mémoire sont de mieux comprendre 1) les mécanismes physiologiques qui limitent les seuils de tolérance aux extrêmes de température et 2) le patron de distribution de la ploïdie chez les clones du crustacé *D. pulex*. Les objectifs spécifiques sont de comprendre l'influence de la composition en acides gras des membranes cellulaires, de la taille cellulaire, de la taille corporelle et de la ploïdie sur la tolérance thermique et la distribution des clones.

1) Mécanismes déterminant les seuils de tolérance thermique

Hypothèse 1 : l'insaturation des membranes cellulaires, qui est influencée par la température d'acclimatation, diminue la tolérance à la chaleur à travers une augmentation de la sensibilité au stress oxydant. Il est prédit que la tolérance à la chaleur diminuera avec l'insaturation des membranes.

Hypothèse 2 : la taille corporelle et la taille des cellules influencent la tolérance au choc de froid par le biais des effets du rapport surface/volume sur le métabolisme cellulaire. Il est prédit que les individus ayant une taille corporelle et des cellules plus grandes auront une meilleure tolérance au choc de froid.

Hypothèse 3 : les processus d'acclimatation à la chaleur, qui permettent une meilleure tolérance aux hautes températures, contribuent à diminuer la tolérance au froid. Il est prédit que la tolérance au froid diminue avec l'augmentation de la tolérance à la chaleur, c'est-à-dire

que le CTmin et CTmax sont corrélés positivement.

2) Distribution de la ploïdie et des clones

Hypothèse 4 : est que les patrons de distribution de la ploïdie et des clones sont liés aux différences de tolérance au froid chez le modèle *D. pulex*. Nous prédisons que les clones provenant d'environnements plus froids et les polyploïdes auront une meilleure tolérance au choc de froid.

Hypothèse 5 : est que les profils d'acides gras des clones varient en fonction de leur ploïdie et de leur origine géographique. Nous prédisons des différences dans les profils d'acides gras entre clones d'origines et ploïdies différentes.

Méthodologie

Animaux et élevage

Les clones de *Daphnia pulex* utilisés proviennent de la collection du laboratoire de France Dufresne. Les clones utilisés pour les expériences varient en fonction de leur origine géographique (tempéré ou subarctique) et de leur ploïdie (diploïde ou triploïde). Les clones sont élevés pendant au moins trois générations dans des conditions standardisées avec une intensité lumineuse de 3000 lux. Ils sont nourris trois fois par semaine de la microalgue *Raphidocelis subcapitata* (anciennement *Selenastrum capricornotum*) avec une concentration finale de 2×10^5 cellules mL^{-1}.

Seuils de tolérance thermique

Deux méthodes sont communément utilisées pour mesurer la sensibilité thermique des êtres-vivants : les courbes de performance thermique (CPT) et les limites critiques de tolérance thermiques minimales (CTmin) ou maximales (CTmax). Les CPTs représentent la

relation entre la température corporelle de l'individu et la performance d'un trait choisi (Sinclair et al., 2016). Les traits étudiés comprennent la croissance, la locomotion, la digestion, la respiration ou la reproduction. Les extrémités des CPTs sont représentées par des températures critiques à partir desquelles la performance du trait correspondant est réduite à zéro, sans nécessairement représenter des températures léthales (Figure 0.3). Malgré plusieurs défauts méthodologiques, les CPTs sont des outils de modélisation largement utilisés (Litchman and Thomas, 2023; Sinclair et al., 2016).

La méthode utilisée ici pour quantifier la tolérance aux chocs thermiques est celle des limites de tolérance thermiques (CT). Bien que les définitions puissent varier, les CT sont mesurés en soumettant les animaux à une rampe de température dont la température de départ et la vitesse sont fixées par l'expérimentateur.ice. Le CT est communément défini comme la température à laquelle la performance d'un trait donné devient nulle ou aussi comme la température à laquelle l'animal devient incapable de soutenir la locomotion et perd l'équilibre. Les repères visuels des CT sont par exemple le début de spasmes musculaires, d'une incapacité respiratoire ou une perte d'équilibre (Chung and Schulte, 2020; MacMillan, 2019; Hazell and Bale, 2011; Lutterschmidt and Hutchison, 1997). Le CTmin est obtenu lors d'un abaissement de la température alors que le CTmax l'est lors d'une hausse de la température. La manière dont le CTmin est mesurée dans cette étude s'apparente à un choc de froid. Celui-ci peut être défini comme une exposition à une baisse rapide de température résultant en une cascade de réponses physiologiques et comportementales (Donaldson et al., 2008). Il peut donc s'apparenter à un extrême thermique dû à la rapidité de sa mise en place.

Les CTmin et CTmax peuvent être influencés par de nombreux facteurs comme les paramètres de la rampe de température, la température d'acclimatation de l'animal, son état de satiété ou l'environnement des parents des individus étudiés (Terblanche et al., 2007; Reid et al., 2022; Cavieres et al., 2020; Nyamukondiwa and Terblanche, 2009; Warriner et al., 2020). Le contrôle de ces paramètres est donc important pour permettre la comparaison des CT entre différents groupes. Dans cette étude, les animaux sont élevés en laboratoire

à plusieurs températures d'acclimatation constantes, avec un nourrissage et une luminosité constante. Avant les mesures de tolérance thermique, les individus sont élevés sur au moins trois générations dans des conditions constantes pour limiter les effets transgénérationnels dus à une éventuelle variabilité des paramètres de l'environnement parental.

Après la réalisation du protocole de CTmin, la taille corporelle et la taille cellulaire de individus ont été mesurées à l'aide du logiciel ToupView (ToupTek Photonics, Chine).

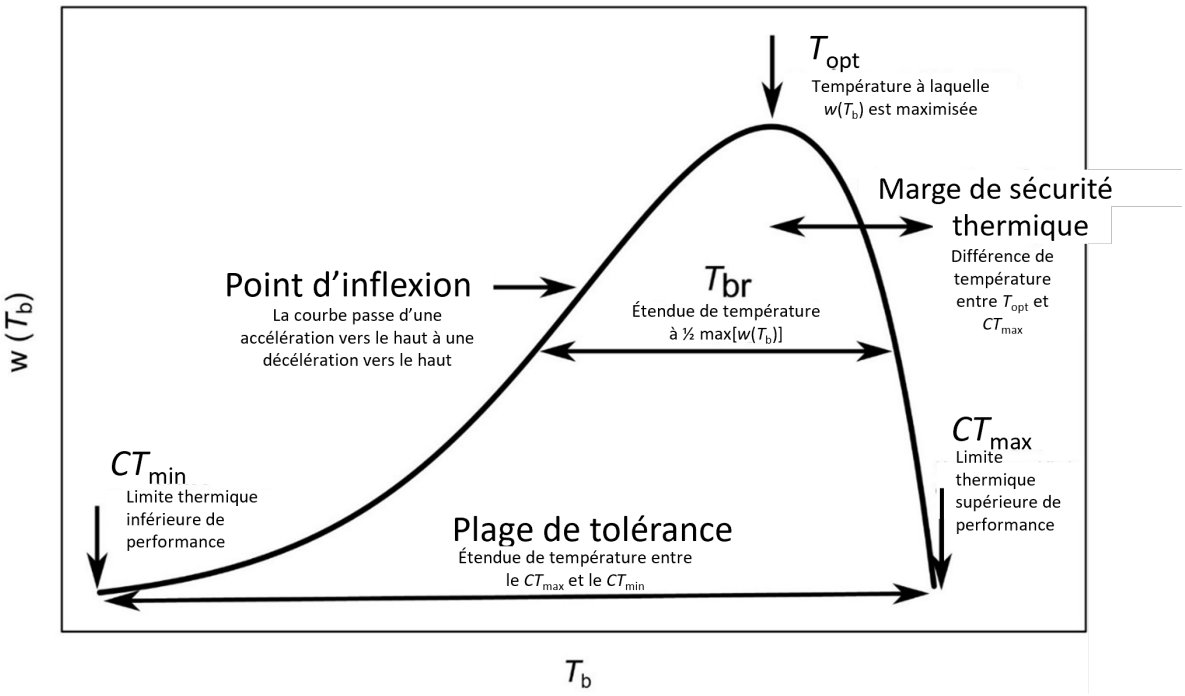

Figure 0.3: Représentation d'une courbe de performance thermique liant la température corporelle T_b à la valeur sélective $w(T_b)$. Les principales caractéristiques de la courbe sont notées (basé sur Huey and Stevenson (1979)). *CTmin* et *CTmax* : seuils thermiques critiques minimaux et maximaux, respectivement ; T_{opt} : optimum thermique ; T_{br} : étendue thermique. Modifié d'après Sinclair et al. (2016).

Analyses de profils d'acides gras

Les individus utilisés pour les analyses des profils d'acides gras ont préalablement été utilisés pour des mesures de CTmax (Bernier, 2020) et stockés à -80 °C. Pour l'extraction,

les acides gras des individus entiers ont été transformés en esters méthyliques d'acides gras à l'aide d'une transestérification directe par catalyse acide (Lepage and Roy, 1984; Christen et al., 2020). Les acides gras ont été séparés par chromatographie à phase gazeuse pour être identifiés par un détecteur à ionisation de flamme (FID) et leur proportion a été calculée ce qui a permis d'obtenir les profils d'acides gras de chaque individu.

ARTICLE I

DOES POLYPLOIDY CONFER A BETTER COLD TOLERANCE ?

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1.1 Abstract

Despite decades of studies on the differential distribution of polyploid organisms, the causes of this pattern have yet to be elucidated. This study aimed to explore some of the possible physiological mechanisms explaining the differential northern distribution of polyploid organisms compared to the one of the diploid parental species in the North American *Daphnia pulex* complex. The critical thermal minimum (CTmin) was measured in *D. pulex* clones of contrasting ploidy (diploid and triploid) and origins (temperate and subarctic climates) reared under low and high temperatures (16 and 24 °C). We also measured body and cell size, two phenotypic traits influenced by polyploidy to test for possible association with CTmin. Triploid clones had higher cold tolerance (i.e. lower CTmin) than diploids when raised at 16 °C but not when raised at 24 °C. No significant association was found between CTmin and body size nor with cell size. We suggest that triploids might express a cold shock resistant phenotype related to higher gene expression and/or lipid content. We show that body and cell size do not influence cold shock tolerance, but we suggest they can still influence chronic cold stress tolerance. Cold tolerance can thus be viewed as one of the possible reasons of polyploid preponderance in subarctic climates, but ecological relevance of cold shock events in their habitat needs to be carefully considered.

Keywords: Temperature, ploidy, *Daphnia*, cell size, body size, CTmin

1.2 Introduction

Polyploidy - a state in which a cell contains more than two sets of chromosomes - is a trait found in multiple living taxa especially in plants but also numerous animal taxa such as fishes, amphibians or arthropods (Mable, 2004). Because past polyploidization events are linked to periods of rapid environmental change and due to its positive effect on genetic variation, polyploidy is considered as a strong evolutionary force (Van de Peer et al., 2020). In species where diploid and polyploid individuals co-exist, differential distribution patterns can often be observed with polyploids being found in environments that are considered as more extreme (higher altitudes or latitudes, colder and drier environments) than that of their diploid counterparts (Rice et al., 2019; Van de Peer et al., 2017; Lorch et al., 2016; Ehrendorfer, 1979). In plants, polyploids are often invasive species that dominate fluctuating and/or marginal environments (Te Beest et al., 2012). Many studies have tried to find explanation for this pattern of differentiated distribution, but no general mechanism has been found and this view is sometimes challenged (Mable et al., 2011). Furthermore, polyploidy is often accompanied by asexual reproduction, making it difficult to disentangle the role of each factor in shaping distribution patterns (Tilquin and Kokko, 2016).

Polyploidy has major consequences on cell physiology, whole-organism phenotype, life history traits, and ecology (Doyle and Coate, 2020; Comai, 2005). The increase in bulk DNA amount that accompanies polyploidy brings increased nuclei and cell size with concomitant changes in cell surface area to volume ratios (Glazier, 2021; Gregory, 2001). These effects are known as nucleotypic effects (i.e. effects on cell that are due to the sole amount of DNA). For example, the ploidy-induced increase in cell surface area in carps lead to changes in metabolic scaling (Zhu et al., 2021).

Considering the observed distributional patterns of polyploids and physiological changes linked to genome duplication, it is of interest to study whether the distribution of ploidy is linked to ploidy-induced physiological changes. One way for polyploidization to take place is

through hybridization between closely related species. Newly formed lineages subsequently go through evolutive processes which can further differentiate them. It can be difficult to distinguish the contributions of hybridization, genome doubling and evolution in setting physiological and ecological differences between diploids and polyploids (Soltis et al., 2016). For example, one hybrid *Daphnia* lineage which dominated a freshwater zooplankton community showed an ability to thrive in winter conditions contrary to other clones (Griebel et al., 2015). As the ploidy of the hybrid lineage is not mentioned, it is difficult to know if its performance was due to hybridization or potential polyploidy.

The observation that polyploid organisms and/or organisms with bigger genomes and cell sizes are more common at higher latitudes and altitudes sparked the following question: are polyploids more tolerant to cold temperatures (Riseth et al., 2020; Hessen et al., 2013) ? In ectotherms, geographical patterns of distribution are often related to their thermal tolerance limits. Cold tolerance increases with latitude and altitude in terrestrial and aquatic ectotherms and matches low temperature extremes measured in the habitat (Holmstrup et al., 2022; Gonzalez et al., 2022; Sunday et al., 2019; Williams et al., 2018). Moreover, some studies have shown that in cold conditions polyploids favourably compete their diploid counterparts thanks to faster development rates (Hermaniuk et al., 2016; Dufresne and Hebert, 1998) or higher metabolic rates (Hermaniuk et al., 2021) and suggest that this cold advantage could be mediated by cell size. Cold stress induces cell membrane depolarization, impairment of energy metabolism, disruption in ion homeostasis and water balance (Reid et al., 2022; Overgaard et al., 2021; Hayward et al., 2014). Larger cells exhibit reduced resource supply and demand due to reduced surface area *per* volume, increased intracellular transport distances, or lower costs of ionic regulation. It also seems that the cost of ion transport relative to cell membrane surface area is lower in bigger cells (Glazier, 2022). Since polyploidization can lead to bigger cell or body sizes in ectotherms (Otto, 2007; Gregory et al., 2000), their larger cells might reduce energetic costs of membrane polarization at low temperatures and confer a better cold tolerance to polyploids.

North American *Daphnia pulex* complex displays a clear pattern of latitudinal distribution of ploidy, with diploids found in temperate and subarctic regions while triploids are found in subarctic and arctic regions (Beaton and Hebert, 1988). Triploid clones originate from hybridization events between closely related species (Dufresne and Hebert, 1994). Several hypotheses were proposed to explain this distribution pattern. Diploids and polyploids do not seem to differ concerning aerobic metabolism (Ratté, 2011) and sensitivity to UV radiation (Martin, 2018). Bernier (2020) explored the thermal tolerance hypothesis, which proposes that distribution is partly related to differences in heat tolerance. The study showed that diploids have a higher heat tolerance than triploids, allowing them to live in warmer environments. As shown by the negative relationship between heat tolerance and cell size (Bernier, 2020), the higher heat tolerance of diploids might be linked to their smaller cell size (higher surface to volume ratio) which reduces oxygen limitation at elevated temperatures. On the other hand, the presence of triploids in colder environments might be linked with a better tolerance to cold. Clones that endure colder temperatures could sustain normal physiological functions and start their reproductive cycle earlier during spring giving them an advantage over less tolerant clones.

This study explores how cold tolerance of *Daphnia* clones may be linked to origin, ploidy and its phenotypic correlates, and how plastic responses to acclimation temperature influence cold tolerance. Specifically, we measure acute cold tolerance of North American *Daphnia pulex* clones differing in ploidy and origin acclimated to 16 and 24 °C. We explore the presence of a relationship between cold shock tolerance and body or cell size. Cold shock can be defined as an "exposure to abrupt temperature changes, or sharp decreases in temperature over a short time period" (Reid et al., 2022). We measure cold shock tolerance using the CTmin metric, sometimes defined as *chill coma* (but see Hazell and Bale (2011)). To our knowledge, no data on tolerance to cold shock is available for *Daphnia*. We propose a new protocol to measure acute cold tolerance in *Daphnia* and small aquatic organisms.

We hypothesized that clonal and ploidy related distribution patterns as well as ability

to acclimate to different temperatures are linked to differences in cold tolerance. We predict that clones originating from colder environments, polyploids and cold acclimated individuals will have better tolerance to cold shock. We hypothesized that cell size influences positively cold shock tolerance through the effects of surface to volume ratio on cell resource demand. We predict that individuals with bigger cells have a better cold shock tolerance.

1.3 Material and methods

1.3.1 *Daphnia* rearing

Several diploid and triploid clones of *Daphnia pulex* from temperate and subarctic North American regions were reared for at least three generations in standardized conditions before the experiments to eliminate potential maternal effects (6 subarctic triploid clones, 4 subarctic diploid clones, 8 temperate diploid clones, see Table 1.1). *Daphnia* were reared in two environmental test chambers at 16 and 24 °C, set on a 12-12h photoperiod and a light intensity of 3000 lux. *Daphnia* were raised in glass jars containing 300 mL of FLAMES culture medium (Celis-Salgado et al., 2008). Each clone was raised in triplicates (3 jars *per* clone *per* temperature). Animals were fed 2.0×10^5 cells mL^{-1} of *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata* or *Selenastrum capricornotum*) three times a week. Populations in each jar were controlled to keep between 10 and 30 individuals *per* jar. One third of the medium was renewed every two weeks.

1.3.2 Cold tolerance test

Cold tolerance was tested by performing a critical thermal minimum (CTmin) challenge. CTmin, sometimes defined as chill coma, is here defined as the lower temperature that prevents locomotion, resulting in the inability of the animal to escape danger or move to a place where conditions are viable. CTmin tests were performed on randomly selected

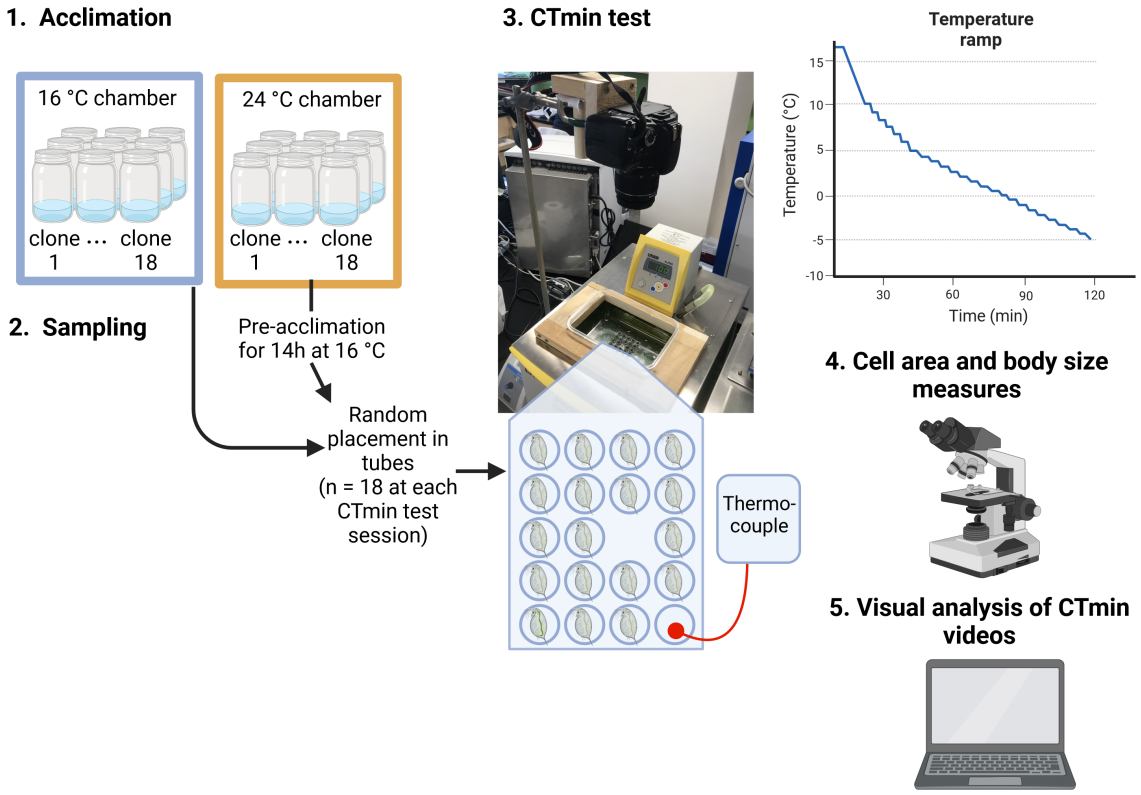

Figure 1.1: Description of the experimental design carried out for CTmin experiments. Acclimation: 18 clones *per* chamber, 3 jars *per* clone. Start of experiments after 3 generations in the same conditions, see section 1.3.1. Sampling: all development stages (males and ephippia-bearing females excluded), whole body size range. CTmin test: repeated more than 30 times until desired sample size reached, see section 1.3.2. Size measures: see section 1.3.3. Visual analysis: see section 1.3.2

female *Daphnia* of different reproductive stages. Selection of individuals was made to ensure that the whole size range was represented in the test sample for each clone. The clones used, their origin, ploidy, and sample size for each rearing temperature are summarized in Table 1.1. Approximately 5 individuals *per* jar were sampled. A maximum of 18 individuals were tested at each CTmin experimental session, which was repeated more than 30 times in order to reach the desired sample size (that is, 5 individuals *per* jar for each clone and each temperature, when possible). A description of the experimental design is represented on Figure 1.1.

Table 1.1: Clones, ploidy, climate, origin and sample size for each temperature for each *Daphnia pulex* clone used for cold tolerance experiments

Clone	Ploidy	Climate	Location	Latitude, Longitude	n 16 °C	n 24 °C
A07	Triploid	Subarctic	Churchill, Manitoba, Canada	58.767638, -93.975239	16	15
C102			Churchill, Manitoba, Canada	58.767638, -93.975239	15	17
IQ12			Kuujjuarapik, Nunavik, Canada	55.276672, -77.776401	16	3
R202			Kuujjuarapik, Nunavik, Canada	55.276672, -77.776401	14	19
SAS-16-17			Kuujjuarapik, Nunavik, Canada	55.276672, -77.776401	15	15
C144			Churchill, Manitoba, Canada	58.767638, -93.975239	15	15
C44	Diploid	Subarctic	Churchill, Manitoba, Canada	58.767638, -93.975239	14	15
R206			Kuujjuarapik, Nunavik, Canada	55.276672, -77.776401	22	17
R210			Kuujjuarapik, Nunavik, Canada	55.276672, -77.776401	15	14
WP2			Kuujjuarapik, Nunavik, Canada	55.276672, -77.776401	13	15
Stukely	Diploid	Temperate	Canton-de-l'Est, Québec, Canada	45.286882,72.420144	14	19
BUS15			Urbana, Illinois, USA	40.129889, -88.206478	16	15
Deeplake			Michigan, USA	42.618166, -85.459009	15	17
KAP-53			Danville, Illinois, USA	40.182782, -87.646184	14	16
Longlake			Michigan, USA	42.551644, -85.377827	13	17
NFL68			Webster, Indiana, USA	39.903509, -84.941463	16	16
SPS100			Homer, Illinois, USA	40.753935, -73.982114	15	15
Ste-Luce			Sainte-Luce, Québec, Canada	48.547571, -68.393969	10	15

Prior to the experiment, *Daphnia* reared at 24 °C were placed for 14h at 16 °C to acclimate them at the temperature of cold-acclimated *Daphnia*. CTmin were performed using a thermostat filled with a mix of ethylene glycol - distilled water (30/70, v/v). *Daphnia* were individually placed inside 650 µL tubes containing 300 µL of a solution of 0.5 mg mL^{-1} bovine serum albumin in FLAMES culture medium. The tube inside which each individual was placed was randomly selected so that *Daphnia* from the same jar would not all be placed on the same row of tubes. The bovine serum albumin increased the surface tension of the solution and kept individual *Daphnia* out of the surface so they were not exposed to air temperature. A thermocouple sensor was placed in one tube to evaluate the temperature inside the tubes.

Tubes were placed in the thermostat set at 16 °C for 10 min to let the individuals acclimate to test conditions. Thermostat temperature was then manually lowered using the following temperature ramp: between 16 and 10 °C: direct decrease (-0.43 °C min^{-1}), between 10 and 5 °C: -1 °C every 6 min 30 s (-0.15 °C min^{-1}), between 5 and -5 °C : -0.5 °C every

3 min 45 s (-0.13 °C min^{-1}). Because of the supercooling process, the medium stayed in a liquid state below 0 °C, allowing the *Daphnia* to continue moving. A minority of tubes (approximately 5%) spontaneously froze during the experiments and were not accounted in further statistical analyses.

Daphnia were filmed using a Panasonic Lumix DC-G9 camera (Panasonic Corporation, Kadoma, Osaka, Japan), equipped with a Panasonic Leica DG Macro Elmarit 45 mm F2.8 ASPH MEGA O.I.S lens (Panasonic Corporation, Kadoma, Osaka, Japan). Videos were shot at 30 frames per second and 1920 × 1080 pixels quality. A second camera was used to record the screen of the thermocouple. Lighting was kept constant throughout all tests. Videos of *Daphnia* and thermocouple screen were synchronized in post processing using DaVinci Resolve 17 (Blackmagic Design, Australia) before visual analysis.

1.3.3 Body size and cell area

Body size and cell area measures were carried out right after CTmin test. Most individuals were alive during the measurements, because the chill coma they were in is a reversible state. ToupView software was used for all microscopic measures (ToupTek Photonics Co., Ltd., China). Measures were done with a microscope (Leica DMLB, Leica, Germany) equipped with a digital camera (Model OMAX A35100U3, Omax Microscope, Seattle, WA, USA) at 2.5 × 10 and 10 × 10 magnification respectively. Body size was measured as the distance between the anterior border of the eye and the tip of the shell spine (Figure 1.2). Cell size was measured on the carapace at 100 × magnification. Cell imprints are easily visualized in this tissue and correspond to well-defined cells. To measure cell area, the mean of ten cell areas was calculated for each individual. Cells located on the posterior and anterior ventral side of the individual were measured. When they were not visible, cells from the center of the carapace were measured (Figure 1.3).

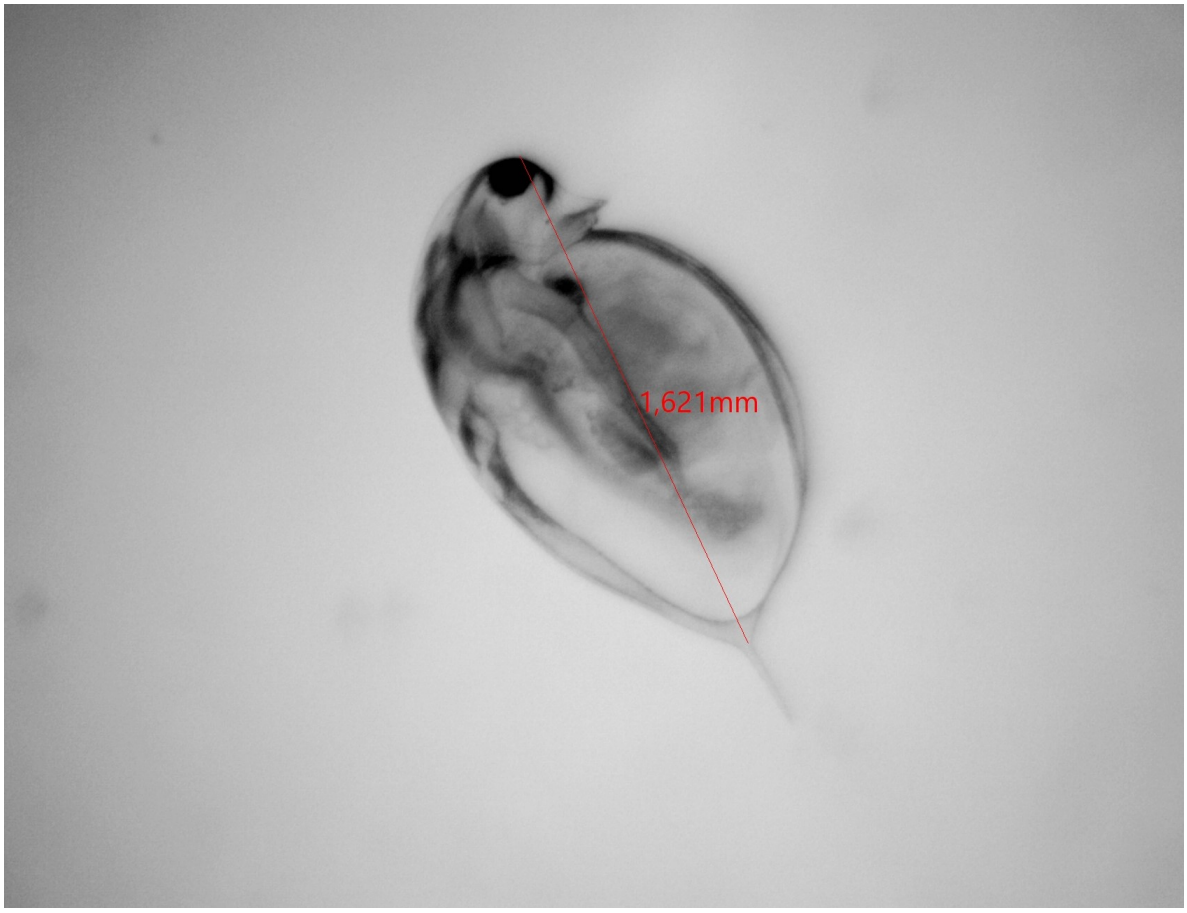

Figure 1.2: *Daphnia pulex* individual observed under 2.5 × 10 magnification. The ruler and body size are highlighted in red.

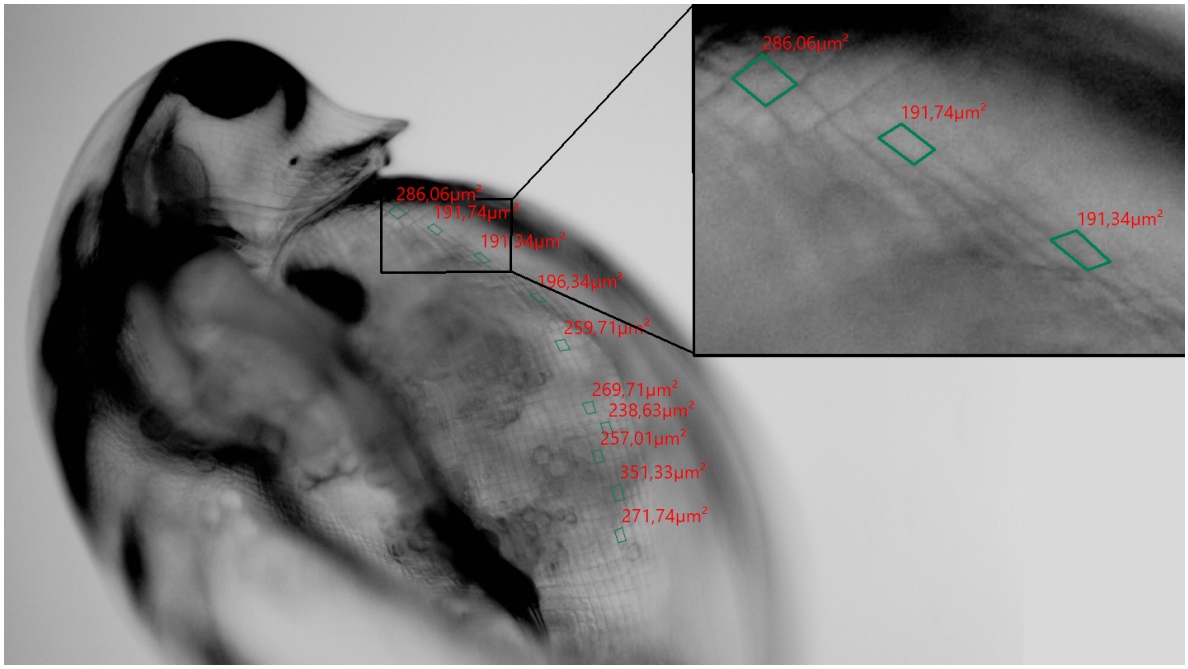

Figure 1.3: *Daphnia pulex* individual observed under 10 × 10 magnification. Ten cell areas are highlighted in red.

1.3.4 Statistical analyses

1.3.4.1 Cold tolerance indicators

Because of the difficulty to detect the precise moment at which *Daphnia* lost their coordination, six different cold tolerance variables (ctmin1 to ctmin6) were measured on each individual (Table 1.2). Means of several combinations of the measured variables were calculated. We chose the mean between ctmin4 and ctmin6 for our CTmin measures as the calculated mean of intra-clone variances was one of the smallest among all measured variables and it respects normality conditions better than ctmin4 for the mixed-effects models.

Table 1.2: Name and description of the different variables measured in Daphnia in order to find the best CTmin indicator. Here, the mean between ctmin4 and ctmin 6 was defined as CTmin.

Variable	Description
ctmin1	First immobilization for at least 30 s with possibility of swimming back up
ctmin2	First immobilization equal or superior to 60 s with possibility of swimming back up
ctmin3	Individual reaches the bottom of the tube for the first time and stays for more than 10 s with possibility of swimming back up
ctmin4	Individual reaches the bottom of the tube without being able to swim back up
ctmin5	First immobilization equal or superior to 30 s without swimming back up
ctmin6	First immobilization equal or superior to 60 s without swimming back up

1.3.4.2 Cold tolerance models

To test the effect of cell area, body size, ploidy (diploid and triploid) and rearing temperature (16 and 24 °C) on cold tolerance, linear mixed-effect models were performed ('lme4' (Bates et al., 2015) and 'lmerTest' (Kuznetsova et al., 2017) packages). Cell area and body size were standardized (mean = 0, SD = 1) in order to allow for a better interpretation of parameter estimates (see Schielzeth (2010) for more details). Standardization introduces the concept of phenotypic standard deviation: the effect size of a standardized variable corresponds to the change in the dependent variable for each change in one unit of standard deviation of the standardized variable. As cell area and body size can covary in ectotherms (Gregory et al., 2000), we tested for multicollinearity by calculating the variance inflation factor (VIF) in a full model containing cell area, body size, ploidy, rearing temperature and their interactions. As VIF values for terms that contained cell area and body size were higher than 5, we decided to treat cell area and body size in separate models in the following steps. Model selection was carried out using a method described in Zuur et al. (2009). First, the best random effect structure was chosen by using a *beyond optimal* model including all fixed variables and their interactions and comparing different random effect structures using Akaike Information Criterion (AIC). All tested random structures and their AIC values for each beyond

optimal model can be found in Table 1.3. The model containing cell area led us to choose a structure with random intercept for Clone and Jar variables while the model containing body size led us to choose a structure with random intercept and slope for Clone and random intercept for Jar. However, as body size was excluded from the corresponding model in the following step, the random slope component was later excluded. After choosing the random structure for each of the two models, the fixed effect structure was chosen using the top-down strategy described by Zuur et al. (2009). First, a full model with all predictors and their interactions was fitted using maximum likelihood (ML) estimation. Then likelihood ratio tests (LRT) were used to drop non-significant predictors (alpha level of 0.05) until the optimal model was reached. The optimal model was then fitted using restricted maximum likelihood (REML). While the interaction between ploidy and rearing temperature was not significant ($p = 0.13$), we chose to keep it in the final model as the interaction was of biological interest to us and Delta AIC for models with and without the interaction was low (ΔAIC = 0.704). The final model chosen to explain cold tolerance included rearing temperature, ploidy and their interaction as fixed effects. Model assumptions were verified graphically. It satisfied the assumptions of heterogeneity of variance, of independence and normal distribution of residuals. All statistical analyses were performed using R 4.2.2 (R Core Team, 2022). Linear mixed models used in this study are shown in Table 1.4.

Table 1.3: AIC and Delta AIC of models used to compare various random effects structures, ranked by Delta AIC. Dependent variable is CTmin, fixed effects include ploidy, rearing temperature (Tacc), cell area (Cell.area, Table 1.3*a*) or body size (Body.size, Table 1.3*b*) and all their interactions. AIC values were calculated using 'bbmle' R package (Bolker, 2017). Models were fitted using REML.

a. Model n°	Fixed effects structure	Random effects structure	AIC	ΔAIC
4	Ploidy × Tacc × Cell.area	(1|Clone) + (1|Jar)	1504.5	0.0
6		(1|Climate/Clone) + (1|Jar)	1506.2	1.8
5		(1+Cell.area|Clone) + (1|Jar)	1507.2	2.7
3		(1|Jar)	1508.4	4.0
2		(1|Clone)	1528.7	24.2
1		No random effects	1556.0	51.5

b. Model n°	Fixed effects structure	Random effects structure	AIC	ΔAIC
5	Ploidy × Tacc × Body.size	(1+Body.size|Clone) + (1|Jar)	1482.1	0.0
4		(1|Clone) + (1|Jar)	1488.9	6.8
6		(1|Climate/Clone) + (1|Jar)	1490.7	8.7
3		(1|Jar)	1493.8	11.7
2		(1|Clone)	1512.2	30.2
1		No random effects	1544.4	62.3

Table 1.4: Formulas of linear mixed models used. Rearing temperature (Tacc), body size (Body.size), cell area (Cell.area) and ploidy. Standardized (mean = 0, SD = 1) input variables are marked with a subscript C. Formulas are written using 'lme4' package terminology (Bates et al., 2015).

Model	Dependent variable	Fixed effects structure	Random effects
Mod1	CTmin	Tacc	(1|Clone) + (1|Jar)
Mod2	CTmin	Body.size_C × Tacc	(1+Body.size_C|Clone) + (1|Jar)
Mod3	CTmin	Cell.area_C × Tacc	(1+Cell.area_C|Clone) + (1|Jar)
Mod4	CTmin	Ploidy × Tacc	(1|Clone) + (1|Jar)
Mod5	Body.size	Tacc × Ploidy	(1|Clone) + (1|Jar)
Mod6	Cell.area_C	Body.size_C × Tacc × Ploidy	(1|Clone) + (1|Jar)

1.4 Results

1.4.1 Body size and cell area

The average body size of *Daphnia* acclimated at 16 °C was 1.41 mm 95% CI [1.36, 1.47] and 1.24 mm [1.20, 1.29] for those acclimated at 24 °C. It ranged from 0.5 to 2.59 mm. The average cell area was 309 μm^2 [298, 319] at 16 °C and 319 [308, 329] μm^2 at 24 °C. It ranged from 95 to 624 μm^2.

The effect of ploidy on body size did not differ significantly between rearing temperatures ($F_{1,83.4}$ = 0.962, p = 0.33, Table 1.5). Ploidy had a significant effect on body size ($F_{1,14.7}$ = 6.270, p = 0.025) as well as rearing temperature ($F_{1,83.4}$ = 7.895, p = 0.006). Body size was lower in warm-acclimated individuals and triploids had a greater body size (Figure 1.4).

Table 1.5: Type III Analysis of Variance Table for the linear mixed model explaining body size of *Daphnia pulex* as a function of rearing temperature and ploidy (Mod5, Table 1.4).

Model term	Num *df*	Den *df*	*F* value	*p* value
Ploidy	1	14.7	6.270	**0.025**
Rearing temperature (Tacc)	1	83.4	7.895	**0.006**
Ploidy × Tacc	1	83.4	0.962	0.330

Note: The *F*-statistics are calculated based on Satterthwaite's degrees of freedom.
Significant terms ($p < 0.05$) are shown in bold.

The effect of body size on cell area changes according to rearing temperature ($F_{1,520}$ = 9.1; p = 0.003; Table 1.6; Figure 1.5; Mod6). For each ploidy and at both rearing temperatures, small-sized *Daphnia* had similar cell area, while larger-sized warm-acclimated individuals had larger cell areas (Figure S.3.6). This effect of body size on cell area was significantly higher at 24 than at 16 °C in diploids (t_{521} = -2.78; p = 0.029; Table 1.8). It was also higher at 24 °C in triploids however the difference of slope estimates was not significant (t_{515} = -1.75; p = 0.300; Figure S.3.6; Table 1.8). Triploids had bigger cell area than diploids regardless of

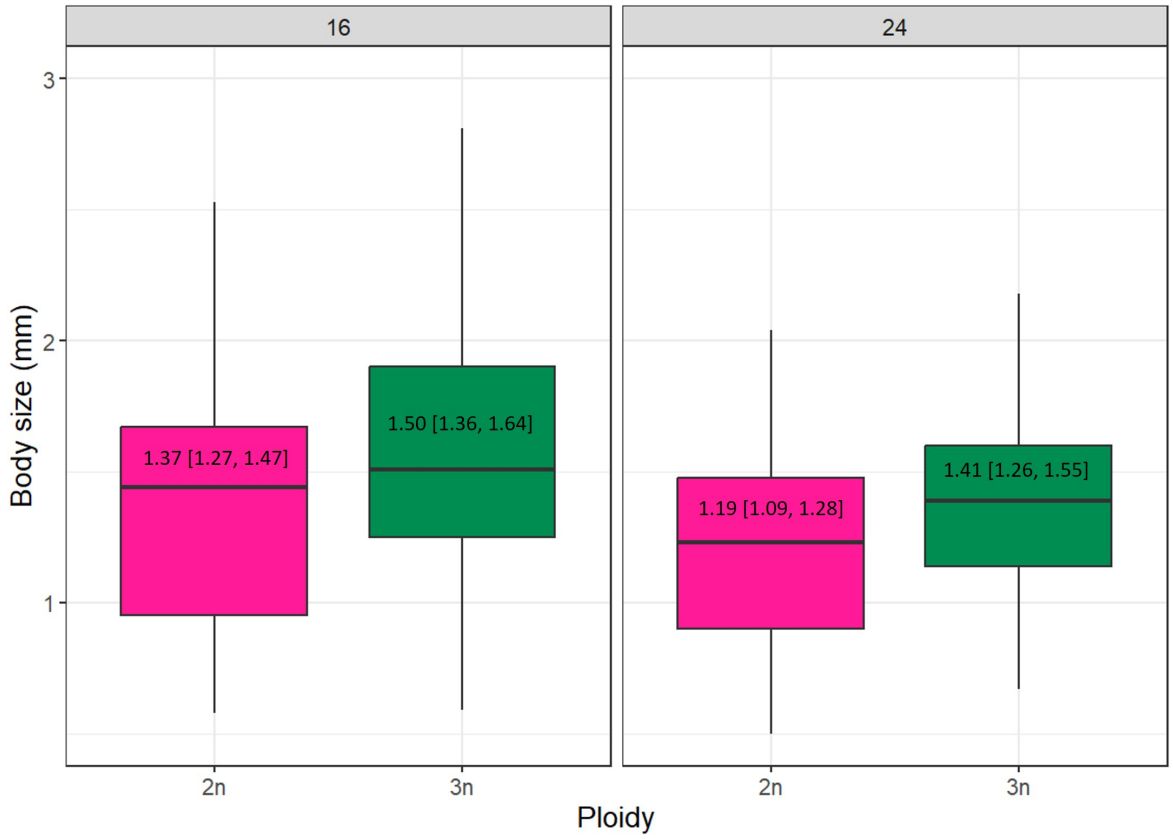

Figure 1.4: Body size (mm) for each ploidy and at both rearing temperatures (Mod5, Table 1.4). Estimated marginal means and 95% CI are shown inside the boxes.

body size and rearing temperature with a difference of 0.73 phenotypic standard deviation in cell area (95% CI [0.43, 1.04]; Table 1.7; Figure S.3.5).

1.4.2 Cold tolerance

A total of 543 *Daphnia* individuals (268 acclimated at 16 and 275 at 24 °C respectively) from 17 clones were tested for cold tolerance (Table 1.1). Significant difference in cold tolerance was found between *Daphnia* reared at 16 and 24 °C ($F_{1,85} = 48$; $p < 0.001$, Mod1). Mean CTmin was -2.44 °C 95% CI [-2.68, -2.20] at 16 °C and -1.66 [-1.90, -1.42] at 24 °C (Figure 1.6). CTmin was below the freezing temperature of the medium, at a temperature that might probably not be found in natural conditions.

Table 1.6: Type III Analysis of Variance Table for the linear mixed model explaining cell area of *Daphnia pulex* as a function of rearing temperature and ploidy corrected for body size (Mod6, Table 1.4).

Model term	Num df	Den df	F value	p value
Body size (Tind)	1	522.75	223.794	**< 0.001**
Rearing temperature (Tacc)	1	82.97	15.383	**< 0.001**
Ploidy	1	15.58	29.971	**< 0.001**
Tind × Tacc	1	520.43	9.100	**0.003**
Tind × Ploidy	1	522.75	0.507	0.477
Tacc × Ploidy	1	82.97	0.191	0.66
Tind × Tacc × Ploidy	1	520.43	0.009	0.926

Note: The F-statistics are calculated based on Satterthwaite's degrees of freedom. Significant terms ($p < 0.05$) are shown in bold. Cell size and body size were centered and scaled.

Table 1.7: Parameter estimates (± SE) of the effect of body size, rearing temperature, ploidy and their interactions on cell area in *Daphnia pulex* (Mod6, Table 1.4).

Effect	Estimate	SE	df	t	p value
Intercept	-0.463	0.090	32	-5.201	**< 0.001**
Body size (Tind)	0.464	0.055	502	8.511	**< 0.001**
Rearing temperature (Tacc)	0.396	0.104	84	3.822	**< 0.001**
Ploidy	0.739	0.155	33	4.772	**< 0.001**
Tind × Tacc	0.228	0.081	521	2.802	0.005
Tind × Ploidy	-0.046	0.089	522	-0.512	0.61
Tacc × Ploidy	-0.080	0.182	83	-0.438	0.66
Tind × Tacc × Ploidy	-0.014	0.147	520	-0.093	0.92

Note: The marginal and conditional coefficients of determination for the model are respectively $R^2_m = 0.46$ and $R^2_c = 0.60$. The model's intercept corresponds to mean body size, Ploidy = 2N and Tacc = 16 °C. Significant estimates are marked in bold. REML was used and the t-statistics are calculated based on Satterthwaite's degrees of freedom. Significant terms ($p < 0.05$) are shown in bold. Body size and cell area were centered and scaled.

There was no evidence that the effect of body size on CTmin varied according to temperature ($F_{1,518.3} = 0.330$; $p = 0.566$, Table 1.9, Mod2). There was no significant relationship between body size and CTmin ($F_{1,15.6} = 0.817$, $p = 0.380$). The change in CTmin for each

Table 1.8: Intercept and slope estimates for cell area as a function of body size for each group of ploidy and acclimation temperature (Mod6, Table 1.4). Comparison of slope estimates between temperatures for each ploidy.

Group	Intercept	95% CI	*df*	Slope	95% CI	*df*	*t*	*df*	*p*
2N 16°C	-0.489	[-0.670,-0.308]	34.1	0.464	[0.356,0.573]	504	**-2.782**	**521**	**0.029**
2N 24°C	-0.106	[-0.288,0.076]	35.2	0.692	[0.574,0.811]	497			
3N 16°C	0.252	[-0.006,0.511]	36.2	0.418	[0.279,0.558]	507	-1.748	515	0.30
3N 24°C	0.557	[0.291,0.823]	36.3	0.633	[0.435,0.831]	520			

Note: Cell area and body size are centered and scaled.

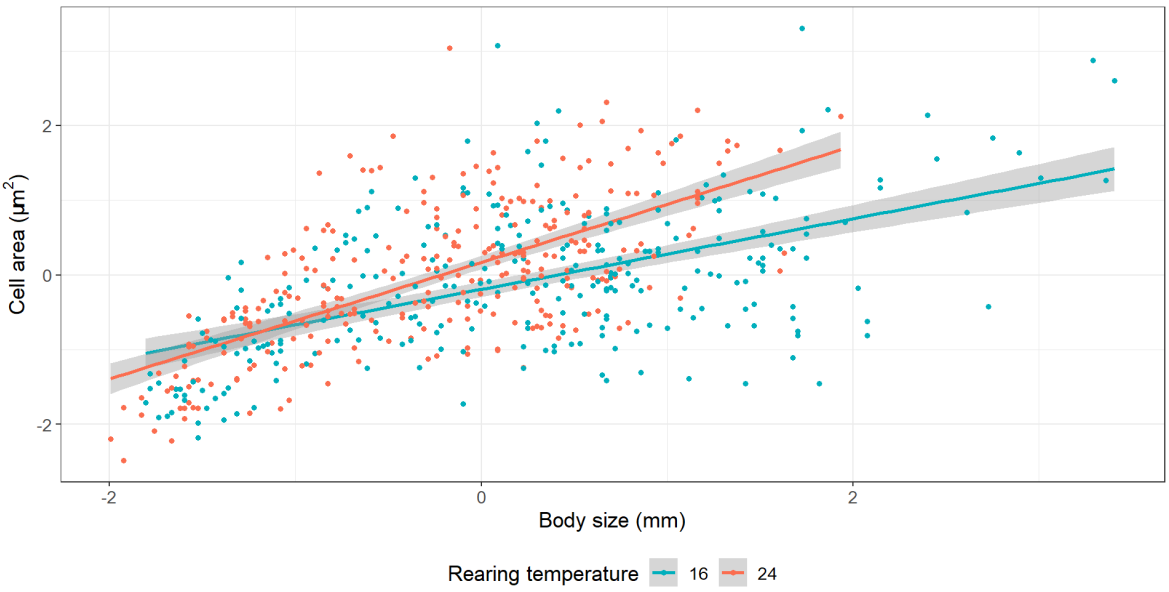

Figure 1.5: Relationship between cell area and body size at 16 and 24 °C. Body size and cell area are centered and scaled. See Table 1.8 for intercept and slope estimates at each temperature and ploidy (Mod6).

phenotypic standard deviation of body size was 0.04 95% CI [-0.13; 0.22] at 16 °C and 0.10 [-0.10 ; 0.29] at 24 °C (Table 1.10). A change in one unit of phenotypic standard deviation corresponds to a change of one unit of body size standard deviation. Rearing temperature had a significant positive effect on CTmin ($F_{1,86.32}$ = 51.69, $p < 0.001$). CTmin increased by 0.81 °C 95% CI [0.59, 1.03] in warm-acclimated individuals ($t_{86.32}$ = 7.19, $p < 0.001$). The marginal and conditional coefficients of determination for the model are respectively

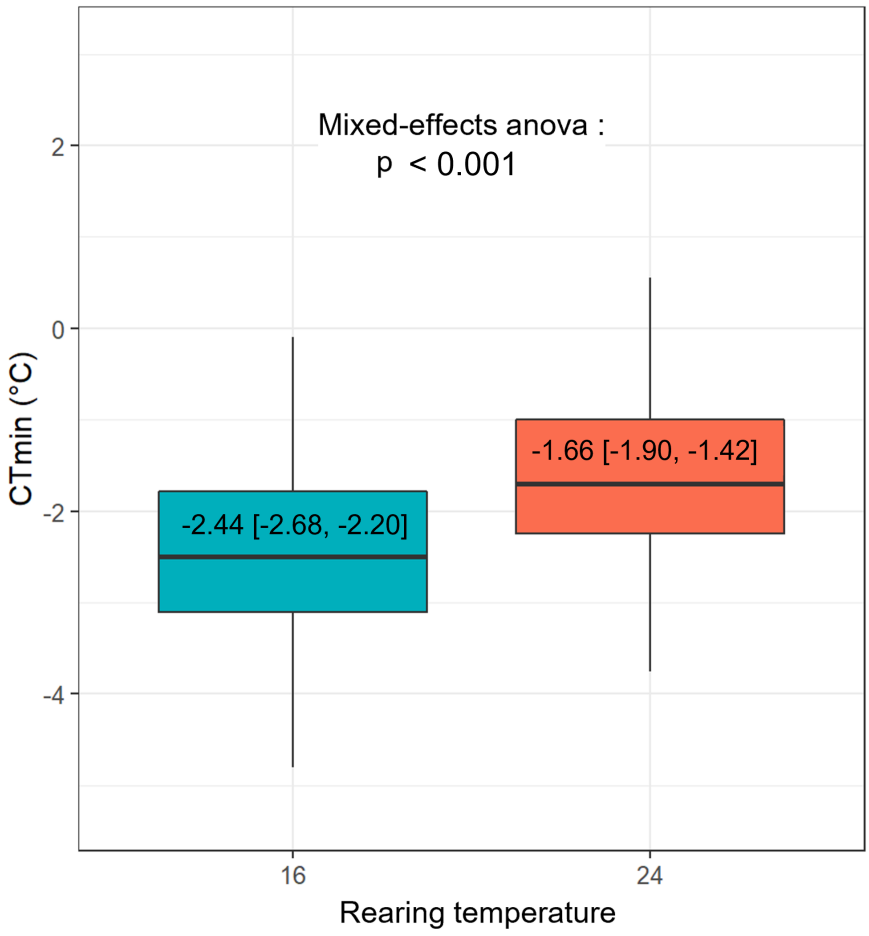

Figure 1.6: CTmin of all tested individuals at each rearing temperature. Means with 95% CIs are shown inside the boxes and p value comes from a LMM (Mod1, Table 1.4).

$R^2_m = 0.12$ and $R^2_c = 0.43$.

Table 1.9: Type III Analysis of Variance Table for the linear mixed model explaining CTmin of *Daphnia pulex* as a function of body size and rearing temperature (Mod2, Table 1.4).

Model term	Num df	Den df	F value	p value
Body size (Tind)	1	15.62	0.817	0.380
Rearing temperature (Tacc)	1	86.32	51.691	**< 0.001**
Tind × Tacc	1	518.33	0.330	0.566

Note: The F-statistics are calculated based on Satterthwaite's degrees of freedom. Significant terms ($p < 0.05$) are shown in bold. Body size was centered and scaled.

Table 1.10: Parameter estimates (± SE) of the effect of body size and rearing temperature and their interactions on CTmin in *Daphnia pulex* (Mod2, Table 1.4).

Effect	Estimate	SE	df	t	p value
Intercept	-2.454	0.124	23.681	-19.870	**< 0.001**
Body size (Tind)	0.042	0.085	24.009	0.496	0.624
Rearing temperature (Tacc)	0.806	0.112	86.316	7.190	**< 0.001**
Tind × Tacc	0.053	0.093	518.333	0.574	0.566

Note: The marginal and conditional coefficients of determination for the model are respectively ${R^2}_m = 0.12$ and ${R^2}_c = 0.43$. The model's intercept corresponds to mean body size, Ploidy = 2N and Tacc = 16 °C. Significant estimates are marked in bold. REML was used and the t-statistics are calculated based on Satterthwaite's degrees of freedom. Significant terms ($p < 0.05$) are shown in bold. Body size was centered and scaled.

There was no evidence that the effect of cell area on CTmin varied according to temperature ($F_{1,436.5} = 0.244$, $p = 0.622$, Table 1.11, Mod3). There was no significant relationship between cell area and CTmin ($F_{1,10.8} = 0.985$, $p = 0.341$). The change in CTmin for each phenotypic standard deviation of cell area was 0.03 °C 95% CI [-0.12 ; 0.19] at 16 °C and 0.08 °C [-0.07 ; 0.23] at 24 °C (Table 1.12). Rearing temperature had a significant positive effect on CTmin ($F_{1,86.81} = 47.61$, $p < 0.001$). CTmin increased by 0.78 °C [0.56, 1.01] in warm-acclimated individuals ($t_{86.81} = 6.90$, $p < 0.001$). The marginal and conditional coefficients of determination for the model are respectively ${R^2}_m = 0.13$ and ${R^2}_c = 0.40$.

Table 1.11: Type III Analysis of Variance Table for the linear mixed model explaining CTmin of *Daphnia pulex* as a function of cell area and rearing temperature (Mod3, Table 1.4).

Model term	Num df	Den df	F value	p value
Cell area (Tcell)	1	10.83	0.985	0.341
Rearing temperature (Tacc)	1	86.81	47.605	**< 0.001**
Tcell × Tacc	1	436.47	0.244	0.622

Note: The F-statistics are calculated based on Satterthwaite's degrees of freedom. Significant terms ($p < 0.05$) are shown in bold. Cell area was centered and scaled.

Table 1.12: Parameter estimates (± SE) of the effect of cell area, rearing temperature and their interactions on CTmin in *Daphnia pulex* (Mod3, Table 1.4).

Effect	Estimate	SE	df	*t*	*p* value
Intercept	-2.438	0.121	25.878	-20.209	**< 0.001**
Cell area (Tcell)	0.034	0.075	28.638	0.451	0.655
Rearing temperature (Tacc)	0.783	0.113	86.810	6.900	**< 0.001**
Tcell × Tacc	0.045	0.092	436.470	0.494	0.622

Note: The marginal and conditional coefficients of determination for the model are respectively R^2_m = 0.13 and R^2_c = 0.40. The model's intercept corresponds to mean cell area, Ploidy = 2N and Tacc = 16 °C. Significant estimates are marked in bold. REML was used and the *t*-statistics are calculated based on Satterthwaite's degrees of freedom. Significant terms ($p < 0.05$) are shown in bold. was centered and scaled.

Effect of ploidy

The model chosen to describe CTmin includes rearing temperature and ploidy and their interaction (Mod4, Table 1.4; Figure 1.7). The geographic origin of the clones had no significant effect on CTmin and thus was not kept in the model. The model's total explanatory power (conditional R^2) was of 0.39 and the part related to the fixed effects alone (marginal R^2) was of 0.18. The effect of ploidy on CTmin was not dependent on rearing temperature ($F_{1,86}$ = 2.32; p = 0.131). Warm acclimation significantly increased CTmin (β = 0.66 °C, 95% CI [0.40, 0.93], $t_{83.82}$ = 4.88, $p < 0.001$). Triploid *Daphnia* had significantly lower CTmin (β = -0.68 °C [-1.11, -0.25], $t_{29.99}$ = -3.10, p = 0.002).

Table 1.13: Type III Analysis of Variance Table for the linear mixed model explaining CTmin of *Daphnia pulex* as a function of ploidy and rearing temperature (Mod4, Table 1.4).

Model term	Num df	Den df	*F* value	*p* value
Rearing temperature (Tacc)	1	86.294	49.463	**< 0.001**
Ploidy	1	15.725	7.112	**0.017**
Tacc × Ploidy	1	86.294	2.320	0.131

Note: The *F*-statistics are calculated based on Satterthwaite's degrees of freedom. Significant terms ($p < 0.05$) are shown in bold.

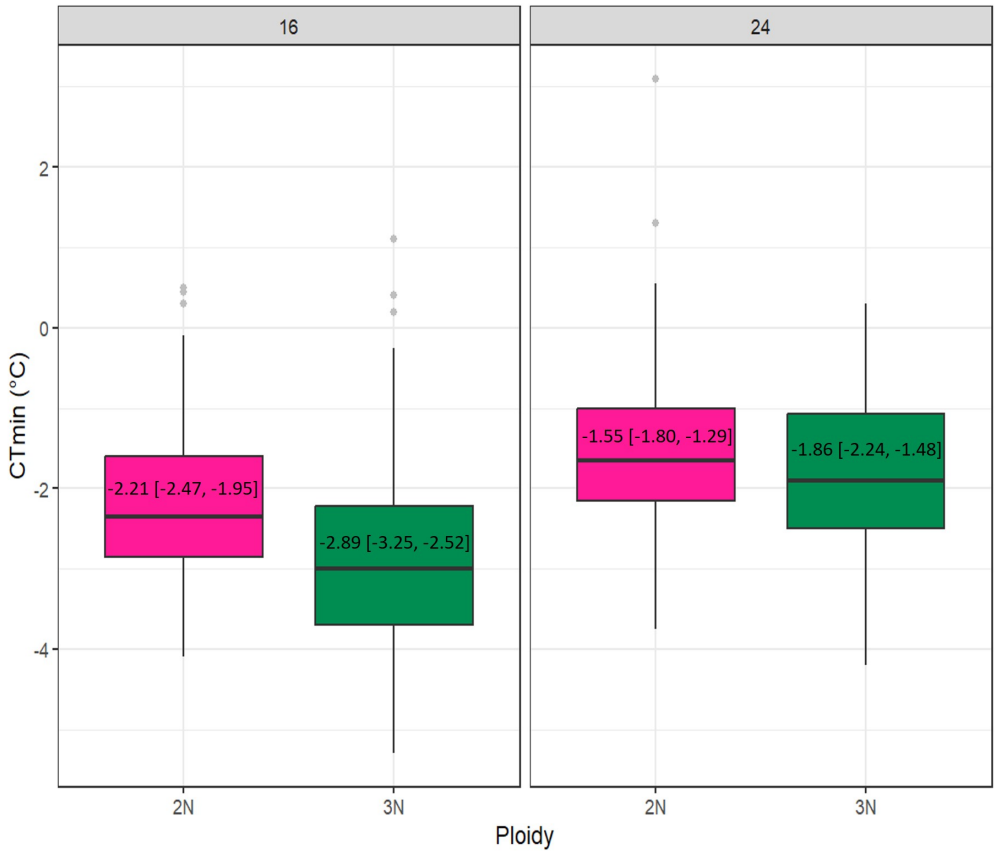

Figure 1.7: CTmin for each ploidy and at both rearing temperatures (Mod4, Table 1.4). Estimated marginal means and 95% CI are shown inside the boxes.

1.5 Discussion

This study aimed to examine to which extent cold tolerance could explain the distribution patterns of clones of the North American *D. pulex* complex by comparing responses to cold shock and testing the hypothesis that body and cell size differences could explain the divergences. Overall, we found a slight advantage of polyploidy for cold shock tolerance, but this was not linked to body or cell size. We discuss potential mechanisms that could lead to differences in cold tolerance and also cold shock in *Daphnia* and therefore influence the distribution.

1.5.1 Effects of acclimation temperature and ploidy on cold shock tolerance

Our results show that *Daphnia* acclimated to low temperatures were more cold tolerant than those raised at higher temperature. This was found in both diploid and triploid clones. While the effect of an 8 °C decrease of acclimation temperature leads to an increase in cold tolerance of less than 1 °C, the difference is significant. A similar result was found for heat tolerance, where an 8 °C increase of acclimation temperature led to an increase in heat tolerance of 1 to 2 °C. The increase in cold tolerance after cold acclimation or rapid cold hardening is a widely observed mechanism in ectotherms (porcelain crab: Ronges et al. (2012); *Drosophila*: Colinet et al. (2012); Enriquez and Colinet (2019); Tarapacki et al. (2021)). Cold acclimation leads to a vast array of physiological changes such as the remodelling of membrane fatty acid composition, the production of cryoprotectants, the upregulation of various proteins or changes in the activities of metabolic enzymes (Enriquez and Colinet, 2019; Overgaard and MacMillan, 2017; Ronges et al., 2012).

We hypothesized that triploid *Daphnia* would be better adapted to cold temperatures, a trait that would be advantageous for them to thrive in subarctic and arctic environments. Thus, we expected that triploids would outperform diploids at cold-shock tests. Our results support this prediction as our data show evidence of a better cold tolerance in triploid *Daphnia*. A few studies also pointed to an advantage of polyploids at cold temperatures. In zebrafish, Hermaniuk et al. (2021) found that warm-acclimated triploids had higher metabolic rates than diploids at acute cold temperature. They interpreted this higher metabolic rate as advantageous for maintaining performance of the animal at low temperature and suggested that this improvement was linked to cell size. Higher development rates have been reported for polyploids in comparison to diploids in *Daphnia* (Dufresne and Hebert, 1998) and tadpoles (Hermaniuk et al., 2016), consistent with a higher metabolic rate in cold-acclimated polyploids. On the other hand, in Atlantic salmon, Riseth et al. (2020) found that triploidy did not give any advantage over diploidy in terms of metabolic capacity or swimming performance at low temperatures. However in their study, triploids are less tolerant of higher temperatures,

consistent with the findings of Bernier (2020) and Hermaniuk et al. (2021) who respectively found a lower CTmax and a lower metabolic rate at high temperature in triploids compared to diploids. Riseth et al. (2020) conclude that other traits contribute to the fact that bigger cells and genomes are observed in animals found at high latitudes.

1.5.2 Physiological determinants of cold shock tolerance

In endotherms and ectotherms, larger body size is often associated with colder environments (Atkinson and Sibly, 1997; Hessen et al., 2013; Vinarskiĭ, 2013; Zamora-Camacho et al., 2014). It was suggested that ploidy-induced increase in cell size would be advantageous in cold conditions (Dufresne and Hebert, 1998; Hermaniuk et al., 2021). Contrary to our expectations, acute cold tolerance was not linked to body size nor cell area in our study. Positive effects of body size on cold tolerance have been found in bumblebees (Gonzalez et al., 2022; Oyen et al., 2016) but this does not apply in other cases such as in ants (Baudier and O'Donnell, 2018) or tropical gobies (Di Santo and Lobel, 2017). The lack of effect of body size on CTmin shown by our data needs to be taken with care. While we selected *Daphnia* of differing body sizes, our sampling was not designed to get the most out of the body size range for all clones at all temperatures. Furthermore, we did not find evidence of a direct relationship between CTmin and cell area as predicted. However, triploids, which had the largest cells, also had the lowest CTmin at both temperatures. Surprisingly, warm-acclimated larger-bodied *Daphnia* had larger cells which is in opposition with the usual pattern temperature-size rule observed in ectotherms where warm-acclimated individuals have smaller cells (Verberk et al., 2020; Ohlberger, 2013). But as pointed by Atkinson et al. (2006), the temperature-size rule does not apply to all cell types. Thus the deviation from the temperature-size rule in our data could be one of many exceptions to the rule. The fact that warm-acclimation led to higher CTmin values but also higher cell size in bigger individuals might have masked the beneficial effect of big cells on cold tolerance that we expected. Further studies could explore the relationship between CTmin and cell size while controlling for body size in order to clearly

verify the absence of link between cell size and CTmin. Our results did not show evidence that body size and cell area play a prominent role in cold shock tolerance. Other unmeasured physiological traits should explain the differences between ploidies in cold acclimated *Daphnia*. We cannot however exclude that cell size or body size could play a role in chronic cold stress tolerance (i.e. exposure to cold lasting for hours to days). Leiva et al. (2019) for example found that ectotherm species with a larger body and larger cells perform better at cold tolerance tests. CTmin is especially enhanced in air-breathing species during long-term trials as opposed to short-term trials as is the case in our study. Bigger cells or a bigger body size could allow resisting to longer periods of cold stress by reducing energetic costs and providing higher energetic reserves without any significant consequences in the ability to resist to cold shocks. Thus, they could benefit triploids to sustain normal functions in colder temperatures. The measurement of tolerance to chronic cold stress of diploid and triploid *Daphnia* clones could help to answer this question. It is also important to keep in mind that other factors than cell surface area can determine the energetic demand of organisms such as cell type and functions (Glazier, 2022).

If not body or cell size, which phenotypic differences led triploids to resist cold shock better than diploids? Polyploidy increases the number of gene copies. This can lead to an increased expression of certain genes, some of which could be helpful to tolerate cold shock. How polyploidization changes gene expression is not so well understood. Some genes might be over-expressed, others downregulated, and the expression of others can stay unchanged due to compensation (Doyle and Coate, 2019). Many changes in gene expression follow cold shock and cold stress. In fish, the expression of some genes is particularly modified (reviewed by Reid et al. (2022)). Amongst them are genes coding for chaperone proteins belonging to the heat shock proteins family, which play an important role in the response to cold stress in ectotherms (Jiang et al., 2021; Štětina et al., 2015; Hayward et al., 2014). In *Drosophila*, HSP genes expression (Colinet et al., 2010) and metabolic changes (Colinet et al., 2012) occurred during recovery from cold stress but not during cold stress itself. Because of the delay between stress and HSP expression and the rate of temperature decrease, it is uncertain whether

Daphnia were able to overexpress HSPs during the trial. It is however possible that due to their higher gene copy number, triploids keep higher baseline levels of genes involved in cold shock response, such as HSPs. Cold stress is also associated with oxidative stress (Reid et al., 2022; Zhang and Wong, 2021) which happens when reactive oxygen species (ROS) are overproduced, and antioxidant defenses are insufficient to counteract their harmful effect. Response to cold stress and cold tolerance have been associated with an antioxidant response, showing the role of oxidative stress and antioxidant defenses in cold tolerance (Reid et al., 2022; Mi et al., 2021; Mucci et al., 2021). HSP and antioxidant responses are among mechanisms that are potentially favored in polyploid *Daphnia* to cope with cold shock. Further testing would be needed to verify the role of these mechanisms in polyploid *Daphnia* cold tolerance.

Another physiological aspect potentially involved in adaptation and acclimation to cold temperatures concerns lipids and membrane fatty acids. Some cases of overwintering *Daphnia* have been observed in clones from subarctic and boreal lakes and these populations were characterized by high body fat content, high fatty acid concentrations and a higher retention of polyunsaturated fatty acids, mainly stearidonic acid (Mariash et al., 2017). According to this study, high lipid reserves constitute a source of energy to survive periods of low food availability and probably help to tolerate cold stress. Higher body fat accumulation in cold-acclimated triploids, which could be favored by their bigger cells, could help them better tolerate cold stress. Fatty acid composition of cell membranes also has an impact on ectotherm cold tolerance through changes in membrane fluidity (Trenti et al., 2022; Enriquez and Colinet, 2019; Hayward et al., 2014; Waagner et al., 2013; Overgaard et al., 2005) which is consistent with the homeoviscous adaptation hypothesis (Ernst et al., 2016). Comparisons of fatty acid profiles of cold-acclimated diploid and triploid *Daphnia* clones found that triploid *Daphnia* tended to accumulate the highest amount of eicosapentaenoic acid (EPA) in their tissues (unpublished data, see article 2). EPA is a polyunsaturated fatty acid that is accumulated at cold temperatures to ensure a adequate membrane fluidity (Zeis et al., 2019; Martin-Creuzburg et al., 2019). These results are not completely comparable with the ones in

the present study as they were carried out on different clones, but they support the hypothesis that changes in fatty acid profile composition following cold acclimation might lead triploids to tolerate cold shocks or cold temperature in general.

1.5.3 Cold shock tolerance in *Daphnia* from an ecological perspective

It would be interesting to know whether cold shock tolerance, measured here as CTmin, can be used as an indicator of cold adaptation in *Daphnia* clones. In that sense, further studies investigating whether CTmin is correlated to chronic cold stress tolerance and growth/reproductive rates at low temperature would be helpful. It is known that various physiological/behavioral traits such as locomotion or reproduction can have different thermal sensitivities (Buckley and Kingsolver, 2021; Dell et al., 2011). Thus it is difficult to assess whether a clone that is able to sustain locomotion at lower temperatures will also be able to complete its life cycle at lower temperatures.

In their natural habitat, *Daphnia* probably do not encounter temperatures as low as their CTmin. As environmental conditions start to deteriorate (e.g., low food availability, temperature decrease), they usually enter diapause (Mariash et al., 2017; Koch et al., 2009). Contrary to aquatic ectotherms, terrestrial ectotherms are more likely to encounter sub-zero temperatures and supercooling of body liquids in order to cope with acute cold has been observed (e.g. Sinclair et al. (2015) ; Berman et al. (2021)). Understanding cold tolerance at temperatures below the freezing point of water in ectotherms is thus valid in a number of species. One might ask if the rate of temperature decrease our individuals had to face is ecologically relevant. Depending on their habitats, populations of aquatic ectotherms face cold shock events. These can be due to natural (e.g., water mixing in shallow or nearshore systems) or anthropogenic reasons (e.g., variations in power plant cooling water discharges) (Szekeres et al., 2016). *Daphnia* populations living in deeper water bodies face rapid temperature changes when undertaking diel vertical migration (Isanta Navarro et al., 2019; Zeis et al., 2019). Populations living in shallow ponds may experience rapid temperature changes,

albeit at a lower rate than in this experience, but which could still be stressful.

1.6 Conclusion

This study is the first to provide CTmin estimates in *Daphnia* clones differing in ploidy and origin. We show that triploids are more resistant to cold shock than diploids. This result supports the hypothesis that polyploids are more cold-adapted and suggests they can colonize and thrive in colder habitats. Body size and cell area were not linked to cold tolerance which is inconsistent with our prediction, but these traits might still be of adaptive significance for chronic cold stress tolerance. We suspect that triploidy could contribute to cold tolerance through 1) increased gene expression, especially particularly for genes involved in heat shock response and antioxidant pathways and 2) lipid content, in terms of quantity and fatty acid composition. It is important to keep in mind that some other aspects of the physiology and ecology of organisms like life-history traits can shape the distribution of North American *D. pulex* clones. We also recommend that future studies on cold tolerance focus on chronic cold stress tolerance rather than acute cold tolerance such as the CTmin metric, as long-term cold stress might be more ecologically relevant in most aquatic ectotherm species.

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ARTICLE II

LINKING FATTY ACIDS, HEAT TOLERANCE AND LOCAL ADAPTATIONS IN DIPLOID AND TRIPLOID *DAPHNIA PULEX* CLONES

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2.1 Abstract

Physiological processes setting thermal tolerance limits in ectotherms are not completely understood and their understanding would be beneficial to estimate the adaptive response to temperature changes. Mitochondrial dysfunctions under heat stress, and particularly ROS overproduction with subsequent oxidative damage, have been proposed as limiting factors for heat tolerance. This hypothesis first proposed in fishes needs to be tested in other groups to validate it as a general mechanism in ectotherms. We use the North American *Daphnia pulex* complex, which makes a remarkable model for comparative physiology as it is composed of clones differing in heat tolerance, ploidies and its range extends through several climatic regions. Here, we analyzed the fatty acid composition of diploid and triploid *D. pulex* clones from temperate and subarctic climates. We linked fatty acid profile with heat tolerance (CTmax), geographic origin, ploidy and cell size in 17 clones acclimated to 16 and 24 °C. Contrary to our expectations, we found no relationship between bulk membrane fatty acid profile properties such as peroxidation index and individual heat tolerance. Eicosapentaenoic acid (EPA) was negatively related to heat tolerance. Temperate clones had a higher thermal plasticity of fatty acid profile than subarctic clones and triploids retained omega-6 polyunsaturated fatty acids in a unique way at high temperature. Overall, these findings suggest that whole-animal fatty acid profiles do not necessarily correlate with CTmax but point to a potential effect of specific fatty acids. We also provide evidence of differential lipid synthesis and deposition in polyploid *D. pulex*.

Keywords: Temperature, ploidy, *Daphnia*, fatty acids, CTmax

2.2 Introduction

Mitochondria are key organelles of aerobic metabolism and their complex network of enzymes is highly sensitive to thermal variation. This led to a growing interest on the role of mitochondrial processes in determining whole-organism thermal limits (Blier et al., 2014). Most research on this topic has been carried out on fishes for which strong evidence suggest that heat-induced heart failure is due to the disruption of cardiac mitochondrial respiration. Heat stress has been linked with changes in catalytic activity of key enzymes of the electron transport system and deficient ATP synthesis in heart mitochondria, thus impairing the ability to support normal cardiac functions (Iftikar et al., 2014; Christen et al., 2018; Michaelsen et al., 2021). In addition to disruption of ATP synthesis, variations in ETS activity can induce reactive oxygen species (ROS) production (Christen et al., 2018).

At moderate concentration, ROS act as signaling molecules for the regulation of several physiological functions (Hulbert et al., 2007; Lane, 2011; Ray et al., 2012). Under normal conditions, ROS homeostasis is maintained by cell antioxidant mechanisms but under stressful conditions such as heat stress, ROS are overproduced and damage proteins, DNA or lipids in a phenomenon known as oxidative stress (Hulbert et al., 2007; Zuo et al., 2015). Oxidative stress causes cell dysfunctions and can lead to cell death (apoptosis). As Hulbert et al. (2007) describe, fatty acids (FAs) which compose cell membrane lipids are not equally susceptible to oxidative damage. Carbon atoms that are most susceptible to ROS attacks are the single-bonded carbons between double-bonded carbons. Saturated and monounsaturated fatty acids are therefore resistant to oxidative damage while polyunsaturated fatty acids are more susceptible. Susceptibility to oxidative damage increases with increasing unsaturation of the fatty acid chain. Thus, differences in membrane fatty acid profiles influence the susceptibility of membranes to oxidative stress.

As membranes play an important role for the regulation of cell functions, their integrity is important for maintaining homeostasis at the organismal level (Casares et al., 2019; Hazel

and Williams, 1990). Membrane fluidity, which is linked to its degree of unsaturation, has impacts on many cellular processes through changes in the activity of membrane-bound proteins and particularly to cellular bioenergetics. This led Hulbert and Else (1999) to describe membranes as metabolic pacemakers. Ectotherms have the ability to modify the composition in lipid classes and fatty acids of their membranes in order to maintain an optimal fluidity under changing temperatures. This is described as the homeoviscous adaptation hypothesis (Ernst et al., 2016; Sinensky, 1974). Low temperature acclimated individuals have a higher unsaturation of mono- and polyunsaturated fatty acid content with shorter-chained FAs (higher fluidity) while warm-acclimated individuals accumulate longer-chained, saturated FAs (lower fluidity).

Mitochondrial ROS production the induced peroxidation due to ROS activity has been proposed as one potential process limiting upper thermal limits in ectotherms. Studies on fish showed that oxidative stress could be directly related to heat-induced cardiac failure (Christen et al., 2018; Banh et al., 2016). Christen et al. (2020) found that in two charr species, the most heat-tolerant individuals were those whose cardiac muscle cell membranes had lower PUFA, and particularly lower eicosapentaenoic acid (EPA) content. In *Daphnia*, oxidative damage of membrane lipids has been shown to be greater in individuals with higher membrane PUFA content, either due to a PUFA-rich diet or to temperature related changes in membrane composition (Yampolsky et al., 2022; Zeis et al., 2019). Along with oxidative stress, PUFA-rich diet and lower acclimation temperature led to elevated membrane unsaturation and a decreased heat tolerance (Martin-Creuzburg et al., 2019). These results support the hypothesis that higher membrane PUFA content and unsaturation increase membrane sensitivity to oxidative stress, which in turn should lower heat tolerance. Despite these evidences, there is still no consensus that ROS production induced by disruptions in mitochondrial respiration and organism thermal limits are mechanistically linked (Chung and Schulte, 2020).

This study explores the links between membrane FA content and limits in acute heat tolerance of clones from the North American *Daphnia pulex* complex. This species complex

is composed of diploid and triploid clones with a distributional pattern of ploidy: diploids are found in temperate and subarctic zones while triploids are found at higher latitudes (Beaton and Hebert, 1988). The use of *D. pulex* clones of contrasted ploidies and origins allows to bridge *Daphnia* to its physiological traits. Bernier (2020) found that polyploids have a lower heat tolerance than diploids. As polyploidy induces increased cell size (Glazier, 2022; Gregory, 2001), thermal tolerance may be at least partly mediated by cell size. We also test whether FA content modulates heat tolerance of *D. pulex* by linking it to CTmax which is a widely used metric to assess maximal critical thermal performance of organisms. FA, also named FA content, is defined here as the proportion of a fatty acid or a group of fatty acids in regards to the total FA content. CTmax is defined as the temperature at which organismal locomotion becomes uncoordinated (Chung and Schulte, 2020; Lutterschmidt and Hutchison, 1997). This temperature may not be directly lethal but leads to what is called "ecological death" (i.e. a temperature at which the animal is unable to escape from lethal condition, Chung and Schulte (2020)). In order to account for acclimated thermal effects, we measured FA content of *Daphnia* acclimated at 16 and 24 °C.

We hypothesize that cell membrane fatty acids unsaturation, which is influenced by acclimation temperature, lowers acute heat stress tolerance through an increased susceptibility to membrane peroxidation. We predict that heat tolerance will be negatively linked to membrane susceptibility to oxidative stress (unsaturation and peroxidation indexes). We hypothesize that clones are adapted to their environmental conditions through their fatty acid profiles. Therefore, we predict differences in the fatty acid profile between clones of differing geographic origins and ploidies.

2.3 Material and methods

2.3.1 *Daphnia* and heat tolerance

Daphnia individuals that were used for fatty acid analyses had been previously reared and used for CTmax analyses and their body and cell size was measured Bernier (2020). 18 clones (5 triploid subarctic, 6 diploid subarctic and 7 diploid temperate) were used. Their names and geographic origins are summarized in Table 2.1. Briefly, each clone was reared for at least three generations in standardized laboratory conditions until the experiments. Each generation was started from the second or third brood. *Daphnia* were raised in environmental test chambers at two temperatures: 16 and 24 °C with a 12:12 photoperiod and 3000 lux of lighting. Three replicate cultures were prepared for each clone and temperature combination. *Daphnia* were raised in glass jars containing 300 mL of FLAMES culture medium Celis-Salgado et al. (2008) and fed $2{,}0 \times 10^5$ cells mL^{-1} of *Raphidocelis subcapitata* (formely known as *Pseudokirchneriella subcapitata* or *Selenastrum capricornotum*) three times a week. A third of the culture medium was renewed every two weeks. Individuals were preserved at -80 °C for the fatty acid measurements.

2.3.2 Fatty acid profiles

The fatty acid extraction protocol was adapted from Lepage and Roy (1984) and Christen et al. (2020). Clones used for fatty acid analysis are summarized in Table 2.1. *Daphnia* were homogenized individually in 500 µL of a methanol-toluene mix (1/1, v/v). The homogenate was transferred in a glass vial, and 500 µL of the methanol-toluene mix (1/1, v/v) was added to the Eppendorf tube to retrieve the rest of the homogenate and transferred to the glass vial. Samples were sonicated for one minute. Fatty acid methyl esthers (FAMEs) were obtained using direct acid-catalysed transmethylation. 1 mL of a 12% methanol-sulfuric acid solution was added to the glass vials containing the homogenate, placed 1h at 90 °C and vor-

texed after 30 min. After the transmethylation, samples were placed on ice 10 min. 500 µL of hexane and 1 mL of distilled water were added to the vials. Vials were vortexed and centrifuged 10 min at 400 *g* at ambient temperature. After centrifugation, the superior organic phase was cautiously collected and transferred to a 1.5 mL gas chromatography vial. Again, 500 µL of hexane and 1 mL of distilled water were added to the vials, samples were vortexed and centrifuged 10 min at 400 *g* at ambient temperature. The superior organic phase was collected and transferred to the respective gas chromatography vials. The collected organic phases were evaporated in a rotative evaporator at 45 °C for 20 min. 50 µL of toluene were added to the vials, and evaporated again at 45 °C for 20 min. 60 µL of toluene was added to each vial. Samples were stored at -80 °C until analysis. FAME separation and quantification was realised using a Trace Ultra 100 gas spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a DB-23 60 m × 0,25 mm capillary column (Agilent Technologies Canada, Mississauga, ON, Canada). Helium was used as carrier gas (constant pressure of 230 kPa). Vaporization temperature was 230 °C with a split injection of 100 mL min^{-1}. The temperature program was set as follows: from 50 to 140 °C (25 °C min^{-1}), 140 °C to 195 °C (3 °C min^{-1}) and a final increase to 225 °C (4 °C min^{-1}) maintained 5 min. FAMEs were detected by flame ionization detection (FID) and were identified by comparison with a Supelco 37 standard (Sigma-Aldrich Co. LLC, Saint-Louis, Missouri, USA).

2.3.3 Statistical analysis

Peroxidation index (PI) and unsaturation index (UI) are metrics that are commonly used to quantify sensitivity of cellular membranes to peroxidation and membrane unsaturation respectively. They were calculated according to Hulbert et al. (2007). The values are commonly presented without units:

$$\begin{aligned} UI = (1 \times \%monoenoics) + (2 \times \%dienoics) + (3 \times \%trienoics) \\ +(4 \times \%tetraenoics) + (5 \times \%pentaenoics) + (6 \times \%hexaenoics) \end{aligned} \tag{2.1}$$

Table 2.1: Clones, ploidy, climate and origin of the *Daphnia pulex* clones used for fatty acid analysis. Sample size is $n = 5$ for each clone at each temperature except for A38-10, C145-01, NFL68 and R206 at 24 °C for which $n = 4$.

Clone	Ploidy	Climate	Location	Latitude, Longitude
A5-7m	Triploid	Subarctic	Churchill, Manitoba, Canada	58.767638, -93.975239
A38-10			Churchill, Manitoba, Canada	58.767638, -93.975239
C145-01			Churchill, Manitoba, Canada	58.767638, -93.975239
IQ12			Kuujjuarapik, Nunavik, Canada	55.276672, -77.776401
R202			Kuujjuarapik, Nunavik, Canada	55.276672, -77.776401
A06-06	Diploid	Subarctic	Churchill, Manitoba, Canada	58.767638, -93.975239
B104-05			Churchill, Manitoba, Canada	58.767638, -93.975239
C44			Churchill, Manitoba, Canada	58.767638, -93.975239
R206			Kuujjuarapik, Nunavik, Canada	55.276672, -77.776401
R210			Kuujjuarapik, Nunavik, Canada	55.276672, -77.776401
WP2			Kuujjuarapik, Nunavik, Canada	55.276672, -77.776401
BUS15	Diploid	Temperate	Urbana, Illinois, USA	40.129889, -88.206478
BUS79			Urbana, Illinois, USA	40.129889, -88.206478
KAP-53			Danville, Illinois, USA	40.182782, -87.646184
NFL68			Webster, Indiana, USA	39.903509, -84.941463
SPS100			Homer, Illinois, USA	40.753935, -73.982114
Stukely			Bolton-Ouest, Quebec, Canada	45.286882, 72.420144
Ste-Luce			Sainte-Luce, Quebec, Canada	48.547571, -68.393969

$$\begin{aligned} PI = {} & (0.025 \times \%monoenoics) + (1 \times \%dienoics) + (2 \times \%trienoics) \\ & +(4 \times \%tetraenoics) + (6 \times \%pentaenoics) + (8 \times \%hexaenoics) \end{aligned} \tag{2.2}$$

To test for the effects of fatty acid profile, cell size and rearing temperature (16 and 24 °C) on heat tolerance (CTmax), linear mixed-effect models were performed. Cell size and fatty acid proportions values were standardized (mean = 0, SD = 1) in order to make the interpretation of regression coefficients easier (see Schielzeth (2010) for more details). Three models were fitted to explain CTmax in relation to fatty acid profile, rearing temperatures and cell size (ModA to ModC, Table 2.2). The first model includes fatty acid profile as fixed effect and fatty acid profile-by-clone and jar as random effects. The second model adds rearing temperature and its interaction with fatty acid profile as fixed effect and keeps the same random effects structure. For the first two models, fixed and random structure

were chosen to be biologically relevant. The third model included fatty acid profile, rearing temperature, their interaction, and cell size as fixed effects and clone and jar as random effects. For the third model, the fixed and random effect structures were chosen following a method described in Zuur et al. (2009). The chosen random effect structure was the one with the lowest Akaike Information Criterion (AIC) and which did not lead to singular fit issues. The fixed effects structure was chosen using maximum likelihood ratio tests and includes cell size, which has a significant effect on CTmax (Bernier, 2020).

To evaluate changes in fatty acid profiles between temperatures for each group of ploidy and origin and each fatty acid profile component, a linear mixed-effects model was used with rearing temperature, origin and ploidy, and the interaction between both variables as fixed effects. Random effects were clone and jar. When significant interactions were found, we performed *post hoc* tests ('emmeans' package, Lenth (2022)). Linear mixed-effects models were computed with 'lme4' (Bates et al., 2015) and 'lmerTest' (Kuznetsova et al., 2017) packages. Model conditions were verified using diagnostic graphs. All statistical analyses were performed using R 4.2.2 (R Core Team, 2022). Models used to explain CTmax are shown in Table 2.2.

Table 2.2: Linear mixed models used to assess the relation between heat tolerance, fatty acid (FA) composition, acclimation temperature (Acc.Temp) and cell size (Cell.size). CTmax is the dependant variable in ModA to ModC. Standardized (mean = 0, SD = 1) input variables are marked with a subscript C. ClimPloidy : factor with three levels (temperate diploid, subarctic diploid, subarctic triploid). Formulas are written using 'lme4' package terminology (Bates et al., 2015).

Model	Fixed effects	Random effects
ModA	FA_C	(1+FA_C|Clone) + (1|Jar)
ModB	$FA_C \times$ Acc.Temp	(1+FA_C|Clone) + (1|Jar)
ModC	$FA_C \times$ Acc.Temp + $Cell.size_C$	(1|Clone) + (1|Jar)
ModD	$FA_C \times$ Acc.Temp $\times$ ClimPloidy	(1|Clone) + (1|Jar)

2.4 Results

2.4.1 Heat tolerance and fatty acid profiles

A linear mixed-effects model (ModA, Table 2.2) showed significant relationships between several fatty acids and CTmax (Table 2.3). There was a positive correlation between few saturated fatty acids and CTmax, while there was a negative relationship between some mono- and polyunsaturated fatty acids and CTmax. Model results for all fatty acids and each model can be found in supplementary material (Table S.4.5 to S.4.9).

The strongest relationship found was between EPA and CTmax with a change of -0.72 °C in CTmax for every change in one standard deviation of EPA (Figure 2.1). The range (mean ± 95% CI) of EPA content of cell membranes was more pronounced at 16 than at 24 °C (16 °C : 0.012 [0.0105, 0.0138], min = 0, max = 0.034 ; 24 °C : 0.004 [0.0032, 0.0043], min = 0, max = 0.011).

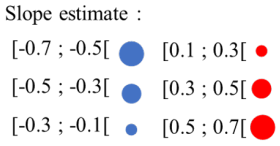
Three linear mixed models showed a significant relationship between heat tolerance and EPA content (Table 2.4). The model that includes rearing temperature and its interaction with EPA content (ModB, Table 2.2) shows that the effect of EPA content on CTmax is temperature-dependent (Table 2.4b). At 24 °C, there was no significant relationship between EPA content and CTmax while the negative relationship was significant at 16 °C. This EPA effect on CTmax is significant even when cell size is included in the model (ModC, Table 2.2).

2.4.2 Fatty acid profile, ploidy and origin

Mixed-effects models showed differential effects of acclimation temperature on fatty acid profiles according to geographic origins and ploidies, with temperate diploids showing higher contrasts (i.e. higher plasticity) between temperatures for several fatty acids (Figure

Table 2.3: Relationship between heat tolerance and some standardized fatty acid profile descriptors. The magnitude and sign of the relationship is given by the size and color of the circles. Corresponding values for slope estimates are given in the legend. Only fatty acids with a significant effect on CTmax and which validated statistical conditions are shown. A lmm (ModA) was used for the analysis, Marginal and conditional *R*-squared values are presented. SFA : total saturated fatty acid, MUFA : total monounsaturated fatty acid, EPA : C20:5n3, DPA : C22:5n3.

	CTmax		
Fatty acid	Est.	R^2_m	R^2_c
EPA		0.25	0.83
EPA/DPA		0.22	0.81
Total MUFA		0.06	0.83
C15:1		0.04	0.82
C22:0		0.02	0.82
C20:0		0.03	0.81
C18:0		0.03	0.82
Total SFA		0.04	0.84



2.2, ModD Table 2.2). Temperate diploid clones accumulated less saturated fatty acids (SFAs) and more polyunsaturated fatty acids (PUFAs) at 16 than at 24 °C (SFAs: t.ratio(47.2) = -5.2, $p < 0.001$; PUFAs: t.ratio(47.1) = 6.1, $p < 0.001$). The difference between acclimation temperatures was not significant for subarctic diploids (SFAs: t.ratio(67.1) = -2.2, $p = 0.24$; PUFAs: t.ratio(66.7) = 1.1, $p = 0.90$) and subarctic triploids (SFAs: t.ratio(62.7) = -1.4, $p = 0.72$; PUFAs: t.ratio(62.3) = -0.68, $p = 0.98$). Peroxidation and unsaturation indexes followed the same pattern with temperate diploids showing a significant difference between temperatures whereas subarctic diploids and triploids showed no difference (UI: temperate diploids t.ratio(46.1) = 6.7, $p < 0.001$; subarctic diploids t.ratio(66.6) = 1.9, $p = 0.39$; subarctic triploids t.ratio(61.8) = 0.86, $p = 0.95$. PI : temperate diploids t.ratio(46.1) = 6.3, $p < 0.001$; subarctic diploids t.ratio(66.6) = 1.5, $p = 0.69$; subarctic triploids t.ratio(61.8) = 0.73, $p = 0.98$). For PUFAs, differences were mainly driven by alpha-linolenic acid (ALA, C18:3n3) and stearidonic acid (SDA, C18:4n3) for which temperate diploids showed the highest diver-

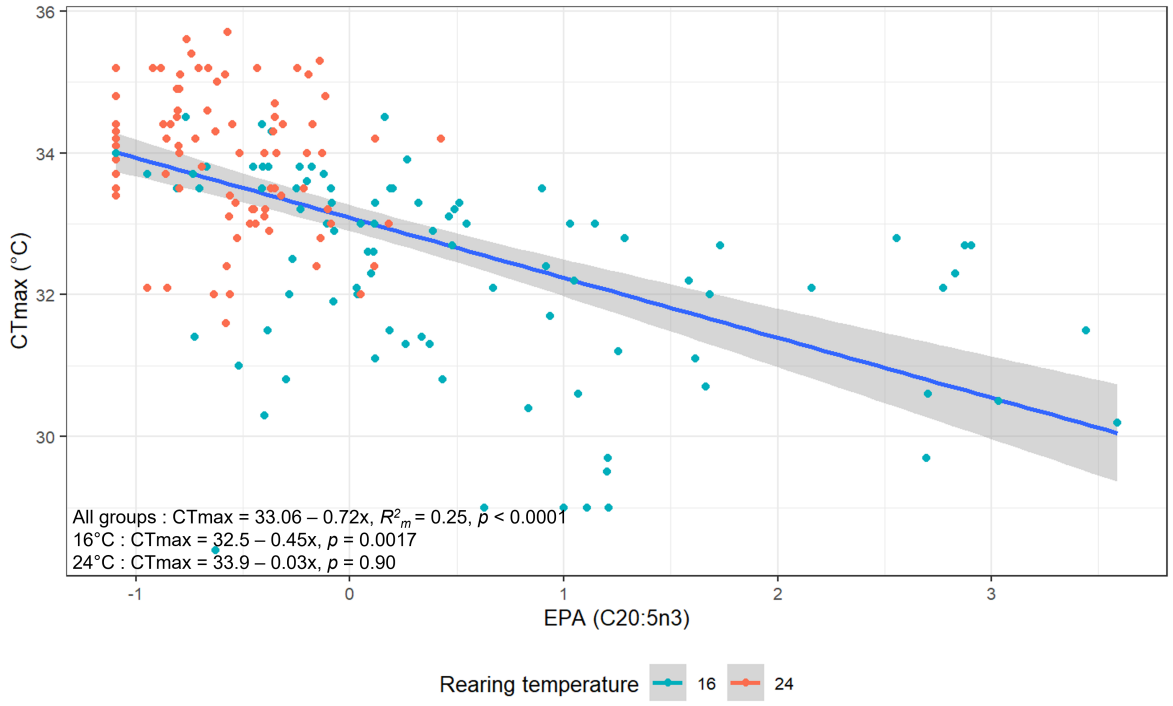


Figure 2.1: Relationship between CTmax and EPA content at 16 and 24 °C. Equations and p values for slope estimates are shown at the bottom left (models used : ModA for all groups, ModB for 16 and 24 °C). R^2_m is the marginal R-squared value.

gences according to temperature. Although differences between groups were not significant, temperate diploids seemed to accumulate more C18:3n3 and C18:4n3 than subarctic clones at 16 °C. A noteworthy observation is that triploid clones showed a unique pattern for omega-6 (n-6) fatty acids as their n-6 content was significantly higher at 24 °C compared to temperate and subarctic diploid clones. This is driven by the content of the two major n-6 PUFAs, gamma-linolenic acid (GLA, C18:3n6) and arachidonic acid (ARA, C20:4n6). Eicosapentaenoic acid (EPA, C20:5n3) content was low at 24 °C for all groups but is higher for subarctic triploids than temperate diploids at 16 °C (Figure 2.2).

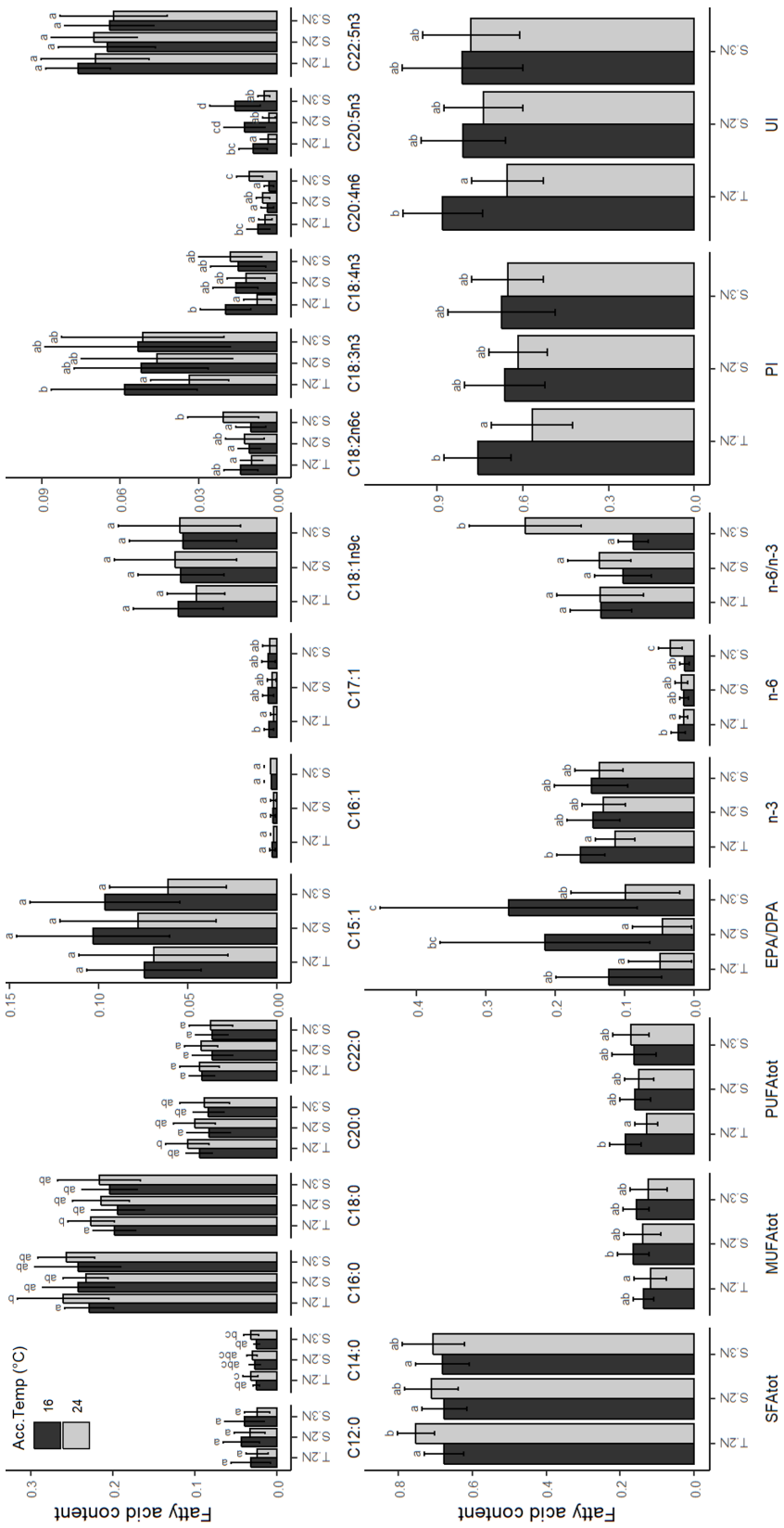


Figure 2.2: Relationship between fatty acid content, acclimation temperature, origin and ploidy. Letters indicate significant differences for each group for each fatty acid.

Table 2.4: Results of (a) linear mixed-effect models that test heat tolerance in response to EPA content (C20:5n3), acclimation temperature (Tacc) and cell size (Tcell). Estimates ± SE for fixed effects are shown. Bold *p* values indicate estimates significantly different from zero. (b) *Post-hoc* pairwise comparisons between slopes and slope differences from zero.

(a) Model and fixed effects	Term	Estimate ± SE	*p*
ModA : C20:5n3	Intercept	32.06 ± 0.22	**< 0.001**
	C20:5n3	-0.73 ± 0.11	**< 0.001**
ModB : C20:5n3 × Tacc	Intercept	32.51 ± 0.24	**< 0.001**
	C20:5n3	-0.45 ± 0.12	**< 0.010**
	Tacc(24)	1.41 ± 0.18	**< 0.001**
	C20:5n3 × Tacc(24)	0.42 ± 0.23	0.08
ModC : C20:5n3 × Tacc + Tcell	Intercept	32.54 ± 0.22	**< 0.001**
	C20:5n3	-0.38 ± 0.08	**< 0.001**
	Tacc(24)	1.39 ± 0.18	**< 0.001**
	Tcell	-0.29 ± 0.07	**< 0.001**
	C20:5n3 × Tacc(24)	0.54 ± 0.22	**0.02**

(b)	EPA (C20:5n3)				
	Slope	Estimate	95% CI	df	*p*
ModB	16	-0.45	[-0.70, -0.19]	20.1	**0.002**
	24	-0.03	[-0.50, 0.44]	124.8	0.90
	Slope	Estimate	95% CI	df	*p*
ModC	16	-0.38	[-0.53, -0.23]	158	**< 0.001**
	24	0.15	[-0.29, 0.59]	157	0.49

2.5 Discussion

This study shows that eicosapentaenoic acid might play a specific role in heat tolerance in an aquatic microcrustacean. We also provide the first evidence of differences in the regulation of fatty acid deposition in clones that differ in ploidy levels.

2.5.1 Heat tolerance and fatty acid profiles

We hypothesized that acute heat tolerance limits are set by damages to cell structures induced by mitochondrial ROS overproduction under heat stress. As membrane PUFAs are particularly targeted by ROS and considering that PUFA content is variable across individuals and species, we predicted that heat tolerant individuals would possess membranes with low susceptibility to oxidative stress. Peroxidation (PI) and unsaturation (UI) indexes are metrics that estimate membrane susceptibility to peroxidation and the density of double bonds in the membrane respectively (Hulbert et al., 2007). Thus, we anticipated heat tolerance to be negatively correlated to PI and UI. Our results do not support the hypothesis, as we found no correlation between each of the indexes and acute heat tolerance in *D. pulex*. Few studies have explored the link between heat tolerance and PI. In two charr species and their hybrids, Christen et al. (2020) found that heat tolerance decreases with increasing PI and UI of cardiac muscle membranes. In brook charr (*Salvelinus fontinalis*), St-Pierre (2022) found no relationship between heat tolerance, PI and UI in heart mitochondrial fatty acids. We suspect that if there is an effect of PI on heat tolerance, it might be more evident in interspecific studies than intraspecific comparisons. To our knowledge, no other study linked heat tolerance and PI in invertebrates but some other studies showed relationships between membrane fatty acid profiles and heat tolerance. In *D. magna*, negative relationships of heat tolerance with UI (Martin-Creuzburg et al., 2019) or PUFA content (Sarrazin and Sperfeld, 2022; Werner et al., 2019) have been found. These results alone may suggest that heat tolerance is modulated by adjustments in membrane fluidity through changes in the content of different fatty acids. Indeed, studies that directly measured membrane fluidity between different temperature and/or diet treatments in *Daphnia* showed a negative effect of membrane fluidity on heat tolerance (Martin-Creuzburg et al., 2019; Coggins et al., 2017). A possible explanation for the discrepancies between our results and results from other studies is that lipid composition of specific organs (like heart in fish, Christen et al. (2018)) may set thermal tolerance limits of ectotherms leading whole-body lipid or fatty acid composition to be poorly linked to thermal

tolerance.

Membrane lipid composition plays a role in temperature acclimation through changes in fluidity (Hazel and Williams, 1990). It is however also conceivable, according to our results and previous studies, that a driver of lipid composition is the resistance to damages from ROS overproduction. It is therefore necessary to further explore the relationships between heat tolerance, lipid peroxidation and antioxidant defences, and how it intertwines with membrane adaptation for fluidity maintenance. Several studies linking lipid peroxidation and heat tolerance have shown contrasting results, with either a negative effect of lipid peroxidation levels on heat tolerance or no effects. In *Daphnia*, higher levels of lipid peroxidation have been linked with a lower heat tolerance (Zeis et al., 2019; Yampolsky et al., 2022). Opposite to these results, Coggins et al. (2017) found higher levels of lipid peroxidation in warm-acclimated heat tolerant individuals. They hypothesized that lipid peroxides can serve as a protection or a signal for antioxidant pathways. Martin-Creuzburg et al. (2019) found no effect of lipid peroxidation levels on heat tolerance. The level of peroxidation of lipids depends on the content of vulnerable fatty acids, on the intensity and duration of oxidative stress and on the buffering capacity of antioxidant enzymes. The markers of lipids peroxidation alone are however poor indicators of stress tolerance since different organisms undergoing equivalent oxidative stress but with different relative proportions of fatty acids will accumulate different content of lipid peroxides. In other occasions the products of lipids peroxidation might depend on the duration of oxidative stress instead of intensity. In these conditions, fatty acid profile may better display the potential tolerance to oxidative stress.

Among the various fatty acids we measured, we found a strong negative relationship between eicosapentaenoic acid (EPA) content and CTmax, with most heat tolerant individuals having the lowest EPA content. This may point to a unique role of this fatty acid in heat tolerance. EPA is a n-3 PUFA with a 20-carbon chain and five *cis* double bonds that contributes to membrane fluidity. It is also highly sensitive to peroxidation. Supplementation in C20 PUFA-rich diet or temperature changes has been shown to increase lipid peroxidation

in ectotherms (*Daphnia* : Yampolsky et al. (2022); fish : Ostbye et al. (2011)). Here we show that individual heat tolerance is linked to the content of a ROS-sensitive PUFA. We observe however that the relation between EPA and CTmax is partly driven by the effect of acclimation temperature. We observe a higher accumulation of EPA in low temperature acclimated *Daphnia* which is consistent with the homeoviscous adaptation hypothesis and other findings that show a higher retention and requirement of EPA for growth and development at low temperatures (Isanta Navarro et al., 2019; Sperfeld and Wacker, 2012). Thus, it is likely that acclimation temperature acts on membrane fatty acids content to modulate fluidity, by relative content of EPA, impacting oxidative stress susceptibility and heat tolerance. In a study on *Daphnia*, Werner et al. (2019) did not find any relationship between heat tolerance and EPA content. However their samples had much lower EPA content than ours (inferior than 1%) and they only used one acclimation temperature (15 °C). The range of EPA values may consequently, have been too small to establish a relationship, such as in our population reared at 24 °C. Martin-Creuzburg et al. (2019) explored the causal link between heat tolerance and membrane fatty acids by supplementing *Daphnia* with different amounts of EPA under three different acclimation temperatures. In *Daphnia* fed an EPA-rich diet, membranes were more fluid and heat tolerance was reduced, supporting a mechanistic link between EPA content and heat tolerance without determining if this link relates to membrane fluidity or to susceptibility to peroxidation. When looking at the content of other unsaturated fatty acids or at the overall peroxidation index, it suggests EPA might operate through other metabolic and cellular mechanisms.

EPA supplementation promotes ROS accumulation and cell death in human cancer cells (Fukui et al., 2013). In other cases, its supplementation reduces ROS levels, enhances the activity of antioxidant enzymes and counteracts the detrimental effects of nutrient excess (Sergi et al., 2021; Xiao et al., 2022). EPA metabolism generates anti-inflammatory mediators contrary to other fatty acids such as arachidonic acid (ARA) which generates pro-inflammatory factors (Crupi and Cuzzocrea, 2022). These properties show the complex relationship between fatty acid polyunsaturation and sensitivity to peroxidation. At high temperature, the

anti-inflammatory benefits of EPA might not be the same as in physiological conditions.

2.5.2 Temperature-dependant plasticity of fatty acid profile

To our knowledge, this study is the first to measure fatty acid profiles in diploid and triploid *Daphnia*. We found that clones of different geographic origins and ploidies respond differently to changes in acclimation temperature. The plasticity of the fatty acid profile is highest in diploid clones from temperate origins, while diploid and triploid clones of subarctic origin show a lower, mainly non-significant plasticity. We hypothesize that temperate clones live in conditions where temperatures fluctuate more and thus have a higher capacity of homeoviscous acclimation. Subarctic clones might live under less fluctuating temperatures and have a lower capacity to adjust their fatty acid profile. One might advocate that the fatty acid profile of subarctic clones should be adapted to cold conditions, with a higher membrane unsaturation and higher PUFA retention. While subarctic clones tend to retain more PUFAs at high temperatures, they do not differ from temperate clones at low temperature. Subarctic clones are thus disadvantaged at high temperatures but do not gain any advantage over temperate clones at low temperatures, at least in terms of fatty acid profile. We suggest that minimal temperatures encountered by temperate and subarctic clones can be of the same magnitude, while temperate clones must also face high temperatures not encountered in subarctic regions. In *Daphnia*, genotype-dependent differences in lipid composition have already been observed. In a single *Daphnia* population consisting of several clones, Werner et al. (2019) found differences in PUFA accumulation between clones. These differences may come from a varying capacity to either assimilate, or synthesize C20 unsaturated fatty acids from C18 precursors (Werner et al., 2019; Yampolsky et al., 2022).

We found that triploid clones retain omega-6 PUFAs in a unique way, as they over-accumulate major omega-6 PUFAs (gamma-linolenic acid, C18:3n6 and arachidonic acid, C20:4n6) at high temperatures. We cannot explain this pattern. Comparative studies on ploidy and lipid composition in ectotherms have focused on farmed bivalve and fish species,

where contrasting results have been found. Several studies report no differences in fatty acid profiles between ploidies (bivalves : Arjona et al. (2008) and Tan et al. (2021) ; fish : Nuez-Ortin et al. (2017)). In cases where differences in fatty acid profiles between diploids and triploids were found, the likely explanation lies in differences in reproductive status, as triploid females were sterile and allocated fatty acids differently than reproductive diploid females (Manor et al., 2014; do Nascimento et al., 2017; Everson et al., 2021). The differences between ploidies could hardly be explained by differences in reproductive mode as both triploids and diploids reproduce asexually under laboratory conditions. In turbot fish muscle (*Scophthalmus maximus*), Aydin et al. (2022) found differences in n-6 PUFAs retention but in their case it was diploid individuals that retained more n-6 PUFAs and not triploids.

Heat tolerance of *Daphnia* measured by Bernier (2020) and fatty acid profiles vary in a different way between ploidies and origins. Heat tolerance is significantly lower for triploids across all temperatures, and temperate and subarctic diploids do not differ. If heat tolerance differences observed in clones of different ploidies was linked to fatty acid profile, we would expect pronounced differences between the fatty acid profile of triploids and diploids at all acclimation temperatures. Here, we only show a significant difference in omega-6 PUFAs at high temperature, where triploids accumulate more of these fatty acids. Interestingly, St-Pierre (2022) found a negative relationship between heat tolerance and arachidonic acid, an omega-6 PUFA in brook charr.

2.6 Conclusion

In summary, our results suggest a significant role of fatty acids in heat tolerance, but the mechanisms involved might be multiple and complex. We observed no correlation between membrane sensitivity to oxidative damage and heat tolerance. We show a negative relationship between EPA and heat tolerance. Heat tolerance in *Daphnia* might be dependent on the failure of specific organs and the whole body profile of fatty acids might then be a poor pre-

dictor since whole-animal fatty acid profile might not be representative of organ-specific fatty acid profile. Whole-body thermal sensitivity might be dictated by the thermal sensitivity of specific organs. We found that temperate clones have a higher thermal plasticity of their fatty acid profile than subarctic clones. This result suggests local adaptations of clones of different climatic regions to their environmental conditions. In changing and variable environments, we can expect that temperate diploid clones will have a better capacity to adapt. We also compared for the first time the fatty acid profiles of diploid and triploid clones and observed over-accumulation of omega-6 fatty acids in triploids at high temperatures showing specific adaptation of fatty acid metabolism associated to ploidy.

2.7 Funding

This work was supported by NSERC Discovery Grants to FD and PUB (RGPIN-2019–05992).

CONCLUSION GÉNÉRALE

Les objectifs principaux de ce mémoire étaient de mieux comprendre 1) les mécanismes physiologiques qui limitent les seuils de tolérance aux extrêmes de température et 2) les patrons de distribution des clones du crustacé *D. pulex*. Les objectifs spécifiques étaient de comprendre l'influence de la composition en acides gras des membranes cellulaires, de la taille cellulaire, de la taille corporelle et de la ploïdie sur la tolérance thermique et la distribution des clones.

Mécanismes déterminant les seuils de tolérance thermique

L'hypothèse était que l'insaturation des membranes cellulaires, qui est influencée par la température d'acclimatation, diminue la tolérance à la chaleur à travers une augmentation de la sensibilité au stress oxydant. Il était prédit que la tolérance à la chaleur diminuerait avec l'insaturation des membranes. Mes résultats ont montré une relations significative entre la tolérance à la chaleur et un acide gras polyinsaturé, l'acide eicosapentaénoïque (EPA), suggérant que des acides gras bien spécifiques pourraient influencer la tolérance à la chaleur. Je n'ai pas observé de corrélation entre la tolérance à la chaleur et les indices généraux de sensibilité des membranes aux dommages oxydatifs, à savoir les indices de peroxydation et d'insaturation. Comme chez les poissons, la tolérance à la chaleur chez les daphnies pourrait dépendre de la défaillance d'organes spécifiques (Christen et al., 2020; St-Pierre, 2022). Le profil d'acides gras de l'animal entier, qui pourrait ne pas être représentatif du profil d'acides gras d'organes bien spécifiques, pourrait alors être un mauvais prédicteur de la tolérance à la chaleur. Outre la sensibilité des membranes à la peroxydation, la capacité antioxydante influencerait la tolérance à la chaleur en contrôlant la quantité de dérivés réactifs de l'oxygène. Le choc thermique à haute température chez le même modèle d'étude a engendré une augmentation de l'expression de l'enzyme catalase, une enzyme antioxydante (Bernier, 2020). Cela suggère donc que les hautes températures augmentent le stress oxydant et induisent une

réponse antioxydante. Il aurait été intéressant de connaître la capacité antioxydante de chacun des individus et de la relier à sa tolérance thermique.

L'acclimatation à des températures froides chez les ectothermes augmente l'insaturation de leurs membranes cellulaires. Mes résultats vont dans ce sens. L'augmentation de l'insaturation cause une plus grande sensibilité au stress oxydant lié à des chocs thermiques à haute température (Zeis et al., 2019). Les chocs thermiques à basse température peuvent aussi engendrer du stress oxydant (Reid et al., 2022; Lu et al., 2019). La mise en relation des valeurs de tolérance au froid et leur profil d'acides gras a montré que la majorité des paramètres du profil d'acide gras n'étaient pas corrélés significativement au CTmin. Au sein d'une même température d'acclimatation, aucune relation significative n'a été trouvée. Une amélioration significative de la tolérance au froid avec l'augmentation du contenu en EPA a été mise en lumière (Figure 2.3 ; Table S.5.10). Il est bien connu que les acides gras membranaires des ectothermes sont davantage insaturés à basse température afin de maintenir une fluidité des membranes adéquate. L'EPA pourrait jouer un rôle dans la tolérance au froid mais les mécanismes sous-jacents qui en feraient un acide gras avantageux sont incertains. Une relation similaire a été observée entre le CTmax et l'EPA, où la tolérance à la chaleur diminuait avec la proportion d'EPA. On ne peut pas exclure que la significativité des relations soit due à un effet conjoint de la température d'acclimatation sur la tolérance thermique et le profil d'acides gras sans qu'il n'existe de relation causale entre la tolérance thermique et le contenu en EPA. La mesure du CTmin et du profil d'acides gras sur les mêmes individus aurait amené une meilleure résolution et permis d'évaluer la significativité des relations avec plus de confiance.

Une perspective de recherche serait de continuer à explorer le lien entre l'EPA et la tolérance thermique. En ce sens, les expériences où le contenu en EPA de l'alimentation est contrôlé permettrait (tel que Martin-Creuzburg et al. (2019)) de mieux établir les relations de causalité entre le contenu en EPA et les seuils de tolérance thermique. En même temps, d'autres aspects du métabolisme des lipides pourraient être étudiés. La peroxydation

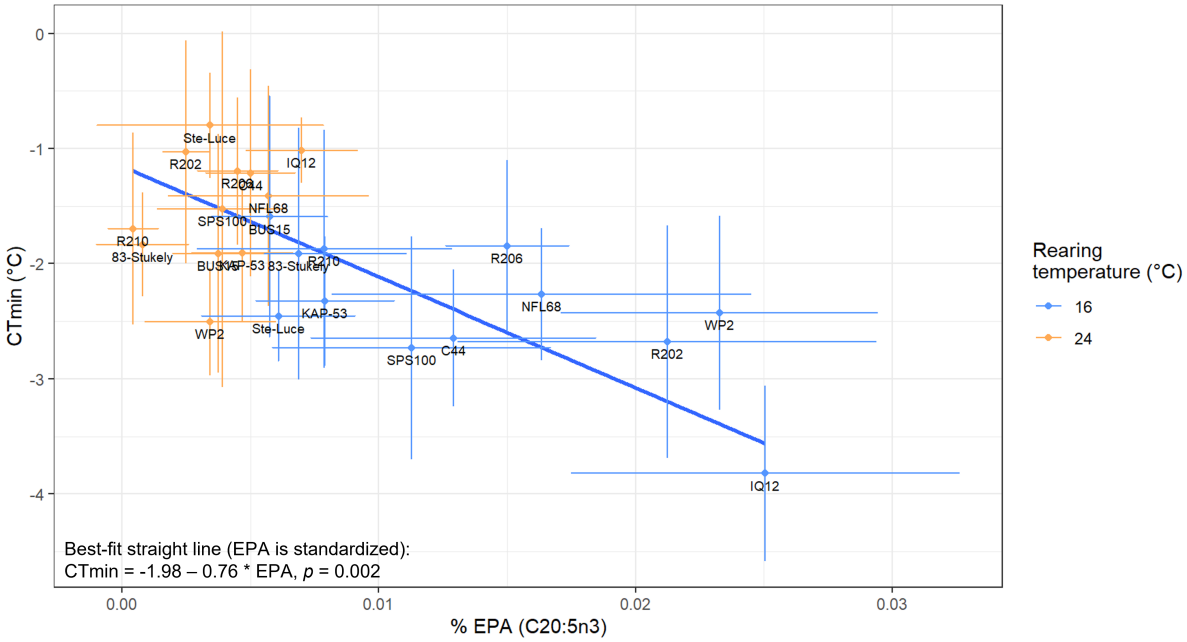


Figure 2.3: Relation entre le CTmin et le contenu en EPA, toutes températures combinées. Une régression de York a été utilisée. En bas à gauche : équation et valeur de *p* pour l'estimé de la pente. L'équation correspond à un modèle où les valeurs d'EPA sont standardisées.

des acides gras polyinsaturés produit des dérivés tels que les malondialdéhydes, les hydroperoxydes des lipides, le 4-hydroxy-2-nonenal (4-HNE) qui ont des effets négatifs sur les fonctions cellulaires. Il serait possible d'associer la production de ces dérivés lors d'un stress thermique aux seuils de tolérance thermique. Peu d'études lient à ce jour la tolérance au froid aigu avec la production de DRO et la capacité antioxydante, et il serait intéressant d'explorer cette avenue chez les daphnies.

La taille corporelle et la taille cellulaire sont des traits qui sont souvent liés à la tolérance thermique chez les ectothermes. De plus, une taille corporelle et/ou cellulaire plus grandes sont souvent associées aux environnements froids (Verberk et al., 2020; Bergmann, 1847). Mon hypothèse était que la taille corporelle et la taille des cellules influencent la tolérance au choc de froid par le biais des effets du rapport surface/volume sur les processus physiologiques qui gouvernent la tolérance thermique. Il était prédit que les individus ayant une taille corporelle et des cellules plus grandes auraient une meilleure tolérance au choc de froid. Je

n'ai pas trouvé de lien significatif entre le CTmin et la taille corporelle ou la taille des cellules. De grosses cellules ou une grande taille corporelle ne semble présenter aucun avantage direct ou indirect pour la résistance à un choc thermique à basse température. Les processus qui gouvernent la capacité à supporter un extrême thermique froid sont donc à chercher ailleurs. Le CTmax était plus élevé chez les daphnies possédant de petites cellules, ce qui était cohérent avec l'hypothèse d'une limitation de la tolérance thermique par les contraintes liées à l'oxygène (Bernier, 2020). L'absence de lien pour le CTmin renforce l'idée que les processus qui gouvernent le CTmin et le CTmax sont différents (Leiva et al., 2019). Le CTmin inférieur des clones polyploïdes acclimatés à basse température représente une observation sur laquelle s'appuyer pour des recherches futures. La polyploïdie a des effets profonds sur la physiologie (Doyle and Coate, 2019), qui peuvent se traduire en une différence de tolérance thermique. Malgré l'absence de lien entre la taille et le CTmin, rien n'exclut qu'une taille plus grande favorise la tolérance à des conditions froides moins intenses et d'une plus longue durée. Les études telles que celles de Leiva et al. (2019) rappellent que la durée d'exposition au stress thermique est un facteur important. Ainsi, des individus de grande taille et/ou avec de grandes cellules pourraient être avantagés dans des conditions de basse température constante.

L'augmentation de la variabilité climatique pose la question des liens entre tolérance à basse et haute température. L'hypothèse était que les processus d'acclimatation à la chaleur, qui permettent une meilleure tolérance aux hautes températures, contribuent à diminuer la tolérance au froid chez les daphnies. Tel que prédit, la tolérance au froid diminuait (le CTmin augmentait) avec l'augmentation de la tolérance à la chaleur, c'est-à-dire que le CTmin et le CTmax étaient corrélés positivement (Figure 2.4, Table S.5.11). La relation était significative dû à l'effet de la température d'acclimatation sur le CTmin et le CTmax. Mes résultats sont cohérents avec l'ensemble des études qui montrent que les CTmin et CTmax des ectothermes augmentent avec la température d'acclimatation. On peut donc suggérer que les effets des chocs thermiques sur les individus et les populations dépendront de leur statut d'acclimatation. L'acclimatation à un environnement chaud limiterait la résistance à une vague de froid extrême. Des processus adaptatifs pourraient en revanche permettre aux populations

d'augmenter leur plage de tolérance thermique. Par leur mode de reproduction, les daphnies peuvent se reproduire en conditions favorables et créer des œufs de dormance lorsque les conditions se dégradent. Cette forte plasticité pourrait interférer sur les phénomènes évolutifs d'une manière qui est difficile à prédire (Buckley and Kingsolver, 2021).

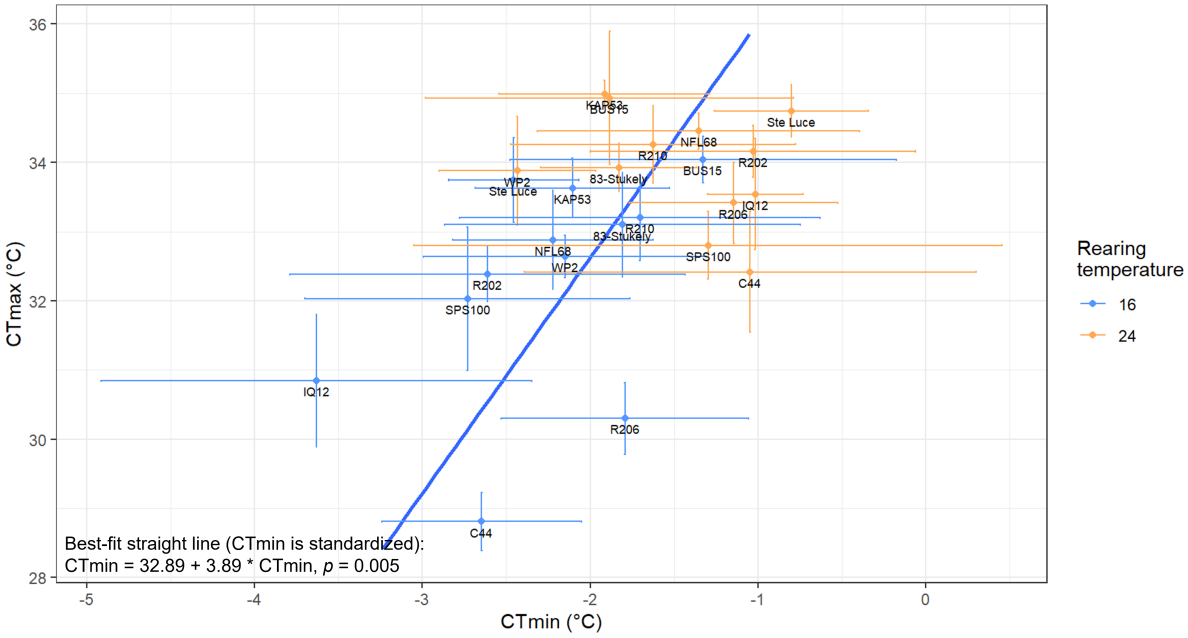


Figure 2.4: Relation entre le CTmax et le CTmin, les deux températures d'acclimatation sont combinées. Une régression de York a été utilisée. En bas à gauche : équation valeur de *p* pour l'estimé de la pente.

2) Distribution de la ploïdie et des clones

Le second objectif était de tester l'hypothèse selon laquelle la distribution des clones et de la ploïdie sont liés à une variabilité de la tolérance au froid. J'avais prédit que les clones provenant d'environnements plus froids et les clones polyploïdes auraient une meilleure tolérance au choc de froid, mesurée par la méthode du CTmin. Les daphnies triploïdes étaient plus résistantes au choc de froid que les diploïdes. L'origine géographique n'avait pas d'effet significatif sur le CTmin. Ces résultats suggèrent que l'une des explications possibles de la prépondérance des clones triploïdes dans les hautes latitudes est leur capacité à mettre en

place un phénotype résistant au froid. Si la résistance au choc de froid des triploïdes se traduit aussi en une meilleure résistance à des températures froides chroniques (plusieurs heures à plusieurs jours), leur présence en hautes latitudes pourrait s'expliquer par une plus grande capacité à croître et à se reproduire en milieu froid. Je suggère que la triploïdie pourrait contribuer à l'acclimatation au froid par 1) la modification de l'expression génique, en particulier pour les gènes impliqués dans la réponse au choc thermique et dans les voies antioxydantes et 2) la teneur en lipides, en termes de quantité et de composition en acides gras. Il est important de garder à l'esprit que d'autres aspects de la physiologie, de l'écologie ou de l'histoire glaciaire ont pu façonner la répartition des clones nord-américains de *D. pulex*.

À ma connaissance, aucune mesure de CTmin chez des daphnies n'avait jusque-là été réalisée. Cette étude a décrit un nouveau protocole de mesure du seuil de tolérance thermique inférieur chez des ectothermes aquatiques de petite taille. Plusieurs constats ont été faits. La tolérance au froid des daphnies étudiées était bien inférieure à ce qui était attendu, les individus étant actifs à des températures inférieures à 0 °C. Les clones de *D. pulex* ont donc une grande résistance au froid. Les conditions expérimentales ont fait en sorte que le milieu de culture restait liquide même en-dessous du point de congélation. La congélation spontanée de plusieurs tubes pendant les expériences a indiqué que les daphnies étaient incapables de survivre à la formation de glace. En milieu naturel, le milieu de vie des daphnies congèle très probablement avant l'atteinte de températures similaires à celle des expériences. Les valeurs absolues de CTmin ne représentent donc pas des données immédiatement transposables à des conditions réelles. En revanche, la comparaison du CTmin entre groupes d'origines géographiques, ploïdies ou températures d'acclimatation reste pertinente. Elle a permis ici de montrer l'avantage de la polyploïdie en conditions froides. Des indices de tolérance au froid chronique représentent probablement mieux des conditions naturelles mais peuvent prendre plusieurs jours ou semaines à être obtenus, comme les paramètres d'histoire de vie. Le protocole de mesure utilisé a l'avantage d'être réalisable de manière rapide, à l'échelle de quelques heures. Nous recommanderions tout de même de comparer des données de CTmin à des indices de tolérance au froid chronique pour voir s'ils sont corrélés.

L'une des hypothèses était que les profils d'acides gras des clones varient en fonction de leur ploïdie et de leur origine géographique. Les clones des régions tempérées présentent une plus grande plasticité thermique de leur profil d'acides gras que les clones subarctiques. En conditions froides, les clones tempérés ont des profils d'acides gras similaires aux clones subarctiques. En conditions chaudes, ils accumulent davantage d'acides gras saturés. Ce résultat suggère qu'il peut exister des adaptations locales des clones de différentes régions climatiques à leurs conditions environnementales. Dans des environnements changeants et variables, on peut s'attendre à ce que les clones diploïdes tempérés aient une meilleure capacité d'adaptation par une plus grande plasticité de leur profil d'acides gras. Cette plasticité pourrait être due à l'expérience d'une plus grande gamme de températures dans leur habitat d'origine. Nous avons également comparé pour la première fois les profils d'acides gras des clones diploïdes et triploïdes et observé une suraccumulation d'acides gras oméga-6 chez les triploïdes élevés à des températures élevées, ce qui montre une caractéristique spécifique du métabolisme des acides gras associée à la ploïdie et influencé par la température d'acclimatation. Nous ne pouvons pas expliquer ce phénomène qui pourrait être dû à une multitude de caractéristiques propres aux ectothermes et des recherches seraient nécessaire pour mieux le comprendre.

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ANNEXE A

ARTICLE 1

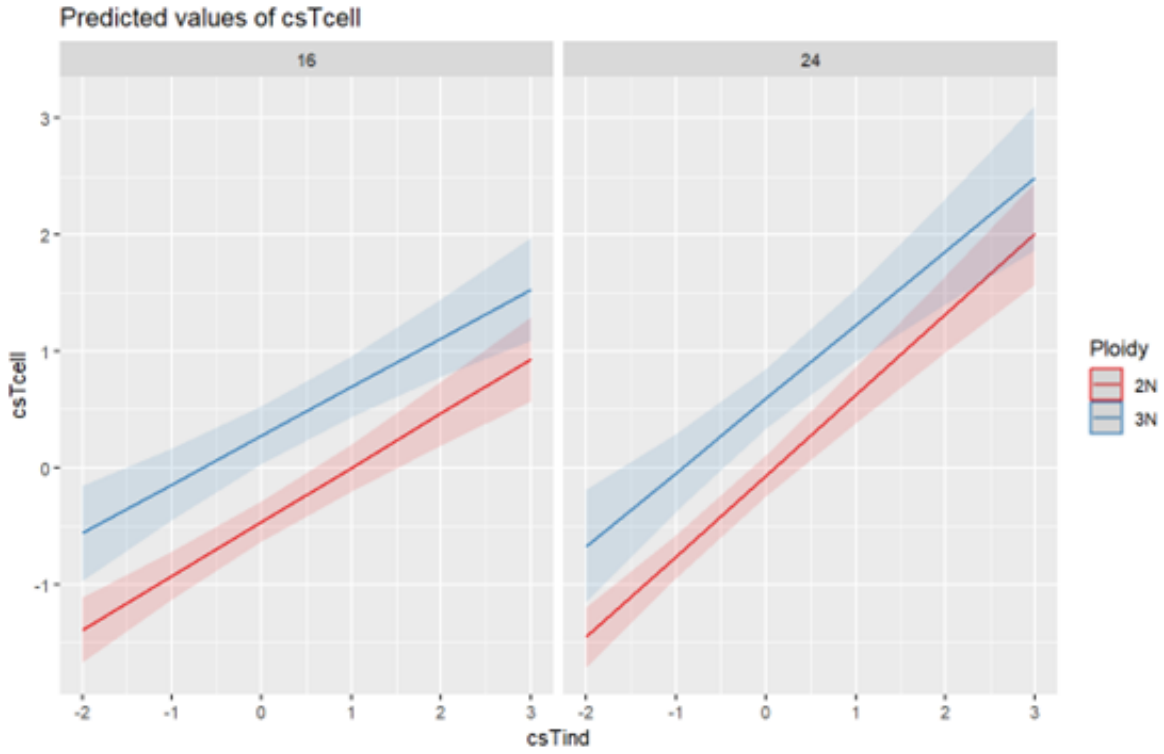

Figure 3.5: Interaction plot of cell size (csTcell) explained by body size (csTind), acclimation temperature (Tacc) for each ploidy (2N : diploid ; 3N : triploid) for Mod6. Prefix cs means that the variable was centered and scaled. Table 1.8 shows slope estimates from the relationship between cell area and body size (both standardized) for each group.

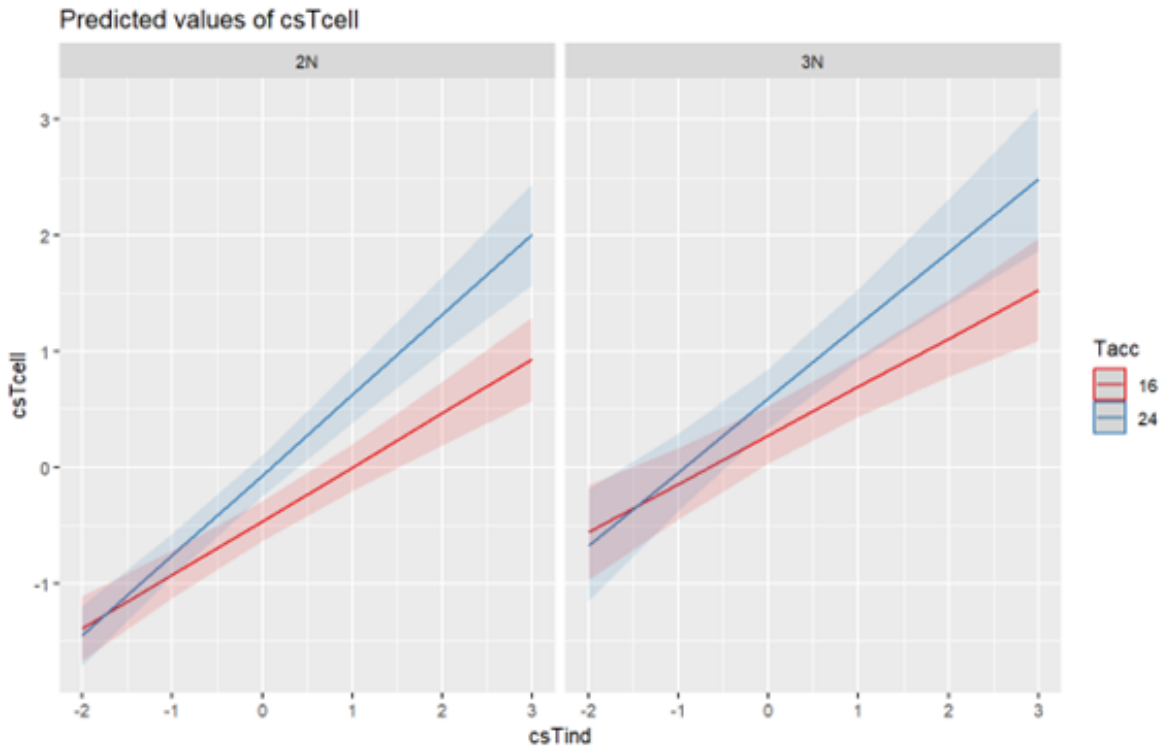

Figure 3.6: Interaction plot of cell size (csTcell) explained by body size (csTind), acclimation temperature (Tacc) for each ploidy (2N : diploid ; 3N : triploid) for Mod6. Prefix cs means that the variable was centered and scaled. Table 1.8. Slope estimates of the relationship between cell area and body size (both standardized) for each group. Comparison of slope estimates between temperatures for each ploidy.

ANNEXE B

ARTICLE 2

Table 4.5: Results of (a) three linear mixed-effect models that test heat tolerance in response to fatty acid profile descriptors, acclimation temperature and cell size. (b) Post-hoc pairwise comparisons between slopes and slope differences from zero for the two acclimation temperatures when a significant interaction was found. First two models (ModA and ModB) : intercepts vary by clone and by jar, effects of fatty acid profile are allowed to vary by clone. Third model (ModC) : intercepts vary by clone and by jar.

(a) Fatty acid	Model fixed effects formula	Term	Estimate ± SE	p	R^2_m
Saturated fatty acids (SFA)	SFA	Intercept	33.03 ± 0.25	**<2e-16**	0.04
		PUFA	0.31 ± 0.10	**0.0075**	
	SFA*Tacc	Intercept	32.28 ± 0.25	**<2e-16**	0.33
		SFA	0.20 ± 0.13	0.132	
		Tacc(24)	1.69 ± 0.14	**<2e-16**	
		SFA * Tacc(24)	-0.20 ± 0.13	0.125	
	SFA*Tacc+Tcell	Intercept	32.40 ± 0.23	**<2e-16**	0.39
		SFA	0.19 ± 0.09	0.04	
		Tacc(24)	1.43 ± 0.14	**<2e-16**	
		Tcell	-0.34 ± 0.08	**1.2e-05**	
		SFA * Tacc(24)	-0.21 ± 0.12	0.09	
Monounsaturated fatty acids (MUFA)	MUFA	Intercept	33.03 ± 0.25	**<2e-16**	0.06
		MUFA	-0.35 ± 0.08	**0.0057**	
	MUFA*Tacc	Intercept	32.36 ± 0.25	**<2e-16**	0.35
		MUFA	-0.35 ± 0.12	**0.0121**	
		Tacc(24)	1.63 ± 0.13	**<2e-16**	
		MUFA * Tacc(24)	0.28 ± 0.08	**0.0204**	
	MUFA*Tacc+Tcell	Intercept	32.43 ± 0.22	**<2e-16**	0.41
		MUFA	-0.35 ± 0.09	**2e-4**	
		Tacc(24)	1.40 ± 0.13	**<2e-16**	
		Tcell	-0.31 ± 0.07	**3.54e-5**	
		MUFA * Tacc(24)	0.30 ± 0.12	**0.011**	
Polyunsaturated fatty acids (PUFA)	PUFA	Intercept	33.05 ± 0.24	**<2e-16**	0.007
		PUFA	-0.12 ± 0.11	0.29	
	PUFA*Tacc	Intercept	32.23 ± 0.25	**<2e-16**	0.33
		PUFA	0.03 ± 0.11	0.81	
		Tacc(24)	1.74 ± 0.13	**<2e-16**	
		PUFA * Tacc(24)	0.02 ± 0.13	0.86	
	PUFA*Tacc+Tcell	Intercept	32.34 ± 0.22	**<2e-16**	0.40
		PUFA	0.03 ± 0.08	0.73	
		Tacc(24)	1.50 ± 0.13	**<2e-16**	
		Tcell	-0.37 ± 0.08	**3.86e-6**	
		PUFA * Tacc(24)	0.06 ± 0.13	0.66	
Total omega-3 (n3tot)	n3tot	Intercept	33.06 ± 0.25	**<2e-16**	0.01
		n3tot	-0.15 ± 0.10	0.17	
	n3tot *Tacc	Intercept	32.23 ± 0.25	**<2e-16**	0.32
		n3tot	0.04 ± 0.11	0.71	
		Tacc(24)	1.73 ± 0.13	**<2e-16**	
		n3tot * Tacc(24)	-0.02 ± 0.14	0.91	
	n3tot *Tacc+Tcell	Intercept	32.35 ± 0.22	**<2e-16**	0.40
		n3tot	0.01 ± 0.08	0.87	
		Tacc(24)	1.50 ± 0.13	**<2e-16**	
		Tcell	-0.36 ± 0.08	**4.45e-6**	
		n3tot * Tacc(24)	0.05 ± 0.13	0.69	

Continued

(a) Fatty acid	Model fixed effects formula	Term	Estimate ± SE	*p*	R^2_m
Total omega-6 (n6tot)	n6tot	Intercept	33.0 ± 0.25	**<2e-16**	3.4e-7
		n6tot	8.7e-4 ± 0.11	0.99	
	n6tot *Tacc	Intercept	32.31 ± 0.25	**<2e-16**	0.29
		n6tot	0.18 ± 0.12	0.14	
		Tacc(24)	1.56 ± 0.12	**<2e-16**	
		n6tot * Tacc(24)	-0.17 ± 0.14	0.24	
	n6tot *Tacc+Tcell	Intercept	32.38 ± 0.22	**<2e-16**	0.40
		n6tot	0.13 ± 0.11	0.26	
		Tacc(24)	1.43 ± 0.12	**<2e-16**	
		Tcell	-0.37 ± 0.07	**2.57e-6**	
		n6tot * Tacc(24)	-0.02 ± 0.14	0.90	
Omega-3/omega-6 (n3/n6)	n3/n6	Intercept	33.02 ± 0.26	**<2e-16**	0.02
		n3/n6	-0.22 ± 0.16	0.19	
	n3/n6 *Tacc	Intercept	32.30 ± 0.26	**<2e-16**	0.27
		n3/n6	-0.02 ± 0.12	0.86	
		Tacc(24)	1.52 ± 0.13	**<2e-16**	
		n3/n6 * Tacc(24)	-0.05 ± 0.15	0.76	
	n3/n6 *Tacc+Tcell	Intercept	32.30 ± 0.23	**<2e-16**	0.39
		n3/n6	0.12 ± 0.08	0.15	
		Tacc(24)	1.47 ± 0.14	**<2e-16**	
		Tcell	-0.37 ± 0.08	**2.84e-6**	
		n3/n6 * Tacc(24)	-0.23 ± 0.14	0.12	
C20:5n3/C22:5n3 (EPA/DPA)	EPA/DPA	Intercept	32.04 ± 0.22	**<2e-16**	0.22
		EPA/DPA	-0.66 ± 0.07	**0.0077**	
	EPA/DPA *Tacc	Intercept	32.47 ± 0.23	**<2e-16**	0.38
		EPA/DPA	-0.44 ± 0.11	**0.0012**	
		Tacc(24)	1.40 ± 0.15	**7.95e-16**	
		EPA/DPA*Tacc(24)	-0.30 ± 0.19	0.1114	
	EPA/DPA*Tacc+Tcell	Intercept	32.53 ± 0.22	**<2e-16**	0.46
		EPA/DPA	-0.41 ± 0.07	**2.95e-8**	
		Tacc(24)	1.28 ± 0.15	**3.44e-14**	
		Tcell	-0.30 ± 0.07	**4.95e-5**	
		EPA/DPA*Tacc(24)	0.34 ± 0.18	0.054	

(b) Fatty acid profile							
MUFA (ModB)	*Contrast*	Estimate	±	SE	*df*	*t* ratio	*p*
	16 – 24	-0.283	±	0.124	156	-2.279	0.0240
	Slope	Estimate	±	SE	*df*	lower.CL	upper.CL
	16	-0.348	±	0.127	33.3	-0.605	-0.0902
	24	-0.065	±	0.113	25.3	-0.297	0.1667
MUFA (ModC)	*Contrast*	Estimate	±	SE	*df*	*t* ratio	*p*
	16 – 24	-0.300	±	0.120	156	-2.511	0.013
	Slope	Estimate	±	SE	*df*	lower.CL	upper.CL
	16	-0.347	±	0.093	148	-0.531	-0.163
	24	-0.047	±	0.080	159	-0.204	0.112

Table 4.6: Results of three linear mixed-effect models that test heat tolerance in response to unsaturation and peroxidation indexes, acclimation temperature and cell size. First two models (ModA and ModB) : intercepts vary by clone and by jar, effects of fatty acid profile are allowed to vary by clone. Third model (ModC) : intercepts vary by clone and by jar.

Fatty acid	Model fixed effects formula	Term	Estimate ± SE	p	R^2_m
Unsaturation index (UI)	UI	Intercept	33.04 ± 0.24	**<2e-16**	0.01
		UI	-0.18 ± 0.10	0.09	
	UI*Tacc	Intercept	32.24 ± 0.25	**<2e-16**	0.32
		UI	-0.02 ± 0.11	0.88	
		Tacc(24)	1.73 ± 0.14	0.03	
		UI * Tacc(24)	0.08 ± 0.13	0.53	
	UI*Tacc+Tcell	Intercept	32.35 ± 0.22	**<2e-16**	0.39
		UI	-0.03 ± 0.09	0.77	
		Tacc(24)	1.49 ± 0.14	0.09	
		Tcell	-0.36 ± 0.07	**4.76e-6**	
		UI * Tacc(24)	0.10 ± 0.13	0.42	
Peroxidation index (PI)	PI	Intercept	33.05 ± 0.24	**<2e-16**	0.003
		PI	-0.08 ± 0.10	0.45	
	PI*Tacc	Intercept	31.24 ± 0.24	**<2e-16**	0.31
		PI	0.09 ± 0.11	0.38	
		Tacc(24)	1.73 ± 0.13	**<2e-16**	
		PI * Tacc(24)	-0.009 ± 0.13	0.95	
	PI*Tacc+Tcell	Intercept	32.33 ± 0.22	**<2e-16**	0.40
		PI	0.06 ± 0.08	0.45	
		Tacc(24)	1.53 ± 0.13	**0.02**	
		Tcell	-0.35 ± 0.08	**6.65e-6**	
		PI * Tacc(24)	0.04 ± 0.12	0.77	

Table 4.7: Results of (a) three linear mixed-effect models that test heat tolerance in response to saturated fatty acids, acclimatation temperature and cell size. (b) Post-hoc pairwise comparisons between slopes and slope differences from zero for the two acclimation temperatures when a significant interaction was found. First two models (ModA and ModB) : intercepts vary by clone and by jar, effects of fatty acid profile are allowed to vary by clone. Third model (ModC) : intercepts vary by clone and by jar.

(a) Fatty acid	Model fixed effects formula	Term	Estimate ± SE	p	R^2_m
C12:0	C12:0	Intercept	33.06 ± 0.24	**<2e-16**	0.01
		C12:0	-0.17 ± 0.10	0.15	
	C12:0 *Tacc	Intercept	32.35 ± 0.24	**<2e-16**	0.31
		C12:0	-0.14 ± 0.11	0.26	
		Tacc(24)	1.62 ± 0.13	**<2e-16**	
		C12:0 * Tacc(24)	0.31 ± 0.14	**0.03**	
	C12:0 *Tacc+Tcell	Intercept	32.4 ± 0.21	**<2e-16**	0.42
		C12:0	-0.19 ± 0.07	**0.01**	
		Tacc(24)	1.42 ± 0.13	**<2e-16**	
		Tcell	-0.37 ± 0.08	**<2.1e-6**	
		C12:0 * Tacc(24)	0.20 ± 0.13	0.12	
C14:0	C14:0	Intercept	33.02 ± 0.25	**<2e-16**	0
		C14:0	-0.03 ± 0.08	0.74	
	C14:0*Tacc	Intercept	32.16 ± 0.26	**<2e-16**	0.31
		C14:0	-0.23 ± 0.14	0.09	
		Tacc(24)	1.79 ± 0.14	**<2e-16**	
		C14:0 * Tacc(24)	0.15 ± 0.16	0.32	
	C14:0*Tacc+Tcell	Intercept	32.30 ± 0.23	**<2e-16**	0.39
		C14:0	-0.11 ± 0.13	0.40	
		Tacc(24)	1.54 ± 0.14	**<2e-16**	
		Tcell	-0.34 ± 0.08	**1.72e-5**	
		C14:0 * Tacc(24)	0.06 ± 0.15	0.67	
C16:0	C16:0	Intercept	33.03 ± 0.25	**<2e-16**	0
		C16:0	0.05 ± 0.07	0.49	
	C16:0*Tacc	Intercept	32.23 ± 0.26	**<2e-16**	0.31
		C16:0	-0.05 ± 0.09	0.59	
		Tacc(24)	1.69 ± 0.13	**<2e-16**	
		C16:0 * Tacc(24)	-6e-3 ± 0.12	0.96	
	C16:0*Tacc+Tcell	Intercept	32.35 ± 0.22	**<2e-16**	0.40
		C16:0	0.01 ± 0.08	0.91	
		Tacc(24)	1.48 ± 0.13	**<2e-16**	
		Tcell	-0.36 ± 0.08	**6.33e-6**	
		C16:0 * Tacc(24)	-0.05 ± 0.11	0.67	
C18:0	C18:0	Intercept	33.03 ± 0.25	**<2e-16**	0.03
		C18:0	0.25 ± 0.08	0.01	
	C18:0*Tacc	Intercept	32.29 ± 0.25	**<2e-16**	0.32
		C18:0	0.17 ± 0.12	0.15	
		Tacc(24)	1.66 ± 0.13	**<2e-16**	
		C18:0 * Tacc(24)	-0.18 ± 0.13	0.16	
	C18:0*Tacc+Tcell	Intercept	32.39 ± 0.23	**<2e-16**	0.39
		C18:0	0.18 ± 0.10	0.07	
		Tacc(24)	1.42 ± 0.13	**<2e-16**	
		Tcell	-0.34 ± 0.08	**1.26e-5**	
		C18:0 * Tacc(24)	-0.15 ± 0.12	0.20	

Continued

(a) Fatty acid	Model fixed effects formula	Term	Estimate ± SE	*p*	R^2_m
C20:0	C20:0	Intercept	33.05 ± 0.24	**<2e-16**	0.03
		C20:0	0.24 ± 0.08	**0.0028**	
	C20:0*Tacc	Intercept	32.36 ± 0.24	**<2e-16**	0.34
		C20:0	0.35 ± 0.11	**0.0015**	
		Tacc(24)	1.60 ± 0.12	**<2e-16**	
		C20:0 * Tacc(24)	-0.36 ± 0.12	**0.0024**	
	C20:0*Tacc+Tcell	Intercept	32.40 ± 0.22	**<2e-16**	0.42
		C20:0	0.29 ± 0.10	**0.003**	
		Tacc(24)	1.43 ± 0.13	**<2e-16**	
		Tcell	-0.33 ± 0.08	**2.36e-5**	
		C20:0 * Tacc(24)	-0.31 ± 0.11	**0.007**	
C22:0	C22:0	Intercept	33.05 ± 0.24	**<2e-16**	0.02
		C22:0	0.22 ± 0.07	**0.0028**	
	C22:0*Tacc	Intercept	32.33 ± 0.24	**<2e-16**	0.33
		C22:0	0.32 ± 0.10	**0.0013**	
		Tacc(24)	1.60 ± 0.12	**<2e-16**	
		C22:0 * Tacc(24)	-0.27 ± 0.11	**0.0139**	
	C22:0*Tacc+Tcell	Intercept	32.37 ± 0.22	**<2e-16**	0.42
		C22:0	0.27 ± 0.09	**0.002**	
		Tacc(24)	1.46 ± 0.13	**<2e-16**	
		Tcell	-0.31 ± 0.08	**7.82e-5**	
		C22:0 * Tacc(24)	-0.22 ± 0.11	**0.05**	

Continued

(b) Saturated fatty acids							
C12:0	*Contrast*	Estimate	±	SE	*df*	*t* ratio	*p*
(ModB)	16 – 24	-0.309	±	0.146	157	-2.114	0.036
	Slope	Estimate	±	SE	*df*	lower.CL	upper.CL
	16	-0.137	±	0.120	18.1	-0.389	0.116
	24	0.172	±	0.138	43.6	-0.107	0.452
C20:0	*Contrast*	Estimate	±	SE	*df*	*t* ratio	*p*
(ModB)	16 – 24	0.362	±	0.124	134	2.906	0.004
	Slope	Estimate	±	SE	*df*	lower.CL	upper.CL
	16	0.348	±	0.111	44.3	0.125	0.572
	24	-0.013	±	0.089	25.7	-0.195	0.168
C20:0	*Contrast*	Estimate	±	SE	*df*	*t* ratio	*p*
(ModC)	16 – 24	0.313	±	0.116	154	2.684	0.008
	Slope	Estimate	±	SE	*df*	lower.CL	upper.CL
	16	0.288	±	0.098	157	0.094	0.482
	24	-0.024	±	0.075	156	-0.173	0.124
C22:0	*Contrast*	Estimate	±	SE	*df*	*t* ratio	*p*
(ModB)	16 – 24	0.274	±	0.118	123	2.322	0.022
	Slope	Estimate	±	SE	*df*	lower.CL	upper.CL
	16	0.324	±	0.100	35.5	0.121	0.526
	24	0.049	±	0.089	27.6	-0.134	0.233
C22:0	*Contrast*	Estimate	±	SE	*df*	*t* ratio	*p*
(ModC)	16 – 24	0.216	±	0.111	154	1.950	0.053
	Slope	Estimate	±	SE	*df*	lower.CL	upper.CL
	16	0.269	±	0.087	153	0.098	0.440
	24	0.053	±	0.075	150	-0.096	0.201

Table 4.8: Results of (a) three linear mixed-effect models that test heat tolerance in response to monounsaturated fatty acids, acclimation temperature and cell size. (b) Post-hoc pairwise comparisons between slopes and slope differences from zero for the two acclimation temperatures when a significant interaction was found. First two models (ModA and ModB) : intercepts vary by clone and by jar, effects of fatty acid profile are allowed to vary by clone. Third model (ModC) : intercepts vary by clone and by jar.

(a) Fatty acid	Model fixed effects formula	Term	Estimate ± SE	*p*	R^2_m
C15:1	C15:1	Intercept	33.02 ± 0.25	**<2e-16**	0.04
		C15:1	-0.31 ± 0.09	**7e-4**	
	C15:1*Tacc	Intercept	32.35 ± 0.25	**<2e-16**	0.32
		C15:1	-0.23 ± 0.13	0.10	
		Tacc(24)	1.64 ± 0.13	**<2e-16**	
		C15:1 * Tacc(24)	0.27 ± 0.13	**0.04**	
	C15:1*Tacc+Tcell	Intercept	32.40 ± 0.22	**<2e-16**	0.41
		C15:1	-0.23 ± 0.09	**9e-3**	
		Tacc(24)	1.42 ± 0.13	**<2e-16**	
		Tcell	-0.34 ± 0.08	**7.98e-6**	
		C15:1 * Tacc(24)	0.18 ± 0.12	0.15	
C18:1n9c	C18:1n9c	Intercept	33.03 ± 0.25	**<2e-16**	0.01
		C18:1n9c	-0.15 ± 0.11	0.19	
	C18:1n9c*Tacc	Intercept	32.26 ± 0.26	**<2e-16**	0.30
		C18:1n9c	-0.11 ± 0.13	0.38	
		Tacc(24)	1.65 ± 0.13	**<2e-16**	
		C18:1n9c * Tacc(24)	0.07 ± 0.14	0.62	
	C18:1n9c*Tacc+Tcell	Intercept	32.35 ± 0.22	**<2e-16**	0.39
		C18:1n9c	-0.10 ± 0.09	0.26	
		Tacc(24)	1.47 ± 0.13	**<2e-16**	
		Tcell	-0.35 ± 0.08	**1e-5**	
		C18:1n9c * Tacc(24)	0.10 ± 0.12	0.37	

(b) Monounsaturated fatty acids							
C15:1 (ModB)	*Contrast*	Estimate	±	SE	*df*	*t* ratio	*p*
	16 – 24	-0.270	±	0.137	152	-1.968	0.051
	Slope	Estimate	±	SE	*df*	lower.CL	upper.CL
	16	-0.231	±	0.13	24.4	-0.504	0.042
	24	0.039	±	0.13	28.7	-0.228	0.306
C15:1 (ModC)	*Contrast*	Estimate	±	SE	*df*	*t* ratio	*p*
	16 – 24	-0.183	±	0.129	156	-1.42	0.158
	Slope	Estimate	±	SE	*df*	lower.CL	upper.CL
	16	-0.233	±	0.089	161	-0.409	-0.056
	24	-0.049	±	0.097	153	-0.241	0.142

Table 4.9: Results of (a) three linear mixed-effect models that test heat tolerance in response to polyunsaturated fatty acids, acclimation temperature and cell size. (b) Post-hoc pairwise comparisons between slopes and slope differences from zero for the two acclimation temperatures when a significant interaction was found. First two models (ModA and ModB) : intercepts vary by clone and by jar, effects of fatty acid profile are allowed to vary by clone. Third model (ModC) : intercepts vary by clone and by jar.

(a) Fatty acid	Model fixed effects formula	Term	Estimate ± SE	p	R^2_m
C18:2n6c	C18:2n6c	Intercept	33.01 ± 0.25	**<2e-16**	0
		C18:2n6c	-0.05 ± 0.09	0.58	
	C18:2n6c *Tacc	Intercept	32.21 ± 0.25	**<2e-16**	0.31
		C18:2n6c	-0.05 ± 0.14	0.71	
		Tacc(24)	1.71 ± 0.13	**<2e-16**	
		C18:2n6c * Tacc(24)	0.08 ± 0.16	0.61	
	C18:2n6c *Tacc+Tcell	Intercept	32.36 ± 0.22	**<2e-16**	0.40
		C18:2n6c	0.03 ± 0.11	0.80	
		Tacc(24)	1.45 ± 0.13	**<2e-16**	
		Tcell	-0.37 ± 0.08	**2.88e-6**	
		C18:2n6c * Tacc(24)	0.07 ± 0.14	0.59	
C18:3n3	C18:3n3	Intercept	33.01 ± 0.26	**<2e-16**	0.02
		C18:3n3	-0.22 ± 0.11	0.07	
	C18:3n3*Tacc	Intercept	32.21 ± 0.26	**<2e-16**	0.31
		C18:3n3	-0.06 ± 0.12	0.62	
		Tacc(24)	1.67 ± 0.13	**<2e-16**	
		C18:3n3* Tacc(24)	-0.01 ± 0.14	0.92	
	C18:3n3*Tacc+Tcell	Intercept	32.35 ± 0.22	**<2e-16**	0.40
		C18:3n3	-0.03 ± 0.08	0.73	
		Tacc(24)	1.47 ± 0.13	**<2e-16**	
		Tcell	-0.36 ± 0.08	**7.17e-6**	
		C18:3n3 * Tacc(24)	0.03 ± 0.12	0.82	
C20:5n3	C20:5n3	Intercept	33.06 ± 0.22	**<2e-16**	0.25
		C20:5n3	-0.73 ± 0.11	**5.36e-5**	
	C20:5n3*Tacc	Intercept	32.51 ± 0.24	**<2e-16**	0.37
		C20:5n3	-0.45 ± 0.12	**0.0016**	
		Tacc(24)	1.41 ± 0.18	**9.15e-13**	
		C20:5n3 * Tacc(24)	0.42 ± 0.23	0.0766	
	C20:5n3*Tacc+Tcell	Intercept	32.54 ± 0.22	**<2e-16**	0.43
		C20:5n3	-0.38 ± 0.08	**1.39e-6**	
		Tacc(24)	1.39 ± 0.18	**2.23e-12**	
		Tcell	-0.29 ± 0.07	**8.81e-5**	
		C20:5n3 * Tacc(24)	0.54 ± 0.22	**0.0187**	
C20:4n6	C20:4n6	Intercept	32.02 ± 0.25	**<2e-16**	0
		C20:4n6	0.05 ± 0.10	0.596	
	C20:4n6*Tacc	Intercept	32.31 ± 0.25	**<2e-16**	0.31
		C20:4n6	0.24 ± 0.11	**0.0280**	
		Tacc(24)	1.58 ± 0.12	**<2e-16**	
		C20:4n6 * Tacc(24)	0.28 ± 0.14	**0.0428**	
	C20:4n6*Tacc+Tcell	Intercept	32.39 ± 0.22	**<2e-16**	0.41
		C20:4n6	0.23 ± 0.10	**0.03**	
		Tacc(24)	1.43 ± 0.12	**<2e-16**	
		Tcell	-0.36 ± 0.08	**4e-6**	
		C20:4n6 * Tacc(24)	-0.20 ± 0.14	0.17	

Continued

(a) Fatty acid	Model fixed effects formula	Term	Estimate ± SE	*p*	R^2_m
C22:5n3	C22:5n3	Intercept	33.05 ± 0.25	**<2e-16**	0.01
		C22:5n3	0.16 ± 0.08	**0.045**	
	C22:5n3*Tacc	Intercept	32.28 ± 0.25	**<2e-16**	0.32
		C22:5n3	0.32 ± 0.09	**0.0021**	
		Tacc(24)	1.64 ± 0.12	**<2e-16**	
		C22:5n3 * Tacc(24)	-0.25 ± 0.11	**0.0246**	
	C22:5n3*Tacc+Tcell	Intercept	32.32 ± 0.22	**<2e-16**	0.41
		C22:5n3	0.27 ± 0.08	**2e-3**	
		Tacc(24)	1.52 ± 0.13	**<2e-16**	
		Tcell	-0.30 ± 0.08	**1e-4**	
		C22:5n3 * Tacc(24)	-0.20 ± 0.11	0.07	

(b) Polyunsaturated fatty acids							
C20:5n3	*Contrast*	Estimate	±	SE	*df*	*t* ratio	*p*
(ModC)	16 – 24	-0.534	±	0.228	157	-2.345	0.020
	Slope	Estimate	±	SE	*df*	lower.CL	upper.CL
	16	-0.446	±	0.123	158	-0.703	-0.189
	24	-0.030	±	0.238	157	-0.502	0.442
C20:4n6	*Contrast*	Estimate	±	SE	*df*	*t* ratio	*P*
(ModB)	16 – 24	0.281	±	0.167	22.9	1.684	0.11
	Slope	Estimate	±	SE	*df*	lower.CL	upper.CL
	16	0.241	±	0.115	36.2	0	0.475
	24	-0.040	±	0.121	7.92	-0.312	0.239
C22:5n3	*Contrast*	Estimate	±	SE	*df*	*t* ratio	*p*
(ModB)	16 – 24	0.249	±	0.116	135	2.151	0.03
	Slope	Estimate	±	SE	*df*	lower.CL	upper.CL
	16	0.32	±	0.10	40.2	0.123	0.519
	24	0.07	±	0.09	26.5	-0.108	0.252

ANNEXE C

CONCLUSION GÉNÉRALE

Table 5.10: Results of York regressions that test CTmin in response to fatty acid profile. Slope estimates are presented with their *p-value*. See note below for details on the *chisq* metric.

Fatty acid	Term	Estimate ± SE	Slope *p-value*	*Chisq*	*Chisq p-value*	df residual
Saturated fatty acids	Intercept	-1.89 ± 0.20		0.57	0.95	22
	Slope	1.0 ± 0.30	**0.003**			
Monounsaturated fatty acids	Intercept	-1.99 ± 0.21		0.61	0.92	22
	Slope	-1.02 ± 0.30	**0.003**			
Polyunsaturated fatty acids	Intercept	-1.79 ± 0.19		1	0.46	22
	Slope	-0.98 ± 0.31	**0.005**			
Total omega 3	Intercept	-1.75 ± 0.18		0.84	0.68	22
	Slope	-0.97 ± 0.30	**0.004**			
Total omega 6	Intercept	-1.97 ± 0.15		1	0.46	22
	Slope	0.41 ± 0.17	**0.02**			
Omega-3/omega-6	Intercept	-2.14 ± 0.18		0.80	0.73	22
	Slope	-0.81 ± 0.21	**8e-4**			
EPA/DPA	Intercept	-2.16 ± 0.22		0.76	0.78	22
	Slope	-1.09 ± 0.37	**0.008**			
UI	Intercept	-1.74 ± 0.18		0.93	0.55	22
	Slope	-0.93 ± 0.30	**0.005**			
PI	Intercept	-1.65 ± 0.17		1.21	0.23	22
	Slope	-0.69 ± 0.25	**0.01**			
C12:0	Intercept	-2.49 ± 0.37		0.90	0.59	22
	Slope	-2.04 ± 0.73	**0.01**			
C14:0	Intercept	-1.73 ± 0.26		0.68	0.87	22
	Slope	1.48 ± 0.54	**0.01**			
C16:0	Intercept	-0.76 ± 0.70		0.42	0.99	22
	Slope	3.39 ± 1.64	**0.05**			
C18:0	Intercept	-1.85 ± 0.22		0.63	0.91	22
	Slope	1.26 ± 0.43	**0.008**			
C20:0	Intercept	-1.98 ± 0.26		0.61	0.92	22
	Slope	1.44 ± 0.61	**0.03**			
C22:0	Intercept	-2.13 ± 0.32		0.45	0.99	22
	Slope	1.74 ± 0.90	0.07			
C15:1	Intercept	-1.85 ± 0.15		1.26	0.18	22
	Slope	-0.53 ± 0.22	**0.02**			
C18:1n9c	Intercept	-2.17 ± 0.24		0.67	0.87	22
	Slope	-1.14 ± 0.43	**0.01**			
C18:2n6c	Intercept	-1.94 ± 0.18		0.83	0.69	22
	Slope	0.73 ± 0.27	**0.01**			
C18:3n3	Intercept	-1.99 ± 0.21		0.68	0.86	22
	Slope	-1.02 ± 0.34	**0.006**			
C20:5n3	Intercept	-1.98 ± 0.16		0.84	0.68	22
	Slope	-0.76 ± 0.22	**0.002**			
C20:4n6	Intercept	-1.94 ± 0.14		1.14	0.30	22
	Slope	0.26 ± 0.11	**0.04**			
C22:5n3	Intercept	-2.07 ± 0.39		0.38	1	22
	Slope	2.42 ± 1.53	0.13			

Table 5.11: Result of York regression that test CTmax in response to CTmin. Slope estimate is presented with its *p-value*. See note below for details on the *chisq* metric.

	Estimate ± SE	Slope p-value	*Chisq*	*Chisq p-value*	df residual
Intercept	34.9 ± 0.37		4.16	**1.88e-10**	22
Slope	0.85 ± 0.20	**2e-4**			

Note : «The goodness of fit metric *chisq* is a weighted reduced chi-squared statistic. It compares the deviations of the points from the fit line to the assigned measurement error standard deviations. If x and y are indeed related by a straight line, and if the assigned measurement errors are correct (and normally distributed), then *chisq* will equal 1. A *chisq* > 1 indicates underfitting : the fit does not fully capture the data or the measurement errors have been underestimated. A *chisq* < 1 indicates overfitting : either the model is improperly fitting noise, or the measurement errors have been overestimated» Sturm (2021).