IMPACT DES VAGUES DE CHALEUR SUR LE MÉTABOLOME DES MOULES BLEUES *MYTILUS EDULIS* JUVÉNILES DIPLOÏDES ET TRIPLOÏDES

Mémoire présenté

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RÉSUMÉ

Sous l'effet des changements climatiques, l'intensité, la fréquence, et la durée des vagues de chaleur marines sont en augmentation, représentant une menace importante pour la conchyliculture. La triploïdisation est de plus en plus utilisée dans l'industrie aquacole pour son potentiel de redirection des investissements énergétiques de la reproduction vers d'autres processus physiologiques, tels que la croissance somatique ou la tolérance au stress. Cependant, la thermotolérance des moules triploïdes face aux vagues de chaleur est encore peu explorée. Cette étude vise à étudier les réponses métaboliques et métabolomiques de *Mytilus edulis* (Linnaeus, 1758) juvéniles diploïdes et triploïdes face aux vagues de chaleur, la triploïdie ayant été induite par pression hydrostatique et par traitement chimique. Les individus de chaque ploïdie et méthode d'induction ont été exposées pendant trois semaines à des conditions de contrôle (18-21°C) et de vague de chaleur (24-27°C). La mortalité et les taux de consommation d'oxygène ont été mesurés dans chaque groupe, et des analyses métabolomiques ciblées ont été réalisées par chromatographie sur phase liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS) afin d'évaluer leurs réponses physiologiques et moléculaires. Aucune mortalité significative n'a été observée, et les mesures respirométriques indiquent que 24°C se situe dans la plage de tolérance thermique des juvéniles. Aucune différence claire n'a été observée entre les niveaux de ploïdie ou les méthodes d'induction au niveau physiologique. Toutefois, les analyses métabolomiques ont révélé des différences d'abondance en acides aminés protéinogènes entre moules diploïdes et triploïdes. Des signatures de stress thermique ont été observées dans chacun des groupes, impliquant les voies énergétiques, de signalisation, et de réparation cellulaire. De faibles différences ont été observées dans la réponse au stress thermique entre individus diploïdes et triploïdes issus d'un traitement par pression hydrostatique. En revanche, la triploïdie induite par traitement chimique provoque des différences marquées chez les moules dans le délai et l'intensité de leur réponse face aux vagues de chaleur, comparativement à celles pour lesquelles la triploïdie a été induite par choc de pression. Les résultats de cette étude suggèrent que la triploïdisation par choc de pression est une approche prometteuse pour produire des moules à croissance rapide sans réduire leur tolérance aux vagues de chaleur, et pouvant potentiellement leur conférer une meilleure résistance au stade adulte.

Mots clés : Triploïdie, Vagues de chaleur, Métabolomique, *Mytilus edulis*, Bivalve, Respirométrie, Juvénile.

ABSTRACT

Marine heatwaves (MHWs) are increasing in intensity, frequency, and duration under climate change, posing a significant threat to bivalve aquaculture. Triploidization is increasingly used in the aquaculture industry for its potential to redirect energy from gonadal development toward other physiological processes, such as somatic growth or stress mitigation. However, triploid mussels' thermotolerance to heatwave remains poorly understood. This study investigated the metabolic and metabolomic responses of juvenile diploid and triploid *Mytilus edulis* families to MHWs, and compared triploids produced by hydrostatic pressure or 6-dimethylaminopurine (6DMAP) treatments. Mussels from each ploidy and induction method were exposed for three weeks to control (18–21 °C) or heatwave (24–27 °C) conditions. Mortality and oxygen consumption rates were recorded, and targeted metabolomic analyses were performed using liquid chromatography–tandem mass spectrometry (LC-MS/MS) to assess their physiological and molecular responses to MHWs. No significant mortality occurred in any treatment, and respirometry indicated that 24°C remained within the functional thermal tolerance window of juvenile *M. edulis*, with no differences between ploidy levels or induction methods. Metabolomic analyses revealed differences in proteinogenic amino acids of diploid and triploid mussels. Clear signatures of thermal stress were detected across all groups, involving energetic, signalling, and stress-repair pathways. While only minor differences were detected between diploids and pressure-induced triploids in their thermal stress responses, 6DMAP-induced triploids exhibited distinct temporal and intensity patterns in metabolomic responses to heat stress. These findings suggest that triploidization by hydrostatic pressure is a reliable approach for producing fast-growing mussels without compromising their tolerance to marine heatwaves, and may even confer enhanced resistance at the adult stage compared to diploids.

Keywords: Triploidy, Marine heatwaves, Metabolomics, *Mytilus edulis*, Bivalves, Respirometry, Juvenile.

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AAAs	Acides aminés aromatiques, Aromatic Amino Acids
ADP	Adénosine Diphosphate, Adenosine Diphosphate
AEC	Charge énergétique adénylique, Adenylate Energy Charge
AMP	Adénosine Monophosphate, Adenosine Monophosphate
ATP	Adénosine Triphosphate, Adenosine Triphosphate
BCAAs	Acides aminés à chaine ramifiée, Branched Chain Amino Acid
CS	Scénario contrôle, Control Scenario
DFO	Ministère des Pêches et des Océans Canada, Department of Fisheries and Oceans Canada
FAA	Acides aminés libres, Free amino acids
FAD	Flavine adénine dinucleotide (forme oxydée), Flavin adenine dinucleotide (oxidized form)
FADH	Flavine adénine dinucleotide (forme réduite), Flavin adenine dinucleotide (reduced form)
FAO	Organisation pour l'alimentation et l'agriculture, Food and Agriculture Organization
GABA	Acide γ-aminobutyrique, γ-aminobutyric acid

GC-MS	Chromatographie en phase gazeuse couplée à la spectrométrie de masse, Gas Chromatography coupled to Mass Spectrometry
HSP	Protéine de choc thermique, Heat Shock Protein
HWS	Scénario de vague de chaleur, Heatwave Scenario
LC-MS/MS	Chromatographie en phase liquide couplée à la spectrométrie de masse en tandem, Liquid Chromatography coupled to Tandem Mass Spectrometry
MHW	Vague de chaleur marine, Marine Heatwave
MTBE	Ether methylique de tert-butyle, Methyl tert-butyl ether
NAD	Nicotinamide adénine dinucléotide (forme oxydée), Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adénine dinucléotide (forme réduite), Nicotinamide adenine dinucleotide (reduced form)
NADP	Nicotinamide adénine dinucléotide phosphate (forme oxydée), Nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	Nicotinamides adénine dinucléotide phosphate (forme reduite), Nicotinamide adenine dinucleotide phosphate (reduced form)
OCR	Taux de consommation d'oxygène, Oxygen consumption rate
ROS	Espèces réactives de l'oxygène, Reactive Oxygen Species
sPLS-DA	Analyse discriminante par moindres carrés partiels parcimonieuse, sparse Partial Least Squares – Discriminant Analysis
T1	Time point 1
T2	Time point 2

T3	Time point 3
TCA	Cycle de l'acide tricarboxylique, Krebs cycle, Citric acid cycle
TFE	2,2,2-trifluoroéthanol, 2,2,2-trifluoroethanol
UV	Ultra-violet
VIP	Importance de la variable dans la projection, Variable importance in the projection
XP	Traitement par choc de pression hydrostatique, Hydrostatic pressure shock treatment
6DMAP	6-diméthylaminopurine, 6-dimethylaminopurine

INTRODUCTION GÉNÉRALE

1. LA CONCHYLICULTURE EN CONTEXTE DE CHANGEMENTS CLIMATIQUES

L'expansion de l'élevage d'animaux aquatiques débute autour des années 1960s (Stickney, 2004; FAO, 2024) et devient, à partir de la fin du 20^{e} siècle, un secteur majeur de la production mondiale de protéines animales (Stickney, 2004). En 2022, la production aquacole dépasse pour la première fois celle de la pêche, représentant ainsi 51 % du poids total des récoltes d'animaux aquatiques (FAO, 2024). Cette tendance devrait se poursuivre dans les décennies à venir, avec une augmentation estimée de 57 % de la production aquacole entre 2018 et 2050, en réponse à la croissance démographique mondiale (Boyd et al., 2022). La mariculture, en particulier, se présente comme une solution prometteuse pour garantir un accès durable aux protéines animales, à condition qu'elle soit accompagnée de mesures de conservation appropriées (Duarte et al., 2009; Costello et al., 2020). En outre, elle offre un potentiel pour atténuer les effets de la surpêche qui représente un défi majeur (Sumaila & Tai, 2020; FAO, 2024).

La mariculture de bivalves est l'activité aquacole avec la plus faible empreinte environnementale, ce qui en fait l'une des sources de protéines les plus durables à l'échelle mondiale, et la catégorie d'aliments aquatiques (blue foods) émettant le moins de CO_2 (Parodi et al., 2018; Gephart et al., 2021; Tamburini et al., 2025). À titre d'exemple, les émissions de gaz à effet de serre liées à la production de bivalves sont dix fois moins importantes que celles issues de la production de bœuf, et représentent un tiers de celles émises par la culture de saumon (MacLeod et al., 2020). Par ailleurs, la conchyliculture fournit d'importants avantages écosystémiques, notamment dans les zones côtières, souvent sujettes aux rejets anthropiques de nitrates et de phosphates (Filgueira et al., 2015). Grâce à leur capacité de filtration, les bivalves jouent un rôle central dans la régulation de l'eutrophisation en capturant les microalgues contenant ces nutriments (Lindahl et al., 2005; Guyondet et al., 2022). Ce processus favorise une meilleure pénétration lumineuse dans la colonne d'eau

jusque vers le fond, permettant le développement de macroalgues et d'herbes marines, espèces ingénieures clés servant d'habitat pour une grande diversité d'organismes (Newell & Koch, 2004; Petersen et al., 2016). Par ailleurs, la filtration exercée par les bivalves contribue à limiter la fréquence des efflorescences algales toxiques et des infections microbiennes (Gallardi, 2014). L'ensemble de ces services écosystémiques fournis par la conchyliculture contribue ainsi au maintien et à l'enrichissement de la biodiversité dans les zones côtières. De plus, l'élevage de bivalves agit également sur la séquestration du carbone (Sun et al., 2025b). À court terme, il favorise le développement des herbiers marins, dont la photosynthèse permet une absorption significative du CO_2. À plus long terme, les moules participent à la séquestration du carbone à travers la formation de leur coquille, composée de carbonate de calcium ($CaCO_3$), via le processus de calcification (Tang et al., 2011) (Figure 1). Une étude récente indique que, sur un cycle de 17 mois, une ferme de bivalves de 0,25 ha pourrait séquestrer jusqu'à 55 tonnes de carbone (Vaher et al., 2024). Toutefois, certains modèles suggèrent que le carbone océanique stocké par l'aquaculture de bivalves ne représenterait qu'environ 0,001 % des émissions anthropiques de CO_2 au Canada (Zavell et al., 2023). Cependant, contrairement à la reforestation ou aux pratiques d'agriculture de conservation, la captation du carbone par les bivalves, bien que modeste, assure une séquestration minérale permanente (Filgueira et al., 2015; Jones et al., 2022).

Enfin, les bivalves représentent une source nutritionnelle particulièrement élevée. À titre illustratif, 13 g de moule suffiraient à atteindre un indice nutritionnel équivalent à celui de 100 g de bœuf (Tamburini et al., 2025). Cette densité nutritionnelle élevée confère donc aux bivalves un intérêt stratégique dans le cadre d'une alimentation à la fois saine et durable, malgré leur utilisation encore relativement limitée à grande échelle, principalement due à des limitations économiques et culturelles (Tamburini et al., 2025).

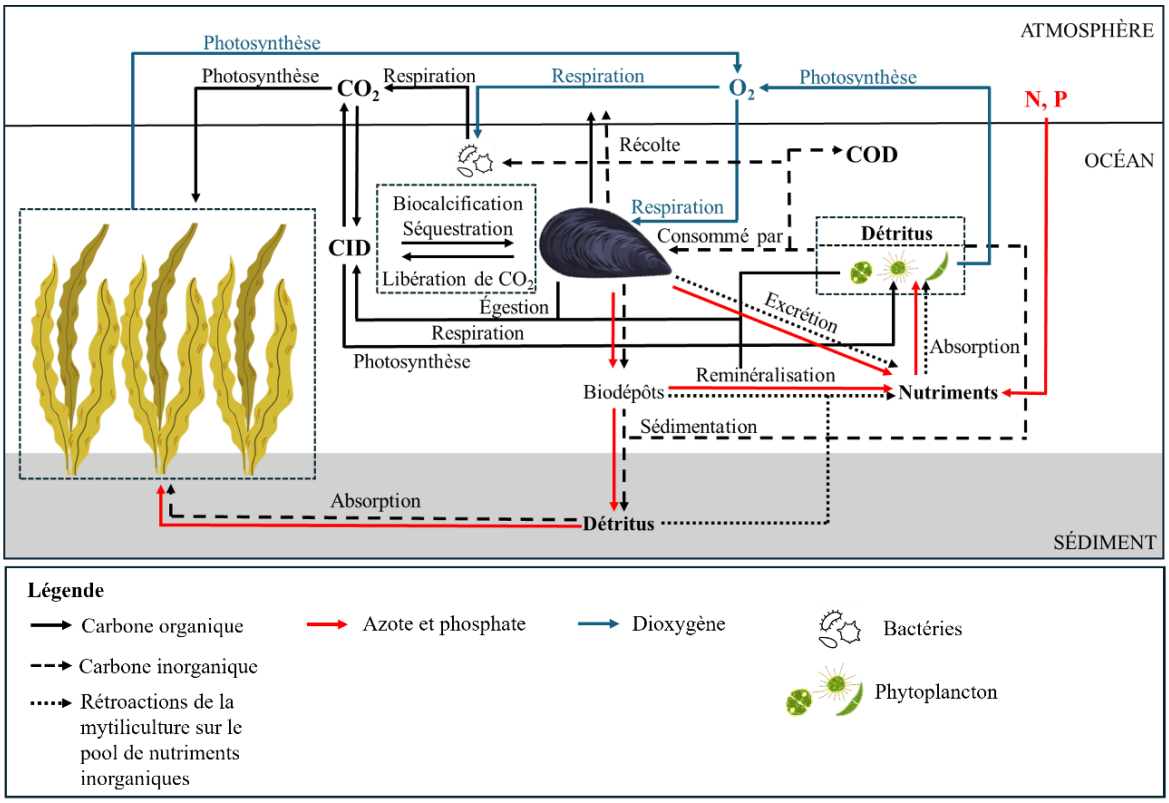

Figure 1. Flux de nutriments et interactions biologiques dans un système mytilicole (inspiré de Filgueira et al., 2015). COD : carbone organique dissous ; CID : carbone inorganique dissous.

2. *MYTILUS EDULIS*

Mytilus edulis (Linnaeus, 1758), *Mytilus galloprovincialis* (Lamarck, 1819), et *Mytilus trossulus* (Goulde, 1850), constituent le complexe d'espèces *Mytilus edulis*, regroupant les trois espèces de moules prédominantes dans l'hémisphère Nord (McDonald et al., 1991; Regan et al., 2024). Ces taxons constituent des espèces majeures de la conchyliculture, en raison de leur importance économique et écologique (FAO, 2020). La répartition géographique de la moule bleue, *M. edulis* couvre les eaux tempérées à tempérées froides de l'Atlantique Nord, de l'Arctique, ainsi que certaines régions méditerranéennes (Hayward & Ryland, 2017). En tant qu'espèce eurytopique, capable de tolérer de fortes variations de

salinité, de dessiccation et de température, elle occupe une large gamme d'habitats, s'étendant des zones supralittorales aux secteurs subtidaux supérieurs (Hayward & Ryland, 2017). *M. edulis* est particulièrement prisée en aquaculture, constituant la deuxième espèce la plus importante au Canada, tant en termes de volume de production que d'apports économiques (Statistics Canada, 2023). Différentes techniques sont utilisées pour l'élevage de moules, notamment la culture sur bouchots, sur radeaux, ou en suspension sur filières, cette dernière méthode étant la seule pratiquée au Canada en raison de sa capacité à être submergée sous le couvert de glace (Kamermans & Capelle, 2019; Mascorda Cabre et al., 2021; DFO, 2023).

M. edulis possède un cycle de vie bentho-planctonique, marqué par une phase planctonique lors des premiers stades de développement, suivi d'une phase benthique après fixation (Figure 2). Dans l'hémisphère Nord, *M. edulis* atteint sa maturité sexuelle au début de l'été, avec une période de ponte s'étalant généralement de mai à août (Lander et al., 2010; DFO, 2003). La fécondation de l'ovule déclenche les premières divisions cellulaires, menant en 4 à 5 heures à la formation d'un embryon cilié mobile (DFO, 2003). Environ 24 à 48 heures après fécondation, l'embryon se développe en une larve trochophore, amorçant la sécrétion de sa première coquille, puis en larve D après 3 à 4 jours (Bayne, 1976). Lorsque la larve atteint le stade véligère, une nouvelle coquille est sécrétée par le manteau, et un mode d'alimentation exogène débute par la filtration de petites particules *via* les cils du velum (Bayne, 1976; Helm et al., 2004). Ce stade perdure généralement pendant 2 à 3 semaines, jusqu'à l'apparition d'un pied extensible caractéristique du stade pédivéligère, lequel permet à la larve de se fixer à un substrat jusqu'à sa métamorphose en post-larve, puis en juvénile (Seed, 1969).

La métamorphose marque le passage de la phase planctonique à la phase benthique et déclenche la formation de filament branchiaux et de palpes labiaux, ainsi que de la sécrétion d'une coquille dissoconque et de filaments de byssus, caractéristiques de l'installation benthique du juvénile (Nelson, 1928; Bayne, 1976). La byssogénèse, *ie* la sécrétion continue de byssus, permet à l'organisme de se fixer à de nombreux types substrats et d'adopter un

mode de vie sessile (Brenner & Buck, 2010). Après avoir atteint les 2,5 mm, les juvéniles deviennent majoritairement benthiques et se déplacent uniquement par reptation sur substrat à l'aide de leur pied afin de former des colonies (Seed & Suchanek, 1992). Ce comportement grégaire permet de réduire l'exposition aux vagues et aux courants et fournit donc une meilleure adhérence au substrat comparativement à une fixation isolée (Seed & Suchanek, 1992). Le stade juvénile constitue une étape transitoire ou l'individu présente des caractéristiques physiques similaires à celles de l'adulte, lui conférant un meilleur taux de survie qu'à l'état larvaire (Rayssac et al., 2010), mais demeure encore sexuellement immature. L'âge d'obtention de la maturité sexuelle peut varier en fonction des conditions environnementales, mais est généralement atteint entre la fin de la première année et la deuxième année (Seed, 1969; Sprung, 1983).

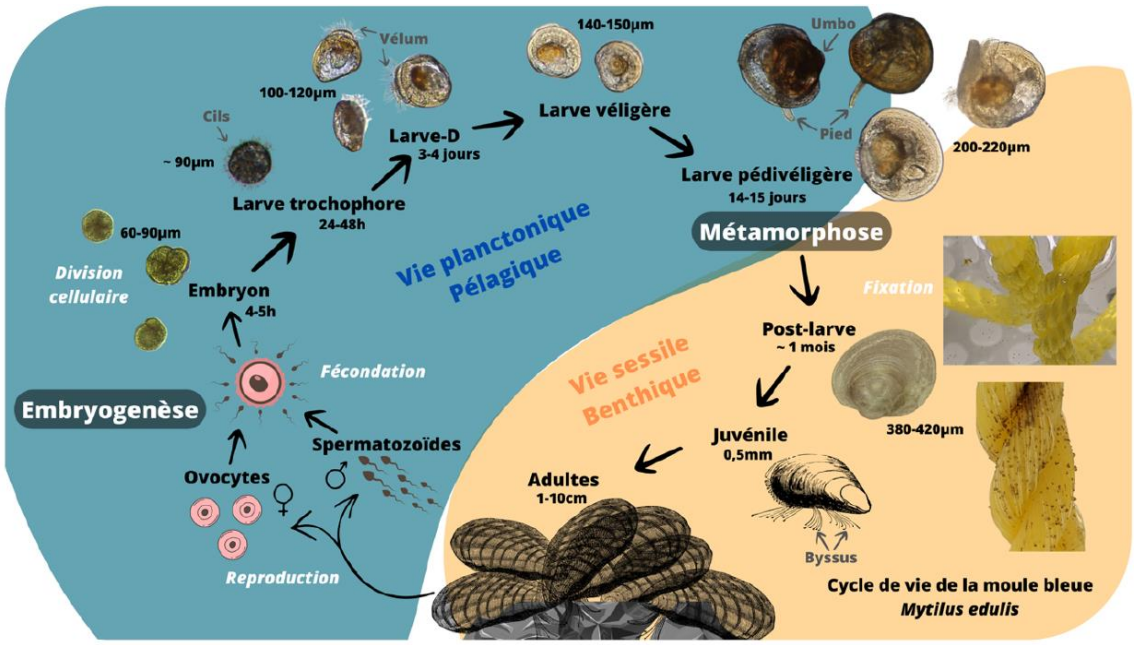

Figure 2. Cycle de développement de la moule bleue *Mytilus edulis* (Veillard, 2025).

3. TRIPLOIDÏE

La gamétogénèse est un processus fortement énergivore, pouvant représenter jusqu'à 50 à 90% de l'investissement énergétique chez *M. edulis* (Bayne et al., 1983; Rodhouse et al., 1984; Lemaire et al., 2006). Elle est généralement suivie d'une expulsion des gamètes associée à de grandes pertes énergétiques affaiblissant considérablement l'individu (Clements et al., 2018; Mredul et al., 2024).

La triploïdie, c'est-à-dire la présence de trois lots de chromosomes dans son patrimoine génétique, peut se produire de manière occasionnelle à l'état naturel, ou être induite en laboratoire (Otto & Whitton, 2000; Osterheld et al., 2021). La triploïdie est généralement associée à une faible fertilité chez les bivalves, et les individus concernés sont le plus souvent stériles (Allen Jr & Downing, 1990; Komaru et al., 1995; Kiyomoto et al., 1996). En effet, chez les moules, l'induction de triploïdie résulte le plus souvent en un biais sur le sexe ratio tourné majoritairement vers la production de mâles, ainsi qu'en une altération de la gamétogénèse interrompue prématurément (Kiyomoto et al., 1996; Brake et al., 2004; Osterheld et al., 2024). Cette dernière est alors généralement suivie d'une dégénération des spermatocytes, initiée par les cellules de Sertoli et les hémocytes (Kiyomoto et al., 1996; Osterheld et al., 2024). Selon Chung et al. (2007) et Osterheld et al. (2024), la réabsorption des gamètes en fin de saison de ponte chez les triploïdes pourrait servir de réserves énergétiques sous forme de lipides et de glycogène. Ainsi, les triploïdes octroient moins d'énergie à la fonction de reproduction comparativement aux individus diploïdes (Kiyomoto et al., 1996; Brake et al., 2002; Osterheld et al., 2024), permettant une réallocation énergétique vers la croissance somatique, comme observé chez plusieurs espèces de bivalves (Ruiz-Verdugo et al., 2000; (Brake et al., 2004) et la production de byssus (Osterheld et al., 2023).

Les bivalves triploïdes présentent donc généralement une croissance plus rapide que les diploïdes, à la fois à l'état larvaire et benthique (Shpigel et al., 1992; Abele & Puntarulo, 2004; Walton et al., 2013; Osterheld et al., 2021; Brianik & Allam, 2023). Brake et al. (2004)

ont observé chez les moules bleues triploïdes une augmentation de la masse sèche des tissus pouvant atteindre 62,8 % ainsi qu'une croissance de la coquille jusqu'à 10,9 % supérieure à celle des diploïdes. Chez les bivalves, la triploïdie contribue donc à une augmentation du taux de croissance en évitant les pertes énergétiques liées à la gamétogénèse et à l'expulsion des gamètes (Allen Jr & Downing, 1991). Ces avantages en font donc une technique efficace et à faible impact environnemental pour favoriser l'amélioration des rendements. De plus, un second facteur pouvant expliquer la présence d'une croissance accrue chez les individus triploïdes serait la présence de taux d'hétérozygotie plus importants chez ces derniers (Allendorf & Leary, 1984; Nell, 2002). En effet, plusieurs travaux ont mis en évidence une corrélation entre un taux élevé d'hétérozygotie, un faible taux métabolique de base et une croissance rapide chez la moule bleue (Tremblay et al., 1998a; Tremblay et al., 1998b; Alberts et al., 2014; Myrand, 2002; Myrand et al., 2009a; Myrand et al., 2009b). Enfin, un gigantisme cellulaire a été observé par Guo et al. (1996) et Wang et al. (2002) chez les bivalves triploïdes. Ce phénomène, caractérisé par un volume cellulaire accru et l'absence de mécanisme compensatoire du nombre de cellules, pourrait également contribuer à une croissance plus rapide chez les triploïdes (Guo & Allen Jr, 1994).

Chez *M. edulis*, Osterheld et al. (2023) ont également observé une augmentation de 65% de la quantité de byssus chez les triploïdes ainsi qu'un accroissement de la rigidité de leurs filaments comparativement aux observations faites sur le diploïdes. Ceci contribue donc également à l'amélioration des rendements en mariculture, dont les pertes liées au détachement des moules de leurs structures peuvent représenter jusqu'à 28% de la production totale (Bourque & Myrand, 2006). Ces détachements sont accrus durant et après la période de ponte, lorsque les individus sont affaiblis (Lachance et al., 2008; Hennebicq et al., 2013), car la production de byssus peut nécessiter entre 8 et 15% des investissements énergétiques (Griffiths & King, 1979; Hawkins, 1985). Enfin, l'utilisation d'organismes triploïdes en mariculture, dont les capacités reproductives sont limitées, permet de réduire le risque de pollution génétique des populations sauvages locales associé à la production en milieu naturel (Grigorakis, 2010).

La triploïdie est induite par manipulation de la méiose peu après fécondation (Piferrer et al., 2009) (Figure 3). En effet, chez la moule bleue, le développement des œufs après la ponte est bloqué au stade métaphase de la méiose I (Colas & Dubé, 1998). La fécondation de l'œuf par un spermatozoïde déclenche la reprise de la méiose qui entraîne l'extrusion successive de deux corps polaires (Colas & Dubé, 1998). La triploïdie peut donc être induite post-fecondation par choc physique (changement de température ou de pression) ou par traitement chimique, de manière à supprimer la division cellulaire en empêchant l'extrusion de l'un des deux corps polaires, aboutissant à l'obtention de trois chromosomes homologues, l'un étant hérité du père et les deux autres de la mère (Figure 3) (Chaiton & Allen Jr, 1985; Desrosiers et al., 1993; Nell, 2002). La 6-(dimethylamino)purine (6-DMAP) est couramment utilisée pour les traitements chimiques puisqu'elle est peu coûteuse, soluble dans l'eau, facilement manipulable et moins toxique que son alternative, la cytochalasine B (CB) (Desrosiers et al., 1993; Nell, 2002). Cette technique possède un fort taux de réussite chez *M. edulis* (Brake et al., 2004; Osterheld et al., 2021). Le 6-DMAP est un inhibiteur de protéine kinase (Neant & Guerrier, 1988; Dubé et al., 1991; Dufresne et al., 1991; Szöllösi et al., 1991). Son utilisation perturbe la condensation de la chromatine, empêche la formation du fuseau mitotique en bloquant l'organisation des microtubules astraux, et bloque la progression méiotique, inhibant ainsi l'extrusion du corps polaire (Dufresne et al., 1991; Szöllösi et al., 1991; Desrosiers et al., 1993). La triploïdisation par choc de pression consiste en une élévation abrupte de la pression hydrostatique autour de l'œuf pendant plusieurs minutes (Piferrer et al., 2009). Les mécanismes sous-jacents de cette méthode restent peu documentés, mais le changement de densité cytoplasmique induit par la pression exercée favoriserait l'inhibition de la capacité de nucléation du centrosome, ainsi que la dépolymérisation des microtubules du fuseau méiotique, entraînant la *rétention du corps polaire (*Salmon, 1975; Zhu et al., 2007; Gao et al., 2018).

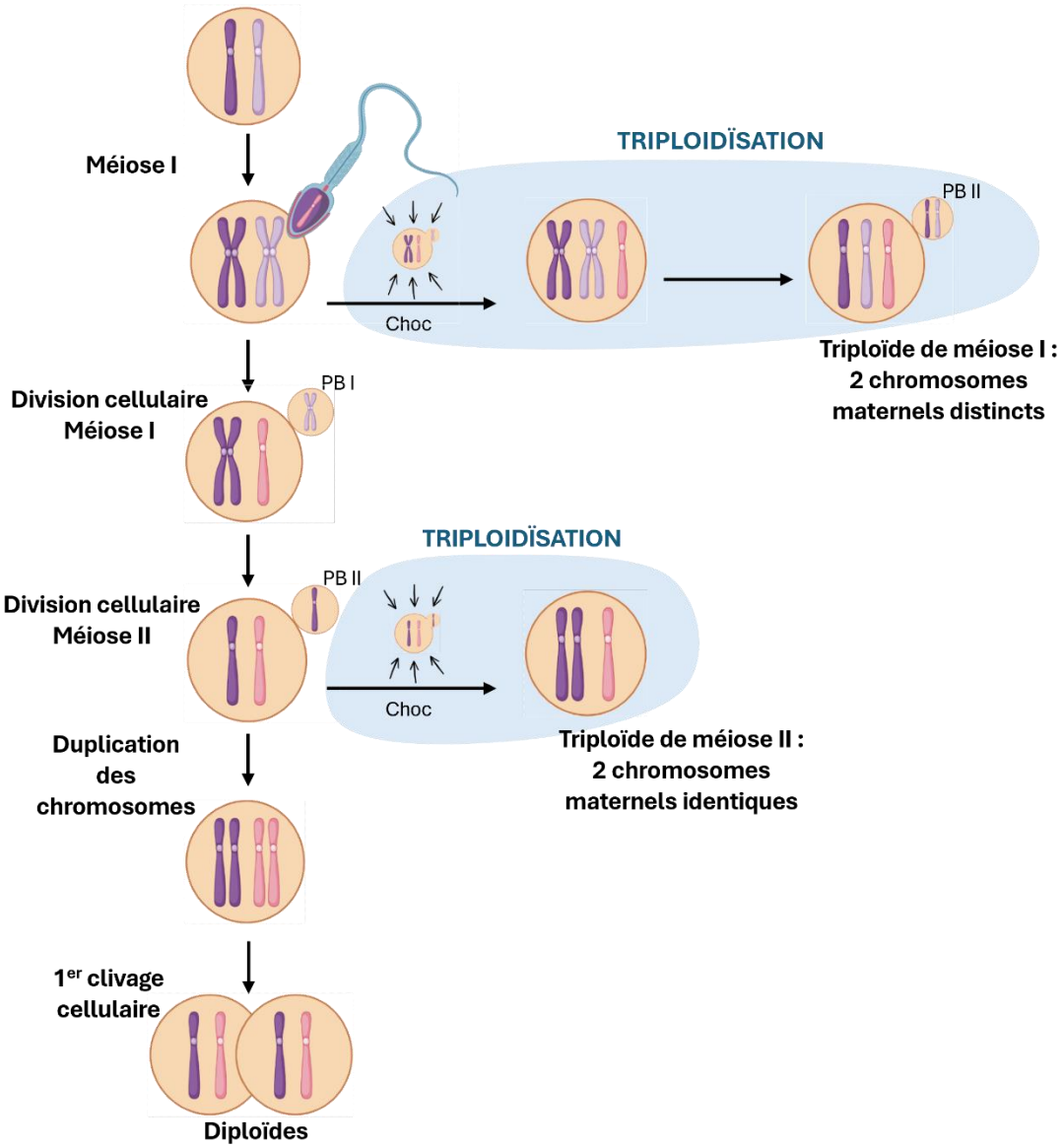

Figure 3. Induction de triploïdie chez les bivalves par inhibition de l'extrusion du premier (PBI) ou du second (PBII) corps polaire (inspiré de Piferrer et al., 2009). Réalisé avec BioRender.com

4. BUDGET ÉNERGÉTIQUE ET STRESS

Les organismes vivants peuvent être décrits comme des systèmes thermodynamiques ouverts, dont l'intégrité structurelle et le fonctionnement reposent sur des sources d'énergie extérieures et un flux énergétique constant (Sokolova, 2013). Chez les animaux, l'énergie extérieure est apportée par la nutrition et peut être convertie en énergie utilisable par la cellule, soit sous forme de composés à haut potentiel énergétique, soit sous forme de gradient ionique (Skulachev et al., 2012). Cette énergie est ensuite utilisée lors de processus servant à remplir plusieurs fonctions physiologiques, notamment le maintien somatique, la croissance et la reproduction. Les dépenses associées au maintien somatique incluent l'énergie nécessaire au fonctionnement de processus cellulaires fondamentaux, ainsi qu'à certaines fonctions vitales comme la respiration, l'excrétion ou encore l'alimentation (Sibly & Calow, 1986). En raison des limites physiologiques des systèmes digestif et métabolique, la quantité d'énergie qu'un organisme est capable d'assimiler demeure fondamentalement restreinte (Sokolova et al., 2012). Par ailleurs, contrairement aux autres fonctions physiologiques, les coûts de maintien somatique ne peuvent être réduits en deçà d'un seuil minimal, au risque de mettre en péril la survie de l'organisme (McCracken et al., 2020). Ainsi, le taux métabolique standard, ou taux métabolique de base désigne l'énergie minimale nécessaire à un organisme ectotherme au repos et en l'absence de digestion pour assurer sa survie (Withers & Withers, 1992; Guderley & Pörtner, 2010).

De plus, l'organisme peut stocker les excédents d'énergie assimilée sous forme de lipides, mais également de glucides ou de protéines, fournissant ainsi un capital énergétique mobilisable en période de stress ou de restriction alimentaire (Sokolova et al., 2012). Les lipides contiennent la plus grande quantité d'énergie (≈39 $kJ \cdot g^{-1}$), comparativement aux protéines (≈20.4 $kJ \cdot g^{-1}$) et glucides (≈17.5 $kJ \cdot g^{-1}$) (Gnaiger, 1983). Ces molécules sont ensuite catalysées pour permettre la formation d'adénosine triphosphate (ATP), qui est la

principale monnaie énergétique dans la cellule (Hochachka & Somero, 2002; Sokolova, 2021) (Figure 4). Environ 90 % de l'ATP est produit dans les mitochondries par métabolisme aérobie, via la phosphorylation oxydative de l'adénosine diphosphate (ADP) (Alberts et al., 2014) mais les glucides et acides aminés peuvent également être oxydés en anaérobie en cas de besoin (Hochachka & Somero, 2002).

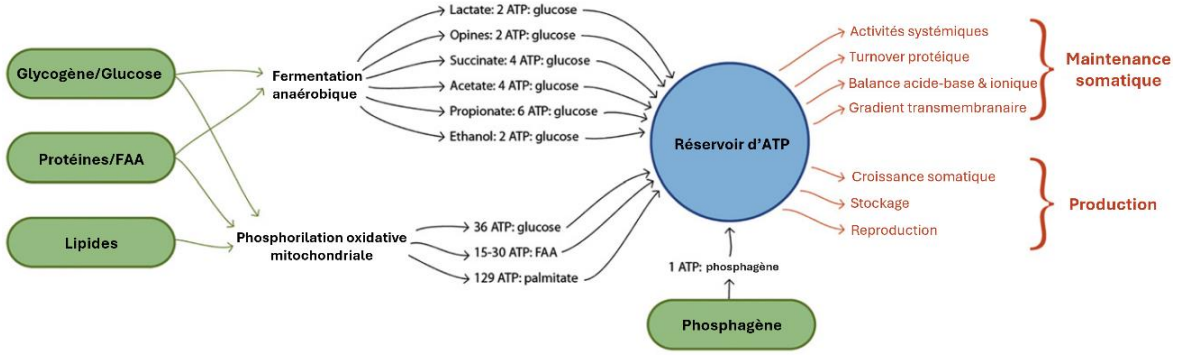

Figure 4. Principales voies de production et de consommation de l'ATP chez les invertébrés aquatiques (traduit de Sokolova et al., 2012). FAA : acides aminés libres

Le stress peut être défini comme l'exposition d'un organisme à un facteur de contrainte biotique ou abiotique, appelé stresseur, entraînant un décalage d'un ou de plusieurs processus biologiques par rapport à leur points d'équilibre homéostatique (Kültz, 2020). Parmi les facteurs abiotiques, la température exerce une influence déterminante sur les processus métaboliques et les équilibres énergétiques des organismes ectothermes (Pörtner, 2002). Lorsqu'elle s'écarte de la zone optimale, elle peut perturber ces équilibres, notamment en modifiant les coûts énergétiques associés au maintien cellulaire, et la capacité globale de production d'énergie de l'organisme (Pörtner, 2010). Cette perturbation peut entraîner un stress bioénergétique, déséquilibrant l'énergie disponible et les besoins énergétiques de l'organisme (Pörtner, 2001; Pörtner, 2002; Pörtner, 2010; Pörtner et al., 2017). La courbe aérobie de performance (SFP) représente l'énergie excédentaire disponible après couverture des coûts liés au taux métabolique standard (Sokolova, 2021). Cette énergie peut ensuite être allouée à des fonctions physiologiques telles que la croissance somatique ou la reproduction,

faisant de la SFP un indicateur pertinent pour évaluer le bilan énergétique d'un organisme (Guderley & Pörtner, 2010).

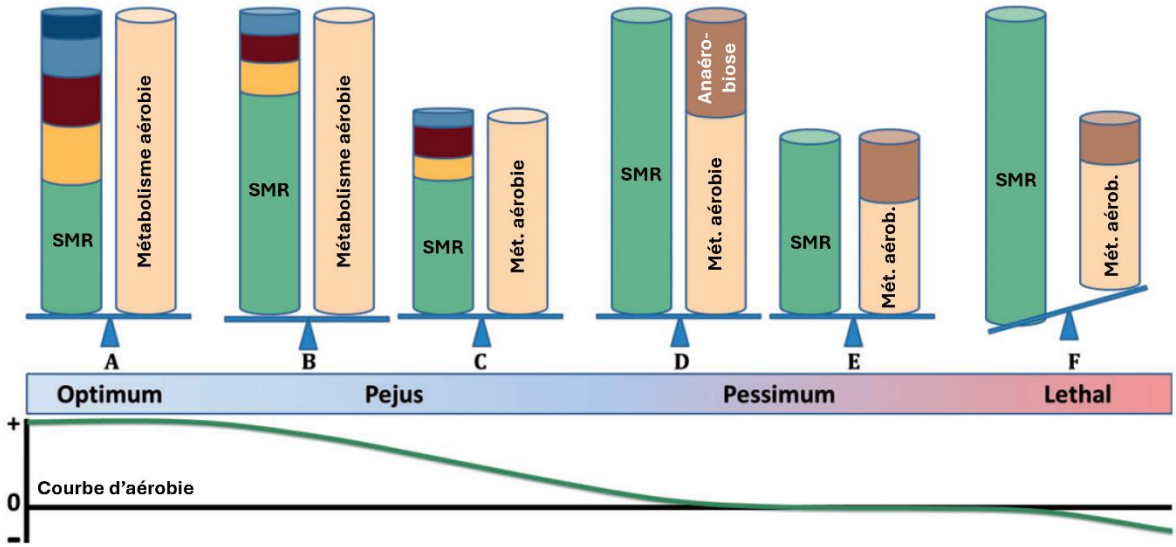

Figure 5. Schématisation du concept de tolérance au stress limitée par l'énergie (traduit de Sokolova, 2013). SMR : Taux métabolique standard; Jaune : activité; rouge : reproduction/maturation; bleu clair : croissance/développement; bleu foncé : stockage des réserves

En particulier, le concept de tolérance thermique limitée par l'oxygène (OCLTT) a été développé pour illustrer la manière dont la capacité d'un organisme à assurer un approvisionnement adéquat en oxygène devient un facteur limitant de sa performance physiologique lorsque la température s'éloigne de sa zone optimale (Pörtner, 2001; Pörtner, 2002; Pörtner et al., 2017). Selon ce concept, illustré dans la Figure 5 la courbe aérobie peut être découpée en 4 fenêtres : 1) en conditions thermiques optimales, la balance énergétique est positive. L'énergie excédentaire, une fois les coûts de maintenance somatique couverts, est maximale, et peut être allouée aux fonctions de performance telles que la croissance, la reproduction ou le stockage sous forme de lipides et de glycogène. 2) Lorsque la température s'éloigne de l'optimum, l'organisme entre dans la zone pejus, caractérisée par une diminution progressive des performances physiologiques. Dans cette zone, les coûts de maintenance cellulaire, et donc le taux métabolique standard, augmentent, tandis que la capacité

métabolique maximale diminue en raison de limitations dans l'approvisionnement en oxygène. Ce double effet réduit la marge énergétique disponible pour les fonctions non essentielles, entraînant un état de stress modéré. 3) Si la température continue de s'éloigner de l'optimum, l'organisme atteint la zone de pessimum. L'intégralité de l'énergie aérobie produite est alors mobilisée pour assurer le maintien somatique, rendant la SFP quasi nulle. L'organisme compense partiellement par l'activation de voies anaérobies, qui permettent une production plus rapide, mais moins efficace d'ATP, et dont les sous-produits sont potentiellement toxiques. Cette stratégie permet une survie temporaire, souvent limitée à quelques jours ou semaines, dans l'attente d'un retour à des conditions plus favorables. 4) Enfin, au-delà de certains seuils critiques, l'organisme entre dans la fenêtre létale, où l'équilibre énergétique est rompu. Les apports énergétiques deviennent insuffisants pour assurer les fonctions de base, et le métabolisme standard ne peut plus être soutenu. Cette situation entraîne des dérèglements homéostasiques majeurs, des dommages cellulaires irréversibles, et conduit généralement à la mort de l'organisme.

5. VAGUES DE CHALEUR

Depuis le siècle dernier, une hausse des températures extrêmes de surface a été constatée à l'échelle mondiale, en particulier dans les zones telles que les grands courants marins occidentaux et les mers semi-fermées comme la Méditerranée, en lien avec les changements globaux (IPCC, 2023). En particulier, l'affaiblissement de la circulation méridienne atlantique (AMOC) expose le nord-ouest de l'Atlantique à un réchauffement océanique particulièrement rapide comparé à la moyenne mondiale, augmentant ainsi le risque d'événements extrêmes tels que les vagues de chaleur marines (Saba et al., 2016; Oliver et al., 2018; Laufkötter et al., 2020; Oliver et al., 2021; Rahmstorf, 2024). Ces dernières sont définies comme des périodes d'au moins cinq jours consécutifs durant lesquelles les températures de surface dépassent le 90e percentile de la moyenne saisonnière locale (Hobday et al., 2016). Du fait de leur faible profondeur, les écosystèmes côtiers sont particulièrement vulnérables aux changements de température rendant des espèces côtières

telles que *M. edulis* particulièrement exposées (Paice & Chambers, 2017; NOAA Fisheries, 2021). Les réponses des moules diploïdes aux stress thermiques ont été largement explorées, depuis les ajustements moléculaires et métaboliques jusqu'aux modifications physiologiques et comportementales (Kittner & Riisgård, 2005; Jones et al., 2009; Marigomez et al., 2017; Clements et al., 2018; Wang et al., 2018; Seuront et al., 2019; Li et al., 2020; Collins et al., 2020; Nguyen & Alfaro, 2020; Azizan et al., 2023; Ericson et al., 2023; Masanja et al., 2023; Talevi et al., 2023; Venter et al., 2023; Xu et al., 2023; Clarke et al., 2025; Papadopoulos et al., 2025). Cependant, les individus triploïdes pourraient être susceptibles de répondre différemment au stress, justifiant une investigation ciblée. En effet, en raison d'une plus grande disponibilité énergétique, ceux-ci pourraient présenter une meilleure résistance face à des conditions de stress (Shpigel et al., 1992; Sun et al., 2025a). Toutefois, certaines études ont au contraire mis en évidence une sensibilité accrue des individus triploïdes à certains stresseurs environnementaux, attribuée à des dérégulations transcriptomiques liées à la présence des chromosomes surnuméraires (Li et al., 2022; George et al., 2023).

Les approches omiques, telles que la transcriptomique, la protéomique ou la métabolomique, sont particulièrement utiles pour étudier les mécanismes moléculaires sous-jacents impliqués dans les réponses physiologiques (Torson et al., 2020; Lu et al., 2023).

6. OBJECTIFS

Le présent projet s'inscrit donc dans le Programme de Partenariats pour les Applications de la Génomique (GAPP) de Génome Canada et fait partie intégrante du programme de génomique des moules triploïdes. Ce dernier est basé sur une approche multi-omique visant à identifier des marqueurs de résistance aux hautes températures et de robustesse des moules de culture triploïdes. Le programme a donc pour but de fournir un lot d'outils omiques permettant d'accélérer l'amélioration des triploïdes.

L'objectif de cette étude est d'analyser les réponses métabolique et métabolomique de moules bleues diploïdes et triploïdes soumises à des conditions de vague de chaleur, en

comparant notamment les moules triploïdes issues d'un traitement par choc de pression à celles obtenues par traitement chimique. Nous testerons donc l'hypothèse selon laquelle les moules triploïdes, indépendamment de la méthode d'induction, pourraient modifier leur transition entre les voies métaboliques aérobie et anaérobie, améliorant ainsi leur résistance aux vagues de chaleur comparativement aux individus diploïdes.

ARTICLE 1
IMPACT OF MARINE HEATWAVES ON THE METABOLOME OF DIPLOID AND TRIPLOID JUVENILE *MYTILUS EDULIS*

1.1 INTRODUCTION

Bivalve aquaculture relies on the ability of bivalves to filter phytoplankton, a function that provides valuable ecosystem services by mitigating eutrophication and contributing to carbon cycling (Filgueira et al., 2015). Furthermore, bivalve aquaculture is recognized as the blue food category with the lowest CO_2 emissions (Gephart et al., 2021) and is widely regarded as one of the highest-quality nutritional sources among all types of aquatic foods (Parodi et al., 2018; Golden et al., 2021). The blue mussel (*Mytilus edulis*) is particularly valued in aquaculture, representing the second most important species in Canada in terms of both production volume and farm-gate value (Statistics Canada, 2023). Prince Edward Island, in particular, plays a central role in this industry, accounting for around 80% of North America's blue mussel production (Statistics Canada, 2023).

1.1.1 Triploidy

Sexual maturation represents one of the main challenges in shellfish aquaculture, as gametogenesis is a highly energy-demanding process, accounting for around 50% to up to 90% of adult mussels' total energy allocation (Bayne et al., 1983; Rodhouse et al., 1984; Lemaire et al., 2006). The subsequent spawning leads to significant energy losses, weakening individuals' stress resistance, decreasing meat quality, and substantially reducing industry productivity (Clements et al., 2018; Mredul et al., 2024). Furthermore, spawning can cause large-scale mortalities in mussels, especially when a major spawning event coincides with

exposure to stressful high temperatures, leading to physiological weakening (Clements et al., 2018; Myrand et al., 2000; Alter et al., 2025).

Triploid organisms, which have three sets of chromosomes instead of two as typically observed in nature. In bivalves, triploidy is often associated with reduced energy investment in reproduction and incomplete gametogenesis (Kiyomoto et al., 1996; Brake et al., 2004; Osterheld et al., 2024), enhancing their physiological resilience by mitigating the weakening typically associated with spawning (Tremblay & Osterheld, 2025). Osterheld et al. (2024) also reported signs of gamete resorption in triploid *Mytilus edulis* following gametogenesis, potentially contributing to the recovery of lipid and glycogen energy reserves. As a result, the energy spared from reproduction may be redirected toward somatic growth and other metabolic demands, such as clearance rates and ultimately leading to enhanced growth (up to 2.6 times higher in 20-30 mm triploids *Mytilus edulis*) (Brake et al., 2004; Osterheld et al., 2023). Additionally, Osterheld et al. (2023) observed a 65% increase in the quantity of byssal thread produced, along with greater thread strength in triploid *Mytilus edulis* compared to their diploid counterparts. Such features contribute to mitigating losses associated with mussel detachment from farming structures, particularly following spawning events (Lachance et al., 2008; Hennebicq et al., 2013). These traits offer valuable economic and ecological benefits by enhancing water filtration and supporting mariculture productivity, contributing to limiting greenhouse gas emissions associated with farming operations (Tremblay & Osterheld, 2025).

Triploidy can be artificially induced in bivalves by inhibiting the extrusion of the polar body during meiosis I or II (Stanley et al., 1981; Chaiton & Allen Jr, 1985; Desrosiers et al., 1993; Osterheld et al., 2021), with several studies reporting higher triploidy induction success and survival rates when targeting the second polar body (Gérard et al., 1999; Guo et al., 1992; Barreto-Hernández et al., 2018). Triploidy can be induced chemically or physically, as summarized in Guo et al. (2009); Piferrer et al. (2009). 6-(dimethylamino)purine (6-DMAP) is widely used to induce triploidy due to its high solubility, ease of handling, and lower user toxicity compared to other agents such as cytochalasin B (Desrosiers et al., 1993). 6-DMAP

is a protein kinase inhibitor that interferes with phosphorylation-dependent processes during meiosis. Among these effects, 6-DMAP inhibits chromatin condensation and disrupts microtubule organization, impairing spindle formation and chromosome migration, thereby preventing cell division (Dufresne et al., 1991; Szöllösi et al., 1991). This is an effective method to induce triploidy, showing 90% success rates when applied during meiosis II (Osterheld et al., 2021). However, 6-DMAP can be toxic to users, prompting hatcheries to explore safer methods such as hydrostatic pressure treatment. The latter is achieved by applying a rapid increase in hydrostatic pressure to the zygotes during the extrusion of the polar body (Piferrer et al., 2009). The underlying mechanisms of this method remain poorly documented, but the pressure-induced change in cytoplasmic density is thought to inhibit centrosome nucleation capacity and promote the depolymerization of meiotic spindle microtubules, leading to the retention of the polar body (Salmon, 1975; Zhu et al., 2007; Gao et al., 2018).

Some studies suggest that triploid bivalves could exhibit greater tolerance to heat stress than diploids, likely due to their higher energy availability (Shpigel et al., 1992; Sun et al., 2025a). Conversely, findings by Li et al. (2022) and George et al. (2023) showed increased sensitivity of triploid oysters to heat stress, which seems to be attributed to gene expression dysregulation.

1.1.2 Marine heatwaves

Since the last century, increases in extreme seawater temperatures have been observed worldwide, particularly in western boundary current systems and semi-enclosed seas, in connection with anthropogenic climate change (IPCC, 2023). The weakening of the Atlantic Meridional Overturning Circulation (AMOC) exposes the Northwest Atlantic to particularly fast rates of ocean warming compared to global averages, thereby increasing the risk of extreme events such as marine heatwaves (MHWs) (Saba et al., 2016; Oliver et al., 2018; Laufkötter et al., 2020; Oliver et al., 2021). These are defined as periods during which seawater temperatures exceed the 90th percentile of the local seasonal average for a minimum

of five consecutive days (Hobday et al., 2016). Coastal ecosystems are especially sensitive to temperature changes owing to their shallow depths (Paice & Chambers, 2017; NOAA Fisheries, 2021).

As ectothermic sessile organisms living in temperate coastal areas, mussels are particularly exposed to thermal stress. Consequently, these organisms have developed an array of behavioural, physiological, and molecular strategies to withstand temperature variability. Typically, valve closure is commonly employed by mussels as a short-term mechanism to preserve homeostasis and limit energy expenditure under thermal stress (Kittner & Riisgård, 2005; Grimmelpont et al., 2024; Clarke et al., 2025). Under longer exposures, aerobic metabolism commonly increases with rising temperatures, a relationship observable at both molecular and physiological levels (Anestis et al., 2010; Collins et al., 2020; Somero, 2002). In response to heatwaves, energy production often relies on multiple metabolic pathways, including the Tricarboxylic Acid cycle (TCA, or Krebs cycle, releasing energy through acetyl-CoA oxidation) and fatty acid β-oxidation (catabolic process of fatty acid to generate acetyl-CoA) (Muznebin et al., 2022; Ericson et al., 2023). This results in accelerated adenosine triphosphate (ATP) production through aerobic metabolism, visible at the physiological level as an increase in the oxygen consumption rate (OCR) (Matoo et al., 2021). When the temperature rises beyond a certain threshold, the metabolism may shift from aerobic to anaerobic pathways to lower energy costs and sustain rapid energy production in the short term (Widdows, 1976; Lewis & Cerrato, 1997). However, this strategy is less efficient than aerobic metabolism and results in the accumulation of toxic by-products, making it unsustainable on a long-term basis (Ellington, 1983; Pörtner, 2001; Pörtner et al., 2017). Additionally, molecular processes such as the upregulation of heatshock proteins and the production of key amino acids and antioxidant molecules may be employed to mitigate oxidative stress, balance intracellular osmolarity, and restore cellular integrity compromised by increasing temperatures (Meng et al., 2013; Hu et al., 2022b; Azizan et al., 2023). However, extreme heatwave events can cause severe damage that may be beyond repair and be responsible for mass mortality in shellfish populations, particularly during the reproductive season (Petes et al., 2007; Harley, 2008; Seuront et al., 2019; das Chagas Pereira

et al., 2024). As a temperate species, *M. edulis* is well adapted to temperature fluctuations between 5 and 20 °C, but shows signs of stress response beyond these temperatures (Zittier et al., 2015; Talevi et al., 2023). Several studies established that the upper lethal limit temperature for *Mytilus edulis* is around 29 °C, although this value may vary depending on population, season, geographic location, and interacting stressors (Jones et al., 2009; Clarke et al., 2025). Thus, the increasing frequency and intensity of MHWs (Plecha et al., 2021; Von Kietzell et al., 2022) is becoming a major challenge for mussel aquaculture (Lattos et al., 2022; Seuront et al., 2019).

Metabolomics is a powerful tool allowing "rapid, unbiased and simultaneous measurements" of many metabolites, thus providing a holistic view of the metabolome of an organism, along with its responses to environmental stressors at the molecular scale (Viant, 2007). This study aims to combine molecular (metabolomics) and physiological (oxygen consumption) measurements to provide a multi-scale comprehension of diploid and triploid mussels' responses to heatwaves. We tested the hypothesis that triploids, regardless of the induction method, are able to modify their energetic investments and their transition from aerobic to anaerobic pathways, thereby influencing their resistance to heatwaves. To complement this study, lipidomic analyses were conducted prior to the experiment to assess any potential differences in fatty acid composition between groups. Fatty acids can represent an important form of energy storage in bivalves, particularly in larvae and juveniles (Holland, 1978; Pernet et al., 2007; Wenk, 2010; Whyte et al., 1987) and play a key role in maintaining membrane fluidity and homeostasis, especially under environmental stress (Fokina et al., 2017; Guinle et al., 2025; Pernet et al., 2007; Wenk, 2010). Any differences in the initial fatty acid composition between groups could therefore influence their tolerance to thermal stress, as well as their metabolomic responses. Triploid oysters, for instance, have been shown to contain higher levels of polyunsaturated and essential fatty acids compared to diploids (Fu et al., 2024; Qin et al., 2018), suggesting that similar patterns might occur in mussels.

1.2 METHODS

1.2.1 Specimen spawning, triploidization, and husbandry

Spawning

Blue mussels (*Mytilus edulis*) were produced in August and September 2023 at the Université du Québec à Rimouski's wet laboratory facility (Pointe-au-Père, QC, Canada), as described in Osterheld et al. (2021) and Osterheld et al. (2023), using broodstock from cultured stocks provided by Atlantic Aqua Farms from Prince Edward Island, Canada. Recently, mussels from this area were genotyped and revealed weak population stratification between different sites without introgression of *M. trossulus,* confirming the large predominance of *M. edulis* (Regan et al., 2024). Briefly, spawning was induced in isolated individuals by thermal shock in filtered (1 µm), ultraviolet (UV)-treated seawater to collect sperm and eggs separately (Rayssac et al., 2010). Two full-sibling families were produced for the experiment. The first family (Family 1) contained triploids obtained by pressure treatment (XP) and their diploid controls, and the second family (Family 2) included triploids from both chemical (6DMAP) and pressure treatments, as described below. These ploidy/induction methods (diploid, XP, and 6DMAP) are hereafter referred to as "groups". In each family, fertilization was carried out using gametes from one male and one female, with a ratio of 10 spermatozoa per oocyte. Fertilized eggs were then filtered through a 20 µm mesh and rinsed with 18°C filtered (1 µm) UV-treated seawater to remove excess sperm and prevent polyspermy.

Triploidy induction

For the chemical treatments, triploidy was induced by exposing fertilized eggs to of 400 µM of a 6-dimetylaminopurine (6-DMAP) (Sigma-Aldrich, MO, USA) solution dissolved in 18°C filtered (1 µm) UV-treated seawater (Brake et al., 2002). The treatment was administered 21 min post-fertilization, during the extrusion of the second polar body, and

lasted 10 min (Osterheld et al., 2021). Following the 10 min incubation, fertilized eggs were rinsed with 18°C filtered (1 µm) UV-treated seawater to remove remaining 6-DMAP.

For the pressure treatments, triploidy was induced using a 75 mL pressure vessel and hydraulic press as described by Small et al. (2021). For the production of the two families used in this experiment, different conditions were tested (58.6 and 62.0 MPa; 15 and 18 min post-fertilization; and 5 min of treatment, Table 1). Only treatments with over 90% triploid level and survival ≥ 15% between the veliger and the pediveliger stages (5-20 days post-f) were retained for this study (Table 1).

Husbandry and transportation

Following each treatment, the fertilized eggs from each group and family were transferred to separate 25 L flat-bottom tanks maintained at 18°C. Larvae were reared as described by Rayssac et al. (2010) and fed every two days 30 cells/µL with a mixture of algae containing *Pavlova lutheri* (CCMP 459), *Nannochloropsis occulata* (CCMP525), *Chaetoceros neogracilis* (CCMP1317), *Tisochrysis lutea* (CCMP 1324), and *Tetraselmis suecica* (CCMP 904) at a ratio of 1:1:1:1:1 equivalent biomass according to culture algal dry weight. Once over 50% of the population reached the pediveliger stage, the larvae from each group were transferred into 15 L flow-through downweller systems maintained at 18°C-20°C and 27 PSU and fed continuously with the same algal diet at 5000 cells mL^{-1}. Water flow rates were kept at 18 L $hour^{-1}$ and aeration was provided with air stones in both facilities.

When most of the individuals reached ≥ 2 mm shell length, 10 mussels per group were preserved in -80°C for lipidomic analyses. The others were placed in humidified, open Ziploc bags to preserve humidity and minimize desiccation, then transported for 8h in a cooler and transferred to Dalhousie University's Aquatron laboratory (Halifax, NS, Canada) for experimentation. Upon arrival, individuals from each group were placed in seawater, in similar conditions to those in Rimouski's wet laboratory, and acclimated for four weeks before starting the experiment. No significant mortalities were observed during the transport and acclimation in any of the groups.

Ploidy measurements

Ploidy was assessed on 5-days-old larvae following the method described by Osterheld et al. (2021), and reassessed on 10-month-old juveniles to confirm consistent ploidy levels throughout rearing and ensure that no differential survival favored diploids between the larval and juvenile stages. The initial ploidy measurements were performed on a pool of approximately 200 larvae per group, while for juveniles, ploidy was assessed individually on tissue samples (~10 mg) collected from 15 individuals per group. Each sample was gently homogenized in 500 µL of ice-cold pH 8 Galbraith's buffer (Galbraith et al., 1983) using a 7 mL loose pestle Douce tissue grinder (Wheaton, NJ, USA). After filtration through a 35 µm mesh, the homogenate was collected into a flow cytometry tube containing 25 µL of 1 mg/mL propidium iodide (PI) solution (Sigma, MO, USA) and 2-µm fluorescing beads (Fluoresbrite, Polysciences) as an internal standard. After 15 min of incubation, stained nuclei were analyzed using a CytoFLEX flow cytometer (Beckman Coulter, IN, USA) for 12 s at a flow rate of 10 µL minute^{-1}. Doublets were excluded from the analysis based on PI fluorescence area versus width plots (610 nm/20 nm bandpass), following the method described by da Silva et al. (2005). The diploid peak in the PI fluorescence histogram was identified using individuals from the diploid group of the first family, along with additional mussels from a commercial stock known to be diploids.

Table 1. Conditions of triploidy induction, triploidy induction success and survival for each group (XP : hydrostatic pressure; 6DMAP : 400 µM 6-dimetylaminopurine; post-F : post-fertilization).

	Family 1		***Family 2***	
	Diploids	*Triploids XP*	*Triploids XP*	*Triploids 6DMAP*
Pressure (PSI)	-	62.0	58.6	-
Time post-F (min)	-	18	15	21
Duration (min)	-	5	5	10
% Triploids (larvae)	0	88	93	99
'% Triploids (juvenile)	0	100	73	100
% Survival (larvae)	100	35	15	93

1.2.2 Experimental design

Experiments were carried out asynchronously for each family. A total of 360 individuals per family were used (i.e., 180 from each group). Mussels from each group were sorted by size to the nearest 0.1 millimeter using callipers, and individuals larger than 5mm were selected for the experiment. Mussels from each group were divided into twelve 9 L tanks (i.e., 30 mussels per tank) in a flow-through filtered seawater system at 18°C. For each family and group, three replicate tanks were exposed to a control temperature scenario (CS), and three others were exposed to a heatwave scenario (HWS) (Figure 6). Water flow rates were kept at 18L h^{-1} per tank and aerated with air stones. Mussels were fed under similar conditions to those before for the duration of the study.

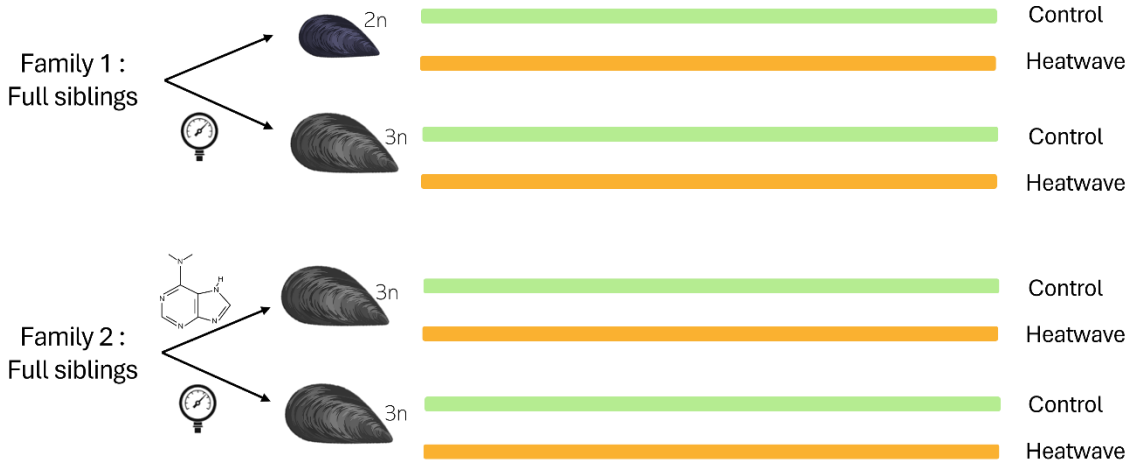

Figure 6. Triploidy induction method and experimental designs applied to each family.

Experimental heatwave conditions were designed (Figure 7) using temperature data collected during a heatwave recorded in 2022 at Atlantic Aqua Farms mariculture sites (Orwell Cove, PE, Canada). Prior to the experiment, all groups were held at 18°C for two days for acclimation. In the heatwave scenario (HWS), seawater temperature was subsequently increased by 1.5°C/day until reaching 24°C over four days. This temperature was then maintained for 48 hours to allow for a second acclimation period. Meanwhile, tanks from the CS were kept at 18°C. Thereafter, temperatures oscillated daily between 24°C and

27°C in the HWS and between 18°C and 20°C in the CS for 22 days, simulating natural diurnal temperature cycles.

Ten individuals from each tank were physically separated from others in mesh bags, resulting in 30 individuals per group for each temperature condition, to be monitored daily for mortality. To assess mortality, individuals displaying open valves were gently stimulated to induce closure. Mussels that failed to maintain valve closure for a few seconds were considered dead and subsequently removed from the experimental tank.

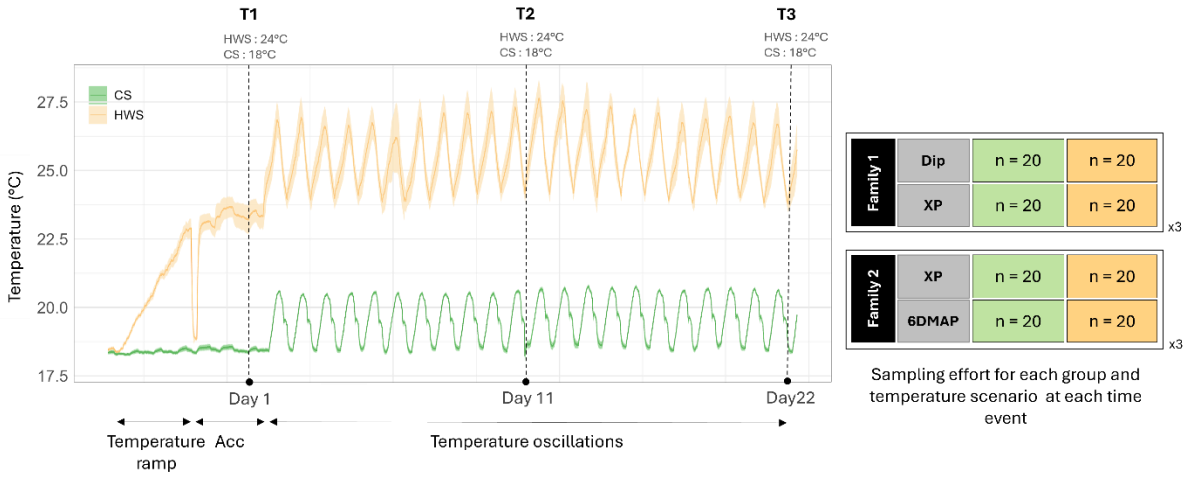

Figure 7. Experimental temperature profiles for control (CS) and heatwave (HWS) scenarios.

On days 1, 11, and 22 of temperature oscillations (corresponding to T1, T2, and T3, respectively), 5 mussels per tank were randomly collected (i.e., 15 individuals per group for each temperature scenario, resulting in a total of 60 mussels) and used for respirometry measurements. After concluding the respirometry, shell lengths were measured along the longest axis to the nearest 0.01 mm using callipers, and the tissue was dissected and rapidly collected into 2 mL soft tissue homogenizing tubes (Precellys 2 mL Soft Tissue Homogenizing Ceramic Beads Kit, Bertin Technologies SAS, Montigny-le-Bretonneux, France) and immediately flash-frozen in liquid nitrogen and preserved in -80°C freezer. Individuals that died (n=3) during respirometry measurements were excluded from the respirometry and metabolomics datasets. At each sampling point, (i.e., T1, T2, and T3)

respirometry measurements and tissue collection were performed at approximately the same time of day (~9:00 am), during the lower-temperature phase of the daily cycle. For Family 1, metabolomics samples from T1 were lost due to a power outage; consequently, only data from T2 and T3 are presented for this family.

1.2.3 Monitoring physico-chemical parameters

Seawater feeding the units was heated using immersion heaters, and both temperature and fluorescence, used as a proxy for food concentration, were monitored in the header tanks and adjusted every 10 s using multi-channel monitoring and control units (Point Four Remote Interface Unit 3, InWater Technologies). Target fluorescence values were established to maintain algal concentrations (5000 cells mL^{-1}, PAMAS S4031GO) in each tank. HOBO loggers (Onset HOBO MX2201) recorded temperature in each tank every minute for the duration of the experiment.

1.2.4 Respirometry

At each of the 3 sampling points (T1, T2, and T3), the 30 selected individuals from each temperature scenario were placed in individual 5 mL closed respirometry chambers filled with seawater from their tank (18°C or 24°C water, respectively, for control and heatwave-exposed mussels). Measurements were taken immediately after placing the mussels in the chambers. Oxygen uptake for 30 min as a proxy for aerobic metabolic rate. Chambers from each temperature scenario (30 mussels per temperature scenario and 2 empty chambers filled with water from the tanks for control) were placed in a rotating water bath to maintain stable temperature. Temperature probes were placed in the water baths, and each chamber was equipped with an optical dissolved oxygen sensor connected to a PreSens Precision Sensing 4-Channel Oxygen Meter (OXY-4 SMA). A total of 8 oximeters were used, recording and logging dissolved oxygen concentrations every 10 s on the PreSens Measurement Studio 2 software. After 30 min, individuals were delicately removed from the chambers, measured, and dissected for tissue sampling. The water volume in each chamber was measured to the

nearest 0.1 mL using graduated cylinders. The sampled mussels averaged 7.70 ± 2.44 mm length for diploids and 9.44 ± 3.40 mm for XP triploids in family 1, and 9.82 ± 2.87 mm and 11.2 ± 3.52 mm for 6DMAP and XP triploids, respectively, in family 2. Individual oxygen consumption rates (OCR_i; mg O_2 h^{-1}) were calculated using the following equation (Casas et al., 2018):

$$OCR_i = [(h - h') \times Vol] \quad (1)$$

Where h is the slope of the linear regression of the decrease in dissolved oxygen concentration (DOC) over time (mg O_2 L^{-1} h^{-1}) in the chamber containing the mussel, and h' is the average decline in DOC in the control chambers. Vol represents the volume (L) of water measured in the chamber. OCR_i was standardized to 1 g of dry tissue from each mussel (OCR_w; mg O_2 h^{-1} g^{-1}) according to Casas et al. (2018) :

$$OCR_w = OCR_i \times \left(\frac{DW_{std}}{DW_{exp}}\right)^b \quad (2)$$

Where OCR_i is the OCR of the experimental mussel, DW_{std} indicates the standardized dry weight (DW_{std} = 1 g), and b is the allometric exponent used to standardize OCR_i to dry weight. In this study, b = 0.67 was applied as the coefficient for blue mussels (Jones et al., 1992). DW_{exp} represents the dry weight of the experimental mussel (g), calculated shell length measurements using a length–weight relationship curve (Appendix I).

1.2.5 Lipidomics

Lipidomic extraction were performed at UQAR/ISMER. The homogenizing tubes containing the sample were taken and 1 mL of 2,2,2-trifluoroethanol (TFE) at 100% was added. Samples were vortexed for 30 s and centrifuged for 1 min at 2,460 g. The supernatants (1 mL) were pooled in 15 mL falcon tubes. Then, 500 µL of a 5/1 (v/v) mix of methyl tert-butyl ether (MTBE) and TFE was added to the homogenizing tubes, vortexed for 30 s, and centrifuged for 1 min at 2,460 g. This extraction was performed three times, and supernatants (500 µL) were pooled in 15 mL falcon tubes. The cartridge of the Captiva EMR-

Lipid was rinsing with 3 mL of 5/1 (v/v) mix of MTBE/TFE and drained by pulling a vacuum to the manifold to obtain a flux of 1 drops each 3 to 5 s. The filtrate was collected in 15 mL falcon tube. Sample was dried using a SpeedVac (SPD2010, Savant) overnight without heating. Sample reconstitution was performed by adding 250 µL of a 3/1 (v/v) mix of MTBE / Methanol HPLC grade (MeOH). 50µL of each reconstituted sample was transferred in 8 mL transesterification vials following and 800µL of toluene and 3 mL of 12% sulphuric acid methanol solution were added. Vials were heated at 90°C for 1 hour in a dry bath under the hood with vortex during 15 min each. When the vials were cooled to room temperature, 3 mL of H2O nano and 800 µL of hexane were added in the transesterification vials, vortexed and centrifuged for 10 min at 2,750 g. The supernatant was transferred into 1.5 mL GC vial and dried with SpeedVac (SPD2010, Savant). Lipids were separated and quantified using a GC-FID 8890 (G3540A, Agilent Technologies). Two microliters of the sample were injected, and the chromatography separation was performed with a DB-23, 120 m (2 x 60 m), 250 µm x 0.15 µm (Agilent Technologies).

1.2.6 Metabolomics

Extraction

Metabolite extraction and quantification were performed at IsoBiokem Laboratories (Rimouski, Québec, Canada). Samples from respirometry measures were first freeze-dried at -50°C for 48 hours, weighed to later normalize metabolite concentrations, and homogenized at 6000 rpm for 30 s at -4°C using a 3D tissue homogenizer (Precellys 24 with cryolis cooling unit, Bertin Technologies SAS, Montigny-le-Bretonneux, France). 500 µL of 1/1 (v/v) mix of TFE/LC-MS grade water was added to the 3 mL Agilent Captiva EMR–Lipid solid phase extraction cartridge (part number 5190-1003 – Agilent 155 Technologies), and 750µL of a 1/1 (v/v) mix of TFE/LC-MS grade water was added to the homogenizing tubes. The samples were vortexed for 30 s and centrifuged at 7500 rpm for 5 min at 4°C. Three extraction cycles (500 µL, with 1 mL of ACN for the last one) were performed, and the filtrates were pooled in a 15 mL Falcon tube. Then, the cartridge was rinsed with 3 mL of a 1/1 (v/v) mix of TFE/

LC-MS grade water and drained using a high vacuum for 30 seconds. Samples (in the Falcon tube) were dried with SpeedVac overnight without heating. Samples were reconstituted by adding 50 µL of LC-MS grade water followed by 200 µL of LC-MS grade ACN. One hundred microliters of each reconstituted sample was transferred to two vials amber HPLC vial with insert: one vial was for positive analysis and the other for the negative analysis. Compounds valine-d8 (10 µg/mL) and pyruvate-d3 (10 µg/mL) were used as internal standards to quantify the compounds in positive and negative ion modes, respectively.

Analysis

Liquid chromatography – mass spectrometry analysis was performed using a high-performance liquid chromatographer (HPLC 1260 Infinity II, Agilent Technologies, Santa Clara, CA, USA) coupled to a mass spectrometer (6420 Triple Quad, Agilent Technologies) in positive and negative ionization mode to detect targeted metabolites. The absolute quantification of these metabolites was assessed with the MassHunter QQQ quantitative (Quant-my-Way) software (Agilent Technologies) and normalized by dry weight to obtain absolute concentrations (in ng mL^{-1}).

1.2.7 Statistical analyses

All analyses were performed separately on each family as the experiments were performed successively.

The effect of ploidy and of the method of induction of triploidy on the lipidomic profiles of juveniles before heatwave exposure was estimated by permutational analyses of variance (PERMANOVA). The PERMANOVA was performed on R (4.1.1) and RStudio (4.5.0) using the *adonis2()* function (number of permutation = 999, distance method = Euclidiean) in the *vegan* package (Oksanen et al., 2025).

The effect of the factors (*Temperature scenario, Sampling time,* and *Induction method* or *Ploidy*) on OCRs was analyzed using Bayesian Generalized Linear Mixed Models (GLMs) in R (4.4.1) and RStudio (4.5.0). Models used the *stan_glmer()* function from the *rstanarm*

package (Goodrich et al., 2020) and fitted a Gamma distribution as the data distribution was not normal. GLMs were run for 4 chains, with 1000 iterations of warmup and 200 iterations, and the factor *Sampling time* was used as a random intercept. To assess model assumptions of data normality and homoscedasticity, the Shapiro–Wilk and Levene's tests were used, respectively. Alternative model structures, varying in their combination of fixed and random effects, were assessed for predictive accuracy using PSIS-LOO and WAIC, performed respectively with the *loo_compare()* and *waic()* functions from the *loo* package. These comparisons identified the combination of fixed effects that best explained OCR variation. In our study, the models including *Temperature scenario* and *Induction method* (or *Ploidy*) as fixed effects and *Sampling time* as a random intercept were selected as the most predictive models based on LOO and WAIC comparisons. Based on this selection, additional sub-models were constructed for each level of *Sampling time* separately, retaining *Temperature scenario* × *Induction method* (or *Ploidy*) as fixed effects.

The impact of exposure to heatwaves on metabolomic data was assessed by multivariate statistics. Data were centered and transformed to produce heatmaps on MetaboAnalyst (v 6.0, http://www.metaboanalyst.ca) (Pang et al., 2024), with hierarchical clustering based on Ward's method and Euclidean distance. R (4.1.1) and RStudio (R.4.5.0) were used to produce Principal Component Analyses (PCA) and perform a permutational analysis of variance (PERMANOVA) to assess the effect of *Temperature scenarios*, *Sampling time*, and *Induction method (or Ploidy)* on the response of 48 metabolites in all 293 samples. The PERMANOVA was performed using the *adonis2()* function (number of permutations = 999, distance method = Euclidean) in the *vegan* package (Oksanen et al., 2025). When differences were significant ($p \leq 0.05$), multiple pairwise comparisons were conducted using the *pairwise.adonis()2* function to identify which groups differed significantly. An additional pairwise PERMANOVA was conducted on the combined factors *Temperature scenario – Sampling time (i.e., sampling event) – Ploidy (or Induction method)* to explore interaction effects across all treatment combinations. Simper analyses were conducted to assess which metabolites significantly differed between groups using the *vegan* package. Complementarily, the variables that best discriminated between groups were identified by

sparse Partial Least Squares Discriminant Analyses (sPLS-DA) using the *splsda()* function from the *mixOmics* package. Metabolite enrichment analyses were conducted in MetaboAnalyst using over-representation analysis (ORA) based on the 'pathway-associated metabolite set', which includes 99 metabolite sets derived from standard human metabolic pathways. MetaboAnalyst version 6.0 is an advanced web-based analytical platform for high-throughput visualization of lipidomic data.

Adenylate Energy Charge indices (AEC) were calculated as follows :

$$AEC = ([ATP] + 0.5[ADP]) / ([ATP] + [ADP] + [AMP]) \quad (3)$$

Where ATP, ADP, and AMP refer to adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate, respectively (Atkinson and Walton, 1967).

All plots on R were made using the *ggplot2* package.

1.3 RESULTS

1.3.1 General data

No significant mortalities were observed in any of the groups during the experiment (df = 5; p-value = 0.3). Furthermore, the lipidomics analyses did not reveal any differences in fatty acid contents between groups before the heatwave exposition (df = 3; Pseudo-*F* = 0.9995; P-perm = 0.418) (Annexe I).

A significant proportion of individuals remained inactive (closed valves) throughout the duration of the respirometry measures, representing 62% of the experimental population, and were therefore excluded from the analysis. For both families, the statistical model revealed no significant effect of the sampling period; however, there was strong evidence for increased OCR during the heatwave scenario. Specifically, the global mean OCR values approximately doubled under heatwave conditions: the global mean rose from 0.17 ± 0.19 to 0.31 ± 0.25 mg O_2 h^{-1} g^{-1} in Family 1, and from 0.25 ± 0.11 to 0.49 ± 0.31 mg O_2 h^{-1} g^{-1} in Family 2, between

control and heatwave scenarios, respectively (Figure 8). In Family 1, a lower average increase was detected in triploids (0.05 mg O_2 h^{-1} g^{-1}) compared to diploids (0.22 mg O_2 h^{-1} g^{-1}), with moderate certainty (Figure 8A).

The highest OCR values were recorded in Family 2 during the heatwave scenario, with mean rates of 0.45 ± 0.28 and 0.53 ± 0.34 mg O_2 h^{-1} g^{-1} for triploids produced via pressure (XP) and chemical (6DMAP) induction, respectively (Figure 8B). The highest OCRs variability was observed in 6DMAP triploids under lasting heatwaves (T2, T3) (Figure 8B).

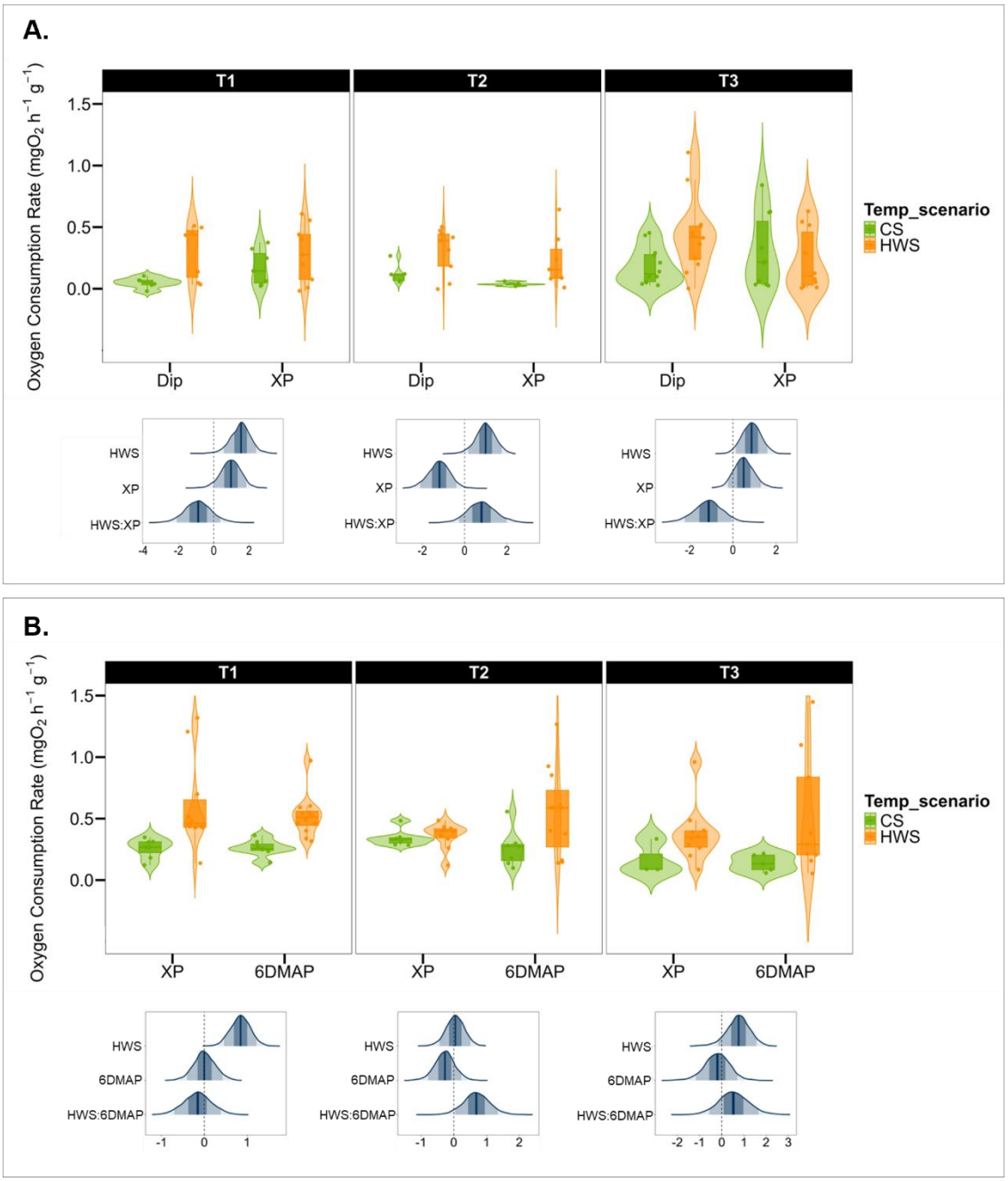

Figure 8. Effect of sampling period, temperature scenario, and ploidy (A), or induction method (B) on oxygen consumption rate. Top graphs : Violin plot of the oxygen consumption rates of diploids and XP triploids (Family 1) (A), and triploids produced via pressure (XP) and chemical (6DMAP) induction (Family 2) (B). Bottom graphs : Posterior distribution plots from Bayesian analysis of the effect of temperature scneario and ploidy (A) or induction method (B) on OCR. CS : control scenario; HWS : heatwave scenario; Dip : Diploids; XP : triploids induced via pressure shock; 6DMAP : triploids induced via chemical treatment.

1.3.2 Metabolomic profiles of triploids VS diploids (Family 1)

Changes in amino acid and energetic metabolite profiles of diploid and triploid mussels exposed to control and heatwave conditions were compared over the sampling times (Table 2). No three-way interaction between ploidy, temperature scenario, and time was observed; however, significant differences in metabolomic profiles were observed between temperature scenarios, between sampling times, and between ploidy levels. These differences were not systematic or similar between sampling times, as interactions between time and ploidy and between time and temperature scenario were observed. Although the permanova did not reveal a significant interaction between ploidy level and temperature scenario, Figure 9A suggests a potential effect that may be masked by significant heterogeneity of dispersion within temperature groups (Permdisp, Table 2).

Table 2. Effects of ploidy, time, and temperature on metabolomic profiles (Permanova results, Family 1).

Factor	P-perm	df	Pseudo-F	Permdisp
Ploidy	**0.027**	1	2.802	0.132
Temperature	**0.001**	1	5.838	0.022
Time	**0.001**	1	22.165	0.009
Ploidy:Temperature	0.187	1	1.404	-
Ploidy:Time	**0.038**	1	2.641	-
Temperature:Time	**0.002**	1	5.748	-
Ploidy:Temperature:Time	0.964	1	0.353	-

Independently of the temperature scenario, no significant metabolomic differences were observed between diploid and triploid mussels at T2 (P-perm = 0.177, Pseudo-F = 1.391); however, a distinct divergence emerged at T3 (P-perm = 0.017, Pseudo-F = 3.177), suggesting time-dependent shifts in their metabolomic profiles (Figure 9A). Therefore, detailed metabolite concentrations of each ploidy level are presented for T3 (Table 3). Independently of the temperature scenario, 16 metabolites out of the 48 measured significantly differed between diploids and triploids at T3, with higher levels in diploids, particularly under the heatwave scenario. The only exceptions were arginine and lysine,

which had higher concentrations in triploids. These 16 metabolites were mainly proteinogenic amino acids (Figure 9A, Table 3).

When comparing temperature scenarios within each ploidy at each sampling period, strong evidence of metabolomic shifts was detected in both ploidies under heatwave conditions at both medium (T2, *P*-perm = 0.008, pseudo-*F* = 3.03) and long-term exposure (T3, *P*-perm = 0.001, pseudo-*F* = 7.09) compared to the control temperature scenario. At T2, methionine was the only metabolite that showed a significant decrease under heatwave conditions in diploids when compared to the control (p-value = 0.016, W = 161), while no metabolites were significantly affected by the presence of heatwave in triploids, despite the overall effect of heat stress on their metabolomic profiles. At T3, 13 metabolites shared by both ploidy levels showed significantly higher concentrations under the heatwave scenario compared to the control (Table 3). The metabolite set enrichment analysis identified 25 biochemical pathways implicated in these differences (Figure 9B), mainly corresponding to aerobic and anaerobic energy metabolisms (Warburg effect, *ie* aerobic glycolysis, glycolysis, tricarboxylic acid cycle (TCA), transfer of acetyl groups into mitochondria, pyruvate metabolism, gluconeogenesis, mitochondrial electron transport chain, and arginine and proline metabolism). Furthermore, octopine, which is not present in human metabolism and therefore not accounted for in the enrichment analysis, was also detected in higher concentrations in diploid and triploid mussels exposed to the heatwave scenario. An additional 9 metabolites, primarily proteinogenic amino acids and intermediates of energetic pathways, showed increased concentrations under the heatwave scenario compared to the control exclusively in diploids (Table 3). At T3, the AEC index was significantly higher under heatwave exposure compared to the control scenario in both ploidy levels (Figures 10A and B). Finally, the amount of Branched-Chain Amino Acids (BCAAs), *i.e.*, the sum of valine, leucine, and isoleucine, also significantly increased at T3 under heatwave compared to the control scenario in diploids (Figures 10A and B).

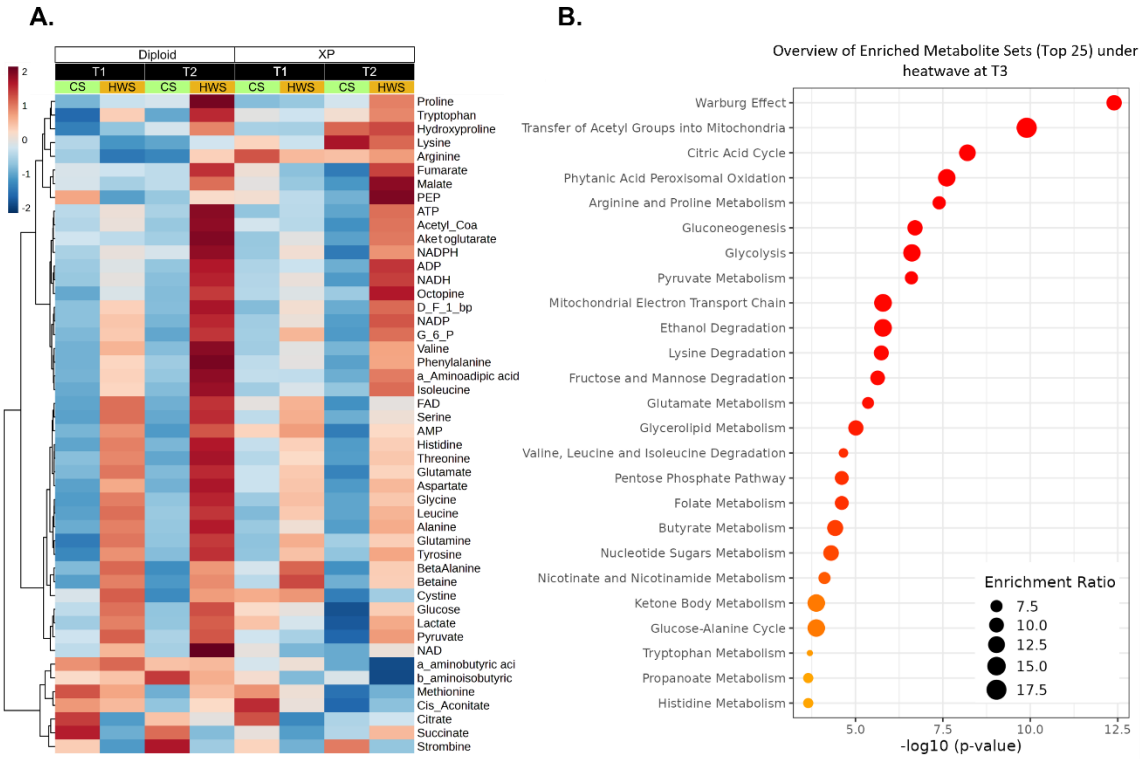

Figure 9. (**A**) Heatmap of mean metabolite concentrations in diploids and XP triploids under control (CS) and heatwave temperature scenarios (HWS) at the sampling points T2 and T3. (**B)** Metabolite Set Enrichment Analysis performed using the 13 shared metabolites that showed increased concentrations under heatwave exposure at T3 in both diploid and triploid mussels. The bubble sizes represent the fold enrichment of the metabolic pathways identified, and the colour gradient corresponds to the P-value.

Table 3. Mean concentrations of the tested metabolites (ng mg^{-1} ± SE) under control and heatwave scenarios in both ploidy levels at T3 (family 1), and the associated Wilcoxon rank-sum test with fdr correction. The last two columns compare values between diploids and triploids independently of the temperature scenario. Significant values are in bold.

	Metabolites	Diploids				XP Triploids				Adj *p*-value	W
		Control	*Heatwave*	Adj *p-value*	W	*Control*	*Heatwave*	Adj *p-value*	W		
Energy transfert	ATP	1446 ± 1442	3968 ± 2691	**0.013**	39	999 ± 848	2686 ± 1736	**0.008**	39	0.370	512
	ADP	8278 ± 7927	25821 ± 15716	**0.007**	26	7059 ± 5302	23364 ± 10574	**4.15E-05**	8	0.882	451
	AMP	4358 ± 1814	4923 ± 3131	0.899	109	4249 ± 1969	3549 ± 1932	0.631	129	0.270	529
	NADP	2824 ± 2138	4937 ± 2187	**0.038**	50	2257 ± 1233	4312 ± 1693	**0.004**	35	0.523	491
	NADPH	540 ± 231	1168 ± 660	**0.011**	34	566 ± 360	791 ± 393	0.115	63	0.370	511
	NAD	1241 ± 886	2044 ± 1393	**0.038**	50	697 ± 389	1023 ± 765	0.374	84	**0.027**	652
	NADH	268 ± 121	636 ± 353	**0.013**	39	276 ± 150	581 ± 273	**0.001**	24	0.958	439
	FAD	160 ± 69	181 ± 124	0.983	106	135 ± 75	106 ± 57	0.631	131	0.060	588
Glycolysis	Glucose	394 ± 208	422 ± 186	0.567	86	279 ± 120	316 ± 141	0.631	95	**0.028**	617
	Glucose-6-phosphate	3785 ± 2397	6778 ± 2790	**0.016**	42	4184 ± 2571	5585 ± 2337	0.389	85	0.595	482
	D-Fructose-1,6-biphosphate	1351 ± 1132	3077 ± 1469	**0.013**	38	1168 ± 1031	2240 ± 1242	**0.028**	49	0.356	515
	Phosphoenol pyruvate	4.2 ± 3.3	9.6 ± 6.9	**0.012**	35	7 ± 6	21 ± 35	0.067	57	0.238	334
	Pyruvate	18.9 ± 19.6	19 ± 11	0.447	78	12 ± 9	17.1 ± 8.3	0.134	65	0.615	479
TCA Cycle	Acetyl-Coa	171 ± 139	323 ± 203	**0.016**	42	142 ± 49	249 ± 100	**0.004**	35	0.719	
	Citrate	238 ± 209	421 ± 222	**0.014**	40	213 ± 128	378 ± 182	**0.023**	47	0.758	463
	Cis-Aconitate	0.56 ± 0.27	0.52 ± 0.31	0.745	115	0.50 ± 0.31	0.41 ± 0.20	0.764	124	0.218	540
	α-ketoglutarate	8.2 ± 5.1	20 ± 38	0.548	84	9.6 ± 7.0	14 ± 13	0.312	81	0.751	405
	Succinate	17.6 ± 23.3	23 ± 10	**0.024**	45	12.8 ± 5.7	104 ± 329	0.165	70	0.515	493
	Fumarate	51.1 ± 31.3	91 ± 32	**0.008**	29	42 ± 19	88 ± 52	**0.002**	29	0.456	501
	Malate	595 ± 349	1111 ± 419	**0.011**	34	568 ± 308	1429 ± 831	**3.27E-04**	18	0.882	420
Anaerobiosis	Lactate	471 ± 293	502 ± 244	0.57	87	331 ± 173	431 ± 202	0.165	71	0.124	562
	Alanine	1442 ± 899	1935 ± 1447	0.567	86	917 ± 550	1230 ± 1291	0.894	107	**0.029**	609
	Strombine	0.16 ± 0.28	0.55 ± 0.96	0.134	64	0.30 ± 0.66	0.47 ± 0.62	0.154	68	0.950	443
	Octopine	57 ± 71	242 ± 385	**0.041**	51	58 ± 61	282 ± 369	**0.002**	29	0.464	371
Proteinogenic AAs	Arginine	474 ± 1005	1671 ± 2637	0.447	78	1851 ± 2518	2084 ± 1842	0.631	95	**0.029**	253
	Aspartate	12599 ± 6108	18223 ± 10963	0.134	64	11486 ± 5188	12022 ± 5767	0.967	111	0.067	556
	Glutamate	6251 ± 2477	7363 ± 3719	0.57	87	5051 ± 2101	4862 ± 2536	0.748	125	**0.027**	626
	Glutamine	1635 ± 802	1988 ± 1927	0.762	114	1319 ± 734	1116 ± 1081	0.300	145	0.124	561
	Glycine	23886 ± 10587	30228 ± 19367	0.639	90	19332 ± 10449	18684 ± 12249	0.796	122	**0.028**	615
	Isoleucine	46 ± 27	100 ± 60	**0.011**	33	34 ± 14	72 ± 52	0.165	71	0.124	562
	Histidine	2408 ± 1228	3171 ± 1915	0.548	84	1819 ± 1024	1827 ± 1681	0.780	123	**0.027**	624
	Leucine	21 ± 12	25 ± 16	0.693	93	14 ± 9	15 ± 15	0.880	119	**0.027**	632
	Lysine	17 ± 26	53 ± 60	**0.05**	53	51 ± 50	142 ± 167	0.147	67	**0.027**	247
	Methionine	50 ± 21	46 ± 35	0.513	128	38 ± 22	23 ± 15	0.079	166	**0.029**	610
	Phenylalanine	139 ± 77	249 ± 149	**0.012**	37	124 ± 53	162 ± 67	0.244	76	0.264	531
	Proline	59 ± 46	305 ± 203	**1.20E-04**	9	37 ± 36	168 ± 116	**3.27E-04**	17	0.124	565
	Serine	2106 ± 1207	2555 ± 1640	0.657	91	1710 ± 1261	1267 ± 1031	0.287	146	**0.050**	594
	Threonine	251 ± 139	371 ± 270	0.513	82	171 ± 83	191 ± 142	0.922	116	**0.036**	603
	Tryptophane	51 ± 51	79 ± 55	0.096	59	39 ± 26	63 ± 44	0.254	77	0.290	524
	Tyrosine	256 ± 130	345 ± 234	0.675	92	208 ± 118	236 ± 212	0.922	116	0.097	573
	Valine	126 ± 76	249 ± 150	**0.026**	46	82 ± 53	136 ± 90	0.147	67	**0.029**	612
Non-proteinogenic AAs	α-aminobutyric acid	4.9 ± 3.2	4.2 ± 4.3	0.567	124	4 ± 4	1.8 ± 1.4	0.631	130	**0.027**	636
	α-aminoadipic acid	61 ± 40	109 ± 77	**0.038**	50	45 ± 18	80 ± 47	**0.022**	46	0.290	524
	β-alanine	75 ± 61	63 ± 51	0.621	121	76 ± 48	50 ± 29	0.162	156	0.958	440
	β-aminoisobutyric acid	0.21 ± 0.10	0.22 ± 0.18	0.814	112	0.15 ± 0.13	0.10 ± 0.09	0.631	129	**0.027**	631
	Betaine	84519 ± 78549	79462 ± 113095	0.497	130	105166 ± 178528	53935 ± 144773	0.165	154	0.194	545
	Cystine	303 ± 164	262 ± 178	0.513	128	266 ± 128	151 ± 77	**0.039**	173	**0.027**	574
	Hydroxyproline	75 ± 32	178 ± 134	**0.011**	36	83 ± 44	209 ± 141	**0.002**	30	0.456	385

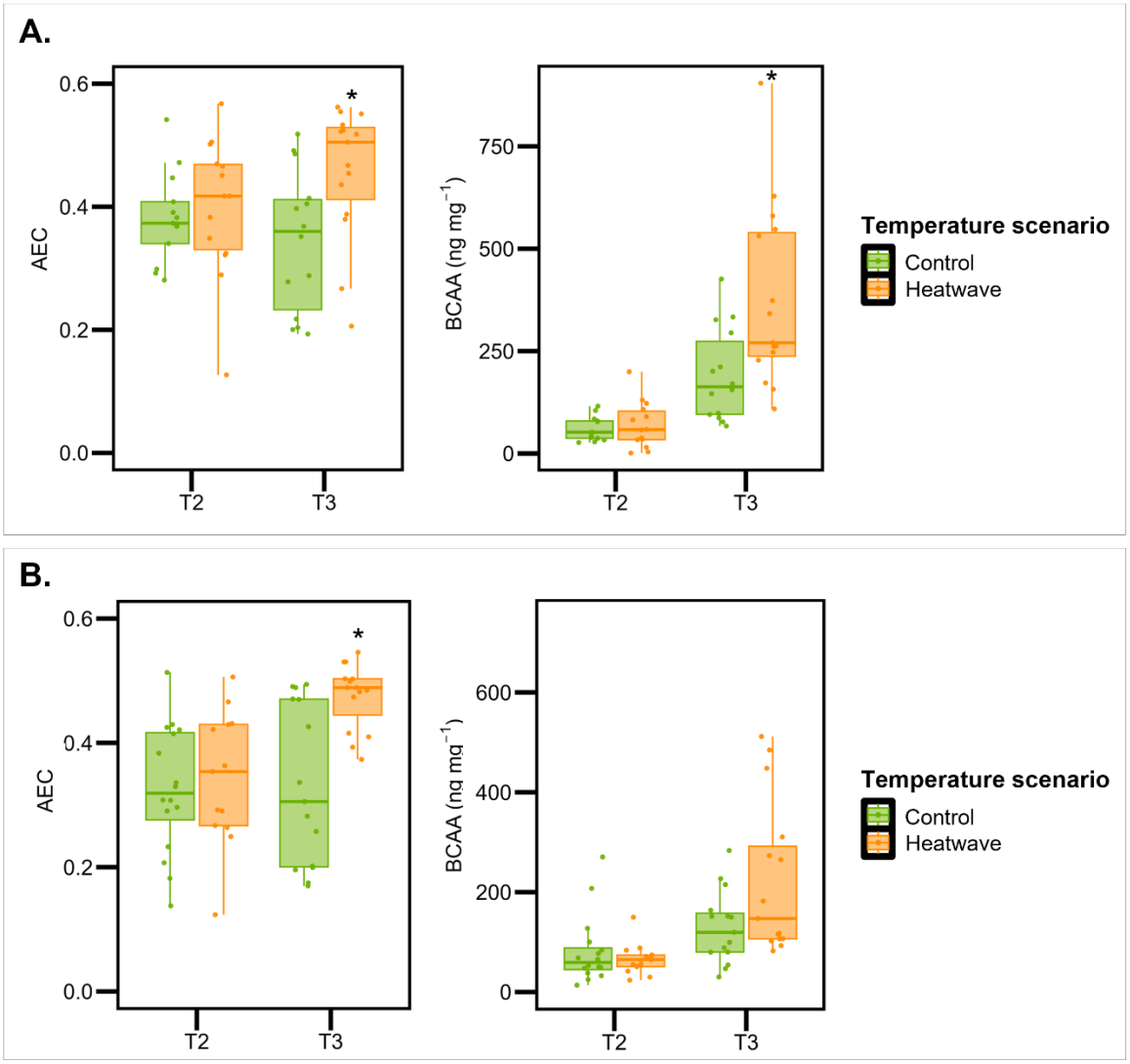

Figure 10. Boxplots of the AEC ratio and BCAAs concentrations (ng/mg) in diploids (**A**) and XP triploids (**B**) under control and heatwave temperature scenarios. Significant difference between the heatwave group and the control group at each time point is indicated by one ($P < 0.05$) or two ($P < 0.01$) asterisks (Wilcoxon test) for each sampling time.

1.3.3 Metabolomic profiles of triploids from pressure VS chemical treatments (Family 2)

Regarding Family 2, no three-way interaction between induction methods to induce triploids, temperature scenario, and sampling time was observed (Table 4). However, significant differences in metabolomic profiles were detected between temperature scenarios and between sampling times. Again, these differences were not systematic or similar between sampling times, as interactions between time and induction method and between time and temperature scenario were observed. Although the permanova did not reveal a significant interaction between induction method and temperature scenario, Figure 11 suggests a potential effect that may be masked by significant heterogeneity of dispersion within temperature groups (Permdisp, Table 4).

Table 4. Effects of induction method, sampling time, and temperature on metabolomic profiles (Permanova results, family 2).

Factor	P-perm	df	Pseudo-F	Permdisp
Induction method	0.568	1	0.731	0.400
Temperature	**0.001**	1	6.699	0.002
Time	**0.001**	2	4.697	0.173
Induction method:Temperature	0.442	1	0.920	-
Induction method:Time	**0.009**	2	2.861	-
Temperature:Time	**0.001**	1	3.425	-
Induction method:Temperature:Time	0.07	1	1.883	-

Both 6DMAP and XP triploids showed similar profiles at the beginning of the experiment (T1), and no differences were observed between control and heatwave scenarios at T1 (Adonis pairwise tests, $p > 0.005$) (Figure 11). Under the control temperature scenario, no evidence of metabolomic differences between triploids from both induction methods were observed at each sampling period (Adonis pairwise test, $p > 0.05$) (Figure 11). However, differences appeared at T2 and T3 between 6DMAP and XP triploids when exposed to the heatwave scenario (Adonis pairwise test, $p < 0.05$) (Figure 11).

When comparing temperature scenarios within each triploidy induction method at each sampling period, an increase in the overall metabolism was observed at T2 in 6DMAP triploids exposed to the heatwave scenario compared to the control, while only a few metabolites were affected by the exposure to heatwave in XP triploids. At T3, the presence of heatwave primarily affected the same metabolites across both induction methods, although concentrations were generally higher in XP triploids (Table 5).

Specifically, 6DMAP triploids exposed to the heatwave scenario and sampled at T2 exhibited an upregulation of key metabolites involved in glycolysis and the TCA cycle (α-ketoglutarate, cis-aconitate, D-fructose-1,6-bisphosphate, FAD, fumarate, glutamine, malate, NAD, NADH), all of which contributed substantially to the temperature-based classification (Figure 12A). At T3, glucose cis-aconitate, FAD and NAD concentrations were higher in 6DMAP triploids exposed to the heatwave scenario compared to those exposed to the control. However, other metabolites involved in energetic pathways, such as glucose-6-phosphate, phosphoenol pyruvate, succinate, fumarate, and malate, were present in lower levels in 6DMAP triploids exposed to the heatwave scenario compared to the control (Figure 12B, Table 5). Conversely, profiles of XP triploids exposed to the heatwave scenario initially exhibited a decrease in several energy-related metabolites at T2 compared to the control, including D-fructose-1,6-bisphosphate, cis-aconitate, acetyl-CoA, ADP, NAD, NADPH (Figure 12A). At T3, no clear differences in glycolytic and TCA cycle intermediates were observed between temperature scenarios in XP triploids, except for an increase in cis-aconitate, FAD and NAD concentrations when exposed to the heatwave (Figure 12B, Table 5). Products of anaerobic metabolism (alanine, lactate) were detected at higher concentrations in both triploid induction methods during the heatwave compared to the control scenario, with their accumulation occurring earlier (Figure 12A and B) and at lower concentrations (Table 5) in 6DMAP triploids than in XP triploids. Lastly, the AEC indices were significantly lower at T3 under the heatwave compared to the control scenario in both induction methods (Figures 13A and B) due to elevated AMP concentrations (Table 5).

Significant changes in the concentrations of various amino acids in response to heatwave exposure were also observed (Figure 11). Total BCAA concentrations significantly increased under the heatwave scenario in both triploidy induction methods compared to the control scenario (Figures 13A and B). More precisely, at T2, isoleucine and valine levels rose in 6DMAP triploids when exposed to the heatwave, whereas only isoleucine increased in XP triploids (Figure 12A), resulting in a smaller overall BCAA increase in the latter. At T3, all three BCAAs increased under heatwave conditions compared to the control scenario in both triploidy induction methods (Figure 12B). A similar pattern was observed for aromatic amino acids (AAAs): at T2, phenylalanine, tyrosine, and tryptophan concentrations rose in 6DMAP triploids exposed to the heatwave scenario when compared to the control, while only tryptophan was affected by temperature in XP triploids (Table 5, Figure 12A). By T3, all tyrosine and tryptophan levels increased under heatwaves compared to the control scenario in both triploidy induction methods (Figure 12B). In terms of osmolytes, heatwave exposure initially triggered increased levels of proline and lysine at T2 in both triploidy induction methods in comparison to the control (Figure 12A), followed by higher concentrations of betaine, proline, and β-alanine at T3 (Figure 12B). XP triploids also showed elevated levels of hydroxyproline under the heatwave scenario at T2 compared to the control (Figure 12A), as well as increased glycine, serine, cystine, and α-aminoadipic acid concentrations at T3 (Figure 12B), while 6DMAP triploids exhibited increased concentrations in all these metabolites under heatwave conditions at both time points, albeit to a lesser extent then XP triploids (Figure 12, Table 5). Lastly, levels of γ-aminobutyric acid (GABA) lowered in XP triploids at T2 under heatwave conditions (Figure 12A) but increased at T3 in both triploidy induction methods, compared to the control scenario (Figure 12B, Table 5).

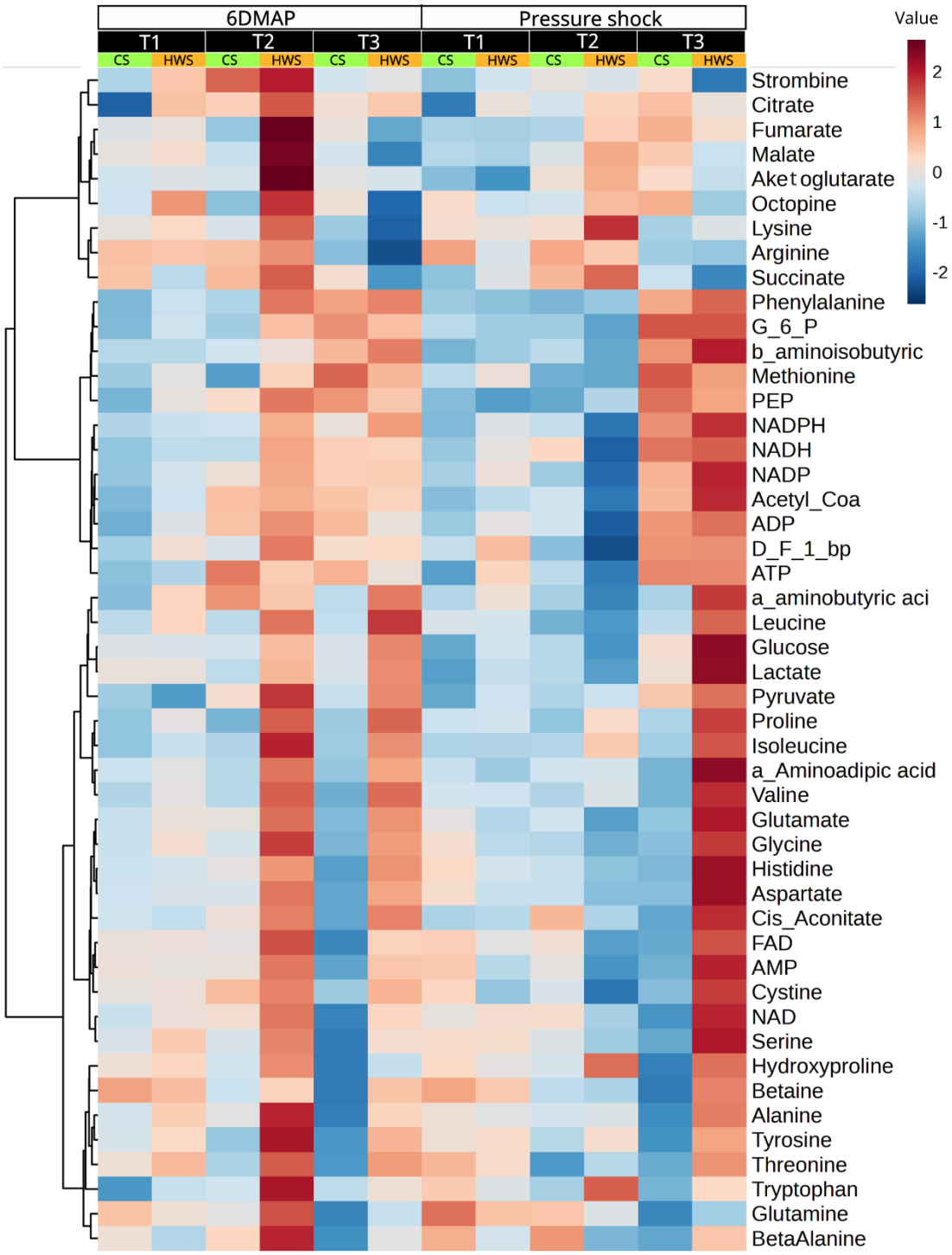

Figure 11. Heatmap of mean metabolite concentrations in 6DMAP and XP triploids under control (CS) and heatwave (HWS) temperature scenarios at each sampling point (T1, T2, and T3).

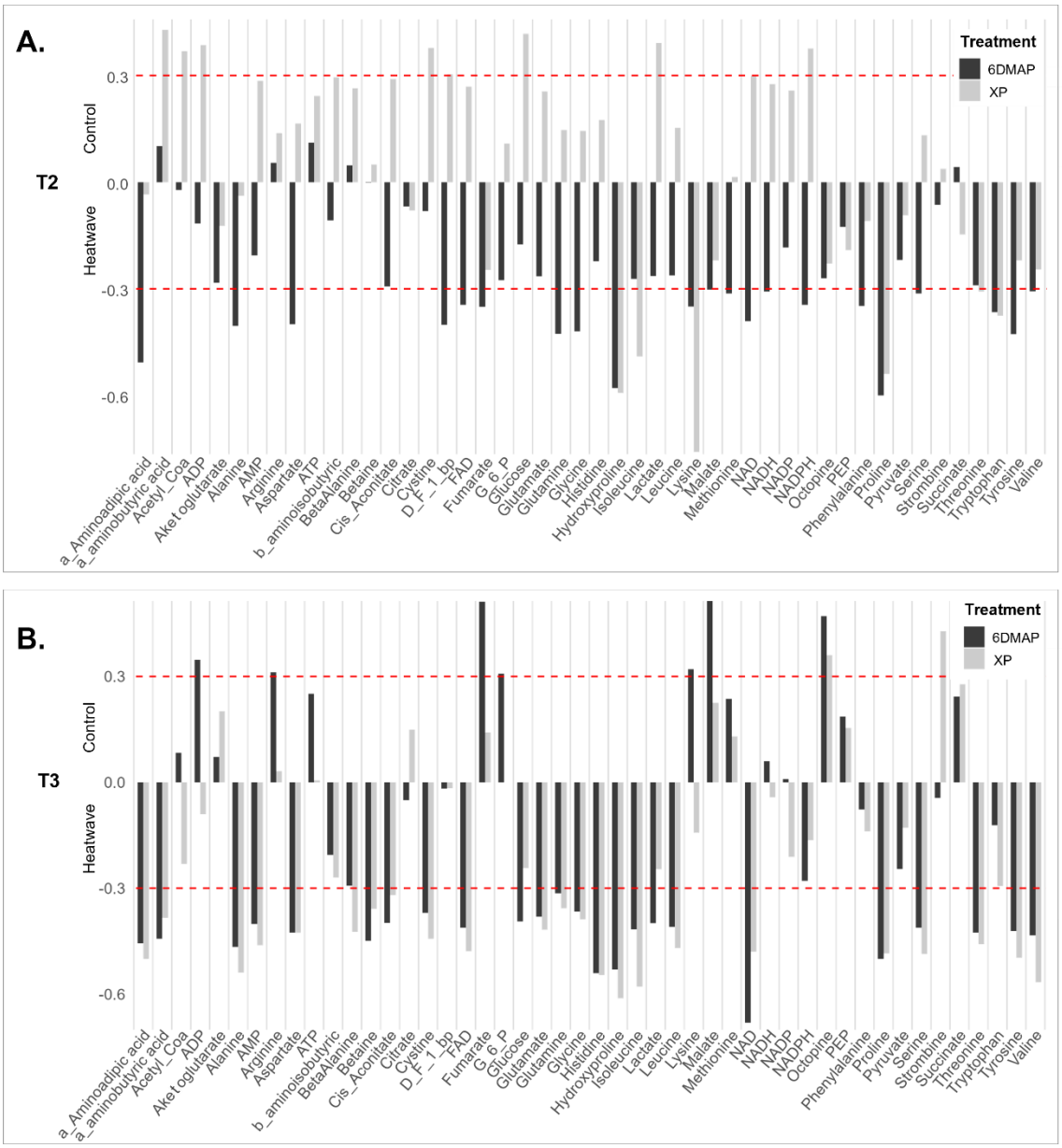

Figure 12. sPLS-DA VIP (variable influence on projection) scores of each metabolite for 6DMAP and XP triploids at T2 (A) and T3 (B). VIP scores ≥ |0.3| were considered as main features (red dashed lines).

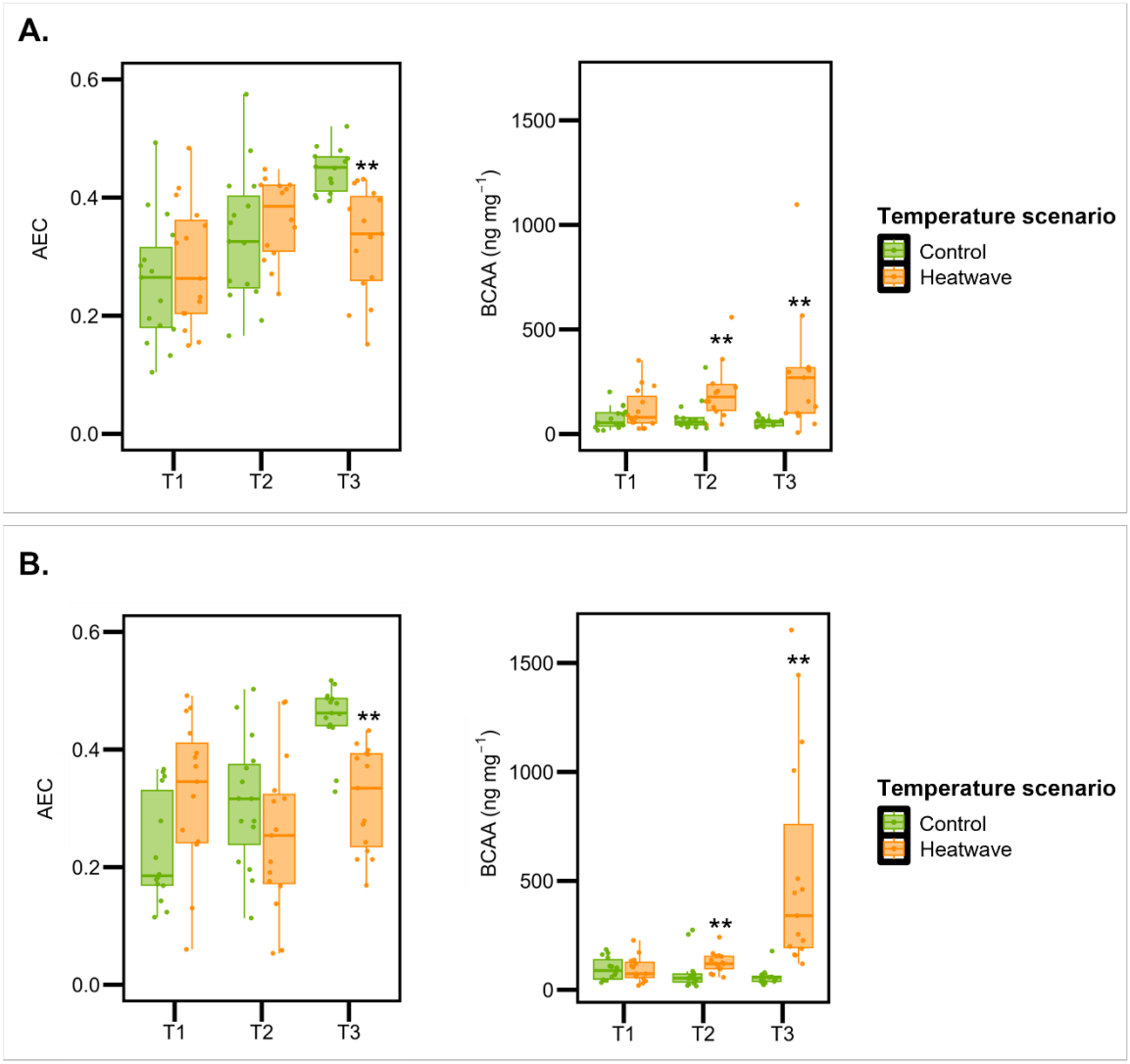

Figure 13. Boxplots of the AEC ratio and BCAAs concentrations (ng/mg) in 6DMAP (**A**) and XP (**B**) triploids under control and heatwave temperature scenarios. Significant difference between the heatwave group and the control group at each time point is indicated by one ($P < 0.05$) or two ($P < 0.01$) asterisks (Wilcoxon test) for each sampling time.

Table 5. Mean concentrations of the tested metabolites (ng/mg ± SE) in 6DMAP and XP triploids (family 2) under control and heatwave scenarios at T3, and the associated Wilcoxon rank-sum test with fdr correction. Significant values are in bold.

	Metabolites	Control	Heatwave			
		Mean	*6DMAP*		*XP*	
				adj_p		*adj_p*
Energy transfert	ATP	1010 ± 809	623 ± 519	0.304	1107 ± 1241	0.452
	ADP	7016 ± 4005	4295 ± 2993	0.144	9370 ± 12448	0.436
	AMP	1727 ± 619	3543 ± 3097	**0.031***	6596 ± 6596	**3.00E-04**
	NADP	1793 ± 1082	1673 ± 959	0.780	3841 ± 6138	0.942
	NADPH	441 ± 360	531 ± 469	0.609	909 ± 1428	0.942
	NAD	221 ± 103	809 ± 474	**0.019***	2488 ± 2942	**4.00E-06**
	NADH	257 ± 164	201 ± 143	0.659	333 ± 359	0.467
	FAD	48 ± 21	105 ± 99	**0.031***	183 ± 171	**3.00E-04**
Glycolysis	Glucose	180 ± 109	348 ± 289	**0.041***	695 ± 1428	0.391
	Glucose-6-phosphate	4189 ± 3352	2533 ± 1728	0.189	4891 ± 5726	0.389
	D-Fructose-1,6-biphosphate	602 ± 467	483 ± 334	0.780	766 ± 810	0.421
	Phosphoenol pyruvate	34 ± 38	19 ± 38	**0.031***	27 ± 33	0.527
	Pyruvate	11 ± 11	18 ± 24	0.372	20 ± 34	0.9
TCA Cycle	Acetyl-Coa	130 ± 76	115 ± 122	0.229	247 ± 333	0.955
	Citrate	332 ± 239	348 ± 412	0.745	288 ± 249	0.511
	Cis-Aconitate	0.18 ± 0.10	0.97 ± 1.34	**0.001*****	1.6 ± 3.0	**3.00E-04**
	α-ketoglutarate	12 ± 9	10 ± 11	0.310	9.3 ± 8.5	0.323
	Succinate	96 ± 216.0	28 ± 50	**0.026***	24 ± 33	**0.028***
	Fumarate	68 ± 47	34 ± 22	**0.024***	61 ± 60	0.099
	Malate	814 ± 710	319 ± 270	**0.024***	629 ± 553	0.148
Anaerobiosis	Lactate	228 ± 153	454 ± 366	0.059	968 ± 2027	0.349
	Alanine	274 ± 109	821 ± 777	**0.02***	1312 ± 1138	**4.00E-06**
	Strombine	0.42 ± 0.57	0.38 ± 1.18	0.059	0.06 ± 0.09	**0.007****
	Octopine	131 ± 184	13 ± 22	**0.001*****	43.0 ± 52	**0.016***
	Arginine	1021 ± 1273	311 ± 982	**0.044***	1020 ± 1309	1
	Aspartate	4607 ± 2035	12010 ± 12051	**0.024***	23666 ± 28375	**5.00E-05**
	Glutamate	2148 ± 105	4835 ± 4857	**0.031***	7506 ± 8208	**3.00E-04**
	Glutamine	207 ± 105	473 ± 593	0.229	382 ± 294	**0.099**
Essential AAs	Histidine	470 ± 265	1523 ± 1242	**0.024***	2919 ± 2620	**5.00E-05**
	Isoleucine	18 ± 12	68 ± 80	**0.026***	97 ± 77	**4.00E-06**
	Lysine	51 ± 71	16 ± 32	**0.031***	82 ± 123	**9.42E-01**
	Methionine	13 ± 7	9.7 ± 8	**0.041***	11 ± 11	**0.148**
	Threonine	88 ± 43	255 ± 266	0.189	265 ± 233	**0.023***
	Tryptophane	23 ± 11	31 ± 31	0.813	33 ± 26	**3.36E-01**
	Valine	36 ± 17	276 ± 369	**0.001*****	432 ± 410	**6.00E-07**
Non-Essential AAs	α-aminoadipic acid	22 ± 10	89 ± 92	**0.031***	270 ± 309	**8.00E-07**
	α-aminobutyric acid	2.1 ± 3.1	18 ± 23	**0.001*****	35 ± 57	**0.002****
	β-alanine	19 ± 11	35 ± 42	0.310	48 ± 40	**0.021***
	β-aminoisobutyric acid	0.33 ± 1.16	0.45 ± 0.56	0.813	0.91 ± 1.35	**3.91E-01**
	Betaine	13811 ± 6078	83181 ± 101555	**0.007****	133899 ± 224415	**2.00E-04**
	Cystine	117 ± 57	266 ± 256	0.168	458 ± 501	**3.00E-04**
	Glycine	8227 ± 3549	18533 ± 19435	**0.026***	26069 ± 29742	**0.001*****
	Hydroxyproline	45 ± 15	126 ± 95	**0.019***	480 ± 398	**6.00E-07**
	Leucine	5 ± 2	33 ± 45	**0.001*****	24 ± 26	**3.00E-04**
	Phenylalanine	127 ± 55	147 ± 158	0.559	164 ± 198	**4.21E-01**
	Proline	21 ± 10	133 ± 144	**0.024***	175 ± 196	**4.00E-06**
	Serine	292 ± 157	722 ± 768	**0.024***	2116 ± 2280	**5.00E-05**
	Tyrosine	72 ± 31	219 ± 231	**0.024***	238 ± 205	**2.00E-04**

1.4 DISCUSSION

This study reveals differences in the metabolomes of mussels exposed to simulated heatwave events, with higher metabolite concentrations observed in diploid mussels compared to triploids produced from the same parents. Pronounced metabolomic effects of heatwave exposure were detected, even in the absence of clear changes in OCRs, with chemically induced triploids exhibiting distinct temporal dynamics and response intensities compared to pressure-shock-induced triploids.

1.4.1 General physiological data

The absence of mortality observed in this study is rather unexpected in the presence of a heatwave event reaching such temperatures (*i.e.* 24 to 27°C) for this species (Clements et al., 2018; Lupo et al., 2021; Clarke et al., 2025). One potential explanation is the use of juveniles rather than adults, as juveniles have been reported to exhibit higher survival rates under heat stress in various mollusk species, such as the soft-shelled clam *Laternula elliptica*, the sea cucumber *Cucumaria georgiana*, the sea urchin *Sterechinus neumayeri*, and the seastar *Odontaster Validus* (Peck et al., 2013), likely due to their lower energetic demands and limited reproductive investment (Clements et al., 2018). However, Guillou et al. (2023) observed greater thermal sensitivity in juveniles larger than 4.5 mm compared to smaller individuals, suggesting that vulnerability to heat stress may be size-dependent. The presence of diurnal temperature oscillations during the simulated heatwave may have contributed to the observed low mortality rates by allowing intermittent periods of physiological recovery. This hypothesis is supported by Widdows (1976), who documented physiological acclimation of *Mytilus edulis* to oscillating temperatures, showing a higher scope for growth at 29°C in mussels exposed to cyclic temperature regimes (21-29°C), compared to those maintained at a constant 29°C.

OCR values are commonly used as a proxy for aerobic metabolism. The metabolic rate of ectothermic organisms such as mussels increases with temperature up to an optimal threshold, beyond which the OCR declines sharply due to physiological constraints (Schulte, 2015; Pörtner et al., 2017). In this study, the average metabolic rate increased with temperature across all groups, indicating that the 24 °C exposure remained within the species' functional thermal range, consistent findings of Widdows (1973); Zittier et al. (2015), where *M.edulis* maintained a positive OCR scope up to ~25°C. However, the pronounced interindividual variability observed in 6DMAP triploids under prolonged heatwave exposure (T2, T3) suggests that repeated exposure to 24–27 °C may have pushed some individuals beyond their optimal thermal range, resulting in uneven physiological responses and early signs of metabolic exhaustion. Consistently, other studies have demonstrated positive correlations between interindividual variability in physiological performances and thermal stress in bivalve populations (Zittier et al.2015; Zhang et al., 2023; Cheng et al., 2025). Nonetheless, the low amount of respiring individuals contributing to these results might be due to the absence of acclimation in the respirometry chambers, and increasing the sample size or adding an acclimation period in a flow through chamber could strengthen the reliability and robustness of these observations.

Lipidomic results did not reveal any differences in fatty acid composition between groups, suggesting that triploidy induction does not appear to affect lipid reserves' accumulation and use at this juvenile stage. Differences observed in metabolite profiles are therefore unlikely to be driven by variations in baseline lipid storage between groups, but rather by metabolic and/or genomic regulations. However, the energy required to withstand this stressful event may have been derived from alternative sources, such as glycogen, which is the main energy reserve in bivalves and has been reported to help maintain mussel condition under thermal stress (Bayne, 1976; Clements et al., 2018; Vodáková & Douda, 2019). In contrast, lipids are primarily recognized for their role in gamete production (Walne, 1970; Holland, 1978; Beninger, 1984), as well as serving as an energy reserve during periods of nutritional stress (Walne, 1970; Beninger & Lucas, 1984).

1.4.2 Metabolomic profiles of diploids VS triploids (Family 1)

The first family revealed distinct metabolic trajectories between diploids and triploids in the absence of thermal stress, with diploid mussels exhibiting higher concentrations of proteinogenic amino acids than triploids over time. Hawkins et al. (1986) reported that fewer heterozygous mussels showed more intense nitrogen metabolism, linked to reduced protein turnover efficiency. These individuals, characterized as "slow growers", displayed high nitrogen recycling efficiency and greater energetic costs. In fact, protein synthesis is a costly process and can increase the cellular ATP demand from 15 to 40 % under normal conditions (Sokolova, 2021). Hawkins et al. (1986) hypothesized, based on Riley (1980)'s findings, that slow-growing mussels store more free amino acids, especially essential amino acids, than fast growers for protein synthesis. This aligns with the lower levels of proteinogenic and essential amino acids observed in triploids in the present study, as triploids tend to be more heterozygous (Allendorf & Leary, 1984) and are generally associated with faster growth rates (Brake et al., 2004; Osterheld et al., 2021; Osterheld et al., 2023; Zhang et al., 2024).

At T2, the decreased concentrations of methionine under heatwave conditions in diploid mussels may reflect its mobilization through the transsulfuration and glutathione synthesis pathways (Nguyen et al., 2018). This mechanism has been previously documented in *Perna canaliculus* as a means to counteract oxidative stress induced by elevated temperatures (Abele & Puntarulo, 2004; Azizan et al., 2023), and could translate either an earlier stress, or a faster response in diploids compared to triploids. Three weeks of exposure (T3) to heat stress disrupted the energetic metabolism of both diploids and triploids, as evidenced by the upregulation of aerobic activity via the TCA cycle, and a concurrent shift toward anaerobic pathways to supplement ATP production when aerobic capacity was exceeded. Particularly, octopine, a fermentation end-product and well-established marker of anaerobic metabolism in bivalves (Ellington, 1983; Müller et al., 2012), increased under these conditions in both ploidy levels at T3. Moreover, elevated levels of branched-chain amino acids (BCAAs) in diploids, along with enhanced activity in the proline and arginine pathways in both diploids and triploids, point to an increased demand for metabolic support and the activation of

protective mechanisms against oxidative stress under heatwaves at T3, especially in diploids. Particularly, BCAAs (valine, leucine, and isoleucine) are essential amino acids involved in protein turnover and cellular signalling (Nair & Short, 2005; Calder, 2006; Holeček, 2018; Wu et al., 2022). These metabolites can also be catabolized to generate acetyl-CoA, feeding into the TCA cycle and thus contributing both to ATP production and to oxidative stress mitigation (Wu et al., 2022). Arginine and proline can both serve as alternative energy substrates via conversion to Krebs cycle intermediates, and as osmoprotective compounds, with proline particularly involved in protein stabilization (Hochachka & Somero, 2002), and arginine contributing to polyamine synthesis, important for maintaining cellular integrity (Wu & Morris Jr, 1998). Changes in AEC index were also observed. The AEC index is a widely used indicator of cellular energy status in adult bivalves (Veldhuizen-Tsoerkan et al., 1991; Maguire, 2007). Values close to 1 indicate high cellular energy levels, whereas low AECs are associated with stress. While the AEC values observed in this study may appear relatively low, it is important to note that this index has been primarily validated for adult stages and may not be directly applicable to earlier life stages (Veillard, 2025). Increased levels of AEC at T3 under heatwave conditions may reflect enhanced ATP production to sustain energy demands in both ploidy levels, but no signs of energetic stress. Therefore, exposure to heatwaves seems to elevate the energetic demands of mussels, with diploids exhibiting a stronger mobilization of energetic metabolites and amino acids. This supports the hypothesis of a lower turnover efficiency in diploids compared to triploids, which would be compensated by intensified protein synthesis and energetic metabolism (Hawking et al 1986). However, previous studies have observed better tolerance to heat stress in diploids than in triploids (George et al., 2023; Keyvanshokooh, 2025), and the higher amino acid concentrations observed in diploids could therefore provide them with a greater capacity to cope with heat stress (Georgoulis et al., 2022; Georgoulis et al., 2023; Zhang et al., 2023). Additional experiments conducted until mortality would help determine whether the observed metabolomic differences are associated with heat stress resistance.

These findings emphasize the key contribution of energy metabolism pathways in mediating responses to heat stress. Thus, results might be different at the adult stage,

especially in diploid mussels, which might show diminished thermal tolerance due to the significant energetic demands of reproduction, especially during spawning seasons (Clements et al., 2018).

1.4.3 Metabolomic profiles of triploids from pressure VS chemical treatments (Family 2)

The decrease in glycolytic intermediates and early TCA cycle products in XP triploids at T2 may indicate an enhanced metabolic flux towards mitochondrial respiration under heatwave exposure. After longer exposure (T3), no significant differences were observed in the concentrations of aerobic metabolites in XP triploids compared to the control temperature scenario, although elevated levels of NAD and FAD may indicate the occurrence of redox stress (Giancaspero et al., 2013). Elevated concentrations of aspartate and alanine reflected signs of early anaerobiosis at T3 in XP triploid mussels exposed to the heatwave (Stokes & Awapara, 1968; Eymann et al., 2020; Chen et al., 2021). In 6DMAP triploids, the first signs of anaerobiosis under heatwave exposure appeared earlier than in XP triploids, with the upregulation of anaerobic glycolysis and alanine-generating fermentation at T2, followed by the accumulation of both alanine and lactate at T3. Furthermore, the accumulation of FAD and NADH, and of early metabolites of glycolysis (glucose) and the TCA cycle (cis-aconitate) at T3, together with the reduced concentrations of succinate, malate, and fumarate, suggest a downregulation of the TCA cycle and a decrease in aerobic metabolic activity in 6DMAP triploids exposed to heatwave conditions compared to the control. Thus, while aerobic metabolism seems sufficient to meet energy demands under heatwave conditions in XP triploids for up to 11 days (T2), 6DMAP triploids initiate a reliance on both aerobic and anaerobic pathways earlier, gradually transitioning toward a predominantly anaerobic metabolic profile after three weeks of exposure (T3). This metabolic shift is further supported by the elevated interindividual variability in metabolic rates observed among 6DMAP triploids exposed to the heatwave, suggesting the adoption of diverse metabolic strategies, driven by differences in individual performance capacities. Additionally, the marked decline in AEC of both XP and 6DMAP triploids after three weeks of heatwave exposure indicates

pronounced energetic stress and impaired ATP regeneration in thermally challenged individuals. These findings are consistent with the observed upregulation of both aerobic and anaerobic pathways in XP and 6DMAP triploids, reflecting an overall increase in energetic demands under prolonged thermal stress (T3). Such metabolic adjustments often promote the excessive generation of ROS species in cells, leading to oxidative stress. ROS are natural by-products of aerobic metabolism, produced by the univalent reduction of O_2 by the mitochondrial respiratory chain (Halliwell & Gutteridge, 1984; Bartosz, 2009). These molecules interact with many proteins, lipids, and nucleic acids, and impair cellular functions (Halliwell & Gutteridge, 1984; Bartosz, 2009). Increased metabolism and thermal stress can disrupt the balance between the production and detoxification of these ROS, ultimately resulting in oxidative stress (Lesser, 2006; Georgoulis et al., 2021; Azizan et al., 2023; Beaudreau et al., 2024). In this context, the increased mobilization of amino acids such as proline and arginine, known for their roles in redox balance and protein stabilization, likely contributes to cellular protection. In parallel, other signalling and cytoprotective compounds are typically activated during heat stress to mitigate ROS-related damages and maintain cellular integrity (Delorme et al., 2021; Ericson et al., 2022; Azizan et al., 2023; Georgoulis et al., 2023; Delorme et al., 2024; Grimmelpont et al., 2024; Clarke et al., 2025).

Aromatic amino acids (AAAs), including tyrosine, tryptophan, and phenylalanine, are essential amino acids that serve as precursors for the synthesis of various hormones and neurotransmitters (Hyland, 2007), and play a key role in mediating stress responses in bivalves (Lacoste et al., 2001; Liu et al., 2018). In this study, AAA concentrations increased over time in mussels exposed to the heatwave scenario compared to those from control, with a more rapid increase in 6DMAP triploids. However, following prolonged heatwave exposure (T3), XP triploids ultimately exhibited higher overall levels. In parallel, γ-aminobutyric acid (GABA), an important neurotransmitter in mollusks (Bai et al., 2012; Nguyen et al., 2021), was also found in elevated concentrations in both XP and 6DMAP triploids after three weeks of exposure to the heatwave (T3), further supporting the involvement of neuroendocrine mechanisms in the response to chronic heat stress.

Along with the activation of neuroendocrine signalling pathways, the accumulation of stress-related metabolites also highlights the involvement of metabolic and osmotic regulatory mechanisms in managing prolonged thermal stress. Similarly to the first family, the retention and buildup of BCAAs during heatwaves could reflect sustained energy production and contribute to thermotolerance. In particular, 6DMAP triploids appeared to initiate BCAA accumulation earlier than XP triploids, especially through the early accumulation of valine, a metabolite known to enhance mitochondrial respiratory efficiency and reduce ROS production (Sharma et al., 2024). In parallel, the accumulation of osmolytes such as betaine, β-alanine, glycine, lysine, and proline likely contributes to maintaining cellular osmotic balance and protecting protein structures during heatwave exposure (Arakawa & Timasheff, 1985; Lin & Timasheff, 1996; Liang et al., 2013), with lysine and proline appearing among the earliest to be mobilized in both XP and 6DMAP triploids. Hydroxyproline, which has recognized antioxidant properties and serves as an efficient energy substrate through its contribution to glycine synthesis (Wu et al., 2019; Hu et al., 2022a), also increased over time under heatwave conditions in triploids from both induction methods. Notably, hydroxyproline, glycine, and proline are key components of collagen, and their elevated levels may indicate enhanced extracellular matrix turnover or repairs in response to heat-induced cellular damage and apoptosis (Phang et al., 2010; Rodríguez et al., 2017). Altogether, these shifts suggest a multifaceted physiological strategy combining metabolic support, redox regulation, and structural maintenance to mitigate the effects of prolonged thermal stress.

The divergent responses to heatwaves observed between 6DMAP and XP triploids, illustrated in Figure 14, may stem from epigenetic modifications induced by the 6DMAP treatment. Phenotypic plasticity is known to be particularly subject to remodeling during periods called “critical windows”, generally occurring during the early developmental stages (Burggren & Reyna, 2011; Safi-Stibler & Gabory, 2020). During these periods, exposure to environmental or chemical stressors may trigger lasting epigenomic modifications, potentially enhancing or impairing the organism's plasticity in coping with future stressors (Ali et al., 2011; Burggren & Reyna, 2011; Safi-Stibler & Gabory, 2020; Wang et al., 2021;

Fallet, 2023; Rodgers & Gomez Isaza, 2023). It is therefore plausible that 6DMAP inductions altered the epigenetic landscape of the treated mussels, resulting in modified thermotolerance compared to XP triploids. However, in the absence of mortality and of comprehensive transcriptomic or proteomic analyses, it remains uncertain whether these changes confer any adaptive benefit. Our results reported a faster upregulation of aerobic ATP production, along with signalling and anti-oxidative pathways in 6DMAP triploids. Similar early activation has been reported in heat-hardened mussels and clams (Georgoulis et al., 2022; Georgoulis et al., 2023; Zhang et al., 2023). However, the overall response was attenuated in 6DMAP triploids relative to XP triploids, potentially indicating reduced resistance. This interpretation aligns with the nature of stress responses associated with hardening, as described by Georgoulis et al. (2021). Furthermore, 6DMAP triploids also exhibited signs of energy depletion by transitioning faster towards partial anaerobiosis compared to XP triploids (Sokolova, 2013; Pörtner et al., 2017), which may be a sign of lower tolerance to heat stress (Dunphy et al., 2018).

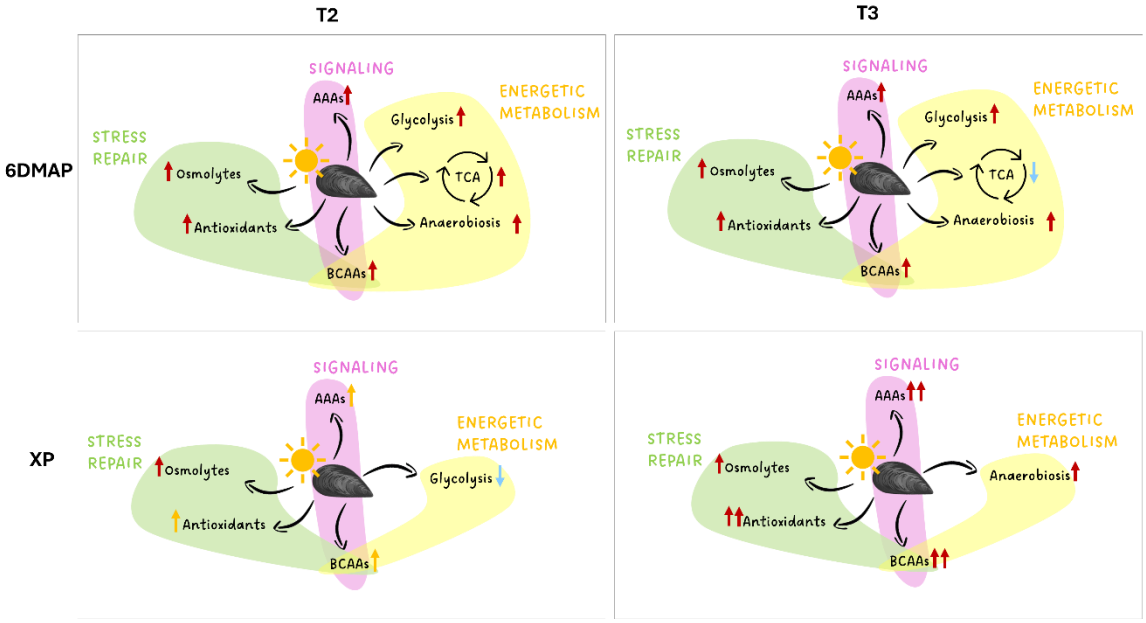

Figure 14. Temporal metabolomic responses of pressure- and chemically-induced triploid mussels to the simulated heatwave. (Yellow arrows : increased concentrations; red arrows : highly increased concentrations; doubled red arrows : very highly increased concentrations; blue arrows : decreased concentrations).

1.5 CONCLUSION

Both diploid and triploid juveniles appeared well adapted to withstand prolonged cyclic heatwaves up to 27 °C, as no significant mortalities were observed. Nonetheless, metabolomic analyses revealed signs of energetic stress in all tested groups, including an upregulation of the TCA cycle followed by a shift to anaerobic pathways after several weeks of exposure. Furthermore, the mobilization of signalling and antioxidant molecules during heatwaves suggests the occurrence of oxidative stress. Despite similar lipid reserves before the experiment, distinct metabolomic profiles were observed between diploids and triploids, which could influence their tolerance under heatwave conditions, despite the absence of OCR and mortality differences between both ploidies. The method used to induce triploidy appeared to influence heat-stress tolerance, as reflected by the metabolome of XP and 6DMAP triploids. Further research is needed to identify the most suitable induction method and to clarify the mechanisms underlying these differences.

CONCLUSION GÉNÉRALE

L'intensité, la durée, et l'étendue des vagues de chaleur marines ont considérablement augmenté depuis ces dernières décennies, atteignant des niveaux records en 2023, notamment dans l'Atlantique Nord (Dong et al., 2025). La triploïdisation apparaît comme une stratégie prometteuse pour améliorer la survie des moules face à ces événements extrêmes. En combinant des approches physiologiques et moléculaires, les travaux réalisés dans le cadre de cette étude ont permis d'améliorer la compréhension des différences métaboliques entre moules diploïdes et triploïdes, ainsi que leur implication dans la réponse au stress thermique, en particulier lors d'épisodes de vagues de chaleur.

Les différences observées entre les métabolomes des individus diploïdes et triploïdes pourraient partiellement expliquer les écarts de performances phénotypiques fréquemment rapportés entre ces deux niveaux de ploïdie, y compris dans la présente étude (Annexe III) (Brake et al., 2004; Barreto-Hernández et al., 2018; Osterheld et al., 2023). Des concentrations plus importantes en acides aminés protéinogéniques ont été mesurées chez les diploïdes, et pourraient traduire une faible efficacité de renouvellement protéique (Hawkins, 1985; Hawkins et al., 1986). Ce phénomène est généralement associé à un faible taux d'hétérozygotie (Hawkins et al., 1986), renforce l'hypothèse de l'hétérozygotie comme facteur contribuant à une croissance accrue chez les triploïdes, particulièrement avant maturation sexuelle (Allendorf & Leary, 1984; Hawkins et al., 1994; Nell, 2002; Wang et al., 2002). Des patrons relativement similaires de réponse aux vagues de chaleur ont été observés chez les jeunes moules diploïdes et triploïdes. Ces résultats pourraient cependant différer au stade adulte, notamment chez les individus diploïdes, dont le métabolisme est susceptible d'être altéré par les coûts physiologiques associés à la gamétogénèse (Clements et al., 2018; Mredul et al., 2024). Une analyse comparative des profils métabolomiques selon le niveau

de ploïdie à ce stade constituerait donc une perspective pertinente pour évaluer les différences de réponses métabolomiques lorsque des investissements reproducteurs sont engagés. Toutefois, l'induction de triploïdie par choc de pression ne semble pas compromettre la résistance au stress et apparaît donc comme une méthode recommandable.

Les familles testées durant cette étude ont démontré une bonne tolérance physiologique aux vagues de chaleur, malgré un temps d'exposition de plusieurs semaines. Cependant, les profils métabolomiques ont permis de révéler, quel que soit la ploïdie ou le traitement d'induction appliqué, une mobilisation des mécanismes de défense au stress, ainsi qu'un épuisement progressif du système énergétique. Chez les triploïdes, le mode d'induction semble influencer à la fois le délai et l'intensité de la réponse au stress, mais des études supplémentaires incluant l'évaluation du seuil thermique létal sont nécessaires pour déterminer quelle méthode d'induction confère une meilleure tolérance aux vagues de chaleur. De plus, effectuer les mesures de respirométrie et les prélèvements métabolomiques à différentes phases du cycle thermique, y compris lors des maxima de température, fournirait une compréhension plus complète des réponses physiologiques et des ajustements métaboliques mis en place par les moules au cours de l'expérience.

Bien que cette étude ne permette pas d'identifier une méthode d'induction clairement plus avantageuse, elle met en évidence l'impact des perturbations environnementales précoces sur la résistance au stress. Cette observation ouvre la voie au remodelage épigénétique au cours des premiers stades de vie comme stratégie complémentaire à l'amélioration de la performance. Certaines études ont exploré le potentiel de cette approche (Rodgers & Gomez Isaza, 2023). En particulier, une combinaison entre triploïdisation et remodelage épigénétique ciblé pourrait offrir aux aquaculteurs une solution prometteuse et durable pour renforcer la tolérance des individus face à l'intensification des vagues de chaleur. Par ailleurs, l'intégration d'analyses omiques complémentaires, telles que la génomique, la transcriptomique ou la métabolomique, couplées à des mesures physiologiques, permettrait de mieux comprendre les mécanismes impliqués dans la réponse au stress et d'identifier des marqueurs génétiques de résistance et/ou de résilience thermique.

En effet, les écarts marqués de phénotypes et de tolérance observés entre populations issues de milieux thermiques contrastés (Watson et al., 2018; Clarke et al., 2025) mettent en lumière le rôle potentiel de facteurs génétiques et/ou épigénétiques dans la modulation de cette réponse. Le durcissement thermique constitue également une stratégie complémentaire, permettant d'induire une tolérance accrue à court terme. Bien documenté chez les espèces ectothermes, ce mécanisme repose sur une exposition brève à un stress sublétal, entraînant une réponse physiologique rapide, notamment via la production de protéines de choc thermique (HSP), permettant à l'organisme de mieux résister à une élévation de température ultérieure (Abele & Puntarulo, 2004; Georgoulis et al., 2021; Zhang & Dong, 2021 ; Georgoulis et al., 2022 ; Dong et al., 2023; Georgoulis et al., 2023; Zhang et al., 2023 ; Georgoulis et al., 2024; Zhang et al., 2025) Bien que ses effets soient généralement transitoires et moins durables que ceux liés à des modifications épigénétiques stables, cette réponse d'acclimatation rapide pourrait être exploitée à court terme, notamment pour anticiper l'arrivée de vagues de chaleur ponctuelles durant les périodes estivales (Zhang et al., 2021).

ANNEXE I

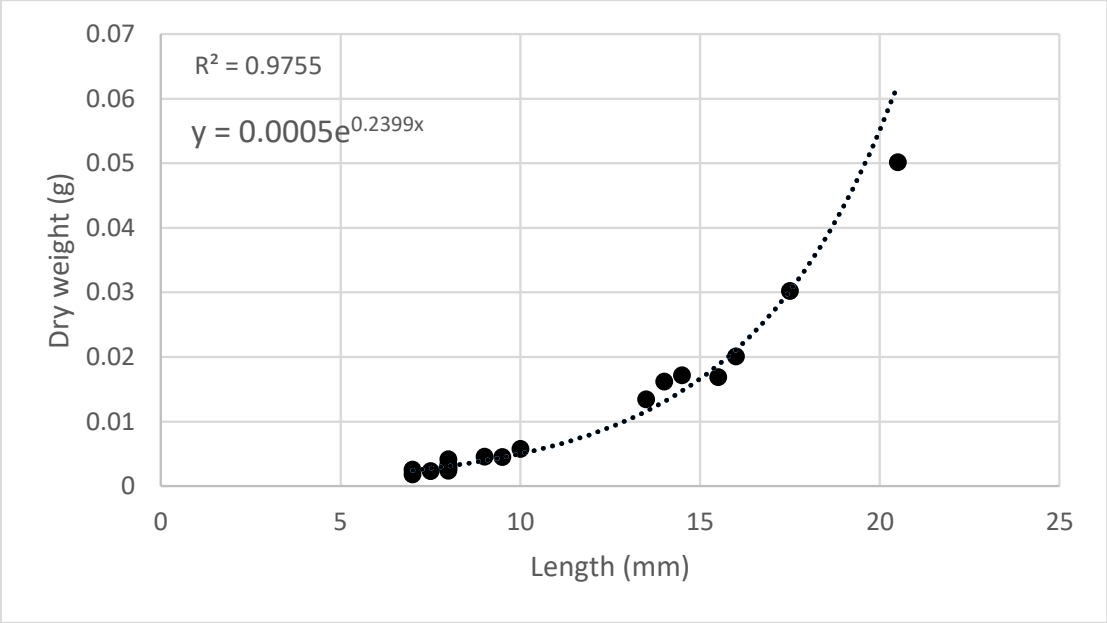

Figure 15. Relation entre la longueur et la masse sèche mesurées chez les juvéniles de *Mytilus edulis*

ANNEXE II

Table 6. Concentrations moyennes des profils d'acides gras dans chacun des groupes avant l'expérience (ng/mg ± SE). SFA: acides gras saturés; MUFA : acides gras monoinsaturés; PUFA : acides gras polyinsaturés; TFM : Total fatty . AA : acide arachidonique, EPA : Acide eicosapentaénoïque; DHA : Acide docosahexaénoïque

	Family 1		***Family 2***	
	Diploids	*Triploids XP*	*Triploids XP*	*Triploids 6DMAP*
Fatty acid				
11:00	0 ± 0	0 ± 0	0 ± 0	0.003 ± 0.007
12:0	0.12 ± 0.15	0.14 ± 0.17	0.22 ± 0.25	0.23 ± 0.17
14:0	2.40 ± 1.66	3.86 ± 2.34	3.97 ± 2.8	4.76 ± 3.24
15:0	0.28 ± 0.18	0.37 ± 0.16	0.45 ± 0.27	0.46 ± 0.21
16:0	38.4 ± 9.21	38.04 ± 10.37	35.69 ± 10.37	27.90 ± 4.29
17:0	0.73 ± 0.77	0.52 ± 0.08	0.64 ± 0.19	0.56 ± 0.11
18:0	18.81 ± 9.19	14.77 ± 9.83	17.84 ± 8.78	10.09 ± 5.74
20:00	0.05 ± 0.09	0.05 ± 0.07	0.1 ± 0.14	0.05 ± 0.06
21:00	0.00 ± 0.01	0.01 ± 0.01	0.017 ± 0.027	0.01 ± 0.02
22:00	0.01 ± 0.02	0.01 ± 0.01	0.03 ± 0.039	0.02 ± 0.03
23:00	0.00 ± 0.00	0.00 ± 0.00	0.002 ± 0.006	0.00 ± 0.00
24:00:00	0.01 ± 0.02	0.00 ± 0.01	0.029 ± 0.04	0.02 ± 0.03
ΣSFA	60.86 ± 17.01	57.77 ± 16.65	59 ± 15.56	44.17 ± 5.87
14:1w5	0.01 ± 0.02	0.03 ± 0.05	0.034 ± 0.062	0.08 ± 0.09
15:1w5	0.00 ± 0.00	0.01 ± 0.02	0.01 ± 0.02	0.03 ± 0.03
16:1w7	9.56 ± 7.75	12.23 ± 10.37	8.27 ± 5.33	10.25 ± 6.31
17:1w7	0.10 ± 0.10	0.12 ± 0.11	0.15 ± 0.16	0.18 ± 1.14
18:1w9	14.85 ± 4.69	13.64 ± 4.13	14.15 ± 3.58	13.68 ± 4.30
20:1w9	4.08 ± 0.14	4.81 ± 1.84	5.05 ± 2.57	6.31 ± 1.64
22:1w9	0.01 ± 0.03	0.02 ± 0.04	0.026 ± 0.038	0.03 ± 0.04
ΣMUFA	28.61 ± 11.60	30.87 ± 11.20	27.69 ± 9.44	30.55 ± 9.26
18:2w6	1.85 ± 1.04	2.04 ± 1.17	2.19 ± 1.64	3.06 ± 1.10
18:3w6	0.07 ± 0.11	0.14 ± 0.17	0.11 ± 0.19	0.22 ± 0.23
18:3w3	0.40 ± 0.47	0.73 ± 0.70	0.58 ± 0.75	1.13 ± 0.74
20:2w6	0.13 ± 0.14	0.63 ± 0.19	0.325 ± 0.1643	0.33 ± 0.17
20:3w3	0.03 ± 0.04	0.04 ± 0.09	0.033 ± 0.035	0.03 ± 0.04
20:3w6	0.22 ± 0.50	0.63 ± 1.37	0.55 ± 1.28	0.29 ± 0.25
20:4w6 (AA)	1.78 ± 1.78	1.55 ± 1.15	2.25 ± 1.83	4.32 ± 2.27
20:5w3 (EPA)	2.95 ± 2.67	3.09 ± 2.17	3.53 ± 2.5	7.58 ± 1.96
22:2w6	0.66 ± 0.67	0.80 ± 0.84	0.7 ± 0.73	1.07 ± 0.96
22:6w4 (DHA)	2.46 ± 2.50	2.18 ± 1.54	3.18 ± 2.55	7.27 ± 2.69
ΣPUFA	10.53 ± 8.31	11.37 ± 6.25	13.31 ± 8.63	25.28 ± 6.00
Σw2	2.63 ± 1.46	3.02 ± 1.47	3.09 ± 1.91	4.45 ± 0.96
Σw3	0.71 ± 0.80	1.53 ± 1.50	2.26 ± 1.63	1.67 ± 1.22
Σw4	1.78 ± 1.78	1.55 ± 1.45	2.25 ± 1.83	4.32 ± 2.27
Σw5	2.95 ± 2.67	3.09 ± 2.17	3.53 ± 2.5	7.59 ± 1.96
Σw6	2.47 ± 2.50	2.18 ± 1.54	3.18 ± 2.55	7.27 ± 2.69
Σw3/Σw6	1.58 ± 1.02	1.48 ± 0,78	1.76 ± 1.38	3.10 ± 1.66
TFM (mg g^{-1})	57.84 ± 68.00	72.59 ± 82.88	89.1 ± 107.04	61.19 ± 53.23

ANNEXE III

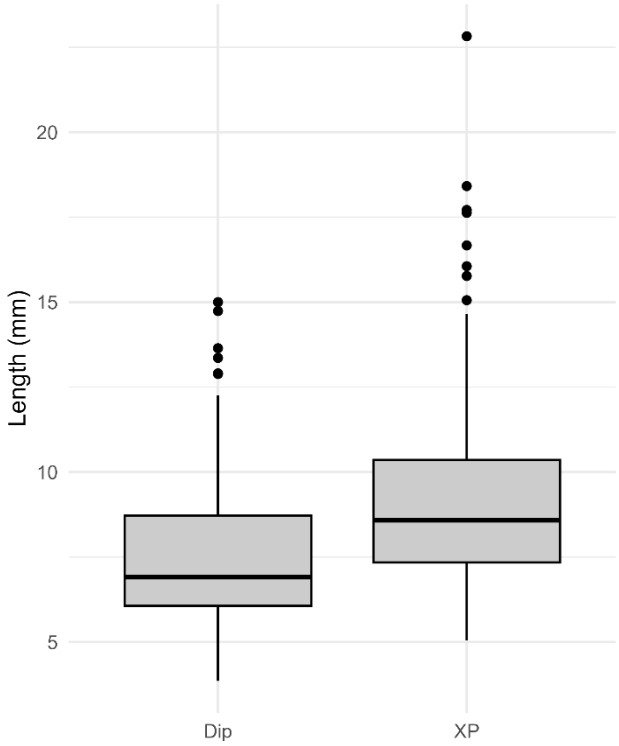

Figure 16. Comparaison de la longueur (mm) entre moules diploïdes (Dip) et triploïdes induites par pression (XP) (*Kruskal–Wallis* $\chi^2_{(1)} = 18.12$, $p = 2.08 \times 10^{-5}$) (Famille 1).

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