Université du Québec
à Rimouski

Les effets des vagues de chaleur marines et de la pêche accidentelle sur la survie et le métabolome de la mye commune *Mya arenaria* (Linnaeus, 1758) et de la mye tronquée *Mya truncata* (Linnaeus, 1758)

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« A society grows great when old men plant trees whose shade they know they shall never sit in » - Greek proverb

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RÉSUMÉ

La biodiversité mondiale subit une contraction sous l'effet des perturbations anthropiques, et les écosystèmes côtiers sont menacés par une gestion inadéquate des pêcheries et par les changements environnementaux qui comprennent l'augmentation des vagues de chaleur marines. Lorsqu'elles agissent conjointement, les perturbations multiples entraînent des effets complexes sur les organismes et sont difficiles à prédire. De plus, la tolérance des espèces d'invertébrés marins comme les palourdes est généralement méconnue, et leur vulnérabilité pourrait être aggravée en raison de leur susceptibilité aux variations thermiques et de leur capacité de dispersion limitée. Deux espèces de palourdes, la mye commune *Mya arenaria* (Linnaeus, 1758) et la mye tronquée *Mya truncata* (Linnaeus, 1758), ont été prélevées de l'estuaire du Saint-Laurent (Québec, Canada) là où leurs répartitions biogéographiques se recoupent. Afin d'évaluer leur tolérance aux vagues de chaleur marines et à la pêche accidentelle, ces espèces ont été assujetties à un design expérimental factoriel croisé comprenant sept températures (2, 7, 12, 17, 22, 27, 32 °C) ainsi que deux intensités de pêche accidentelle (présence, absence). La survie a été mesurée après une période de 12 jours et trois tissus (branchies, manteau, muscle adducteur postérieur) ont été récoltés pour caractériser leur profil métabolomique. La survie était principalement affectée par l'interaction significative entre l'*espèce* et la *température* sans effet de la *pêche*. La tolérance aux vagues de chaleur était nettement différente entre les espèces : 26,9 °C pour *M. arenaria* et 17,8 °C pour *M. truncata*. À la limite supérieure de tolérance de *M. arenaria*, les métabolites [acide α-aminoadipique], [histidine], [phénylalanine], [serine], [thréonine] et [valine] ont augmenté et [aspartate], [acide β-aminoisobutyrique], [FAD], [glutamine], [acétyl-CoA], [ADP], [cis-aconitate], [citrate] et [oxaloacétate] ont diminué dans les trois tissus. Pour *M. truncata*, seul le métabolite [phénylalanine] a augmenté tandis que [glutamine], [glycine] et [succinate] ont diminué dans les trois tissus. Selon les analyses en composantes principales et les analyses discriminantes, le métabolome de chaque tissu changeait en réponse aux vagues de chaleur. Les analyses de réseau ont révélé que *M. arenaria* s'appuie sur un grand nombre de voies cellulaires et emploie principalement des voies d'expression et de réparation de l'ADN et des voies signalétiques. Notre étude a révélé des mécanismes physiologiques pouvant expliquer la survie des espèces d'invertébrés marins en conditions futures de vagues de chaleur marines.

Mots clés: conservation, métabolomique, changements climatiques, physiologie, pêcheries, bivalves, biologie intégrative

ABSTRACT

Biodiversity is currently threatened by several anthropogenic global change stressors and coastal ecosystems are imperiled by both unsustainable fishing practices and changing environmental conditions such as marine heatwaves. The co-occurrence of multiple stressors may produce complex interactions that are difficult to predict without appropriate experimentation. Furthermore, the tolerance of marine invertebrate species such as clams to these stressors is poorly understood, yet their vulnerability may be high due to their acute sensitivity to temperature variations and limited dispersal abilities. Two species of clams, softshell clams *Mya arenaria* (Linnaeus, 1758) and blunt gapers *Mya truncata* (Linnaeus, 1758) were collected from the St-Lawrence Estuary (Québec, Canada) where their biogeographic distributions overlap. To test their tolerance to marine heatwaves and harvesting disturbance, these species were subjected to both stressors in a controlled experimental environment with a fully crossed design comprised of seven temperatures (2, 7, 12, 17, 22, 27, 32 °C) and two harvesting disturbance intensities (with, without). Survival was measured after a 12 d exposure period, and three tissues (gills, mantle, posterior adductor muscle) were collected from surviving individuals for metabolomic profiling. Breakpoint analysis for species survival revealed a significant interaction *species x temperature*, and no significant effect of *harvesting disturbance*. Heatwave tolerance differed between species: 26.9 °C for *M. arenaria* and 17.8 °C for *M. truncata*. At the upper thermal limit of *M. arenaria*, [α-aminoadipic acid], [histidine], [phenylalanine], [serine], [threonine], and [valine] consistently increased whereas [aspartate], [β-aminoisobutyric acid], [FAD], [glutamine], [acetyl-CoA], [ADP], [cis-aconitate], [citrate], and [oxaloacetate] consistently decreased across tissues. At the upper thermal limit of *M. truncata*, only [phenylalanine] consistently increased whereas [glutamine], [glycine], and [succinate] consistently decreased across tissues. Entire metabolome profiles were most significantly impacted by the interactive effect of *species* × *tissue*. Individually, each species' tissue metabolome displayed a unique and gradated response to heatwaves as evidenced by the converging results of principal component analysis and discriminant analysis. Network analysis of species' individual tissues revealed striking differences in pathway utilization at the upper heatwave limit, with *M. arenaria* displaying more abundant pathways and a greater reliance on DNA repair and expression as well as cell signaling pathways. Our study offers an in-depth understanding of physiological underpinnings of survival in marine invertebrates exposed to intensified heatwaves.

Keywords: conservation, metabolomics, climate change, physiology, fisheries, bivalves, integrative biology

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INTRODUCTION GÉNÉRALE

La protection de la biodiversité et des ressources naturelles se fait principalement grâce aux gouvernements qui souhaitent préserver les nombreux services dont l'humain dépend. On compte de nombreuses organisations ayant pour mandat de freiner l'érosion de la biodiversité provoquée par l'action humaine, en identifiant les causes principales: changements climatiques et globaux, fragmentation de l'habitat, espèces exotiques envahissantes (EÉE), et pollution (IPBES 2019; IPCC 2022). En bref, il s'agit d'établir des aires protégées (Chape et al. 2005), de limiter les émissions de gaz à effets de serres selon des engagements volontaires (voir « pledge-and-review » Keohane and Oppenheimer 2016), d'étudier et de mieux gérer les énergies alternatives (Dubash and Florini 2011; Urpelainen and Van de Graaf 2015), d'améliorer les suivis environnementaux (Lepom et al. 2009), et de freiner les EÉE (Genovesi et al. 2015) entre autres. Cela étant dit, la biodiversité mondiale est fragilisée par diverses perturbations environnementales, dont la présence de contaminants (Köhler and Triebskorn 2013), la disparition de 77,8 % de l'habitat sauvage terrestre (Watson et al. 2016), l'effondrement de stocks naturels surexploités (Sala and Knowlton 2006; Essington et al. 2015), l'importation et la prolifération d'EÉE (Pyšek and Richardson 2010), et d'importantes sècheresses, des vagues de chaleurs, et des inondations dont l'intensité est accentuée par le dérèglement du système climatique mondial (Ummenhofer and Meehl 2017; IPCC 2022). Au rythme actuel, peu de perturbations anthropiques vont s'aggraver autant que les changements climatiques provoqués par l'augmentation effrénée de CO_2 atmosphérique d'origine humaine (IPCC 2022). Les études portant sur la conservation de la biodiversité ont historiquement favorisé les mammifères, ou plus globalement les vertébrés, qui bénéficient alors d'une meilleure évaluation des risques posés par les perturbations environnementales à leur égard (Clark and May 2002). En revanche, les invertébrés souffrent d'un manque de

connaissances, ce qui limite notre capacité à évaluer les mêmes risques chez des espèces ayant des fonctions clés au sein des écosystèmes (Cardoso et al. 2011). Si les distributions biogéographiques des populations d'invertébrés sont bornées par les conditions environnementales (Chown and Gaston 1999; Calosi et al. 2010; Bozinovic et al. 2011; Sunday et al. 2012), il s'en suit que des perturbations environnementales mèneront à une redistribution de ces espèces (Sunday et al. 2012; Pinsky et al. 2013). Les changements climatiques anthropiques opèrent graduellement (moyennes annuelles) et rapidement (évènements extrêmes), et ces deux modalités auront chacune des répercussions sur les distributions biogéographiques et la pérennité des espèces individuelles ainsi que sur le fonctionnement écosystémique.

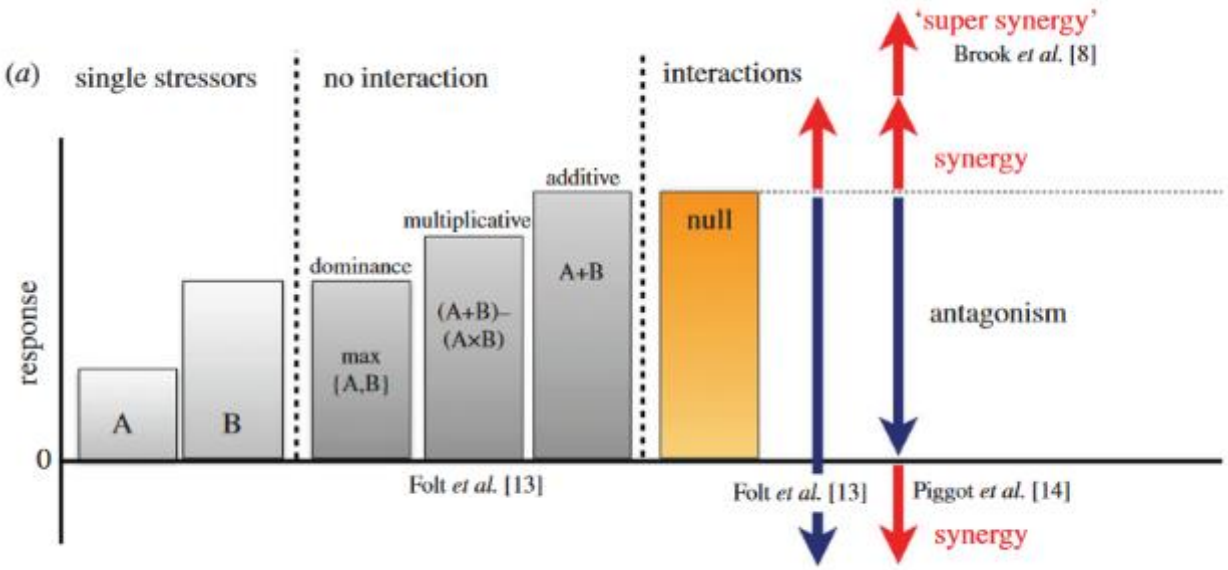
Les changements globaux provoqués par l'augmentation de CO_2 atmosphérique modifient le système de carbonates et le pH des océans (i.e. diminution du pH moyen) (Gruber 2011; IPCC 2022). De plus, les événements climatiques extrêmes comme les vagues de chaleur s'intensifient en termes de durée, fréquence, et amplitude (Meehl and Tebaldi 2004; IPCC 2022). Le Groupe d'experts intergouvernemental sur l'évolution du climat (GIEC) rapporte que la fréquence des vagues de chaleurs marines aurait doublé depuis l'année 1982 (Hoegh-Guldberg et al. 2018). Selon certaines études, ces phénomènes climatiques extrêmes représentent un risque nettement plus élevé qu'un changement graduel des moyennes climatiques, qui lui s'opère sur des plus grandes échelles temporelles (Thibault et Brown 2008; Smale et Wernberg 2013; Frölicher et Laufkötter 2018). À l'échelle écosystémique, ces changements pourraient entraîner la perte d'espèces clés, plus particulièrement celles qui sont incapables de migrer en direction des conditions environnementales plus favorables (Walther et al. 2002; Sunday et al. 2015). Les régions vulnérables aux vagues de chaleur marines ont subi des mortalités massives survenues en raison des conditions extrêmes (Garrabou et al. 2009): le « blob » du Nord-Est de l'océan Pacifique (Bond et al. 2015), la région Nord-Ouest de l'océan Atlantique (Mills et al. 2013), la mer Méditerranéenne (Olita et al. 2007), l'océan Arctique (Simpkins 2017). La grande

mortalité découle de différents facteurs, dont les conditions de température qui dépassaient le seuil de tolérance de chaque espèce (Stillman 2002, 2019; Sorte et al. 2011), l'affaiblissement des défenses immunitaires qui confèrent la protection contre les pathogènes (Harvell et al. 1999), ou encore les effets des cascades écosystémiques (Wernberg et al. 2016) et de la dynamique des réseaux trophiques (Jones et al. 2018; Oliver et al. 2019). D'une part, les phénomènes climatiques extrêmes limitent la répartition biogéographique des espèces (Bozinovic et al. 2011; Smale et Wernberg 2013), et d'autre part, ils poussent l'évolution des seuils de tolérance physiologique (Buckley et Huey 2016; Williams et al. 2016; Grant et al. 2017; Bennett et al. 2021). Dans un cadre d'évaluation des risques et de conservation de la biodiversité, différents experts suggèrent de définir les limites de tolérance des espèces aux conditions environnementales futures (Wikelski et Cooke 2006; Somero 2010; Seebacher et Franklin 2012; Cooke et al. 2013). La redistribution future des espèces dépendra de leurs seuils de tolérance aux conditions futures, l'efficacité de la réponse cellulaire en réponse au stress, ainsi que la plasticité physiologique des mécanismes de tolérance face à ces conditions environnementales.

Les menaces aux écosystèmes marins ne se limitent pas aux effets des changements climatiques; on parle d'une accumulation de perturbations anthropiques : l'acidification des océans, la diminution des concentrations d'oxygène dissout, l'accumulation de contaminants, l'intensification du transport maritime, la diminution des ressources, et la perte et la fragmentation de l'habitat (IPBES 2019; IPCC 2022). Pour évaluer l'impact des perturbations multiples sur les systèmes biologiques, un cadre théorique a été développé pour adresser les effets d'interaction entre perturbateurs, notamment le synergisme et l'antagonisme (Côté et al. 2016; Schäfer and Piggott 2018). L'impact conjoint des perturbateurs peut simplement s'avérer comme la somme des effets simples, mais un phénomène de synergisme dédoublerait les impacts négatifs (Fig. 1). En revanche, l'effet d'antagonisme est également répandu et a été rapporté pour plusieurs études traitant du réchauffement (Jackson et al. 2016). L'impact du réchauffement des eaux pourrait alors être

aggravé ou atténué lorsqu'il agit conjointement à d'autres perturbations, comme la surpêche. La surpêche s'inscrit dans la gestion inadéquate des pêcheries, une problématique très répandue mondialement (Mora et al. 2009; Christensen et al. 2014). Les pêcheries commerciales contribuent significativement aux déclins des populations d'espèces côtières lorsqu'elles emploient des stratégies de pêche non viables (Sala et Knowlton 2006; Essington et al. 2015). Ces types de gestion des stocks exploités peuvent fragiliser les populations naturelles (Lotze et al. 2006), ce qui augmente la vulnérabilité aux perturbations multiples ou successives (Jackson et al. 2001; Folke et al. 2004). Les écosystèmes côtiers sont composés d'espèces commerciales qui contribuent à la sécurité alimentaire forment une composante économique majeure pour une grande partie de la population mondiale (Pauly et al. 2005; Anderson et al. 2011). Ainsi, une attention particulière doit être portée envers la tolérance d'espèces côtières en réponse aux activités de pêche. Cette tolérance devrait intégrer les réponses physiologiques aux effets des changements climatiques afin d'en arriver à une meilleure estimation de la viabilité réelle des populations futures. Le degré de réponse physiologique reflète la tolérance de l'espèce aux perturbations environnementales (Stillman 2002; Compton et al. 2007; Somero 2010; Madeira et al. 2012) et peut être étudié chez différentes espèces conjointement pour identifier celles qui présentent la plus forte vulnérabilité aux effets des changements globaux. La quantification des niveaux de tolérance aux perturbations est alors un outil puissant pour la physiologie de la conservation.

Il existe une panoplie de réponses physiologiques chez les invertébrés (Harley et al. 2006; Poloczanska et al. 2013; Przeslawski et al. 2015) qui leur confère une certaine tolérance aux perturbations environnementales. Ainsi, cette tolérance se construit sur l'intégration des mécanismes physiologiques à chacune des strates de complexité d'un organisme vivant, des mécanismes à l'échelle subcellulaire jusqu'aux mécanismes de l'ensemble du l'individu (Kassahn et al. 2009; Hofmann and Todgham 2010). Cette idée a été formellement

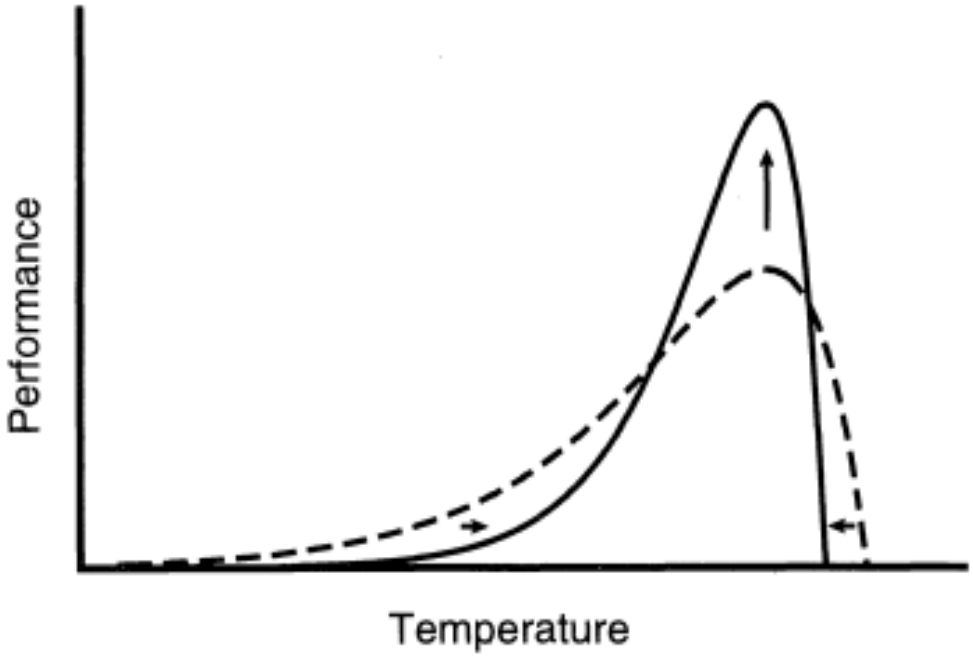


Côté et al. 2016

Figure 1 : Cadre théorique illustrant le degré de réponse aux perturbateurs (*response*) en fonction des effets simples ainsi que différents scénarios impliquant des effets i) sans interactions (dominant, multiplicatif et additif) et des effets avec interaction (synergisme et antagonisme). Tire de Côté et al. (2016).

proposée par Bartholomew (1958). Dans ce contexte, le réchauffement climatique soulève d'importants aspects fondamentaux du fonctionnement de l'invertébré, particulièrement celui de l'acclimatation et l'adaptation à la température. La physiologie d'un invertébré est intimement adaptée à la température environnante (voir poïkilotherme ectotherme; Moyes et Schulte 2015) qui agit directement sur la vitesse des réactions biochimiques (Kingsolver 2009), la disponibilité et la répartition de l'oxygène au sein d'un organisme (Angilletta Jr. 2009), et les propriétés fonctionnelles des membranes biologiques (Hazel et Williams 1990) et des protéines (Somero 1995). Les processus évolutifs agissent sur ces contraintes et poussent vers l'élargissement ou le rétrécissement de l'étendue de températures pour lesquelles l'individu sera adapté (Hochachka et Somero 2002; Angilletta Jr. 2009). Cela fait

naître le concept opposant l'évolution d'espèces généralistes ou spécialistes (eurytolérant ou sténotolérant), qui prétend qu'une fenêtre de tolérance plus large ou plus étroite serait favorable d'un point de vue évolutif (Somero 2005). Les espèces « eurythermes » démontrent une performance plus soutenue que les « stenothermes » en conditions variables de température (Fig. 2; Gilchrist 1995), ce qui suggère qu'elles possèdent une plus grande plasticité des mécanismes de tolérance physiologiques. Pour cette raison, ces espèces seraient moins vulnérables aux effets du réchauffement climatique (Somero 2005; Munday et al. 2013). Quelle



Sinclair et al. 2016

Figure 2 : Courbe de performance thermique illustrant la fenêtre de tolérance, et le niveau de performance d'une espèce généraliste (ligne pointillée) et celui d'une espèce spécialiste (ligne continue). Tiré de Sinclair et al. (2016).

que soit sa classification eurytherme ou sténotherme, chaque espèce démontre la capacité de s'ajuster aux variations de température comprises dans sa fenêtre de tolérance thermique que

l'on définit comme sa plasticité phénotypique (Cossins and Bowler 1987; Angilletta Jr. 2009; Schulte et al. 2011). Lorsque ces conditions sont perdues, on observe un effondrement de la machinerie cellulaire, principalement les enzymes et les membranes (Bowler 1987; Hightower 1991). On parle alors des seuils thermiques, des outils indispensables à la quantification de la vulnérabilité des espèces aux réchauffement climatique. Alors que ces seuils ont historiquement été étudiés sur l'ensemble de l'organisme, des avancées en biochimie offrent une nouvelle perspective de la tolérance thermique par l'emploi des techniques omiques. Ces techniques comportent plusieurs avantages, dont la rapidité et la simplicité, et peuvent permettre de quantifier la réponse du métabolisme cellulaire plus efficacement.

La portée d'une étude traitant du métabolisme dépend largement de l'outil analytique employé, et ce sont les méthodes à haut-débit telle que la métabolomique qui sont à prévaloir puisqu'elles procurent une quantité inégalée d'information biologique liée à l'activité métabolique. L'arrivé de ces méthodes a transformé le champ d'étude en permettant la quantification simultanée des composés présents dans un échantillon pour ainsi dresser un profil exhaustif du système biologique à l'étude (van der Greef et Smilde 2005). La métabolomique (méthode à haut débit dédiée à la quantification de molécules de très petite taille < 1,5 KDa) est un outil innovateur qui saisit la variation métabolique complexe dans un tissu pour fournir un profil métabolomique. Laissant de côté l'approche typique de molécule-par-molécule employé en biochimie, la métabolomique quantifie le profil métabolomique à la recherche de biomarqueurs permettant la détection des maladies liées au dysfonctionnement métabolique (McGarrah et al. 2018; Lent-Schochet et al. 2019). Plus fondamentalement, cette approche se cadre parmi les nombreuses strates d'organisation qui composent la hiérarchie physiologique étudiée en biologie des systèmes (Weckwerth 2003). Les changements dans l'activité métabolique en réponse aux perturbations environnementales entraînent des conséquences sur l'ensemble de l'organisme, comme le vieillissement prématuré (Yang et Ming 2012; Bonomini et al. 2015; Ren et al. 2018) et

l'apoptose (Mason et Rathmell 2011; Andersen et Kornbluth 2013) qui peuvent tous deux être déclenchés par le stress métabolique. On peut alors intégrer la réponse de l'ensemble de l'organisme avec la réponse métabolique pour révéler les maillons faibles d'un système biologique qui imposent une contrainte sur la capacité de tolérance aux perturbations. La métabolomique est utilisée en milieu expérimental afin de détecter les changements métaboliques déclenchés en réponse à la perturbation de température environnementale et de comparer ces réponses chez différentes populations et/ou différentes espèces (Lin et al. 2006; Bundy et al. 2008; Sun et al., 2022). Si les réponses cellulaires sont assujetties aux processus adaptatifs, les réponses métaboliques devraient alors varier selon l'histoire évolutive de chaque espèce vivant dans un milieu qui présente des conditions environnementales uniques. Dans cette optique, mesurer le métabolisme cellulaire revient à caractériser les stratégies adaptives qui fournissent un avantage face aux perturbations environnementales en apportant des modifications métaboliques propres à chaque espèce.

OBJECTIF

L'objectif de cette étude était d'évaluer la réponse de deux espèces d'invertébrés marins aux perturbations multiples : les vagues de chaleur marines et la pêche accidentelle. Le degré de réponse est évalué au niveau physiologique en estimant leurs seuils de tolérance et en caractérisant les mécanismes cellulaires employés.

CONTEXTE EXPÉRIMENTAL

Les espèces choisies pour l'étude sont la mye commune (*Mya arenaria*, Linnaeus, 1758) et la mye tronquée (*M. truncata*, Linnaeus, 1758). Leurs rôles dans les écosystèmes côtiers sont nombreux : filtreurs améliorant la qualité de l'eau (Nakamura et Kerciku 2000), proie des mammifères marins (Fisher and Stewart 1997), et cible des pêcheries (Glude 1955), entre autres. Elles sont distribuées géographiquement là où elles subiraient les impacts des vagues de chaleurs marines et de la pêche accidentelle. Afin d'estimer l'impact de ces

perturbations sur la physiologie de ces deux espèces, nous les avons placées dans un système expérimental à recirculation partielle permettant un contrôle thermique automatique, pour ensuite les assujettir à sept intensités indépendantes de vagues de chaleur marines allant de 2 ºC jusqu'à 32 ºC pour une durée de 12 jours. De plus, une pêche accidentelle a été imitée dans la moitié des bassins du système. Au bout de la période d'exposition, la mortalité a été mesurée et des analyses en métabolomique ciblée ont été employées sur les survivants pour caractériser la variation du profil métabolomique (42 métabolites) et explorer les changements apportés aux voies physiologiques en fonction des traitements.

HYPOTHÈSES

Il était attendu que chaque espèce réponde différemment aux vagues de chaleur marines et qu'un plus grand succès serait prévu pour espèce tempérée *M. arenaria*, une tendance documentée chez des d'autres espèces polaires (Pörtner 2002). Il est également attendu que la pêche accidentelle impacte négativement les deux espèces au même degré. Une interaction synergique négative est prévue pour les deux perturbateurs en raison de leur potentiel d'aggraver le stress métabolique à l'endroit des fonctions bioénergétiques.

Cette étude emploie une approche intégrative pour évaluer les effets des changements climatiques extrêmes sur la physiologie des invertébrés marins, notamment en réponse aux vagues de chaleur marines, et a pour but d'approfondir nos connaissances portant sur les phénomènes cellulaires permettant la survie des espèces face aux changements globaux. On souhaite caractériser le degré de vulnérabilité de chaque espèce qui affronte le réchauffement climatique et la pêche accidentelle en accordant une attention particulière à deux espèces de palourdes d'importance économique et écologique qui fournissent une source alimentaire pour les communautés côtières (alimentation autochtone traditionnelle) et pour les mammifères marins.

CHAPITRE 1

THE COMBINED EFFECTS OF HEATWAVES AND HARVESTING DISTURBANCE ON THE SURVIVAL AND METABOLOME OF SOFTSHELL CLAMS (*MYA ARENARIA*; LINNAEUS, 1758) AND BLUNT GAPERS (*M. TRUNCATA*; LINNAEUS, 1758)

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1.1 INTRODUCTION

The conservation of biodiversity and natural resources is of primary concern to maintain beneficial ecosystem services (Mengist et al. 2020; Weiskopf et al. 2020). Collaborative international efforts to halt the growing threat of ecosystem collapse due to anthropogenic pressures target the major culprits: global climate change, habitat destruction and fragmentation, invasive species, and pollution (IPBES 2019; IPCC 2022), with the first arguably being the most pressing. It is thus important to understand how anthropogenic pressures, particularly global climate change, may impact marine organisms, with cascading effects on biodiversity and ecosystem functioning. Currently, biodiversity conservation

research is largely focused on mammals, or more generally vertebrates (Clark and May 2002) with research focused on invertebrate species being limited (Clark and May 2002; Chen 2021), which has consequent impacts for risk assessments of natural populations that sustain key ecosystem functions (Cardoso et al. 2011). Studying invertebrates from remote Arctic ecosystems presents logistical limitations and, thus they are often poorly understood, yet may face greater rates of environmental change than those of temperate ones (IPCC 2022). The distributions of Arctic invertebrate populations are greatly constrained by environmental conditions (Chown and Gaston 1999; Calosi et al. 2010; Bozinovic et al. 2011; Sunday et al. 2012), thus global change could shift species distributions (Parmesan 1996; Sunday et al. 2012; Pinsky et al. 2013) with potential negative consequences for the long-term persistence of species and ecosystem functioning.

Increased atmospheric CO_2 is driving changes in average ocean temperature conditions (Hansen et al. 2010; IPCC 2022). Moreover, the number of extreme climatic events, such as heatwaves, is becoming more frequent (Meehl and Tebaldi 2004; IPCC 2022). Marine heatwaves (as defined by (Hobday et al. 2016) have doubled in frequency since 1982 (Hoegh-Guldberg et al. 2018). Extreme events such as these may pose an even greater threat to species and communities than gradual, average changes to the ocean, such as ocean warming and acidification (Thibault and Brown 2008; Smale and Wernberg 2013; Frölicher and Laufkötter 2018). Ecosystems may face losses of key species that are unable to adapt or seek out more favourable conditions (Walther et al. 2002; Sunday et al. 2015). Areas that have experienced notable marine heatwaves include the Northeast Pacific Ocean (Bond et al. 2015), the Northwest Atlantic Ocean (Mills et al. 2013), the Mediterranean Sea (Olita et al. 2007), and the Arctic Ocean (Simpkins 2017), at times leading to mass mortality (Garrabou et al. 2009). Such mortality events are likely induced by exposure to temperatures that exceed species' physiological tolerance thresholds (Stillman 2002, 2019; Sorte et al. 2011), increased vulnerability to other drivers, such as pathogens (Harvell et al. 1999), cascading effects on species-interactions (Wernberg et al. 2016), food web dynamics (Jones et al. 2018;

Oliver et al. 2019), and the combined effects of these and likely other factors. According to the climate extreme hypothesis (CEH), extreme climatic events may constrain species ranges (Bozinovic et al. 2011; Smale and Wernberg 2013) and impact the evolution of their physiological tolerance (Buckley and Huey 2016; Williams et al. 2016; Grant et al. 2017; Bennett et al. 2021). Defining the tolerance limits of species is key to predicting the effects of climate warming (Somero 2010; Huey et al. 2012; Bozinovic and Pörtner 2015; Magozzi and Calosi 2015) and should factor into conservation strategies (Somero 2010; Seebacher and Franklin 2012; Lefevre et al. 2021). Future changes in species distributions will depend on the limits of species' tolerances, the extent of their cellular stress response, and their plasticity to environmental perturbations.

Coastal ecosystems harbour species that provide food security and support economies worldwide (Pauly et al. 2005; Anderson et al. 2011). Marine species and the ecosystems that support them face threats of not only to global climate change but also the ensemble of anthropogenic pressures, including unsustainable fisheries management (Mora et al. 2009; Christensen et al. 2014), one of the main drivers of population decline of coastal species (Sala and Knowlton 2006; Essington et al. 2015). Impacts due to over-fishing stocks can weaken species' resilience over time (Lotze et al. 2006) and increase their vulnerability to concomitant environmental disturbances (Jackson et al. 2001; Folke et al. 2004). Understanding coastal species' tolerances to harvesting (including accidental fishing, i.e. bycatch) and integrating their related physiological responses to climate warming could inform future management actions (Stillman 2002; Compton et al. 2007; Somero 2010; Madeira et al. 2012) by allowing the identification of species that are most at risk to the combined impacts of harvesting and global change.

Invertebrate physiological responses to environmental disturbances are diverse (Harley et al. 2006; Poloczanska et al. 2013; Przeslawski et al. 2015) and range from sub-cellular to whole organism-level coordinated responses (Kassahn et al. 2009; Hofmann and

Todgham 2010). Invertebrate physiology is adapted to external temperature (Moyes and Schulte 2015), which drives their biochemical rates (Kingsolver 2009), directly affects availability and behaviour of oxygen within organisms (Angilletta Jr. 2009), and alters the functional properties of both membranes (Hazel and Williams 1990) and proteins (Somero 1995). Adaptive processes underpin the range of temperatures under which organisms can survive (Hochachka and Somero 2002; Angilletta Jr. 2009). The Generalist *vs.* Specialist (eurytolerant *vs.* stenotolerant) paradigm suggests that there should be evolutionary selection for wide *versus* narrow windows of suitable temperature conditions depending on environmental circumstances (Somero 2005). Thermal generalists outperform thermal specialists in highly variable environments (Gilchrist 1995), through greater plasticity of tolerance mechanisms which should make them less vulnerable to climate warming (Somero 2005; Munday et al. 2013; Magozzi and Calosi 2015). Temperature changes can be tolerated within a "thermal window" through an organisms' thermal acclimation, which constitutes phenotypic plasticity (Cossins and Bowler 1987; Angilletta Jr. 2009; Schulte et al. 2011). Beyond this this window, cellular metabolic machinery (i.e enzymes and membranes) is likely compromised (Bowler 1987; Hightower 1991). Knowledge of these thresholds is valuable for predicting a species' vulnerability to climate warming. While temperature thresholds have historically been studied at the whole-organism level, a novel perspective to thermal tolerance may be achieved by focusing on cellular metabolism through metabolomics.

"Metabolomics" is an innovative high-throughput method for measuring small metabolites (< 1.5 kDa in size). Enabling evaluation of the complex metabolic activity occurring in cells *via* metabolite profiling and quantification (McGarrah et al. 2018; Lent-Schochet et al. 2019). Metabolomics allows for identification of biomarkers associated with stressor-related metabolic dysfunction and for integrating these into a hierarchical physiological framework typical of systems biology (Weckwerth 2003). Metabolic responses to environmental stressors may reflect whole-organism changes (Putri et al. 2013), with

examples including stress causing premature aging (Yang and Ming 2012; Bonomini et al. 2015; Ren et al. 2018) and apoptosis (Mason and Rathmell 2011; Andersen and Kornbluth 2013). Evaluation of cellular and whole-organism responses may together indicate failing biological systems that underpin a species' limits for tolerating a given stressor. Metabolomics may also be used to tease apart the underlying metabolic changes associated with temperature responses and potential differences between populations or species (Lin et al. 2006; Bundy et al. 2008; Sun et al., 2022).

This study takes an integrative approach to further understand the effects of extreme climatic events on invertebrate physiology and heightens our understanding of the details surrounding cellular mechanisms these taxa may employ to cope with global change. Specifically, this study examines the response of the temperate softshell clam *Mya arenaria* (Linnaeus, 1758) and the polar blunt gaper *M. truncata* (Linnaeus, 1758) to marine heatwaves and harvesting disturbance. Theory suggests that the temperate species would have greater homeostatic abilities compared to polar species (Pörtner 2002). The impact of these disturbances was assessed in terms of both whole-organism (survival) and cellular (metabolome) responses. These species are critically important to coastal areas as they support fisheries (Glude 1955), are important prey of marine mammals (Fisher and Stewart 1997), and impact water quality (Nakamura and Kerciku 2000) and both inhabit areas that are susceptible to co-occurring heatwaves and harvesting disturbance. We therefore exposed individuals to different acute temperature changes (ranging from 2 to 32 ºC) for a duration of 12 days (within the range of future projected heatwave duration) and a simulated harvesting disturbance event to mimic fishing practices. Mortality was evaluated and tissues (gills, mantle, and posterior adductor muscle) sampled from the surviving individuals to evaluate 42 metabolites. Metabolomics were used to characterize shifts in metabolomic profiles across treatments and investigate changes in physiological pathways in response to each treatment. It was hypothesised that species-specific differences would arise in response to heatwave treatments, with a more favorable outcome for the temperate congener *M.*

arenaria. Harvesting disturbance was predicted to have a negative effect on survival and shift the clam metabolome with compensatory responses of the metabolism, and that the combined effect of both treatments would yield a synergistic negative effect. Findings underline the vulnerabilities of two ecologically, economically, and culturally important clam species to climate warming and harvesting disturbance. Evaluating contrasting responses of these polar and temperate congeners will contribute to the scant literature available for remote and underrepresented species for which metabolomics studies are limited.

1.2 METHODS

1.2.1 Specimen collection, transport, and husbandry

Softshell clams, *M. arenaria* Linnaeus, 1758, and blunt gapers, *M. truncata* Linnaeus, 1758, were collected from August to October 2020 at two locations in the Lower Saint Lawrence Estuary: Métis-sur-Mer, Québec, Canada (48° 40' 4.6092" N, 68° 1' 5.9484" W) and Godbout, Québec, Canada (49° 19' 25.626" N, 67° 35' 17.034" W) respectively. Individuals with shell lengths between 50 and 70 mm were selected, maintained under humid conditions to prevent desiccation, and transported in 20 L containers (200 individuals *per* container) to the Maurice-Lamontagne Institute (MLI) in Mont-Joli, QC, Canada, within 10 h of collection.

Upon arrival at MLI, individuals were checked for overall condition and those that displayed abnormalities (e.g. broken valves) or were unresponsive (i.e. inability to retract siphons or close valves) were excluded from the experiment. Healthy individuals were then gently hand-buried into randomly assigned holding tanks containing ~ 20 cm of washed sand (num. 70, Groupe Bellemare, Trois-Rivières, QC, Canada). Tanks were set up in a semi-closed recirculating system where they were supplied with constant 1 L m^{-1} estuarine water pumped from just offshore into an insulated header tank (750 L) and controlled at 6 °C (ambient temperature at collection sites) by a heat pump (Gell'air, Mont-Joli, QC, Canada). This stabulation phase took place over at least 21 d prior to the experimental phase. Clams

were fed daily (at 16h00) with a commercial algal mixture (Shellfish Diet 1800, Reed Mariculture Inc., San Jose, CA, USA) containing five marine microalgae *per* the manufacturer's recommendations.

1.2.2 Experimental design, system, and protocol

We used a fully crossed factorial experimental design to investigate the combined effect of heatwaves and harvesting disturbance on the survival and tissue-specific metabolome profiles of *M. arenaria* and *M. truncata*. This included seven temperature levels (2, 7, 12, 17, 22, 27, and 32 °C) crossed with two harvesting disturbance conditions (i.e. with harvesting disturbance and without harvesting disturbance), yielding 14 distinct treatment levels for each species. Each treatment level had four independent tank replicates, totaling 56 experimental tanks. Each experimental tank was filled with ~20 cm of sand and set at a constant flow rate of 1 L m^{-1}. Estuarine water was pumped into two header tanks (750 L each) each fitted with a sand filter and held at either a high (35 °C) or low (1 °C) temperature using four independent heat pumps (Gell'air, Mont-Joli, QC). Raceways were used to pump water from the header tanks to the experimental tanks. Temperature was controlled independently in each experimental tank with proportional-integral-derivative controllers (REX-C100, XNY International, Wenzhou, China). Each PID controlled a two-way valve that supplied experimental tanks with a mixture of high and low temperatures to achieve target temperatures.

Clams were acclimated to the experimental system by transferring eight randomly selected individuals of each species (16 total) from the holding tanks to an experimental unit for 14 d prior to beginning experimental treatments. At the time of the transfer, shell morphometrics (length, height, and width) were recorded, clams were labelled, and then hand-buried into experimental units. Average (± standard deviation) lengths, heights and widths were 71.10 ± 7.37 mm, 44.63 ± 4.82 mm, and 29.75 ± 3.89 mm for *M. arenaria* and 59.83 ± 5.04 mm, 38.91 ± 3.81 mm, and 27.53 ± 2.84 mm for *M. truncata*.

To satisfy the definition of "heatwave," (as *per* Hobday et al. 2016) exposure temperatures needed to exceed the 90th percentile calendar day average and be sustained for a minimum duration of 5 d. Of the seven temperature levels, only 22 – 32 °C inclusively were technically considered heatwaves based on the local summer sea surface temperature maxima at each collection location currently around 16-17 °C (Chin et al. 2017; NASA JPL 2021). Temperature treatments 2 - 17 °C were within the annual range of temperatures experienced at the collection sites. This design thus tested the species' physiological responses within and outside the bounds of the species' recent thermal histories, in conditions of increased heatwave intensity projected by various studies.

A temperature exposure duration of 12 d was selected based on recent average heatwave duration of 5 – 7 d in the study region (Lau and Nath 2012; Jeong et al. 2016) and projected increases in heat-wave duration of 3.6 d by 2041 – 2070 (Lau and Nath 2012), of 1 – 10 d by 2040-2069 (Jeong et al. 2016) or 8.4 d °C $^{-1}$ increase in mean global surface temperature (Perkins-Kirkpatrick and Gibson 2017). The temperature level range was chosen to encompass the entire thermal range of both species and beyond with the upper limit (32 °C) selected based on the reported upper lethal temperature limit (LT50-24h) being 30.9 – 34.4 °C for adult *M. arenaria* (Kennedy and Mihursky 1971). The lower temperature tolerance in these species is unknown, but likely approaches the freezing point based on their known distribution. Given experimental system limitations, the lowest temperature used in this study was 2 °C to model a complete temperature-response gradient.

The 12 d heatwave treatment (hereafter, "temperature") included an initial 3 d step-wise ramping period, during which the temperature was increased or decreased at a stable rate to reach the target temperature. Ramping was conducted for 8 h, then paused 16 h overnight and restarted the next day for a total of 24 ramping hours during the 3 d ramping period. This metric was used to determine the ramping rate at each treatment level. From an

initial temperature of 6 °C, the theoretical ramping rates were -0.17, 0.04, 0.25, 0.46, 0.67, 0.88 and 1.08 °C h^{-1} for treatment levels 2, 7, 12, 17, 22, 27 and 32 °C respectively.

The harvesting disturbance treatment (hereafter, "*harvest*") used in the current study was similar to previous studies investigating harvesting disturbance effects for other bivalves: softshell clam (Beal and Vencile 2001), eastern oysters and hard clams (Lenihan and Micheli 2000), *Ruditapes* clams (Beck et al. 2015), venus clams (Ballarin et al. 2003). In the current study, *harvest* consisted of a single non-lethal disturbance where the substrate in each tank was completely turned over, effectively removing clams from the substrate, and exposing them at the surface. This was achieved by emulating professional clam diggers, using a professional grade clam rake with four tines each approx. 25 cm in length (BACK-HOE-4, KB White Company, Marblehead, ME, USA). The treatment was applied immediately following the 3 d ramping period and did not coincide with feeding times.

In all eight tanks exposed to the 32 °C treatment, the exposure period was terminated due to 100 % mortality within 10 d. Tanks that displayed significant temperature variation during either acclimation, ramping, and/or exposure periods were removed from analyses (n = 14). The final experimental units retained for tissue sampling (41 tanks), the resulting mortality and the average physical-chemical parameters during acclimation, ramping, and exposure periods are given in Annexe I.

1.2.3 Monitoring physical chemical parameters

Physical chemical properties of the sea water were monitored using a multiparameter probe (HI-98194, Hanna Instruments, Padova, Italy) to measure temperature, an NBS pH meter (914, Metrohm AG, Herisau, Switzerland) equipped with probe (iUnitrode PT1000, Metrohm AG, Herisau, Switzerland) to measure pH_{NBS}, a refractometer (DD H2Ocean, MOPS Aquarium Supplies, Hamilton, ON, Canada) to measure salinity, and a handheld

oxymeter (FSG02, PyroScience GmbH, Aachen, Germany) to measure dissolved oxygen (DO). Temperature (°C) was measured daily at all stages (stabulation, pre-exposure, temperature ramping, temperature exposure), while pH $_{NBS}$, salinity and dissolved oxygen (%) were measured every 3 d in all stages except temperature ramping. Each experimental unit was equipped with an automatic temperature logger (HOBO 8K Pendant® Temperature/Alarm Data Logger, Onset, Massachusetts, USA) to record data at 15 min intervals during pre-exposure, temperature ramping, and temperature exposure. Average acclimation conditions were ~6 °C, pH ~8.1, and ~100 % DO. Average experimental conditions with standard error (+ standard error; SE) in the pre-exposure phase were 6.20 ± 0.030 °C, 8.08 ± 0.004 pH, and 101.60 ± 0.050 % DO. Average temperature ramping rates (+ SE) were -0.13 ± 0.003, 0.05 ± 0.002, 0.25 ± 0.003, 0.40 ± 0.005, 0.64 ± 0.006, 0.81 ± 0.004, and 0.94 ± 0.007 °C h^{-1} . Average exposure temperatures (+ SE) following ramping were 2.91 ± 0.007, 7.34 ± 0.01, 11.98 ± 0.009, 16.93 ± 0.020, 22.00 ± 0.030, 26.60 ± 0.020 and 31.47 ± 0.030 °C for temperature treatments 2, 7, 12, 17, 22, 27, and 32 °C, respectively. Seawater conditions during pre-exposure, ramping, and exposure periods are summarised in Annexe I.

1.2.4 Mortality assessment and dissection

Mortality was assessed at the end of the experimental phase (12 d). Clams were gently removed from the substrate, their siphons were prodded and the mantle was stroked following Kennedy and Mihursky (1971). Responsiveness was assessed visually based on the ability of individual clams to retract their siphons or adduct the shells around the exposed mantle. The absence of these responses was interpreted as death. Mortality was assessed in each tank and compiled for each treatment level (N = 656 mortality-survival measurements).

The surviving specimens were dissected on ice to obtain tissue samples for metabolomic analyses. Three individuals *per* species were processed from each experimental unit, resulting in 13 – 19 individuals per treatment for *M. truncata* and 12 – 21 individuals

per treatment for *M. arenaria*. Three tissues (i.e gill, mantle, and posterior adductor muscle – hereafter, "muscle") were dissected from each individual to assess tissue-specific metabolomic profiles. Tissues were excised, blotted to remove excess water, weighed, and immediately flash frozen in liquid nitrogen and stored at -80 °C for subsequent analyses.

1.2.5 Metabolite extraction

Metabolite extraction and quantification methodologies were adapted from Hsiao et al. (2018) and performed at Iso-BioKem Laboratories (Rimouski, QC, Canada). First, each sample was freeze-dried for 24 h at -50 °C. Each sample was then separated into two equal parts, weighed, and transferred into tissue homogenizing tubes containing 1.4 mm ceramic beads (Precellys 2 mL Soft Tissue Homogenizing Ceramic Beads Kit, Bertin Technologies SAS, Montigny-le-Bretonneux, France). Samples were then homogenized for 30 sec at 6000 rpm and -4 °C using a 3D tissue homogenizer (Precellys24, Bertin Technologies SAS, Montigny-le-Bretonneux, France). Metabolites were extracted separately in positive and negative phase stock solutions from the two parts of each sample, respectively. The positive phase solution contained 200 mM of ammonium formate (Amm Fm) at pH 3 in liquid chromatography – mass spectrometry grade H_2O (H_2O-MS). The negative phase solution contained 100 mM ammonium acetate (Amm Ac) at pH 9 in H_2O-MS. The internal standards (ISTDs) were phenylalanine-d_8 and fumarate-d_4 for positive and negative phases, respectively. The two sample parts were extracted from homogenized tissues by adding 1600 µL of 50:30:20 acetonitrile:isopropanol:phase stock solution + ISTD (1 µg mL^{-1}) to each tube and vortexing. The tubes were then centrifuged at 31 300 g for 5 min at 4 °C. A volume of 100 µL of the supernatant was transferred to a vial and stored at -80 °C until analysis by liquid-chromatography.

1.2.6 HPLC-QqQ-MS targeted analysis

Liquid chromatography – mass spectrometry analysis was performed using a high-performance liquid chromatographer (HPLC) (1260 Infinity II, Agilent Technologies, Palo

Alto, CA, USA) coupled with a 6420 Triple Quad mass spectrometer (1260 Infinity II, Agilent Technologies, Palo Alto, CA, USA). The column used in both positive and negative modes was the Agilent InfinityLab Poroshell 120 HILIC-Z column (2.7 μm, 100 x 2.1 mm) (Agilent Technologies, Palo Alto, CA, USA). In positive mode, metabolites were eluted from the column according to a gradient mobile phase containing positive phase A (20 mM Amm Fm pH 3, H2O-MS) and positive phase B (20 mM Amm Fm pH 3, 90:10 ACN: H2O-MS), with a flow rate of 500 uL min^{-1} and a sample injection volume of 10 uL. Column temperature was set at 30 °C. The positive linear gradient procedure was conducted as follows: 100 % phase B from 0 - 11.5 min, 70 % phase B from 11.5 – 12 min, and 100% phase B from 12 – 17 min. In negative mode, the gradient mobile phases contained negative phase A (10 mM Amm Ac, 5 μM deactivator pH 9, H2O-MS) and negative phase B (10 mM Amm Ac, 5 μM deactivator pH 9, 90:10 ACN: H2O-MS), with a flow rate of 250 μL min^{-1} and a sample volume of 10 uL. Column temperature was set at 30 °C. The negative linear gradient procedure was conducted as follows: 90 % phase B from 0 – 12 min, 60 % phase B from 12 – 16 min, and 90 % phase B from 16 – 24 min. Unlike in positive mode, these negative phase solutions contained 5 μM of InfinityLab deactivator additive (Agilent, CA, USA). Prior to negative phase analysis, a H_3PO_4 wash (0.5% H3PO4 90:10 ACN: H2O - ultra pure) was run through the pump at 5 mL min^{-1} for 5 min and through the system at 10 μL min^{-1} for ≥ 12 h.

The mass spectrometry electrospray ionization (ESI) parameters for positive and negative phases were identical for gas temperature (340 °C), gas flow (13 L min^{-1}), nebulizer (30 psi), capillary voltage (3500 V), scan type (multiple response monitoring), cycle time (500 ms), MS1 and MS2 resolution (unit), and cell accelerator voltage (7 V). Precursor ions, product ions, retention time, and QQQ parameters for each compound are summarized in Annexe II.

Metabolites were quantified by creating calibration curves designed with standards for each analysed metabolite. First, metabolite stock solutions with concentrations of 1 mg

mL $^{-1}$ were made with specific solvents (0.1% formic acid, 1 M HCl, or 0.1% NH_4OH) for each compound, and a given volume (50 or 500 uL) was frozen at -80 °C, then freeze-dried for 24 h at -50 °C. Metabolites and their corresponding solvents and volumes are summarized in Annexe III. After freeze-drying, 1 mL of extraction solvent with ISTD was added to the freeze-dried stock metabolite and vortexed. From this original concentration, a series of 5-fold dilutions was applied to obtain eight calibration concentrations. The resulting concentrations ranged from 6.4 to 5 10 e^5 ng mL^{-1} for the metabolites alanine, betaine, glycine, proline and sarcosine, and from 0.64 to 5 10 e^4 ng mL^{-1} for the remaining 42 metabolites. Quantification was performed using MassHunter QQQ quantitative analysis (Quant-my-Way) from Agilent Technologies. The metabolites *alanine*, *cystine*, *succinyl-coa*, *glucose*, *NADP*, and *NADPH* displayed more than 50 % missing values across all samples and were removed from analysis.

Stock metabolites were purchased from various manufacturers, including Sigma-Aldrich (St. Louis, MO, USA), Cayman Chem (Ann Arbour, MI, USA), and Cambridge Isotope Laboratories (Tewksbury, MA, USA). The manufacturers for each reagent are summarized in Annexe IV.

1.2.7 Bioinformatics and statistical analyses

All analyses were performed in R (v4.1.1) and RStudio (v1.4.1717) and plots produced with the *ggplot2* package (Wickham 2016).

Temperature, harvesting, and species effects on mortality were assessed in isolation and combined using a generalized linear mixed effects model (glmer) with the variables *temperature* (continuous), *harvest* (categorical), *species* (categorical) as main effects and the random *tank* effect of the experimental units assuming a binomial distribution of the dependant variable (mortality) and a *logit* link function using the *lme4* package (Bates et al. 2015). Goodness of fit was assessed using receiver operator curves with the *pROC* package (Robin et al. 2011). Assuming a non-linear temperature effect, mortality data was fit using a

multiple change points (*mcp*) model whereby temperature changepoints were estimated for each species according to the two-phase curve (plateau – changepoint – binomial curve) using the Bayesian-analysis *mcp* package (Lindeløv 2020). The difference between species' temperature changepoints was evaluated using Savage-Dickey estimates of the Bayes factor with the curves plotted based on the predicted values of the *mcp* models.

Metabolomics data were analysed in different phases. First, a permutation analysis of variance (PERMANOVA) was applied to all 481 samples to test the treatment effects on the response of 42 metabolites using the *adonis2* function (# of permutations = 999, distance method = Euclidean) in the *vegan* package (Oksanen et al. 2020). The fixed independent variables were assumed to be categorical for this analysis (*temperature, harvest, species*) with the *tank* effect as a random variable. The *temperature* treatment was considered as a categorical variable (contrary to the previous mortality analyses) to facilitate multivariate analysis and for ease of interpretation. Assumptions of multivariate homogeneity of group dispersion were evaluated using the *betadisper* function. This assumption was not met for the factors *species* ($F_{1, 479}$, $p < 0.001$) and *tissue* ($F_{2, 478}$, $p < 0.001$). Moreover, we report pseudo-replication in the data structure where tissues (*mantle*, *gill*, *muscle*) were nested within each specimen.

PERMANOVA analyses showed the factor *species* to account for a large proportion of the variation in the metabolome (Table 4). The three *tissue* datasets were thus further split according to *species*, to yield six datasets according to *tissue* × *species* combination and each dataset was treated separately. Prior to evaluating the effects of *temperature* and *harvest* on the metabolome, the missing data in each dataset was imputed according to a random forest method using the *missForest* package (Stekhoven and Bühlmann 2012). Each dataset was then centered log-ratio transformed (CLR) with the *mixOmics* package (Rohart et al. 2017) and centered and Pareto-scaled with the *MetabolAnalyze* package (Gift et al. 2010). The random effect of *tank*, the individual morphometrics (*length*, *width*, *height* in mm), and the

individuals' acclimation behaviour (*buried*) prior to the experiment were controlled by applying a linear mixed-effect model transformation to each metabolite using the *lmm2met* package (Wanichthanarak et al. 2019) to reduce the noise commonly associated with metabolomics studies. Once complete, multivariate normality (kurtosis and skew) of the transformed data was evaluated using the *semTools* package (Jorgensen et al. 2021). The normalizing effect of these transformations is observable in the frequency distributions of each metabolite before and after all steps (see Annexe V). The assumption of multivariate normality was not met before or after transformation in all datasets ($p < 0.05$). The transformation was nonetheless retained due to its beneficial normalizing effect on metabolite histogram distributions (Annexe V).

Transformed metabolite data were analyzed by principal component analysis (PCA) using singular value decomposition with the *prcomp* base function and the *screeplot* function to evaluate the proportion of variation among treatment groups explained by each component and to select the two first components in the models. The two first components explained most of the variation (> 50 % of total) and were plotted using the *ggplot2* package with the *temperature-*, *harvest-*, and the *interaction*-groupings overlaid for visual interpretation (frame type = 't'). If *temperature* groupings were distinctly separate from the 7 °C grouping, we considered there to be an effect of that treatment on the metabolome. If *harvest* groupings were distinctly separate, the same applied. Continuing along this rationale, and once distinct grouping were identified with PCA, a partial least squares discriminant analysis (PLS-DA) was applied using the *mixOmics* package. A k-fold approach and *Area Under the Curve* (AUC) of the receiver operating characteristic curve was used for model cross-validation with the *perf* function in the *mixOmics* package (5-fold, 50 repeats), and classification error rates plotted and evaluated using Mahalanobis and centroid distance measures. Models were not overfit as the classification error rate decreased consistently with each new component until it reached a plateau (Annexe VI). Additionally, the *auroc* function in the *mixOmics* package measured the model's ability to classify samples into their respective treatment

grouping using the AUC. The number of components sufficient to minimize the classification error rate and the AUC are reported with the main results. Generally, three or four components were sufficient to minimize classification error rate *per* the Mahalanobis distance metric (Annexe VI). Following model validation, PLS-DA results were plotted with three components in the projections. The metabolites most involved in group separation were extracted with variable importance in projection (VIP) scores according to the cut-off value (> 1) in every component using the *vip* function in the mixOmics package.

Once the upper temperature response of the metabolome was defined, significantly differentially expressed metabolites (SDMs) (i.e. those that varied significantly among temperatures) were identified. Metabolites that were significantly up- or down-regulated ($p < 0.05$) compared to the 7 °C treatment were selected. This was achieved using unpaired T-tests corrected for false discovery rate (FDR) with the *t_test* function in the *rstatix* package. Selected SDMs were then used for pathway analysis.

Pathway analysis was performed using the *FELLA* package, a diffusion-based algorithm that considers a list of SDMs and places them into knowledge-based biological networks that allow for pathway crosstalk (Picart-Armada et al. 2018). The algorithm was set to 10 000 iterations and output limited to 1000 nodes. A specific network was built by running the diffusion algorithm through a reference network with the input of SDMS tested against a background set of all metabolites assayed in the HPLC-QqQ-MS targeted analysis (with the exception of alanine which is currently undifferentiable from sarcosine). *Crassostrea gigas*, a model bivalve available in the Kyoto Encyclopedia of Genes and Genomes (KEGG; Kanehisa and Goto 2000) was used to build the reference network. In addition to housing the original input metabolites, the resulting network generated a list of significantly triggered metabolites, enzymes, reactions, modules, and pathways. The visual representation of this network was trimmed according to the elements of interest, namely the

input metabolites and pathways, and then improved aesthetically using Cytoscape (Shannon et al. 2003).

1.3 RESULTS

1.3.1 Mortality

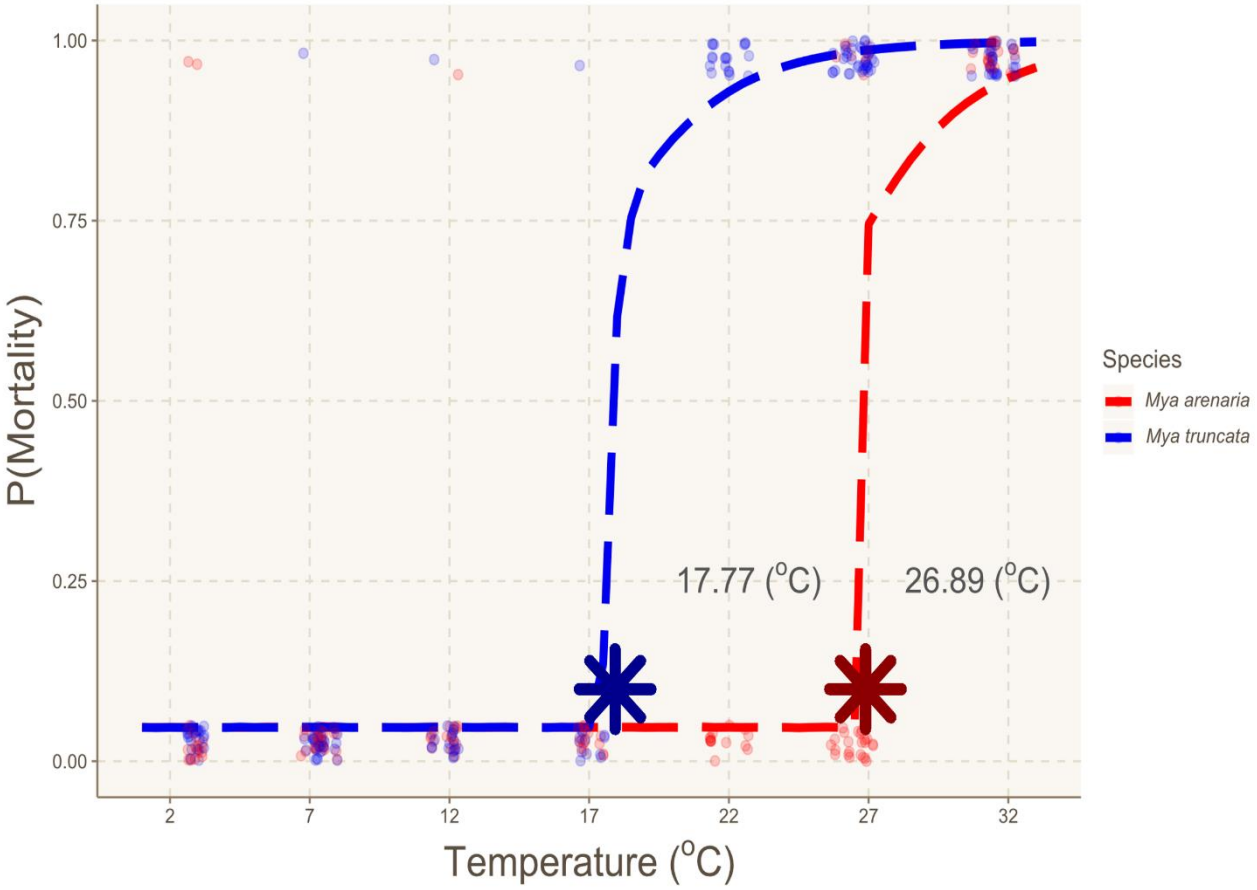
After 12 d of experimental exposure to simulated marine heatwaves, both *M. arenaria* and *M. truncata* experienced significant mortality. In total, 255 *M. arenaria* and 170 *M. truncata* survived exposure to the different temperature conditions, out of an initial 328 individuals *per* species. All clams died at the 32 °C treatment for the temperate species *M. arenaria* and at the 22 - 32 °C treatments for the polar species *M. truncata* (Annexe I). Low temperatures (2 – 17 °C) did not significantly affect mortality for either species (i.e. 100% survival), while a temperature increase beyond 27 °C for *M. arenaria* and 17 °C for *M. truncata* increased mortality considerably. This difference in thermal sensitivity

Table 1: Results of the generalized linear mixed-effects model (GLMER) testing the effects of the fixed variables temperature (T), harvest (H), species (SP), and their interactions on *M. arenaria* and *M. truncata* mortality (N = 656).

	Estimate	SE	Z-value	P_r (>|z|)
Intercept	**-10.22**	**2.47**	**-4.13**	**< 0.001**
T	**0.39**	**0.10**	**3.69**	**< 0.001**
H	2.39	2.87	0.83	0.41
SP	-7.05	4.31	-1.64	0.10
T:H	-0.10	0.13	-0.84	0.40
T:SP	**0.62**	**0.24**	**2.61**	**< 0.01**
H:SP	4.73	4.60	1.03	0.30
T:H:SP	-0.26	0.26	-1.02	0.31

Between species was supported by the presence of a significant *temperature* × *species* interaction (Z-value = 2.61, p = 0.009; Fig. 3, Table 1). Breakpoints for survival were 26.89 °C for *M. arenaria* and 17.77 °C for *M. truncata* (Fig. 3), a difference of 9.11 °C (Savage-Dickey Bayes = 0).

Figure 3: Probability mortality curves predicted for acute thermal shock ranging between 2 °C and 32 °C in *M. arenaria* and *M. truncata.* Curves and breakpoints (⁎) were estimated according to multiple change point (*mcp*) analysis (N = 656).



1.3.2 Metabolite profiles of *M. arenaria* and *M. truncata*

Species and *tissue* were the greatest sources of variation in metabolome profiles. *Temperature* was also a significant driver of metabolome variation in multivariate analyses

whereas the effect of *harvest* did not have consistent effects. We thus focus our results on temperature treatment responses separated by species and tissues.

Significantly up- or downregulated metabolites at temperatures approaching each species' upper thermal limit are presented in figure 4 and are summarized in table 2. Full metabolite level comparisons are available in Annexe VII. All 42 metabolites responded, to some extent, to temperature in *M. arenaria*, whereas in *M. truncata* only 36 metabolites responded to temperature treatments with the remainder (*fad, acetyl-coa*, *α-ketoglutarate*, *cis-aconitate, fumarate,* and *oxaloacetate*) showing stable levels. Significant variation in response to temperature was detected in *M. arenaria* tissues for 6 to 35 metabolites, with between 2 to 21 being downregulated and 4 to 23 upregulated across all tissues and temperature treatments (Tables 2 and 3). *Mya truncata* had between 6 to 21 metabolites that varied significantly among temperature treatments, with between 3 to 12 being downregulated and 3 to 12 being upregulated across all tissues (Tables 2 and 3).

Follow-up analysis of the effects of single variables and their interactions across all 481 clams and 42 metabolites (Table 4), revealed that tissue-specific metabolome responses differed uniquely by species, as indicated by the significant *tissue* × *species* interaction ($F_{2, 421} = 3.87$, $p < 0.01$). The effect of *harvest* on the metabolome varied as a function of *temperature*, as indicated by the presence of a significant interaction between *harvest* and *temperature* ($F_{5, 421} = 2.66$, $p < 0.001$). The model also reported effects of *species* ($F_{1, 421} = 129.83$, $p < 0.001$), *tissue* ($F_{2, 421} = 22.74$, $p < 0.001$), *temperature* ($F_{5, 421} = 5.44$, $p < 0.001$), and *harvest* ($F_{1, 421} = 0.15$, $p < 0.001$) in decreasing order of effect magnitude.

Multivariate PCA analyses showed separation among individuals by *temperature* treatment groups as temperature approached the breakpoint of each species (Fig. 5). For *M. arenaria*, the separation began at 22 and 27 °C with the first (PC1) and second (PC2) components explaining > 65 % of the variation in the metabolite levels for each tissue: 43.77 and 22.25% for mantle (N = 97), 43.74 and 27.82 % for gills (N = 96), and 38.69 and 28.45

% for muscle (N = 97). For *M. truncata*, the separation began at 17 °C with the first and second components explaining > 57 % of variation in the metabolite levels for each tissue: 36.48 and 23.81 % for mantle (N = 63), 35.84 and 28.32 % for gills (N = 64), and 42.41 and 15.51 % for muscle (N = 64). In contrast, the *harvest* groupings did not show a clear or consistent pattern of separation, and the overall effect appeared absent (Annexe VIII).

Table 2: Normalized and multivariate-transformed levels of individual metabolites near the upper thermal limit in *M. arenaria* (27 °C) and *M. truncata* (17 °C). Arrows indicate a significant ($p < 0.05$) increase (green up arrow) or decrease (red down arrow) in the metabolite level as compared to the 7 °C treatment according to unpaired t-tests with FDR correction. Metabolites from positive (POS) and negative (NEG) phase analysis are separate.

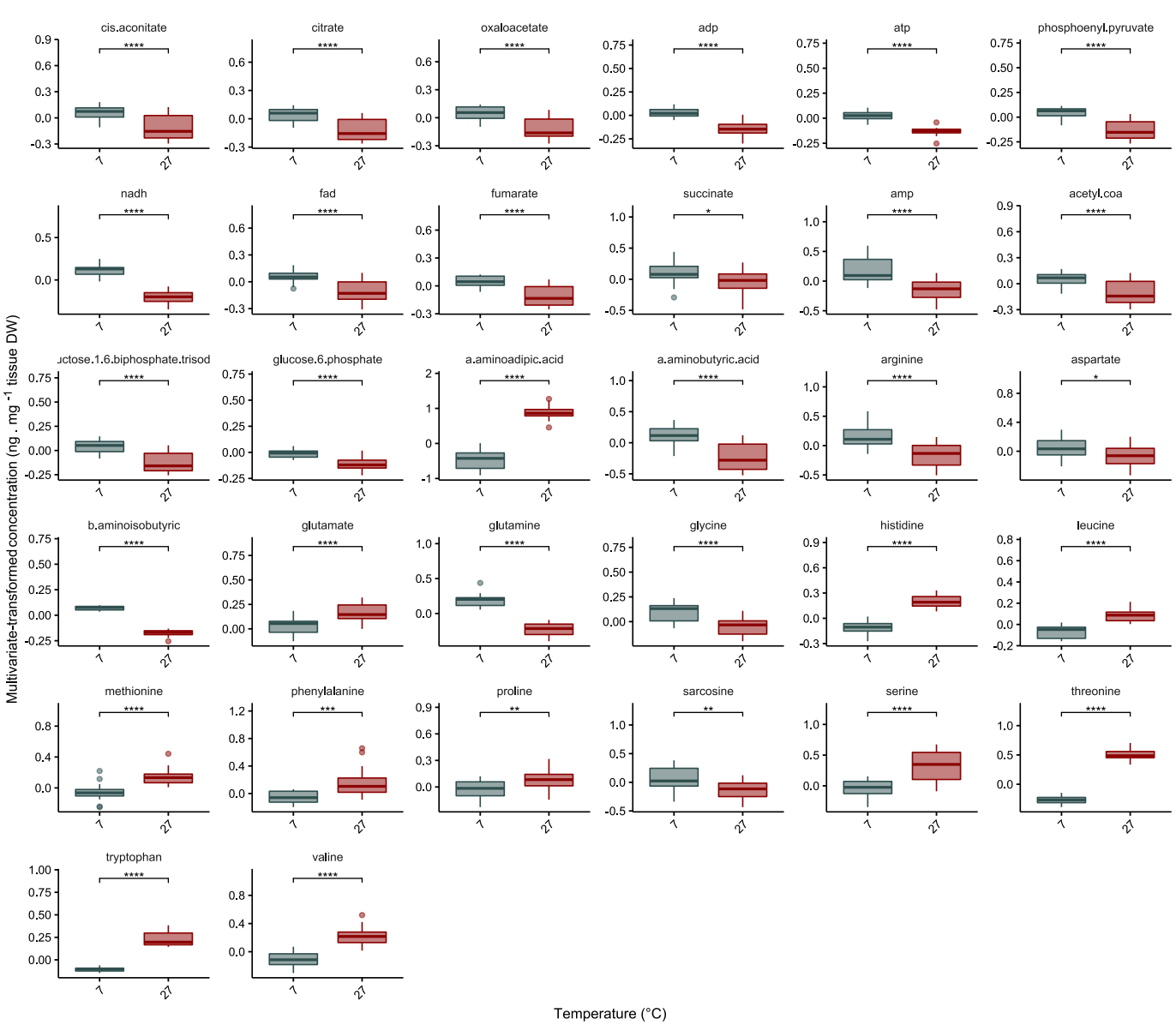
	SPECIES	*M. arenaria*			*M. truncata*		
	TEMPERATURE	27 °C	27 °C	27 °C	17 °C	17 °C	17 °C
	TISSUE	Gills	Mantle	Muscle	Gills	Mantle	Muscle
POS	α-aminoadipic.acid	↑↓	↑	↑↓	↑		
POS	α-aminobutyric.acid	↓		↓	↑		
POS	AMP	↓					↓
POS	arginine	↓	↑↓	↓	↑↓	↓	↓
POS	aspartate	↓	↓	↓	↓	↑	
POS	β-aminoisobutyric	↓	↓	↓		↑	↓
POS	betaine			↓		↓	↓
POS	FAD	↓	↓	↓			
POS	glutamate	↑		↓	↓		
POS	glutamine	↓	↓	↓	↓	↓	↓
POS	glycine	↓		↓	↓	↓	↓
POS	histidine	↑	↑	↑	↑		
POS	hydroxyproline		↓	↓		↑	
POS	isoleucine		↓			↑	↑
POS	leucine	↑	↓	↑		↑	↑
POS	lysine		↑	↑			↑
POS	methionine	↑					
POS	NAD		↓	↓	↓		↓
POS	phenylalanine	↑	↑	↑	↑	↑	↑
POS	proline	↑	↓	↓	↓	↑	↑
POS	sarcosine	↓		↓		↓	
POS	serine	↑	↑	↑	↑	↑	
POS	threonine	↑	↑	↑		↓	↓
POS	tryptophan	↑		↑	↓	↑	↑
POS	tyrosine			↑	↓	↑	↑
POS	valine	↑	↑	↑		↑	↑
NEG	acetyl.coa	↓	↓	↓			
NEG	ADP	↓	↓	↓	↓		
NEG	aketoglutarate		↓				
NEG	ATP	↓	↓		↓		
NEG	cis.aconitate	↓	↓	↓			
NEG	citrate	↓	↓	↓	↓		
NEG	d.fructose.1.6.biphos.trisod.	↓	↓		↓		
NEG	fumarate	↓	↓				
NEG	glucose.6.phosphate	↓	↓	↑			↓
NEG	lactate		↑	↑	↑	↑	
NEG	malate		↓	↑	↓		
NEG	nadh	↓		↓		↓	
NEG	oxaloacetate	↓	↓	↓			
NEG	phosphoenyl.pyruvate	↓	↓				
NEG	pyruvate			↑			↑
NEG	succinate	↓		↑	↓	↓	↓

Table 3: Summary of the number of significantly differentially expressed metabolites (SDM) in response to different temperature treatments across tissues of *M. arenaria* and *M. truncata*. U = upregulated, or more of the metabolite present, and D = downregulated, or less of the metabolite present relative to individuals held at 7 ^{o}C.

M. arenaria	2 ºC	12 ºC	17 ºC	22 ºC	27 ºC
Gills	7 U, 6 D	8 U; 5 D	9 U; 9 D	11 U; 9 D),	11 U; 21 D
Mantle	4U; 2D	13U; 8D	9 U; 4D	23U; 11D	9 U; 21 D
Muscle	8U; 8D	20U; 11D	10U; 9D	10U; 18D	15U; 19D
M. truncata					
Gills	10U; 6D	3U; 3D	9U; 12D	-	-
Mantle	5U; 5D	6U; 8D	12U; 8D	-	-
Muscle	5U; 6D	7U; 7D	9U; 10D	-	-

PLS-DA analyses indicated similar trends (Fig. 6) as those reported above for the PCA analyses. In *M. arenaria*, group separation occurred along the PC1 axes at temperatures as low as 22 °C for the gill and muscle and 27 °C for the mantle. The AUC measures for model classification at 22 and 27 °C were 0.6657 and 0.999, 0.793 and 0.998, and 0.778 and 0.984 for gill, mantle, and muscle, respectively. This indicates good model performance for *M. arenaria* tissues, especially at 27 °C. For *M. truncata*, group separation occurred along PC1 at 17 °C for all tissues. The AUC measure at 17 °C was ~1, 0.959, and 0.923 for gill, mantle, and muscle, respectively. This indicates good model performance for *M. truncata* tissues, especially at 17 °C. Metabolites with VIP scores > 1 were considered to contribute most to group separation in PC1, as reported in Table 5. On this basis, the following metabolites were involved in separating temperature treatments for all three *M. arenaria* tissues: a-aminoadipic acid, histidine, and threonine. The following metabolites were involved in separating temperature treatments based on VIP scores for all three *M. truncata* tissues*:* arginine, glycine, proline, and tyrosine. When comparing similar tissues between species, the VIP metabolites were: (i) a-aminoadipic acid, ATP, glutamine, histidine, and serine for gills;

A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adp
atp
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nadh
fad
fumarate
succinate
amp
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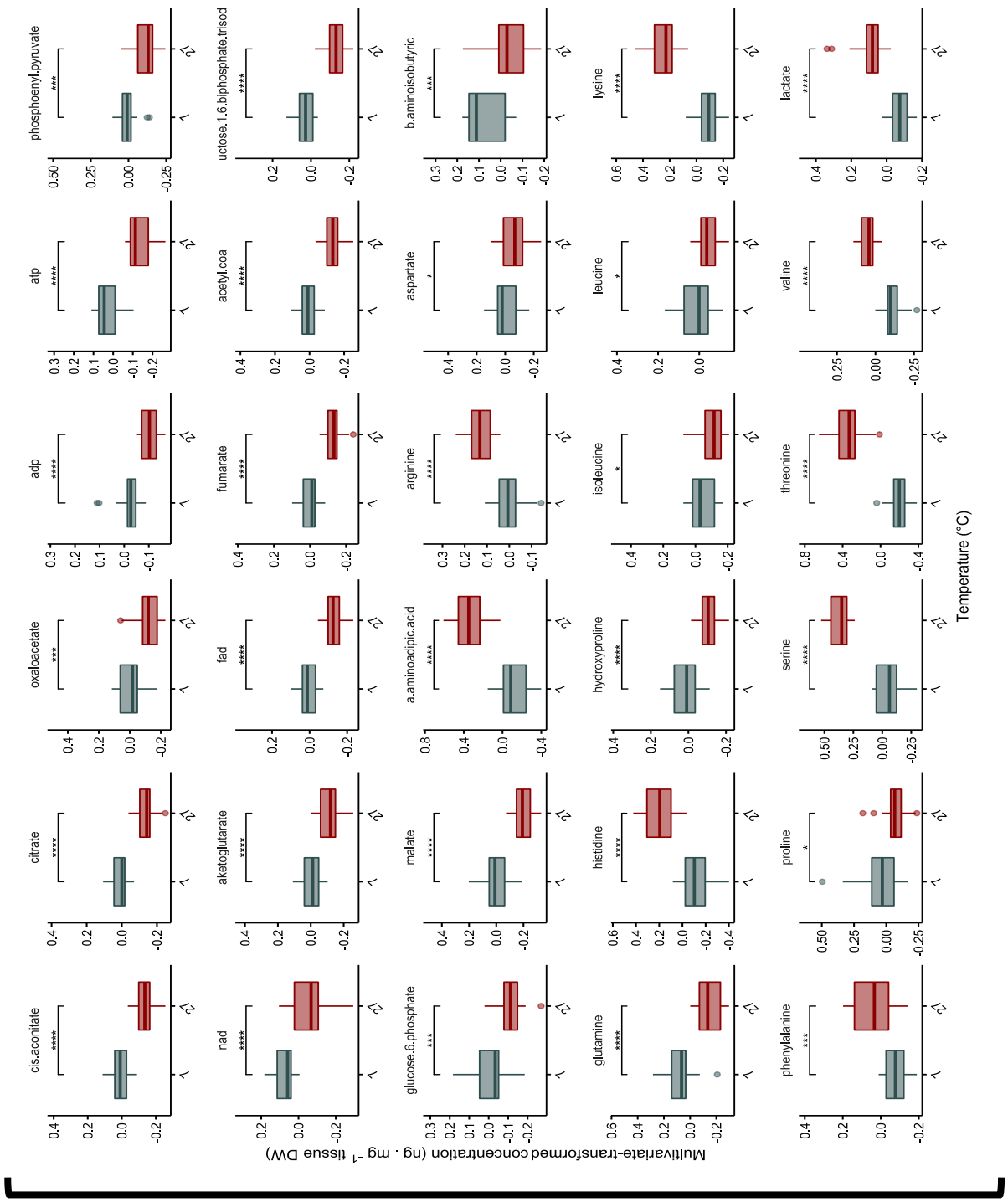
a.aminobutyric.acid
arginine
aspartate
b.aminoisobutyric
glutamate
glutamine
glycine
histidine
leucine
methionine
phenylalanine
proline
sarcosine
serine
threonine
tryptophan
valine
Multivariate-transformed concentration (ng . mg -1 tissue DW)
Temperature (°C)


B
Multivariate-transformed concentration (ng . mg -1 tissue DW)
Temperature (°C)
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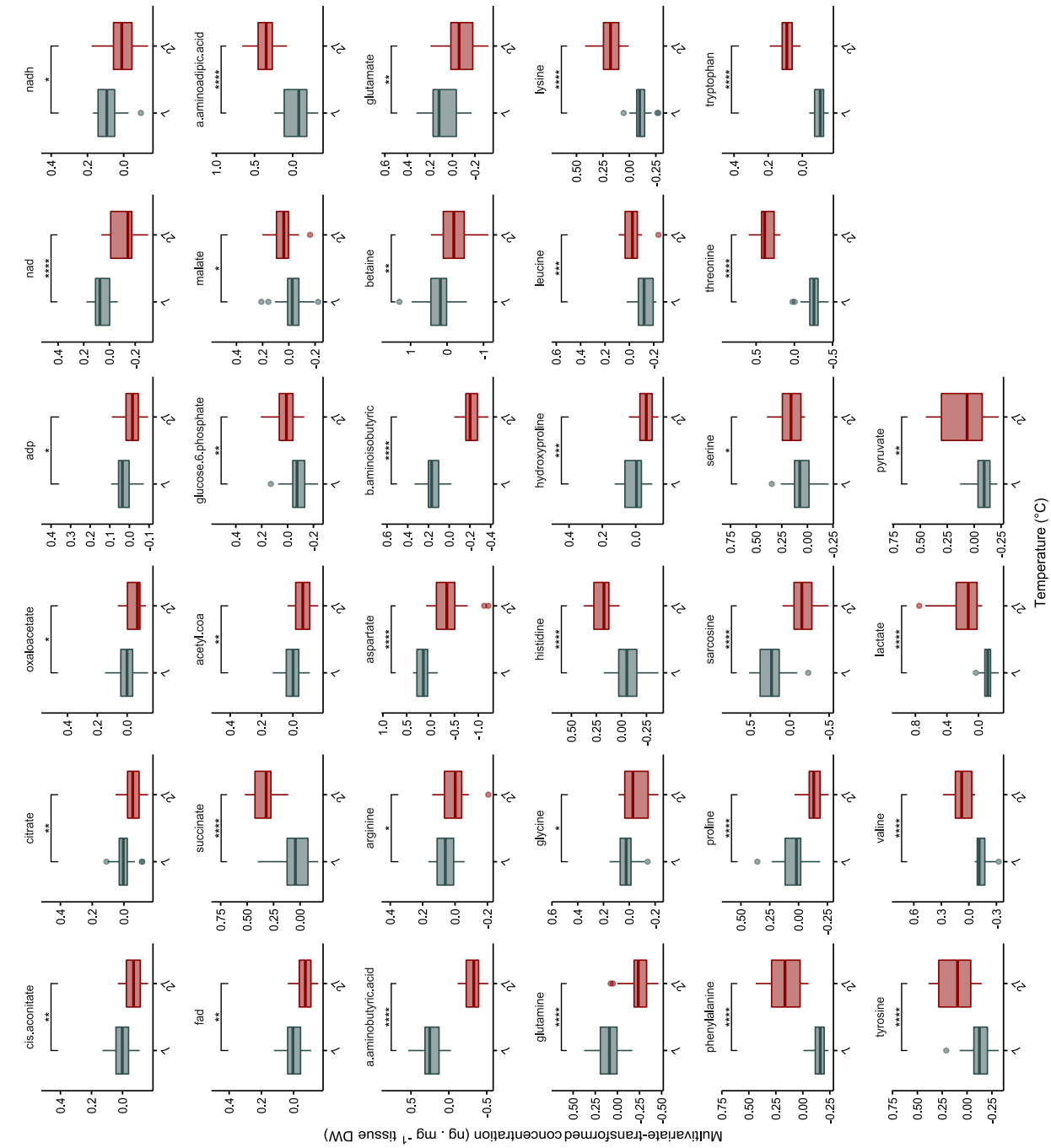
oxaloacetate
adp
atp
phosphoenyl.pyruvate
nad
aketoglutarate
fad
fumarate
acetyl.coa
uctose.1.6.biphosphate.trisod
glucose.6.phosphate
malate
a.aminoadipic.acid
arginine
aspartate
b.aminoisobutyric
glutamine
histidine
hydroxyproline
isoleucine
leucine
lysine
phenylalanine
proline
serine
threonine
valine
lactate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Multivariate-transformed concentration (ng . mg -1 tissue DW)
Temperature (°C)
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adp
nad
nadh
fad
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malate
a.aminoadipic.acid
a.aminobutyric.acid
arginine
aspartate
b.aminoisobutyric
betaine
glutamate
glutamine
glycine
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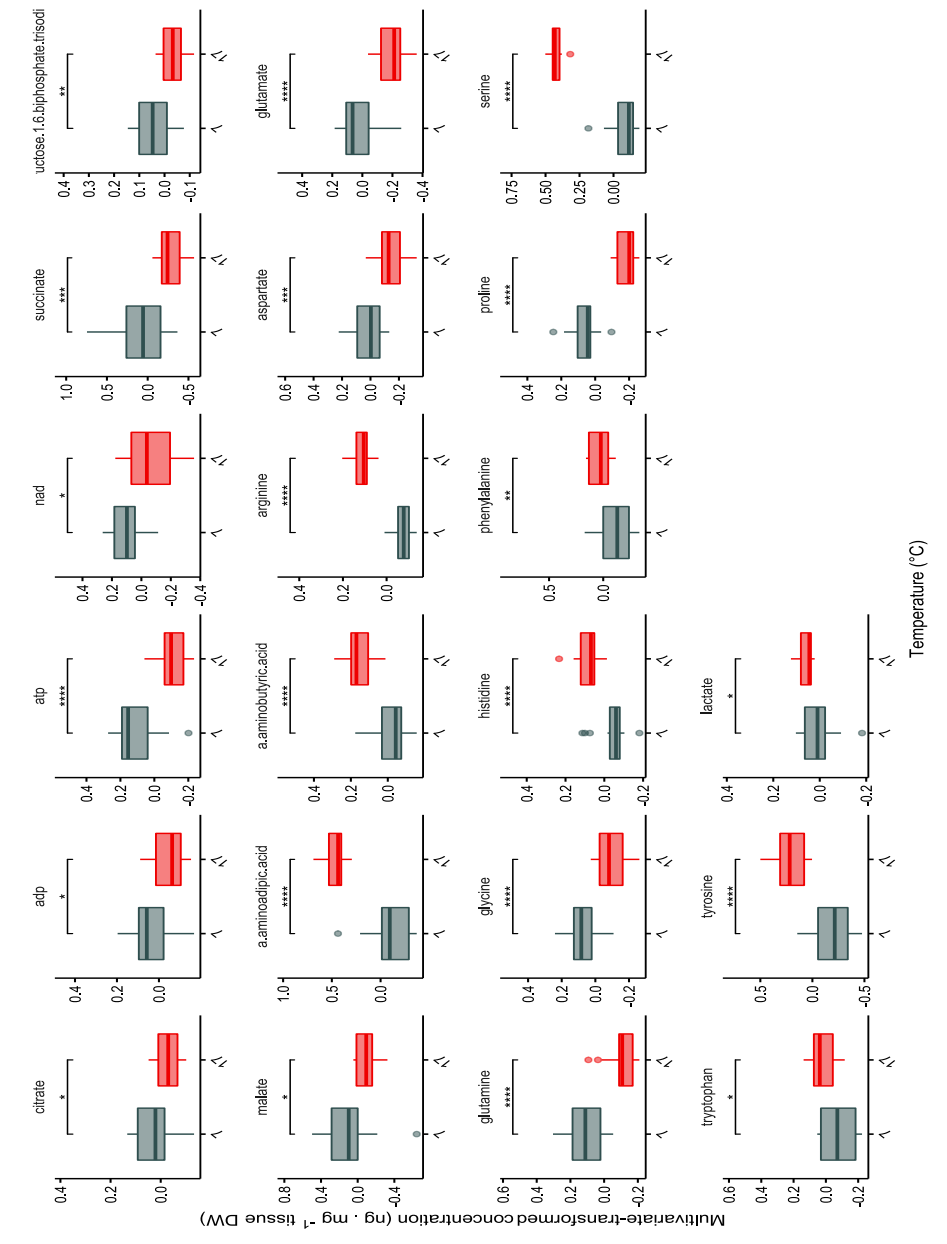
valine
lactate
pyruvate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Multivariate-transformed concentration (ng · mg -1 tissue DW)
Temperature (°C)
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atp
nad
succinate
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malate
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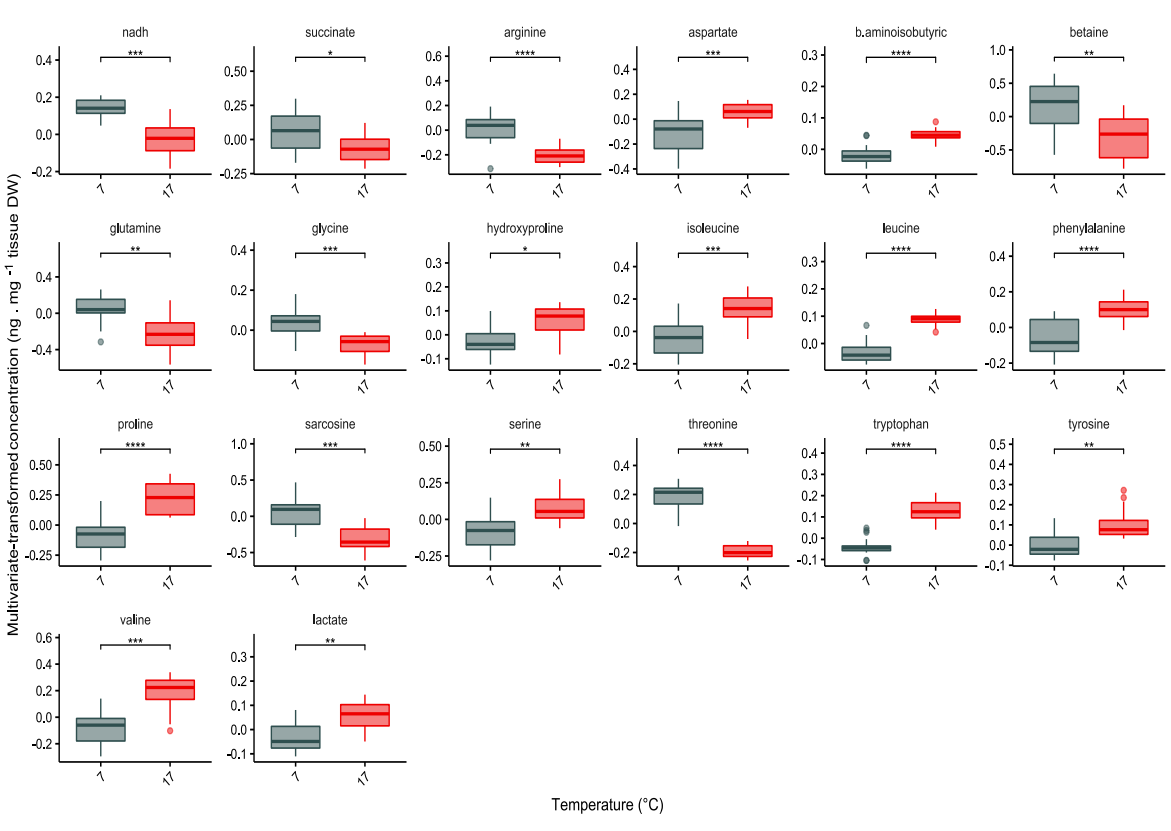
a.aminobutyric.acid
arginine
aspartate
glutamate
glutamine
glycine
histidine
phenylalanine
proline
serine
tryptophan
tyrosine
lactate

E


nadh
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glycine
hydroxyproline
isoleucine
leucine
phenylalanine
proline
sarcosine
serine
threonine
tryptophan
tyrosine
valine
lactate
Multivariate-transformed concentration (ng . mg -1 tissue DW)
Temperature (°C)
7
17

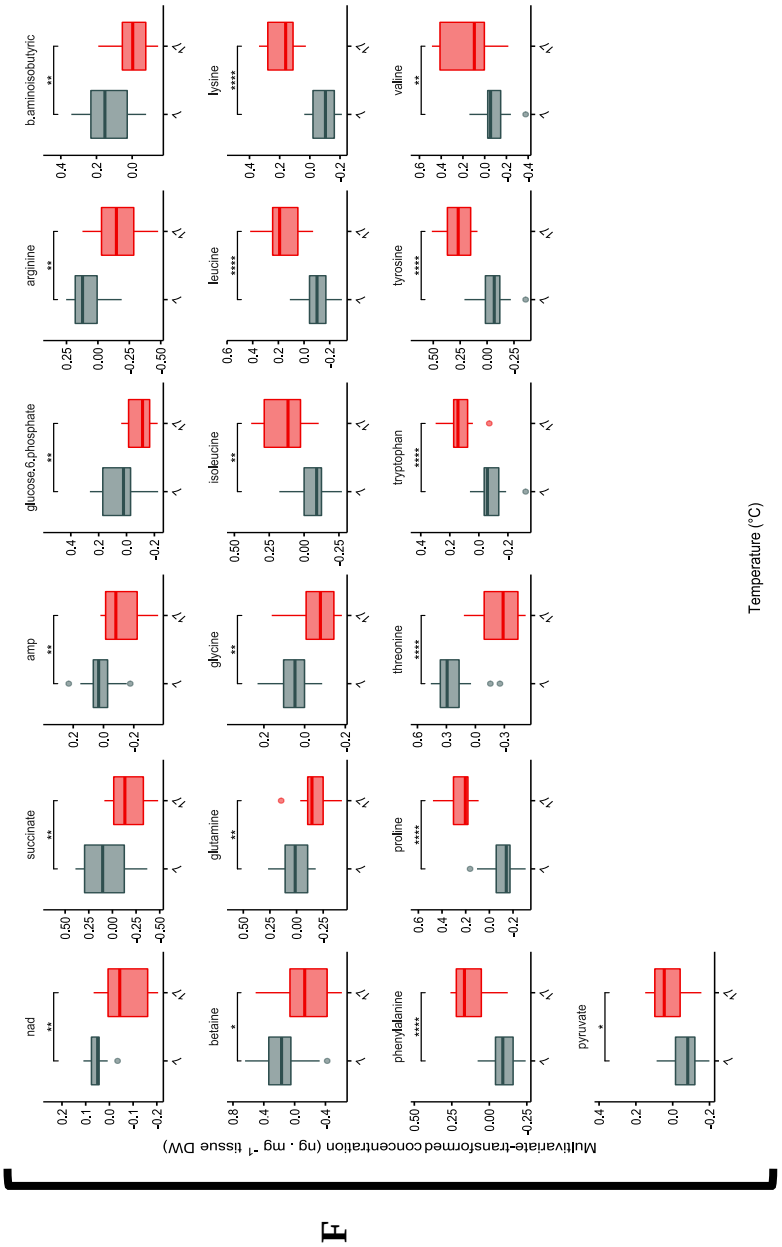


Figure 4: Boxplots of normalised and multivariate-transformed significantly differentially expressed metabolite levels ($p < 0.05$) at the upper thermal limit treatment of *M. arenaria* tissues (27 °C) and in *M. truncata* tissues (17 °C). Gills, mantle, and adductor tissues are reported in panels A, B and C for *M. arenaria* and D, E, and F for *M. truncata*. Significance levels (*, **, ***, ****: < 0.05, < 0.01, < 0.001, < 0.0001) of treatments were tested against the 7 °C treatment according to unpaired t-tests with FDR correction over all temperature comparisons .

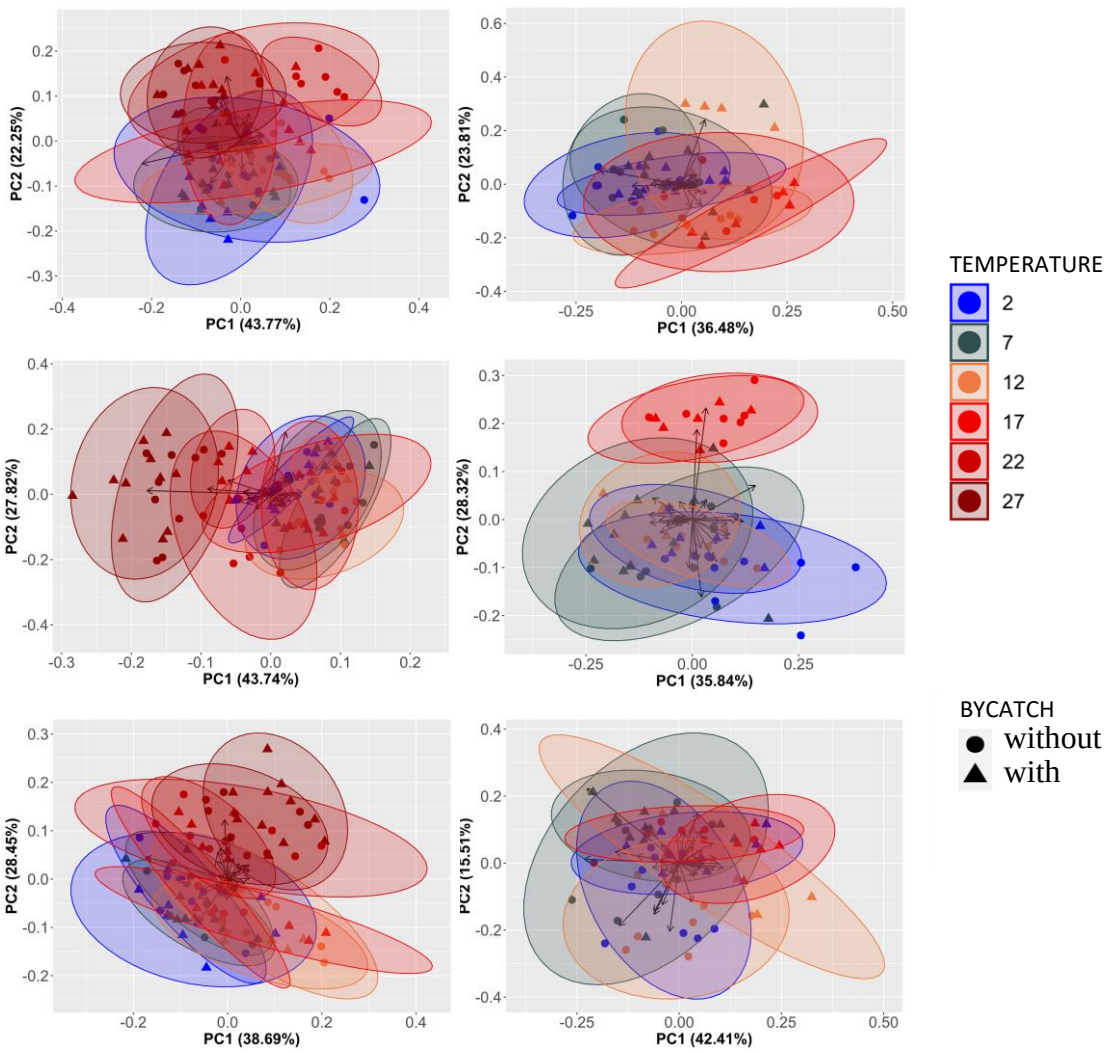


Figure 5: Principal component analyses (PCA) plots displaying the PC1 and PC2 of the targeted metabolome (42 metabolites) separately for different tissues (i.e. mantle, gills, and posterior adductor muscle) in *M. arenaria* (top-left, middle-left, bottom-left) and *M. truncata* (top-right, middle-right, bottom-right). Each plot displays the grouping variable *temperature* (2, 7, 12, 17, 22, and 27 °C, if available) and *harvest* (with, without).

(ii) arginine, serine, and threonine for mantle; and (iii) lysine, NAD, phenylalanine, proline, threonine, tryptophan, and valine for muscle. Full VIP score results for all components are reported in Annexe IX.

Table 4: Results from the permutation analysis of variance (PERMANOVA) with the effects of the fixed variables temperature (T), harvest (H), species (SP), tissue (TS), and their interactions on *Mya* clam metabolome (42 metabolites) (N = 481). Analysis metrics such as degrees of freedom (df), R^2 values, F-statistic value, and associated *P*-values for each treatment term are reported, with significant results highlighted in bold text.

	df	R^2	*F*-value	*P-value*
T	**5**	**0.04**	**5.44**	**< 0.001**
TS	**2**	**0.07**	**22.74**	**< 0.001**
SP	**1**	**0.19**	**129.83**	**< 0.001**
H	**1**	**0.00**	**0.15**	**0.303**
T:TS	10	0.02	1.15	0.197
T:SP	3	0.00	1.01	0.380
T:H	**5**	**0.02**	**2.66**	**< 0.001**
TS:SP	**2**	**0.01**	**3.87**	**< 0.010**
TS:H	2	0.00	1.26	0.239
SP:H	1	0.00	0.52	0.558
T:TS:H	10	0.01	0.53	0.907
T:TS:SP	6	0.00	0.36	0.962
T:SP:H	3	0.01	1.48	0.172
TS:SP:H	2	0.00	0.20	0.937
T:TS:SP:H	6	0.00	0.43	0.922

1.3.3 Selection of candidate metabolites responding to temperature

A list of significantly differentially expressed metabolites (SDMs) was extracted from the upper temperature treatments in each species. These SDM were up- or downregulated at 27 °C in *M. arenaria* and at 17 °C in *M. truncata* (i.e the upper temperature threshold for each species, respectively) with significantly different levels (FDR-corrected $p < 0.05$) (Table 2). A list of SDM was retrieved for each species' tissue and used in pathway analysis.

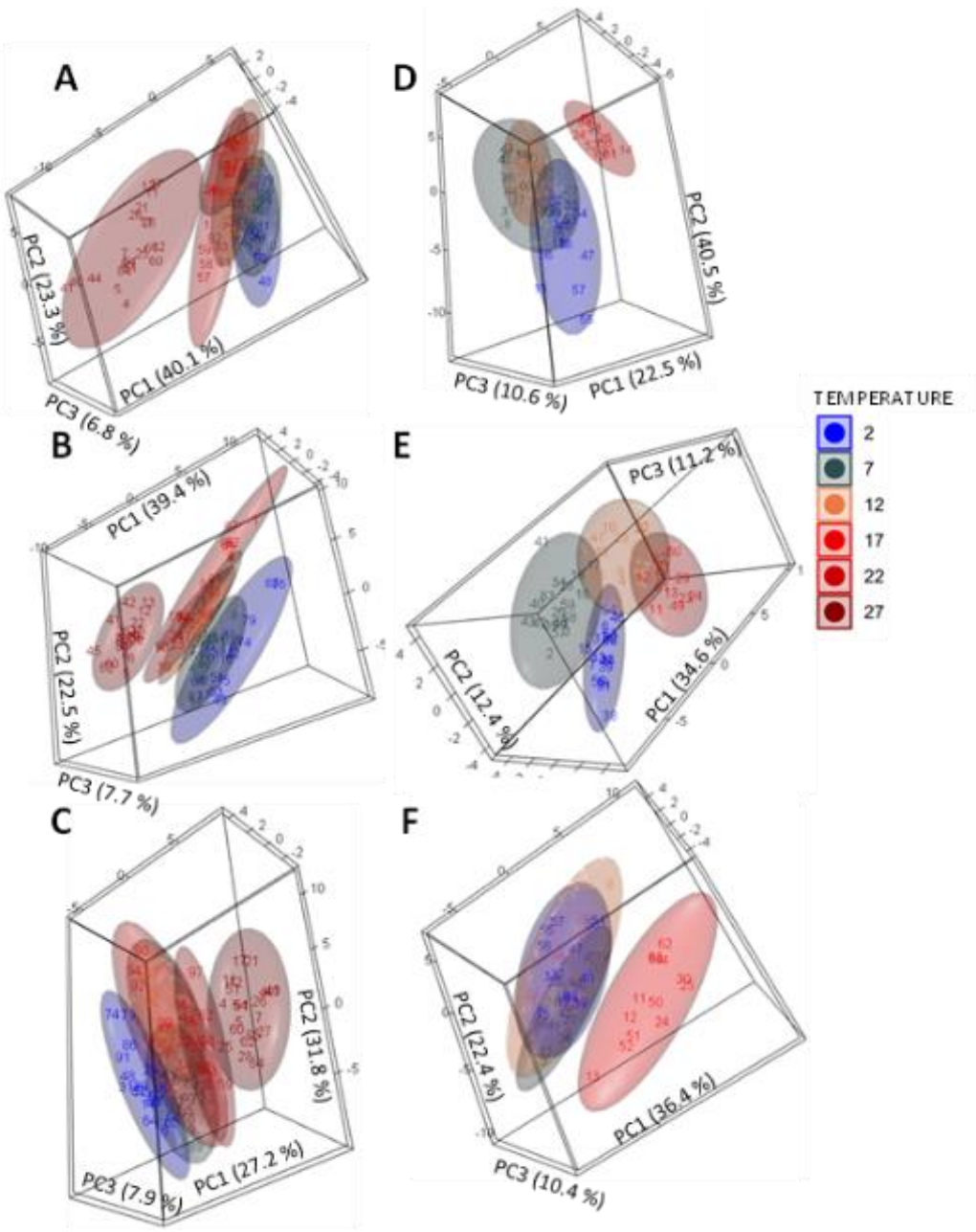


Figure 6: Partial least squares – discriminant analysis (PLS-DA) plots including the three first principal components (% of variation explained) for different temperature treatments in the gills, mantle, and muscle tissues of *M. arenaria* (**A**, **B**, and **C**) and *M. truncata* (**D**, **E**, and **F**).

Table 5: List of most impactful metabolites emerging from the PLS-DA analysis according to VIP scores (> 1) extracted from PC1. Metabolites are organized according to similarities between tissues within and across species' tissues. VIP scores are included in brackets (for full output, see Annexe IX).

	M. arenaria			*M. truncata*		
	Gills	Mantle	Adductor	Gills	Mantle	Adductor
Within species	α-aminoadipic.acid (1.58) threonine (1.67) histidine (1.44)	α-aminoadipic.acid (1.16) threonine (1.36) histidine (1.13)	α-aminoadipic.acid (1.45) threonine (1.79) histidine (1.26)	arginine (1.7) glycine (1.06) proline (2.07) tyrosine (1.48)	arginine (1.79) glycine (1.49) proline (1.5) tyrosine (1.77)	arginine (1.54) glycine (1.13) proline (2.06) tyrosine (1.9)
Gills	α-aminoadipic.acid (1.58) ATP (1.34) glutamine (1.37) histidine (1.44) serine (1.23)			α-aminoadipic.acid (2.1) ATP (1.02) glutamine (1.18) histidine (1.58) serine (2.13)		
Mantle		arginine (1.03) serine (1.47) threonine (1.36)			arginine (1.79) serine (1.13) threonine (2)	
Muscle			lysine (1.67) NAD (1.17) phenylalanine (1.38) proline (1.31) threonine (1.79) tryptophan (1.55) valine (1.18)			lysine (1.6) NAD (1.41) phenylalanine (1.72) proline (2.06) threonine (1.85) tryptophan (1.84) valine (1.57)
Remaining	ADP (1.36) β-aminoisobutyric (1.6) citrate (1.1) d.fructose.1.6.biphosphate.trisodium (1.18) glucose.6.phosphate (1.2) leucine (1.13) NADH (1.47) oxaloacetate (1.01) phenylalanine (1.01) phosphoenyl.pyruvate (1.23) tryptophan (1.62) valine (1.36)	acetyl.coa (1.42) ADP (1.52) aketoglutarate (1.17) ATP (1.49) cis.aconitate (1.4) citrate (1.42) d.fructose.1.6.biphosphate.trisodium (1.4) FAD (1.46) fumarate (1.46) glucose.6.phosphate (1.31) hydroxyproline (1.22) lysine (1.51) malate (1.41) phosphoenyl.pyruvate (1.09)	α-aminobutyric.acid (1.47) aspartate (1.53) β-aminoisobutyric (1.65) cis.aconitate (1.02) FAD (1.06) glutamine (1.15) hydroxyproline (1.05) lactate (1.2)	α-aminobutyric.acid (1.87) aspartate (1.24) glutamate (1.75) succinate (1.27)	betaine (1.25) isoleucine (1.48) lactate (1.11) leucine (1.21) methionine (1.03) phenylalanine (1.62) sarcosine (1.61) tryptophan (1.73) valine (1.5)	AMP (1.15) betaine (1.02) isoleucine (1.46)

1.3.4 Metabolic pathways responding to temperature

Based on species- and tissue-specific networks constructed using SDMs associated with the upper temperature response (Table 2), 13, 24, and 5 pathways were found in *M. arenaria* and 7, 7 and 3 pathways in *M. truncata* for mantle, gills, and muscle tissue, respectively (Table 6). Interestingly, comparison across tissue-specific networks between species revealed that the endocytosis pathway was uniquely altered in the gill tissue of both *M. arenaria* and *M. truncata.* Although species- and tissue-specific networks were built for all temperature treatments using the SDMs at those respective temperatures (Annexe X), these are not elaborated on in the results.

Table 6: Summary of significantly altered pathways ($P < 0.05$) at the upper temperature treatment in mantle, gill, and muscle tissues of *M. arenaria* (27 °C) and *M. truncata* (17 °C) based on a knowledge-based network and diffusion algorithm (*FELLA*).

M. arenaria		
Mantle	Gills	Muscle
Autophagy - animal	Autophagy - animal	Arginine and proline metabolism
Autophagy - other	Autophagy - other	β-Alanine metabolism
Basal transcription factors	Basal transcription factors	D-Amino acid metabolism
Endocytosis	DNA replication	Glycine, serine, and threonine metabolism
Fanconi anemia pathway	Endocytosis	Neuroactive ligand-receptor interaction
Homologous recombination	Fanconi anemia pathway.	
Mitophagy - animal	FoxO signaling pathway	
Nucleocytoplasmic transport.	Homologous recombination	
Phagosome	Mismatch repair	
Phosphatidylinositol signaling system	Mitophagy - animal	
Spliceosome	mRNA surveillance pathway	
TGF-beta signaling pathway	mTOR signaling pathway.	
Wnt signaling pathway	Non-homologous end-joining	
	Nucleocytoplasmic transport	
	Nucleotide excision repair	
	Phosphatidylinositol signaling system	
	Purine metabolism	
	Ribosome biogenesis in eukaryotes	
	RNA degradation	
	RNA polymerase	
	Spliceosome	
	TGF-β signaling pathway	
	Ubiquitin mediated proteolysis	
	Wnt signaling pathway	
M. truncata		
Mantle	Gills	Muscle
ABC transporters	Arginine biosynthesis	ABC transporters
Aminoacyl-tRNA biosynthesis	Biosynthesis of amino acids	Aminoacyl-tRNA biosynthesis
Arginine and proline metabolism	Endocytosis	Valine, leucine, and isoleucine biosynthesis
D-Amino acid metabolism	Neuroactive ligand-receptor interaction	
Glycine, serine, and threonine metabolism	Phenylalanine, tyrosine, and tryptophan biosynthesis	
Phenylalanine, tyrosine, and tryptophan biosynthesis	Taurine and hypotaurine metabolism	
Valine, leucine, and isoleucine biosynthesis	Thiamine metabolism	

1.4 DISCUSSION

This study reports striking differences in heatwave tolerance and metabolomic plasticity between two partly sympatric clam species with different climatic niches, and negligible impact of harvesting disturbance to the degree that it was tested here. These findings have important implications for predicting future biogeographic distributional shifts in changing coastal waters and provide indicators of management strategies with regards to the coinciding of extreme climatic stressors (i.e. heatwaves) and direct human stressors (harvesting disturbance). By integrating mortality and metabolomics analyses, our results suggest that the blunt gaper (*M. truncata*) is more vulnerable to heatwaves than its congener the softshell clam (*M. arenaria*) by nearly 9 °C in peak heatwave intensity and that this difference may be rooted in its cellular physiology. It appears that both species undergo unique metabolic remodeling near their respective thermal limits to withstand short-term environmental temperature extremes. We expand our discussion through a mechanistic interpretation of the observed metabolomic changes by focusing on significant pathway changes detected through a knowledge-based network analysis of different clam tissues. We then discuss the likelihood that tissue-specific metabolic pathways underpin the observed differences in whole-organism thermal stress responses.

1.4.1 Mortality

The observed difference in the upper heatwave tolerance for these species has important ecological implications related to the breadth of their suitable thermal niches and thus their potential global redistribution under ocean warming. *Mya arenaria* was expected to exhibit a wider thermal niche considering its broad biogeographic distribution (Southern Florida – Canadian Maritimes, Canadian Pacific Coast, Northern Europe), while *M. truncata* solely inhabits cooler Arctic and sub-Arctic waters. While no study had previously assessed the upper thermal limit of *M. truncata*, only one study reported the LC50 (24 h) in *M. arenaria* to range from 30.1 to 32.5 °C, indicating that upper thermal limits would be lower

with a longer duration of exposure, as one would expect based on the concept that stress is the integral of intensity and duration of exposure to unfavorable conditions. As thermal limits help define species biogeography (Pörtner and Knust 2007; Calosi et al. 2010; Bozinovic et al. 2011), our results suggest that against a backdrop of future heatwave intensification *M. truncata* may undergo severe population decline under recurrent heatwave events at its southern edge with the potential for local extinction. Examples of such range contractions are also projected for numerous polar invertebrates (Alabia et al. 2018). While our mortality results are useful to define population reduction due to heatwaves and the threat of local population extinction, sub-lethal impacts of heatwaves are also important for predicting potential reductions in individual performance that may influence individuals' fitness and ultimately population dynamics (Beckerman et al. 2002). Sub-lethal impacts of thermal stress can be best characterised by an organism's physiological responses to heatwave exposure nearing a species' tolerance limit.

1.4.2 Metabolome

The exposure of individuals from these two clam species to a simulated heatwave elicited physiological changes at the cellular level that could ultimately affect individual performance and survival. The observed shifts in metabolome profiles with increased temperature in this study suggest a relatively effective coordinated response to heatwaves across several tissues, with distinct molecular signatures associated with species- and tissue-specific responses. Over the range of heatwaves tested, *M. arenaria* displayed a more extensive cellular response as evidenced by metabolome changes at multiple sub-lethal temperature levels, while *M. truncata* exhibited a more limited ability to adjust its metabolome at more extreme temperatures. With the exception of [α aminoadipic-acid], [ATP], and [NAD+], the metabolites most engaged in the species-specific temperature response and tissue-specific responses were amino acids. A growing body of evidence suggests that amino acid metabolism may operate at several levels of stress response to supply cells with bioenergetic substrates (Zurburg and De Zwaan 1981; Wood 2001), to be

regulated for protein turnover (Houlihan 1991; Wood 2001), to form antioxidant defenses against reactive oxygen species (ROS) (Atmaca 2004; Newsholme et al. 2012), and to provide immunological signaling and protection (Grohmann and Bronte 2010; McGaha et al. 2012; Huang et al. 2020). While amino-acid concentrations may be sourced from diet, we will only consider the enzymatic processes regulating their concentrations in the context of cellular stress response.

Concentrations of histidine, a metabolite involved in antioxidant responses (carnosine and anserine; Kohen et al. 1988; Chen et al. 2021; Wang et al. 2021) and inflammatory signalling (histamine; Novák and Falus 1997; Delitheos et al. 2010), significantly increased in all *M. arenaria* tissues and in the gills of *M. truncata.* Heatwave stress may require higher antioxidant activities and inflammatory signalling, which appear to be most engaged in *M. arenaria* and only slightly apparent in *M. truncata* as indicated by pathway analysis.

Glycine, serine, and threonine are metabolites that interact closely through the action of several enzymes and reflect changes in associated methylamines such as sarcosine and betaine (Meister 1965) that were also measured in this study. Our study highlights that changes in glycine, serine, and threonine concentrations play a role in defining differences in species' susceptibility to heatwaves. These metabolites provide energy substrates (Meister 1965), antioxidant protection (Meister and Anderson 1983; Hu et al. 2015), or improved membrane fluidity (Zwingelstein et al. 1998; Chung et al. 2018) mainly by increasing the serine pool.

Branched-chain amino-acids (BCAAs), which include isoleucine, leucine, and valine, accounted for significant changes in certain tissue metabolomes for each species. In both *M. arenaria* and *M. truncata*, the experimental heatwave conditions elicited an increase in valine concentrations of the adductor muscle. BCAAs increased with heatwave intensity in all tissues except in *M. arenaria* mantle, where isoleucine and leucine levels dropped. Changes in BCAA concentrations following high temperature stress have been previously reported in

aquatic ectotherms (Xu et al. 2017; Wang et al. 2019), although the exact mechanisms responsible for these changes are still poorly understood. The catabolism of these amino acids may provide valuable energy substrates such acetoacetate, acetyl- and succinyl-CoA (Chuang 2013), thus (although only temporarily) providing some respite for heatwave-exposed organisms.

Proline, along with hydroxyproline, glutamate, glutamine, arginine, and ornithine (not measured), are interrelated amino acids involved in common pathways (Meister 1965), and three of these were found to contribute significantly to the metabolome heatwave response. In the adductor muscle, proline concentrations were found to decrease in *M. arenaria* and increase in *M. truncata*. Furthermore, both proline and arginine concentrations are impacted in all *M. truncata* tissues, yet they vary in the exact opposite direction. Similarly, proline and glutamate concentrations both decrease in *M. truncata* gills but increases in *M. arenaria* gills. This suggests that these amino acid pairs are linked but that each functions differently across tissues and species. Proline and glutamine may confer alternate sources of energy production under stressful conditions (Phang and Liu 2012) and contribute to general stress response (Natarajan et al. 2012; Liu and Phang 2012), and may be key actors in heatwave response and show evidence of species-specific abilities to withstand environmental changes.

Tyrosine and phenylalanine showed changes under heatwave conditions and are discussed together due to their shared pathway. Phenylalanine significantly altered the metabolome profile of adductor muscle tissue in both species, while tyrosine altered all tissue metabolome profiles in *M. truncata*. These amino acids can be converted to fumarate or acetoacetate, making them either glucogenic or ketogenic. Given previously observed patterns in tissue expression of tyrosine (Nagatsu et al. 1964; Hirashima et al. 2000; Zhang et al. 2014), we suspect that the alternate role of this amino acid in the production of catecholamines (i.e. adrenaline, noradrenaline, dopamine) may confer tolerance to

heatwaves. Gill tissue in *M. truncata* could be unable to supply sufficient substrates for catecholamine production, hampering the effectiveness of its stress response, while *M. arenaria* shows no tyrosine concentration changes in its gills. Differences between species in the efficiency and expression levels of enzymes surrounding catecholamine production could explain the observed differences in heatwave tolerance in these two *Mya* species. Observed phenylalanine concentration increases may indicate stressed individuals were attempting to elevate or maintain dopamine production, implying that organisms that can attain higher dopamine levels may have higher survival chances, albeit with accompanying physiological costs.

The metabolism of the essential amino acid tryptophan and of nicotinic acid (NAD^+) exhibited consistent behaviour in the clam adductor muscle whereby the former increased while the latter decreased with increasing heat stress. The catabolism of tryptophan is dictated by the requirement of two pathways, where the kynurenine pathway produces energy substrates and NAD^+, and the serotonin pathway produces valuable stress-sensitive neurotransmitters (Yao et al. 2011). The relative contribution of tryptophan to either the kynurenine or serotonin pathway could be assessed by dosing the activity of the rate-limiting enzymes of each. That tryptophan concentrations decreased in *M. truncata* gills suggests that these pathways do not keep pace with the tissue needs for NAD+ or protective neurotransmitters in this species.

Lysine is the final amino acid that showed shared behaviour between species. Its levels increased in the adductor muscle and may point to its ketogenic nature because lysine degradation produces acetyl-CoA through the saccharopine pathway (Higashino et al. 1971), ultimately forming α-aminoadipic acid. This metabolite, α-aminoadipic acid, responded significantly to heatwave stress in all *M. arenaria* tissues and in *M. truncata* gills. This intermediary metabolite has been studied as a marker for protein oxidation (Sell et al. 2007; Lee et al. 2019) and shows a pro-oxidant behaviour (Estaras et al. 2020). this suggesting that

M. arenaria tissues undergo significant oxidative stress, while *M. truncata* shows oxidative stress primarily in its gill tissue. This may indicate that *M. arenaria* experiences more oxidative stress under severe marine heatwaves or that *M. truncata* gills are an area with particularly high localized oxidative stress.

Temperature stress likely induced the observed metabolome shift in the gill tissue of both species, with adenosine triphosphate (ATP) as a significant player. ATP levels decreased in gills, and are known to respond to environmental stress, accompanied by decreased adenosine diphosphate (ADP) concentrations (Vetter and Hodson 1982; Calderwood et al. 1985; Caldwell and Hinshaw 1994), consistent with the effects seen here on clams at their respective upper heatwave limits. Decreased concentrations of ATP and ADP suggest metabolic reduction and eventually depression at higher temperatures (Pörtner et al. 1998; Sokolova 2013). Exposed to repeated sub-lethal heatwave events, invertebrates would be expected to accumulate negative effects in invertebrates which may hamper reproductive success at the population level (Sokolova et al. 2012).

1.4.3 Network Analysis

While individual metabolites may serve as biomarkers for heatwave responses, network analyses integrate them and provides a mechanistic understanding of cellular stress responses. Within tissues, some pathways may be grouped into general functional classes: cell signalling, structural turnover, DNA repair and expression, and metabolism. Strikingly, our study did not detect any pathways related to cell signaling in *M. truncata* response to heatwave stress, in contrast to *M. arenaria* that relies heavily on these functions. This suggests that, although these species are phylogenetically closely related, they share no overlap in these cellular functions in response to marine heatwaves.

The only common pathway affected by the heatwave challenge, found across similar tissues, was the endocytosis pathway expressed in gill tissue. This pathway has a unique role in membrane remodelling (Moyes and Schulte 2015) and has demonstrated increased activity

with higher temperatures in fish (Røsjø et al. 1994; Padrón et al. 2000). Endocytosis may also function to recycle defective membrane-bound proteins under heat-stress (Foot et al. 2017; López-Hernández et al. 2020), with corresponding increased protein turnover, as a potential avenue to promote cell remodelling and acclimatisation. Endocytosis appears to be a fundamental tool for the survival of bivalves experiencing intense marine heatwave conditions.

Of all tissues sampled in *M. arenaria*, the gills and mantle appear to have the greatest number of similar pathways in response to heatwave challenge. Apart from the phagosome pathway, all remaining pathways expressed in the mantle are expressed in the gills, suggesting a somewhat comparable response in these tissues and a distinctiveness in the responses level observed in the gills. Of all functional classes expressed in the gills, DNA repair and expression were most prominent in *M. arenaria* gills. Pathways such as DNA replication, homologous recombination, mismatch repair, non-homologous end-joining, nucleotide excision repair, and Fanconi anemia were significantly altered under heatwave stress. The DNA replication pathways act upstream to slow or halt DNA synthesis (Osborn et al. 2002) which aids the heat stress response (Velichko et al. 2012). Mechanisms of DNA repair are ubiquitous as temperature can produce genotoxic effects in aquatic ectotherms (Malev et al. 2010) and may promote cell death (Zhou et al. 2001). Transcription of DNA is stress sensitive (Sadhale et al. 2007) and the export and translation of RNA needs to be preserved to avoid disrupted proteostasis (Jamar et al. 2018). Disposing of defective RNA transcripts is thus essential to coping with severe heat stress (Cherkasov et al. 2013). The amount of DNA repair and expression pathways detected in *M. arenaria* greatly outnumber those of *M. truncata.* The latter species expressed a single pathway in its mantle and muscle, namely the aminoacyl tRNA biosynthesis pathway. Temperature may impact nascent, growing, and mature tRNA (Huang and Hopper 2016), and ROS is known to affect tRNA enzymes and lead to higher occurrences of misfolded and oxidized proteins (Ling and Söll 2010). Heatwave stress appears to affect numerous pathways, especially in *M. arenaria* gills,

ranging from DNA stability and expression, repair, and transcription to export from the nucleus. This may, in part, explain the heatwave tolerance we report in this species. Furthermore, the sensitivity of the pathways expressed in the gill of the more thermotolerant *M. arenaria* points to the effectiveness of these protective mechanisms within the context of marine heatwaves.

Cell turnover mechanisms are important components of cellular stress response, acting to replace damaged enzymes or membrane structures or when cells require increased synthesis of cellular machinery. In *M. arenaria*, the autophagy, mitophagy, and phagosome pathways were all significantly altered in both mantle and gill tissues, while ribosome biogenesis and ubiquitin-mediated proteolysis were altered in gill tissue. Although mitochondria are unquestionably targets of heat stress (Slimen et al. 2014), the exact role of mitophagy in heat stress response is not documented in higher eukaryotes. The phagosome pathway, uniquely expressed in the mantle, is intimately linked to autophagy (Shui et al. 2008), and is affected by heat stress in salmon (Shi et al. 2019) and oyster (Zhang et al. 2022). The first of two pathways uniquely expressed in the gills is ubiquitin-mediated proteolysis, which is involved in routine maintenance and cellular stress response (Schwartz and Ciechanover 1992) and responds to heat shock conditions (Abdelmohsen et al. 2009; Maor-Landaw et al. 2014). The ribosome biogenesis pathway is also uniquely altered in *M. arenaria* gills and is suppressed as an initial signal of nucleolar stress (Rubbi and Milner 2003; Golomb et al. 2014) and showing both downregulation (Cherkasov et al. 2015) and upregulation (Quinn et al. 2011; Mohamed et al. 2014) with heat stress.

Cell turnover mechanisms also appeared important to the heatwave response of *M. truncata*. Amino acid levels can be partly linked to protein degradation and synthesis and valine, leucine, and isoleucine biosynthesis pathways were expressed in mantle and adductor muscle tissues, phenylalanine, tyrosine, and tryptophan biosynthesis pathways in both mantle and gill tissues, and the arginine and amino acids biosynthesis pathways in the gills.

Generally, the synthesis of these individual amino acids may serve a multitude of competing functions, which were addressed in the previous section (*Metabolome*) and will not be readdressed. In short, metabolomics analyses revealed the potential effects of heatwave stress on multiple cell turnover mechanisms. The relative contribution of each pathway to heat stress response and their interactions remains to be determined.

Metabolic pathways are considered as those functioning to provide substrates and cofactors for metabolic activity. Although patterns differ substantially between species, the total number of pathways detected in response to heatwaves are comparable. In *M. arenaria*, purine metabolism and β-alanine metabolism pathways were uniquely expressed in gills and muscle, respectively. Unique to *M. truncata* gills, the pathways of taurine and hypotaurine metabolism and thiamine metabolism are significantly altered. Interestingly, the pathways of arginine and proline metabolism, D-amino acid metabolism, and glycine, serine and threonine metabolism are all expressed in both *M. arenaria* muscle and *M. truncata* mantle tissues. Purine metabolism salvages or synthesizes nucleotides, which may reflect the increased need for gene expression as suggested by the response of ark clams to heat stress (Jiang et al. 2020). Taurine and hypotaurine metabolism are likely recruited in *M. truncata* gills for their roles in tissue repair, immunity, and as potential antioxidants (Aruoma et al. 1988; Salze and Davis 2015). Thiamine metabolism is also significantly altered in *M. truncata* gills and plays a role in oxidative stress mitigation, autophagy, and endoplasmic-reticulum stress (Liu et al. 2017). These findings, and amino acid dynamics, suggest species-specific changes in metabolic pathways under heatwave conditions are present across taxa and are part of complex cellular responses that promote short-term survival of both species.

Cell signalling in *M. arenaria* was affected through the phosphatidylinositol, PI, the TGF-b, and Wnt signaling systems in the mantle and gills; mTOR and FoxO signalling uniquely in gills and neuroactive ligand-receptor interaction signalling in muscle. To a much lesser extent, cell signalling was involved in the *M. truncata* heatwave response through a

single pathway in the gills, the neuroactive ligand receptor interaction signalling pathway. There is growing evidence describing the role of these signalling pathways in general cellular stress response. The PI signaling system can cooperate with endocystosis through the action of PI3K and can impact pro-inflammatory responses (Hawkins and Stephens 2015). The TGF-b gene is involved with inflammatory response and upregulates in heat-stressed fish (Sun et al. 2020; Yang et al. 2021). Wnt related genes have been shown to display different expression patterns under heat stress (Risha et al. 2021; Huang et al. 2021; Yin et al. 2022), and may alter membrane characteristics (Risha et al. 2021). mTOR signalling in gills impacts RNA translation and mTOR transcription increases in ectotherms exposed to increased temperature (Chou et al. 2018; Pandey et al. 2021). FoxO signalling has received considerable attention with respect to oxidative stress (Essers et al. 2005; Lehtinen et al. 2006) and FoxO transcripts are upregulated under heat stress (Eremina et al. 2021). The neuroactive ligand-receptor interaction signalling pathway is poorly understood but has been detected in various stress responses (Liu et al. 2018; Feng et al. 2022), including heat (Kim et al. 2017; Lu et al. 2018). Overall, signalling pathways appear to be central mechanisms in stress response for heatwave-tolerant animals. These pathways provide a framework for studying integrated stress signalling at the main active sites shared between pathways.

One of the unique pathways expressed in *M. truncata,* both in the mantle and the muscle, is the membrane transport pathway of ABC transporters. The role of these membrane proteins has largely been studied in a toxicological framework (Jeong et al. 2017). Their role in environmental stress tolerance is beginning to be explored, such as the observation of increased levels in urchins exposed to extreme environmental changes (Marques-Santos et al. 2017), or significant pathway recruitment in sea cucumbers exposed to heat stress (Huo et al. 2019). Considering the diversity and role of these transmembrane proteins, they merit further investigation.

The clam species evaluated in this study are abundant and integral components of temperate and polar coastal benthic ecosystems. Their ability to withstand environmental stressors is reflected by their survival and cellular stress responses to marine heatwaves. Harvesting disturbance does not appear to negatively impact either species whether alone or when paired with marine heatwaves. Single harvesting disturbance events can be tolerated by these clam species in the short term, but this may not apply for longer or repeated harvesting disturbance events. In light of the growing threat of marine heatwaves to these environments, our study illustrates the complex, species- and tissue-specific responses to a single heatwave event situated within the range of projected future heatwave intensities in the North Atlantic regions of Eastern Canada. Phylogenetically closely related species display marked differences in marine heatwave tolerance which may jeopardize the survival of these populations in the near future. These differences can be explained in part by unique shifts in tissue metabolome at both the individual metabolite and pathway levels. At their respective upper heatwave limits, *M. arenaria* utilises a significantly greater number of signalling and DNA repair pathways when compared to *M. truncata*. These findings will be useful to inform clams species management and conservation practices, for distribution modelling under projected global change conditions, and as a basis for targeting cellular stress response mechanisms for future study of other non-model marine invertebrates in the context of global change stressors.

CONCLUSION GÉNÉRALE

Cette étude a démontré que les deux espèces de palourdes, bien apparentées, ont des capacités de tolérance aux vagues de chaleur nettement différentes. La pêche accidentelle sans récurrence n'impacte pas les espèces à l'étude à court terme, et ne présente pas d'interaction avec les vagues de chaleur marines. Cependant, des évènements répétés pourraient avoir des impacts significatifs sur les populations à l'étude. Notre étude indique que ce sont plutôt les vagues de chaleur marines qui sont les principales menaces. Nous avons défini les seuils de tolérance thermique et la dynamique du métabolome des différents tissus. Le seuil de tolérance de *M. arenaria* est comparativement plus élevé que celui de *M. truncata* : les seuils se situent respectivement autour de 27,89 et 17,99 °C. En-deçà de ces valeurs de température, les espèces ne subissent que très peu de mortalité, ce qui indique qu'elles peuvent tolérer ces conditions pour une durée 12 jours consécutifs au plus. Au niveau physiologique, la réponse de *M. arenaria* a été comparée à celle de *M. truncata* dans les conditions les plus élevées de température, c'est-à-dire 27 et 17 °C. Cette comparaison a permis de comprendre dans quelle mesure et comment les métabolomes des tissus de *M. arenaria* se démarquent de ceux de *M. truncata.* En absolu, un plus grand nombre de métabolites répondent à la vague de chaleur chez *M. arenaria*, ce qui suggère qu'elle apporte un changement plus marqué à son métabolisme pour pallier les effets des eaux marines plus chaudes. Les concentrations de métabolites ont subi des changements qui sont partagés entre les tissus d'une même espèce et entre les deux espèces également. Les variations de concentrations de phénylalanine (augmentation) et de glutamine (diminution) sont similaires autant chez *M. arenaria* que chez *M. truncata* en réponse aux vagues de chaleur, et peuvent ainsi être considérées comme des bioindicateurs fiables pour les deux espèces. De plus, chacune des espèces présente ses propres bioindicateurs. Chez *M. arenaria*, l'augmentation

des concentrations d'acide α-aminoadipique, d'aspartate, d'histidine, de serine, de threonine et de valine, ou la diminution d'aspartate, d'acide β-isobutyrique, de FAD, d'acetyl-CoA, d'ADP, de cis-aconitate, de citrate et d'oxaloacetate est constante parmi les tissus. Chez *M. truncata*, seule la diminution de glycine et de succinate est constante parmi les tissus. L'espèce moins tolérante aux vagues de chaleur, *M. truncata*, semble donc avoir moins de bioindicateurs fiables (2) nous permettant de détecter le phénomène, alors que *M. arenaria* en détient bien plus (14). D'un point de vue mécanistique, ces métabolites font partie d'un ensemble de voies cellulaires, qui ont été explorées dans trois tissus de ces deux espèces. Ces voies se regroupent dans un cadre simplifié, qui traite principalement de quatre classes fonctionnelles, dont la signalétique cellulaire, le renouvellement des structures cellulaires, la réparation et l'expression de l'ADN et le métabolisme. Tous les tissus de *M. arenaria* ont exprimé un plus grand nombre de voies que ceux de *M. truncata*, et une seule voie était partagée par le même tissu de chaque espèce. On la retrouve au niveau des branchies, où la voie cellulaire de l'endocytose a été détectée pour les deux espèces, une fonction cellulaire qui permet de restructurer les membranes cellulaires pour maintenir leur stabilité, entre autres. Les branchies de *M. arenaria* se démarquent pour le nombre absolu élevé de voies cellulaires détectées (24) ainsi que pour un important recrutement de voies liées à la réparation et l'expression de l'ADN (12). Quant à *M. truncata*, la plupart des voies se centraient sur les acides aminés et relevaient du métabolisme. Les vagues de chaleur ont alors suscité des réponses au niveau cellulaire qui sont bien différentes d'une espèce à l'autre. L'écart des taux de survie entre les espèces relève fort probablement de la capacité d'ajustement de leur métabolisme cellulaire. Ces réponses doivent en partie expliquer le succès que connaît *M. arenaria* dans des conditions plus chaudes. Quant à l'effet attendu de la pêche accidentelle, ce traitement n'a pas suscité de réponse physiologique, ce qui suggère que l'impact est négligeable et n'est pas la principale menace pour l'espèce dans la mesure qu'elle a été effectuée dans cette étude.

Selon nos résultats, l'intensification des vagues de chaleur marines aura des conséquences plus immédiates sur *M. truncata* et impactera sa survie à la limite sud de sa répartition biogéographique. En l'occurrence, *M. truncata* pourrait subir une contraction de son aire de répartition bien avant *M. arenaria,* car les conditions du milieu naturel approchent les 16 °C durant l'été autour du site de récolte des individus (observation personnelle). De plus, puisque différentes populations locales peuvent avoir une tolérance thermique variable qui reflète les conditions environnementales historiques de leur habitat, les populations nordiques de *M. truncata* risquent d'avoir une capacité de plasticité thermique plus étroite encore. À la vitesse actuelle de l'intensification et l'augmentation de la fréquence des vagues de chaleurs, qui est particulièrement rapide dans les environnements nordiques où se trouvent un grand nombre de populations de *M. truncata*, celles-ci seraient menacées d'extinction locale. Avant de vivre ces phénomènes d'extinction, il y a raison de croire que cette espèce verra des réductions significatives de ses effectifs dans l'estuaire et le Golfe du Saint-Laurent si les tendances climatiques actuelles se poursuivent. Dans les milieux qui abritent présentement les deux espèces, il se pourrait que seule l'espèce *M. arenaria* soit présente. En raison du statut envahissant de cette espèce sur les côtes de l'Ouest Canadien et de l'Europe, elle aurait la capacité de s'étendre vers latitudes plus élevées. À mesure que les niches nordiques se transforment sous les changements climatiques, *M. arenaria* pourrait alors y trouver un succès après une migration naturelle ou assistée. Au-delà de la réponse de ces espèces, les impacts ressentis des vagues de chaleur peuvent entraîner des répercussions indirectes sur l'écosystème, en impactant les autres espèces qui dépendent des palourdes comme sources d'alimentation. De plus, quelques populations humaines vivant près des côtes exploitent cette ressource et pourraient ne plus y avoir accès. Outre ces conséquences, les palourdes occupent un rôle important dans les cycles biogéochimiques des environnements côtiers, notamment en tant qu'espèces fouisseuses et par leur capacité d'impacter la qualité de l'eau environnante. Ces services seraient for probablement fragilisés par l'absence des palourdes. En bref, la réponse de *M. arenaria* et de *M. truncata* aux vagues de chaleur,

principalement leur seuil de tolérance, est une mesure utile pour prédire la distribution future de ces espèces et d'anticiper les conséquences directes des changements climatiques sur les ressources naturelles.

Les outils permettant l'évaluation des risques et la vulnérabilité des espèces marines continuent de se développer. Pour augmenter la précision des prédictions, les gestionnaires doivent connaître et intégrer le plus grand nombre de risques auxquels font face les écosystèmes. Les organismes chargés de légiférer sur la gestion des populations de palourdes peuvent s'appuyer sur nos résultats pour définir un plan d'action pour la conservation des espèces connaissant l'impact des vagues de chaleur et de la pêche accidentelle. La tolérance thermique des palourdes est également une composante prisée dans la modélisation de la distribution des espèces. En ce sens, les évènements extrêmes de température comme les vagues de chaleur peuvent contraindre les populations de palourdes dans leur distribution avec des impacts plus graves que les augmentations moyennes annuelles des températures. Au niveau écologique, il est d'autant plus important de caractériser la diversité des réponses physiologiques aux changements climatiques chez les invertébrés en raison de leur abondance dans les écosystèmes et leur rôle dans les réseaux trophiques. Les impacts des changements climatiques entraîneront alors des répercussions directes sur les espèces de palourdes et indirectes sur les espèces qui s'y rattachent. Évaluer les seuils de tolérance nous permet d'anticiper les conséquences des changements sur la dynamique des populations, tandis que les mécanismes physiologiques de la réponse cellulaire d'un organisme informent sur les faiblesses du système biologique des palourdes. Cette approche peut être transférée sur de nombreuses espèces, à condition qu'elles puissent aisément être maintenues en conditions de laboratoire. Les types de réponses et les mécanismes physiologiques risquent de se recouper entre espèces et nous souhaiterions que les résultats de notre étude puissent inspirer des études futures en métabolomique environnementale. Globalement, les écosystèmes nordiques comprennent des espèces emblématiques et subissent un réchauffement climatique qui s'aggrave plus rapidement qu'ailleurs dans le monde.

L’importance écologique, commerciale et traditionnelle des palourdes dans ces régions et dans les régions côtières plus globalement ne doit pas être négligée. Cette étude permet de mieux connaître la diversité des réponses physiologiques des palourdes aux effets des vagues de chaleur et permet d’anticiper la redistribution de ces populations de palourdes.

ANNEXES

ANNEXE I

Supplementary Table 1: Summary of mean (+/- SE) seawater conditions in the experimental phase. This includes mortality measurements for *M. arenaria* and *M. truncata*, temperature readings tracked during the acclimation (14 d), ramping (3 d), and exposure (9 d) periods and physical chemical parameters (pH, dissolved oxygen, salinity) tracked during the exposure period. Yellow highlights indicate problematic behaviour in the system and refer to experimental units removed from analysis.

Treatment	Mortality (%)		Average pre-exposure temp. (°C) (N = 1344) + SE		Temperature ramping rates (°C . h $^{-1}$) (N = 288) + SE + R^2			Average exposure temp. (°C) (N = 864) + SE		Exposure period					
	Mya arenaria	*Mya truncata*								Average pH (NBS) + SE		Average DO (%) + SE		Average sal + SE	
2 °C	0	0	6.83	.044	.023	.018	.02	8.32	.035	8.33	-	100.1	-	28	-
	0	0	6.28	.008	-.131	.005	.88*	2.99	.022	8.28	.02	101.5	.9	28	0
	0	0	6.15	.008	-.162	.007	.83*	2.97	.017	8.25	.05	101.7	.5	28	0
	12.5	0	6.00	.008	-.127	.005	.87*	2.70	.018	8.25	.05	100.4	.3	28	0
2 °C x harvest	0	0	6.76	.021	-.188	.012	.54*	3.87	.116	8.26	.03	103.5	1.4	28	0
	0	0	6.24	.008	-.081	.005	.76*	2.70	.014	8.26	.05	100.6	.2	28	0
	12.5	0	6.36	.010	-.121	.006	.79*	2.94	.015	8.30	.02	100.7	.3	28	0
	0	0	5.85	.026	-.152	.008	.79*	3.18	.015	8.29	.03	100.4	.3	28	0
7 °C	0	0	6.04	.016	.037	.005	.41*	7.18	.028	8.27	.04	104.3	.8	28	0
	0	0	6.12	.021	.036	.009	.16*	7.91	.021	8.25	.03	100.8	.5	28	0
	0	12.5	6.01	.024	.109	.009	.62*	7.58	.022	8.29	.02	100.7	.4	28	0
	0	0	6.23	.015	.094	.004	.87*	7.35	.020	8.29	.02	100.9	.3	27.7	.3
7 °C x harvest	0	0	6.29	.012	.040	.003	.61*	7.25	.032	8.27	.02	100.6	.1	28	0
	0	37.5	6.07	.010	.051	.006	.44*	6.79	.008	8.25	.05	101.2	.2	28	0
	0	0	6.04	.019	.054	.005	.60*	7.47	.040	8.21	.05	101.3	.7	28	0
	0	0	6.10	.011	.050	.005	.52*	7.42	.016	8.27	.01	101.5	.3	28	0
12 °C	0	12.5	6.74	.007	.244	.004	.98*	11.40	.023	8.27	.02	102.4	.7	28	0
	0	0	6.23	.011	.238	.004	.98*	11.99	.008	8.25	.06	103.4	.8	28	0
	0	0	6.58	.010	.202	.003	.98*	13.05	.032	8.22	.04	101.8	.9	28	0
	0	0	6.30	.011	.234	.005	.95*	12.07	.010	8.27	.01	100.3	.3	28	0
12 °C x harvest	12.5	87.5	6.34	.051	.247	.003	.99*	12.65	.158	8.30	.02	100.1	.1	28	0
	12.5	0	6.13	.013	.234	.004	.98*	12.22	.027	8.29	.02	100.7	.4	28	0
	12.5	12.5	5.52	.027	.292	.005	.97*	12.22	.016	8.25	.02	101.7	2.4	28	0
	0	25	6.20	.008	.228	.006	.94*	15.52	.112	8.26	.04	102.4	1.4	28	0
17 °C	0	0	6.15	.022	.408	.004	.99*	16.73	.008	8.30	.01	101.2	1.5	28	0
	12.5	12.5	6.22	.025	.431	.008	.97*	17.59	.117	8.23	.03	101.4	.4	28	0
	0	0	6.56	.009	.440	.004	.99*	17.45	.015	8.22	.01	99.9	.7	28	0
	0	75	6.76	.109	.422	.008	.97*	13.51	.128	8.28	.03	102.8	1.3	28	0
17 °C x harvest	0	100	6.42	.013	.491	.025	.81*	19.64	.158	8.26	.06	98.0	1.2	28	0
	0	100	6.32	.036	.393	.056	.34*	16.65	.031	8.27	.01	97.0	1.6	28	0
	0	0	5.49	.027	.366	.017	.83*	16.89	.058	8.19	.02	102.1	1.2	28	0
	0	12.5	6.42	.017	.371	.008	.96*	16.66	.028	8.27	.01	102.7	1.4	28	0

Treatment	Mortality (%) *Mya arenaria*	Mortality (%) *Mya truncata*	Average acclimation temp. (°C) (N = 1344) + SE		*Ramping rates (°C . h $^{-1}$) (N = 288) + SE + R^2			Average exposure temp. (°C) (N = 864)		Exposure period: Average pH (NBS) + SE		Exposure period: Average DO (%) + SE		Exposure period: Average sal + SE	
22 °C	12.5	100	6.65	.017	.661	.009	.98*	22.64	.089	8.25	.04	101.1	.2	28	0
	12.5	87.5	6.41	.015	.541	.050	.56*	20.50	.110	8.26	.01	98.9	1.7	28	0
	12.5	100	6.60	.013	.627	.006	.99*	21.96	.023	8.28	.01	99.1	3.2	28	0
	0	100	6.16	.012	.600	.005	.99*	21.43	.024	8.19	.01	93.3	.9	28	0
22 °C x harvest	0	100	6.41	.021	.710	.011	.98*	22.63	.040	8.27	.02	97.8	2.3	28	0
	0	100	6.48	.016	.641	.007	.99*	23.97	.103	8.30	.01	96.9	2.8	28	0
	0	62.5	5.90	.026	.602	.010	.98*	18.67	.026	8.27	.03	98.0	5.8	27.7	.3
	12.5	100	6.79	.018	.614	.016	.94*	21.32	.058	8.24	0	98.5	3.0	27.7	.3
27 °C	62.5	100	6.48	.009	.833	.005	.99*	26.92	.054	8.24	.01	94.7	.7	27.7	.3
	25	100	6.18	.006	.830	.006	.99*	27.15	.025	8.29	.02	93.7	1.8	27.7	.3
	25	100	5.77	.024	.802	.007	.99*	26.35	.020	8.29	.02	91.4	1.1	28	0
	37.5	100	6.05	.018	.835	.014	.97*	26.16	.044	8.32	.01	88.7	3.0	27.7	.3
27 °C x harvest	0	100	6.54	.008	.776	.006	.99*	26.71	.058	8.27	.02	95.0	.7	28	0
	50	100	6.19	.008	.806	.006	.99*	26.91	.052	8.22	.01	89.2	3.7	27.7	.3
	12.5	100	5.93	.018	.820	.010	.99*	25.75	.037	8.28	.03	88.8	3.9	28.3	.3
	12.5	100	6.02	.013	.787	.006	.99*	26.86	.012	8.26	.03	89.7	1.6	27.7	.3
32 °C	100	100	6.47	.009	.934	.009	.99*	31.59	.053	8.24	.04	91.9	5.4	28	0
	100	100	7.19	.061	1.06	.010	.99*	31.40	.040	8.29	.01	96.5	5.5	27.5	.5
	100	100	5.76	.026	.986	.023	.95*	31.38	.053	8.33	-	91.5	-	28	-
	100	100	6.26	.015	1.01	.008	.99*	30.72	.085	8.27	-	90.1	-	28	-
32 °C x harvest	100	100	6.47	.014	1.01	.009	.99*	31.34	.082	8.29	-	90.5	-	29	-
	100	100	6.34	.012	.941	.013	.98*	32.18	.045	8.33	.01	94.8	1.8	27.5	0.5
	100	100	5.67	.039	.714	.023	.91*	26.82	.182	8.31	.01	84.2	.2	27.5	.5
	100	100	5.92	.038	.855	.015	.97*	31.35	.032	8.27	-	87.8	-	27	-

ANNEXE II

Supplementary Table 2: Precursor ions, product ions, retention time, and QQQ parameters for each compound in positive mode (left) and negative mode (right)

Compound	Precursor Ion	Product Ion	RT (min)	Frag	CE
α-aminoadipic acid	162.1	98	7.987	80	12
α-aminoisobutyric acid	104.1	57	7.147	60	12
Alanine	90.1	44.2	7.309	40	8
AMP	348	136.1	8.194	128	12
Arginine	175.1	70.2	9.745	10	24
Aspartate	134	74	8.846	60	8
β-aminoisobutyric acid	104.1	86.1	7.227	60	4
Betaine	118.1	58	5.837	128	28
Cystine	241	74	10.116	80	24
FAD	786.3	348	8.592	124	12
Glutamate	148.1	84	8.363	80	12
Glutamine	147.1	84	7.858	60	16
Glycine	76.1	30.2	7.738	40	4
Histidine	156.1	110	9.332	80	12
Hydroxyproline	132.1	86.2	7.459	80	12
Isoleucine	132.1	86.2	5.646	60	4
Leucine	132.1	86.2	5.379	60	4
Lysine	147.1	84	10.205	80	16
Methionine	150.1	133	5.934	80	4
NAD	664	428.1	9.687	128	24
Phenylalanine-d8	174	128	4.996	80	8
Phenylalanine	166.1	120.2	4.970	80	8
Proline	116.1	70	6.665	80	12
Sarcosine	89.9	44.1	7.416	40	8
Serine	106	60	7.867	60	8
Threonine	120.1	74.1	7.463	60	4
Tryptophan	205.1	188.3	5.127	80	0
Tyrosine	182.1	165.2	6.275	60	4
Valine	118.1	72	6.476	60	4

Compound Name	Precursor Ion	Product Ion	RT (min)	Frag	CE
Acetyl-CoA	403.6	79	11.963	100	40
ADP	426	79	12.395	128	48
α-ketoglutarate	145.1	101	10.128	60	0
ATP	506	159	13.106	144	44
Cis-aconitate	173	129	11.525	60	0
Citrate	191	111	13.675	80	8
D-fructose-1.6-biphosphate trisodium	339	97	15.089	128	20
Fumarate	115	71	11.596	60	0
Fumarate-d4	117	29	11.604	60	4
Glucose	179	89	2.720	60	4
Glucose-6-phosphate	259	199	13.087	100	4
Lactate	89	43	2.709	60	8
Malate	133	115	11.380	80	4
NADH	664.1	408	10.172	160	32
NADP	742.1	620	14.292	40	4
NADPH	744.1	408	13.949	20	32
Oxaloacetate	131	87	13.194	60	0
Phosphoenol-pyruvate	166.9	78.8	13.926	80	4
Pyruvate	87	43	1.898	60	0
Succinate	117	99	11.281	80	8
Succinyl-CoA	866.6	408	13.998	100	10

ANNEXE III

Supplementary Table 3: Stock metabolites and corresponding solvents and solvent volumes for positive analysis (left) and negative analysis (right)

Compound	Sln stock (mg/mL)	Solvent	Stability	Vol Lvl 8 (µL)
Alanine	1	0.1% FA	Stable	500
AMP	1	0.1% FA	Stable	50
Arginine	1	0.1% FA	Stable	50
Aspartate	1	0.1% FA	Stable	50
Betaine	1	0.1% FA	Stable	50
Cystine	1	1 M HCl	Unstable	50
FAD	1	0.1% FA	Unstable	50
Glutamate	1	0.1% FA	Stable	50
Glutamine	1	0.1% FA	Stable	50
Glycine	1	0.1% FA	Stable	500
Histidine	1	1 M HCl	Stable	50
Hydroxyproline	1	0.1% FA	Unstable	50
Isoleucine	1	0.1% FA	Unstable	50
Leucine	1	0.1% FA	Stable	50
Lysine	1	0.1% FA	Stable	50
Methionine	1	0.1% FA	Stable	50
NAD	1	0.1% FA	Stable	50
Phenylalanine	1	0.1% FA	Stable	50
Proline	1	0.1% FA	Stable	500
Sarcosine	1	0.1% FA	Stable	500
Serine	1	0.1% FA	Stable	50
Threonine	1	0.1% FA	Stable	50
Tryptophan	1	0.1% FA	Stable	50
Tyrosine	1	0.1% FA	Stable	50
Valine	1	0.1% FA	Stable	50
α-aminoadipic acid	1	0.1% FA	Stable	50
α-aminobutyric acid	1	0.1% FA	Unstable	50
β-aminoisobutyric acid	1	0.1% FA	Stable	50

Compound	Sln stock (mg/mL)	Solvent	Stability	Vol Lvl 8 (µL)
Acetyl-CoA	1	0.1% NH_4OH	Stable	50
ADP	1	0.1% NH_4OH	Stable	50
α-ketoglutarate	1	0.1% NH_4OH	Stable	50
ATP	1	0.1% NH_4OH	Unstable	50
Cis-Aconitate	1	0.1% NH_4OH	Unstable	50
Citrate	1	0.1% NH_4OH	Stable	50
D-fructose-1.6-biphosphate trisodium	1	0.1% NH_4OH	Unstable	50
Fumarate	1	0.1% NH_4OH	Stable	50
Glucose	1	0.1% NH_4OH	Stable	50
Glucose-6-phosphate	1	0.1% NH_4OH	Stable	50
Lactate	1	0.1% NH_4OH	Stable	50
Malate	1	0.1% NH_4OH	Stable	500
NADH	1	0.1% NH_4OH	Stable	50
NADP	1	0.1% NH_4OH	Stable	50
NADPH	1	0.1% NH_4OH	Stable	50
Oxaloacetate	1	0.1% NH_4OH	Stable	50
Phosphoenol-pyruvate	1	0.1% NH_4OH	Unstable	50
Pyruvate	1	0.1% NH_4OH	Unstable	50
Succinate	1	0.1% NH_4OH	Unstable	50
Succinyl-CoA	1	0.1% NH_4OH	Unstable	50

ANNEXE IV

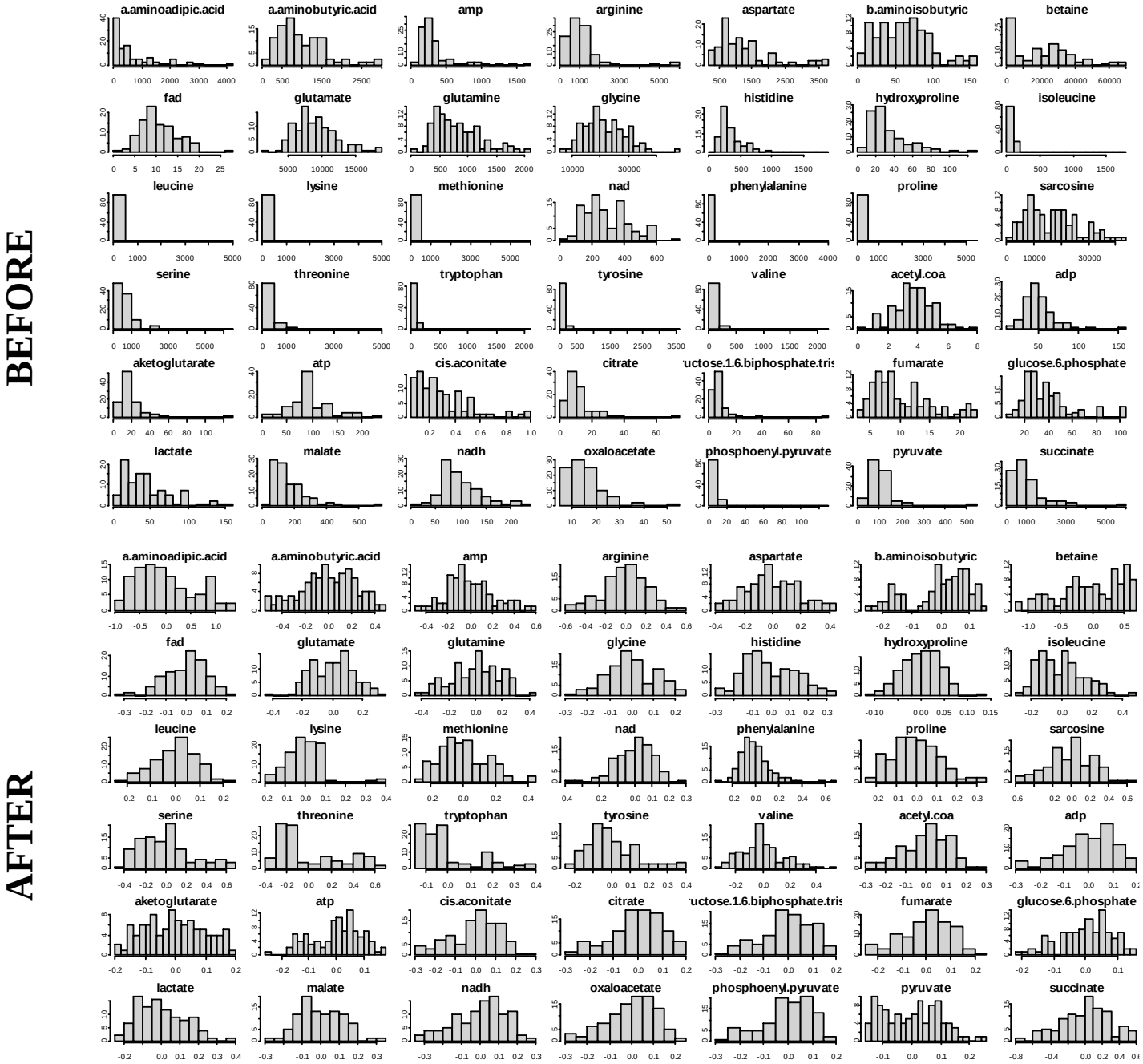
Supplementary Table 4: Stock metabolites manufacturers and corresponding KEGG identifiers for positive analysis (left) and negative analysis (right)

Analysis	Compound	Supplier	Product No	KEGG ID
Pos	α-aminoadipic acid	Sigma	A7275	C00956
Pos	α-aminobutyric acid	Sigma	162663	C02356
Pos	L-Alanine	Sigma	A7627	-
Pos	Adenosine 5'-monophosphate	Sigma	A2252	C00020
Pos	L-Arginine	Sigma	A8094	C00062
Pos	L-Aspartatic acid	Sigma	A8949	C00049
Pos	β-aminoisobutyric acid	Sigma	757454	C05145
Pos	Betaine	Sigma	B2629	C00719
Pos	L-Cystine	Sigma	C7602	-
Pos	FAD	Cayman	23386	C00016
Pos	D-Glutamic acid	Sigma	G101	C00025
Pos	L-Glutamine	Sigma	G8740	C00064
Pos	Glycine	Sigma	G7126	C00037
Pos	L-Histidine	Sigma	H8000	C00135
Pos	Hydroxy-L-proline	Sigma	H54409	C01157
Pos	L-Isoleucine	Sigma	I2752	C00407
Pos	L-Leucine	Sigma	L8912	C00123
Pos	L-Lysine	Sigma	L5501	C00047
Pos	L-Methionine	Sigma	M9625	C00073
Pos	NAD	Cayman	16077	C00003
Pos	L-Phenylalanine	Sigma	P5482	C00079
Pos	L-Phenylalanine-d8	CIL	DLM-372-PK	-
Pos	DL-Proline	Sigma	171824	C00148
Pos	Sarcosine	Sigma	S7672	C00213
Pos	L-Serine	Sigma	S4500	C00065
Pos	L-Threonine	Sigma	T8625	C00188
Pos	L-Tryptophan	Sigma	T0254	C00078
Pos	L-Tyrosine	Sigma	T8566	C00082
Pos	L-Valine	Sigma	V0500	C00183

Analysis	Compound	Supplier	Product No	KEGG ID
Neg	Acetyl CoenzymeA	Cayman	16160	C00024
Neg	Adenosine 5'-diphosphate	Cayman	16778	C00008
Neg	α-ketoglutarate	Sigma	75890	C00026
Neg	Adenosine 5'-triphosphate	Cayman	14498	C00002
Neg	Cis-aconitic acid	Sigma	A3412	C00417
Neg	Citrate (sodium)	Sigma	PHR1416	C00158
Neg	D-fructose-1.6-biphosphate trisodium	Sigma	F6803	C05378
Neg	Fumaric acid	Sigma	800269	C00122
Neg	Fumaric acid-d4	CIL	DLM-7654-PK	-
Neg	D-(+)-Glucose	Sigma	G8270	-
Neg	D-Glucose-6-phosphate	Sigma	G6526	C00092
Neg	L-Lactate (sodium)	Sigma	PHR1113	C00186
Neg	DL-Malic acid	Sigma	M0875	C00149
Neg	NADH	Cayman	16078	C00004
Neg	NADP	Cayman	2145	-
Neg	NADPH	Cayman	9000743	-
Neg	Oxaloacetic acid	Sigma	O4126	C00036
Neg	Phospho(enol)pyruvic acid	Sigma	P7127	C00074
Neg	Pyruvate (sodium)	Sigma	P2256	C00022
Neg	Succinate (sodium)	Sigma	818601	C00042
Neg	Succinyl-CoenzymeA	Cayman	23297	-

ANNEXE V

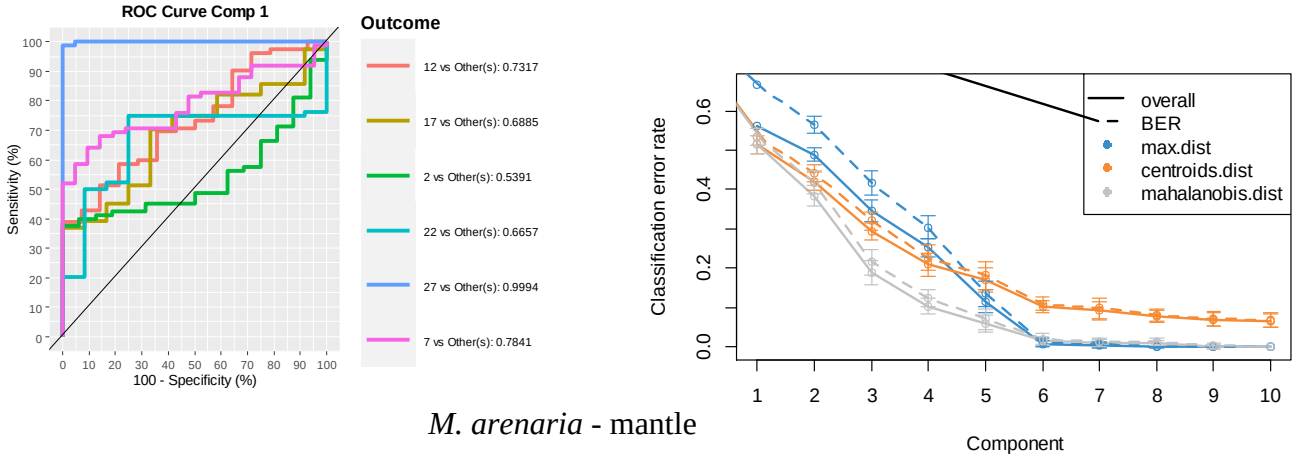
Supplementary Figure 1: Variable histograms for 42 metabolites in the *gills* of *M. arenaria* (N = 97) before (top) and after (bottom) variable transformation (missing value imputation, CLR transformation, centering, scaling, and linear mixed effect model transformation).



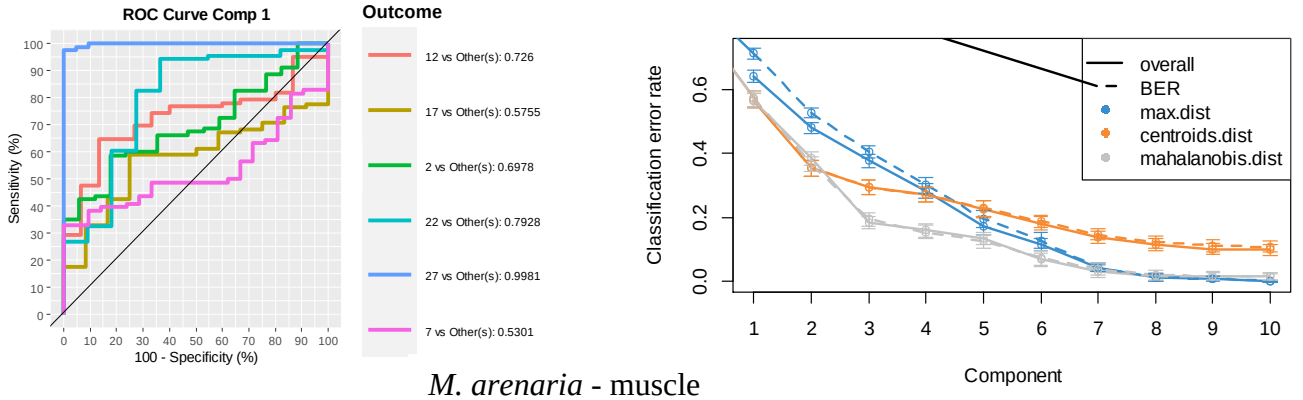
ANNEXE VI

Supplementary Figure 2: Cross validation of PLS-DA models using (left) both area under the curve (AUC) of receiver operating characteristic (ROC) curve and (right) classification error rate with maximum-, centroids-, and mahalanobis- distance measures for all six analyses

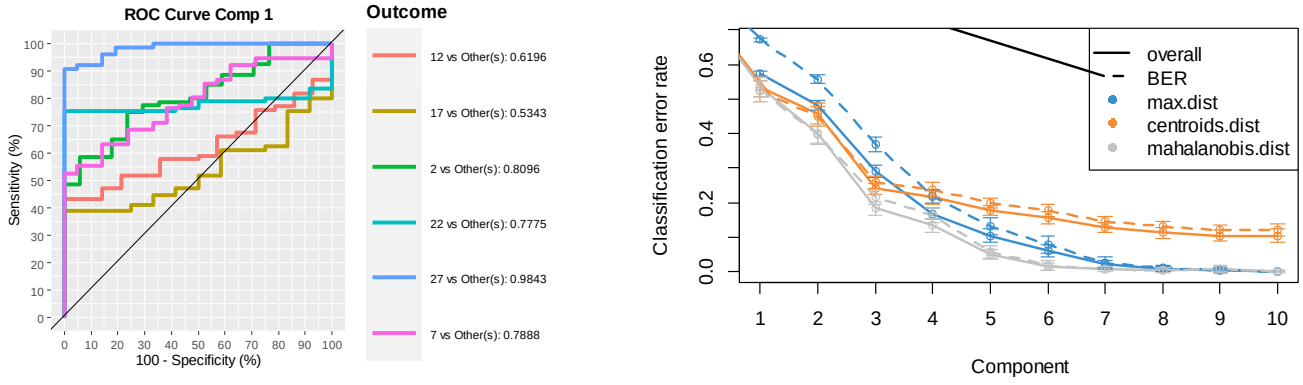
M. arenaria - gills



M. arenaria - mantle

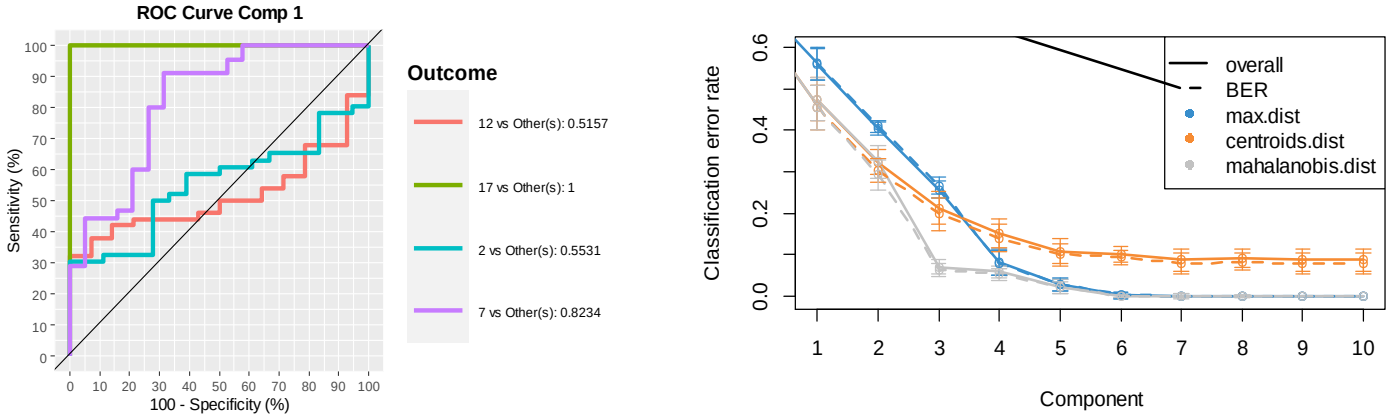


M. arenaria - muscle

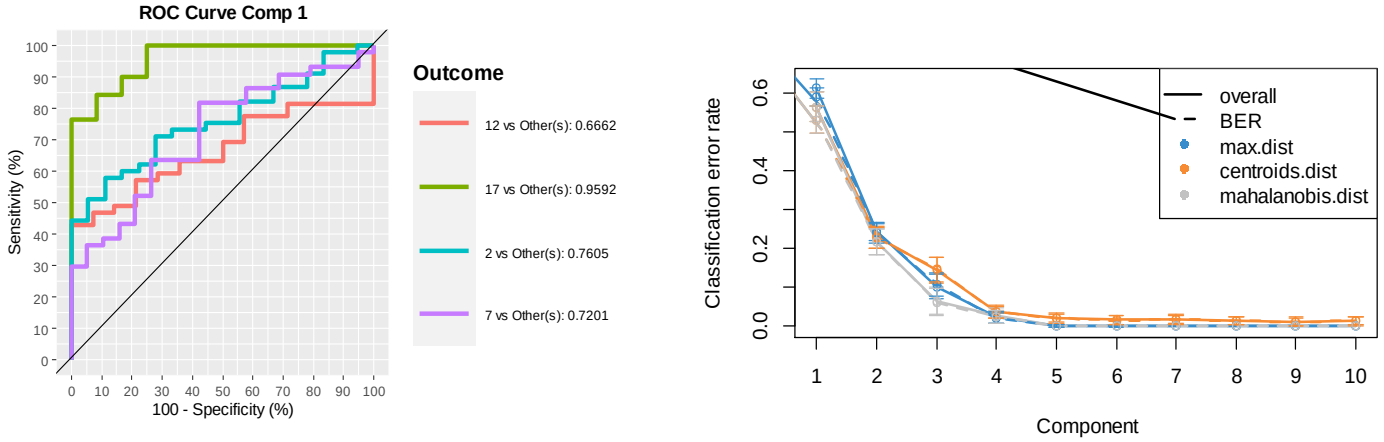


Supplementary Figure 2 (cont.): Cross validation of PLS-DA models using (left) both area under the curve (AUC) of receiver operating characteristic (ROC) curve and (right) classification error rate with maximum-, centroids-, and mahalanobis- distance measures for all six analyses

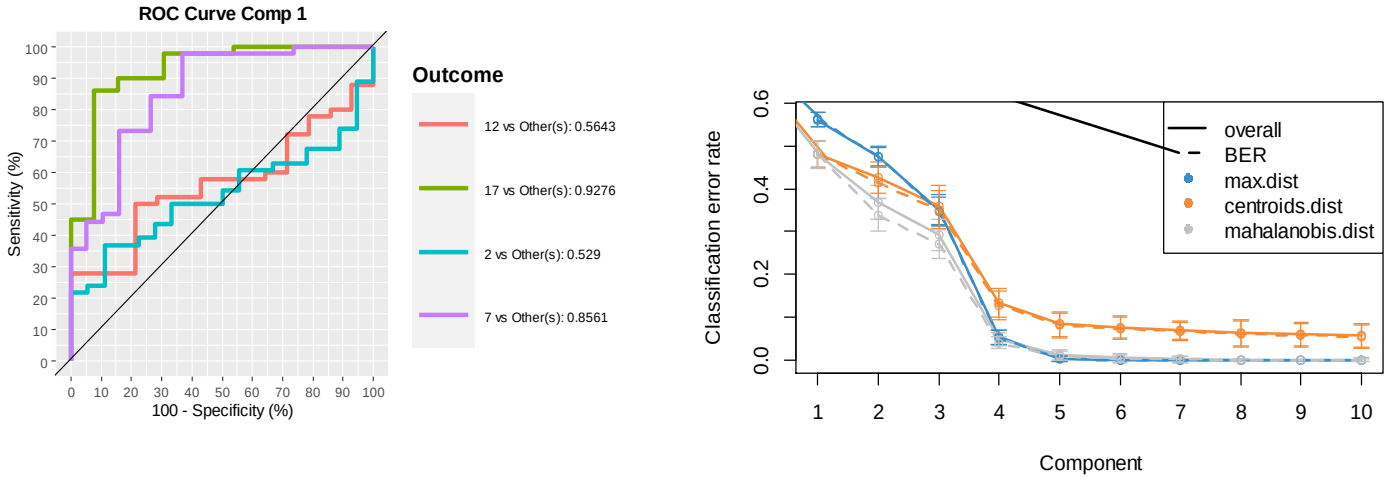
M. truncata - gills



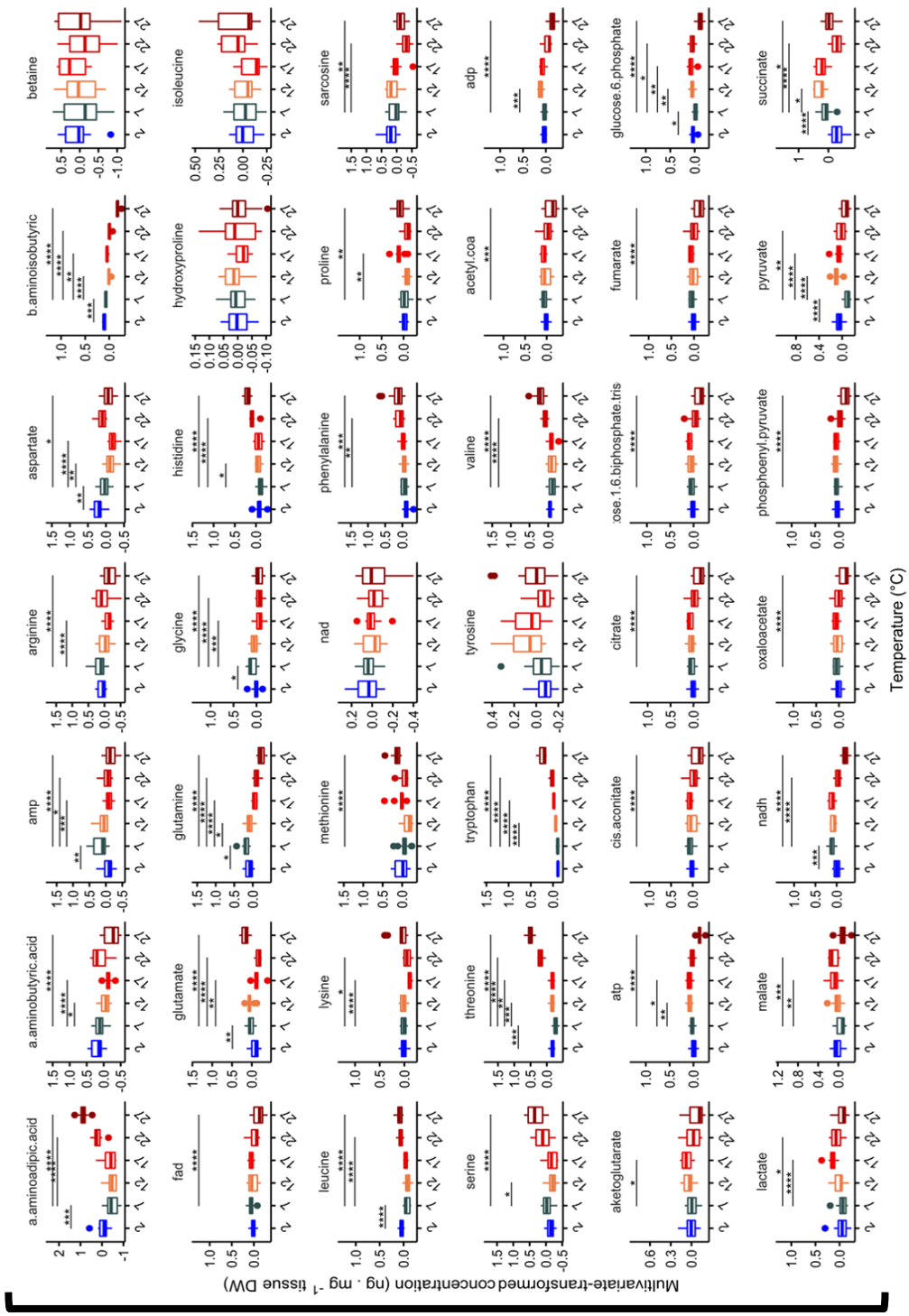
M. truncata - mantle



M. truncata - muscle



ANNEXE VII


A
Multivariate-transformed concentration (ng . mg -1 tissue DW)
Temperature (°C)
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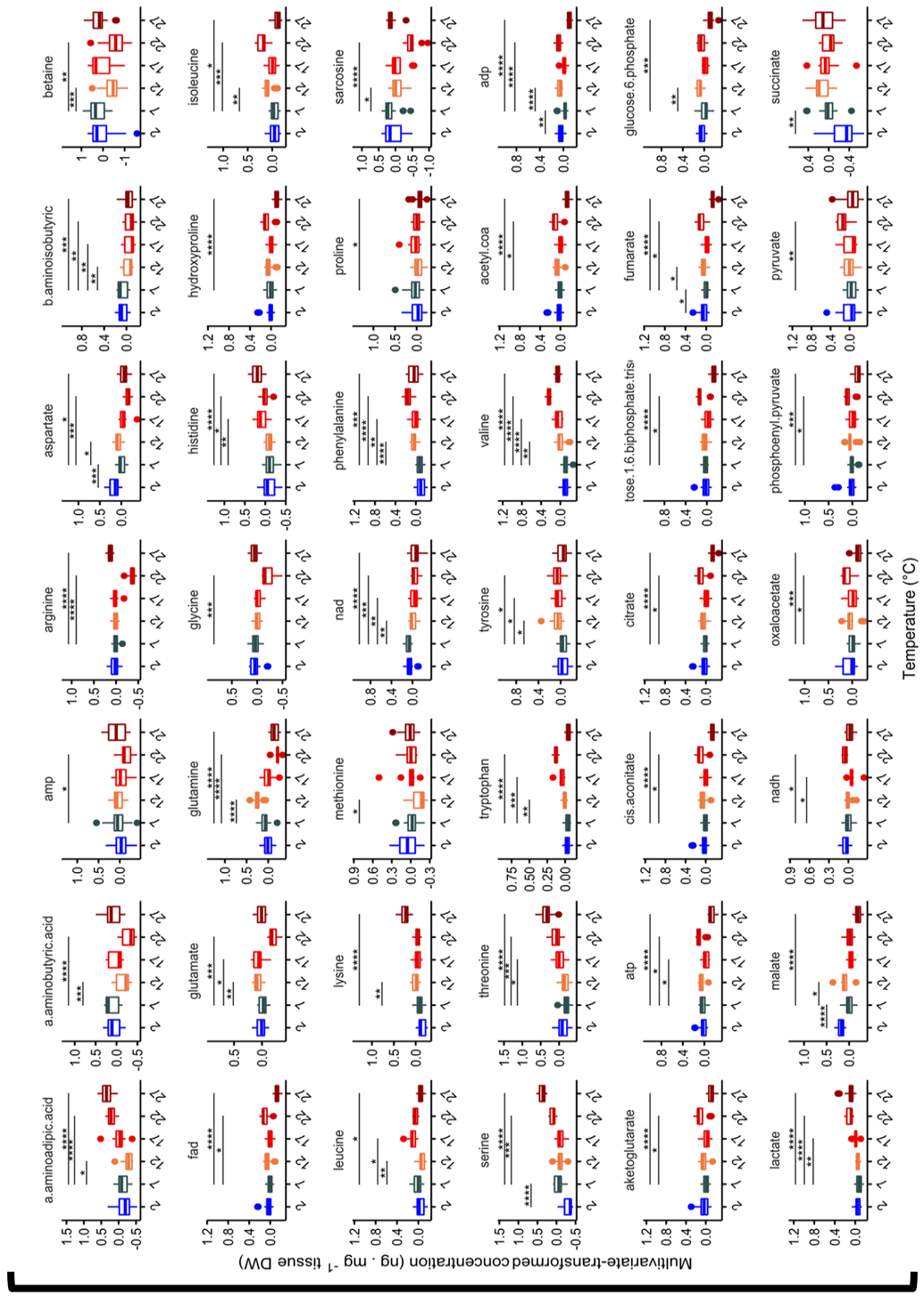
a.aminobutyric.acid
amp
arginine
aspartate
b.aminoisobutyric
betaine
fad
glutamate
glutamine
glycine
histidine
hydroxyproline
isoleucine
leucine
lysine
methionine
nad
phenylalanine
proline
sarcosine
serine
threonine
tryptophan
tyrosine
valine
acetyl.coa
adp
lactate
malate
nadh
oxaloacetate
phosphoenyl.pyruvate
pyruvate
succinate
akétoglutarate
atp
cis.aconitate
citrate
fumarate
glucose.6.phosphate


B
Multivariate-transformed concentration (ng . mg -1 tissue DW)
Temperature (°C)
a.aminoadipic.acid
a.aminobutyric.acid
amp
arginine
aspartate
b.aminoisobutyric
betaine
fad
glutamate
glutamine
glycine
histidine
hydroxyproline
isoleucine
leucine
lysine
methionine
nad
phenylalanine
proline
sarcosine
serine
threonine
tryptophan
tyrosine
valine
acetyl.coa
adp
lactate
malate
nadh
oxaloacetate
phosphoenyl.pyruvate
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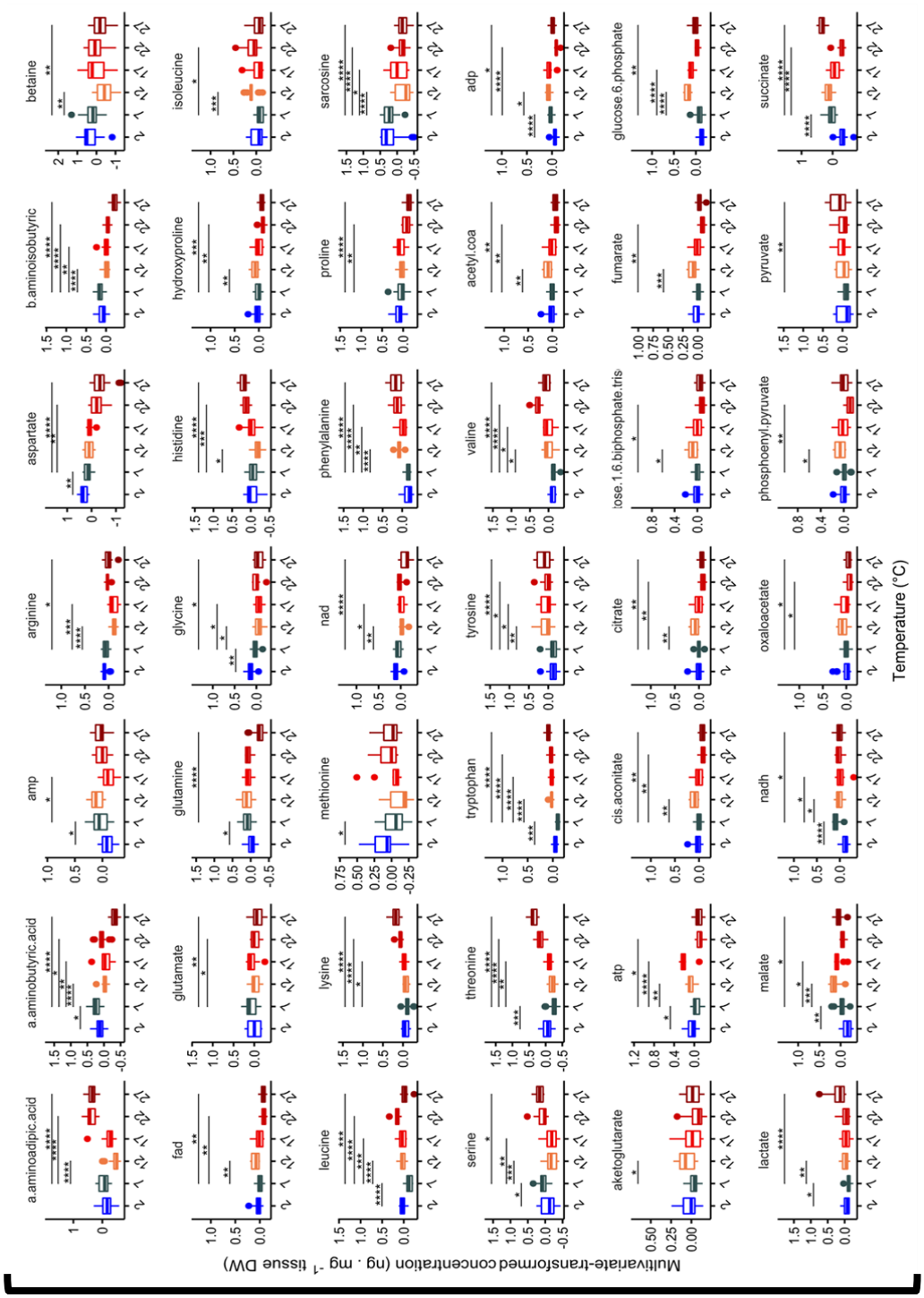
fumarate
glucose.6.phosphate


a.aminoadipic.acid
a.aminobutyric.acid
amp
arginine
aspartate
b.aminoisobutyric
betaine
fad
glutamate
glutamine
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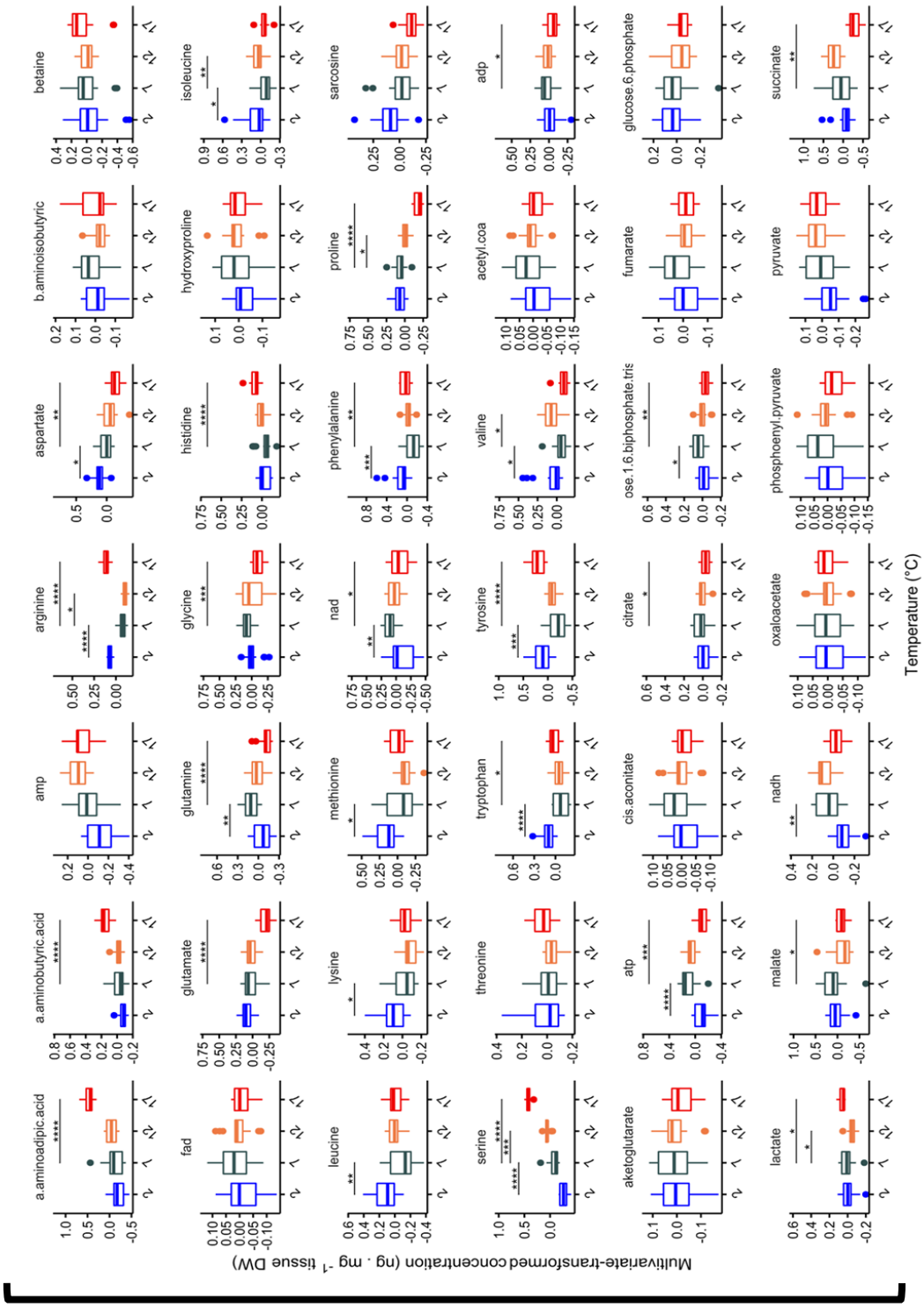
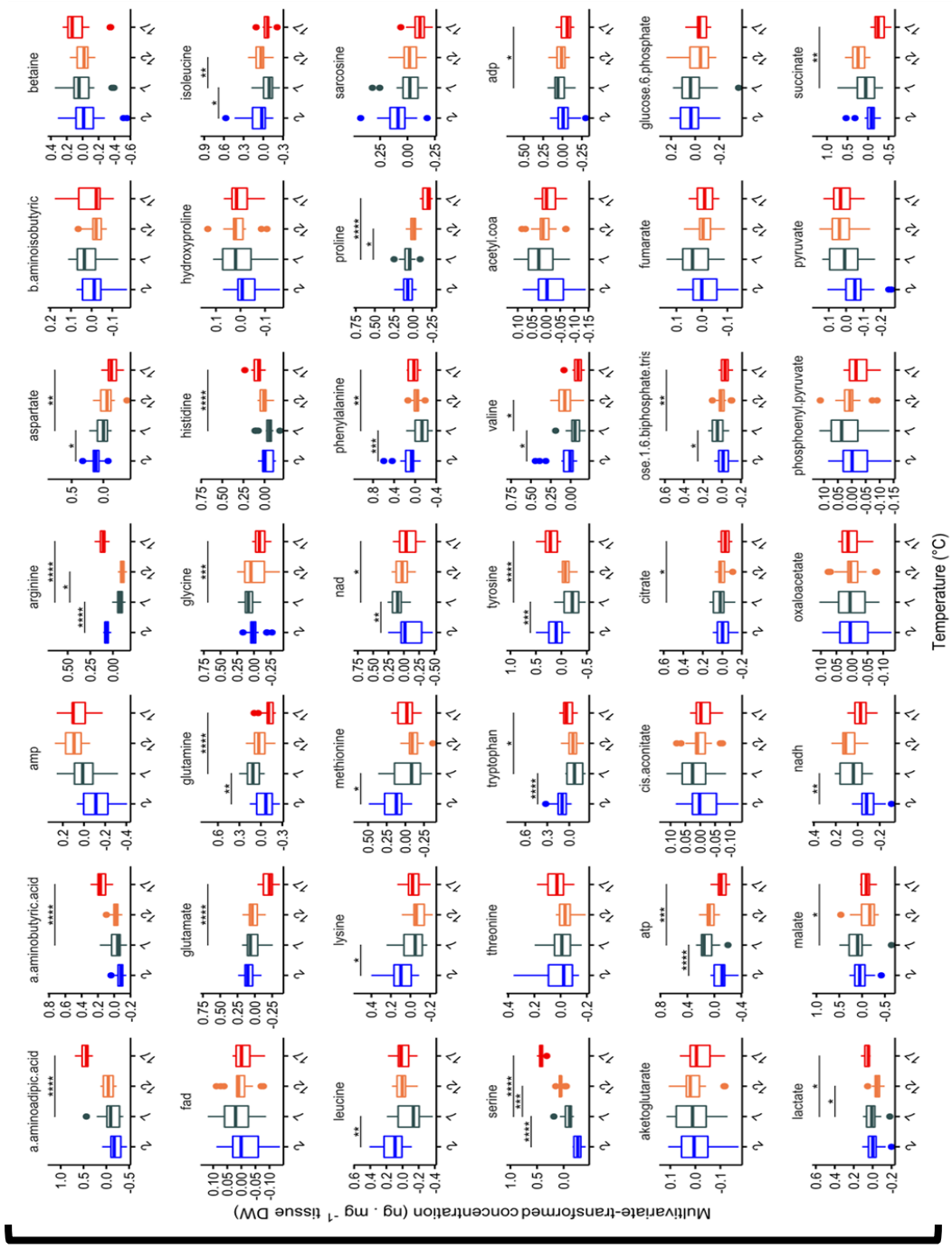
histidine
hydroxyproline
isoleucine
leucine
lysine
methionine
nad
phenylalanine
proline
sarcosine
serine
threonine
tryptophan
tyrosine
valine
acetyl.coa
adp
aketoglutarate
atp
cis.aconitate
citrate
fumarate
glucose.6.phosphate
lactate
malate
nadh
oxaloacetate
phosphoenyl.pyruvate
pyruvate
succinate
Multivariate-transformed concentration (ng . mg -1 tissue DW)
Temperature (°C)

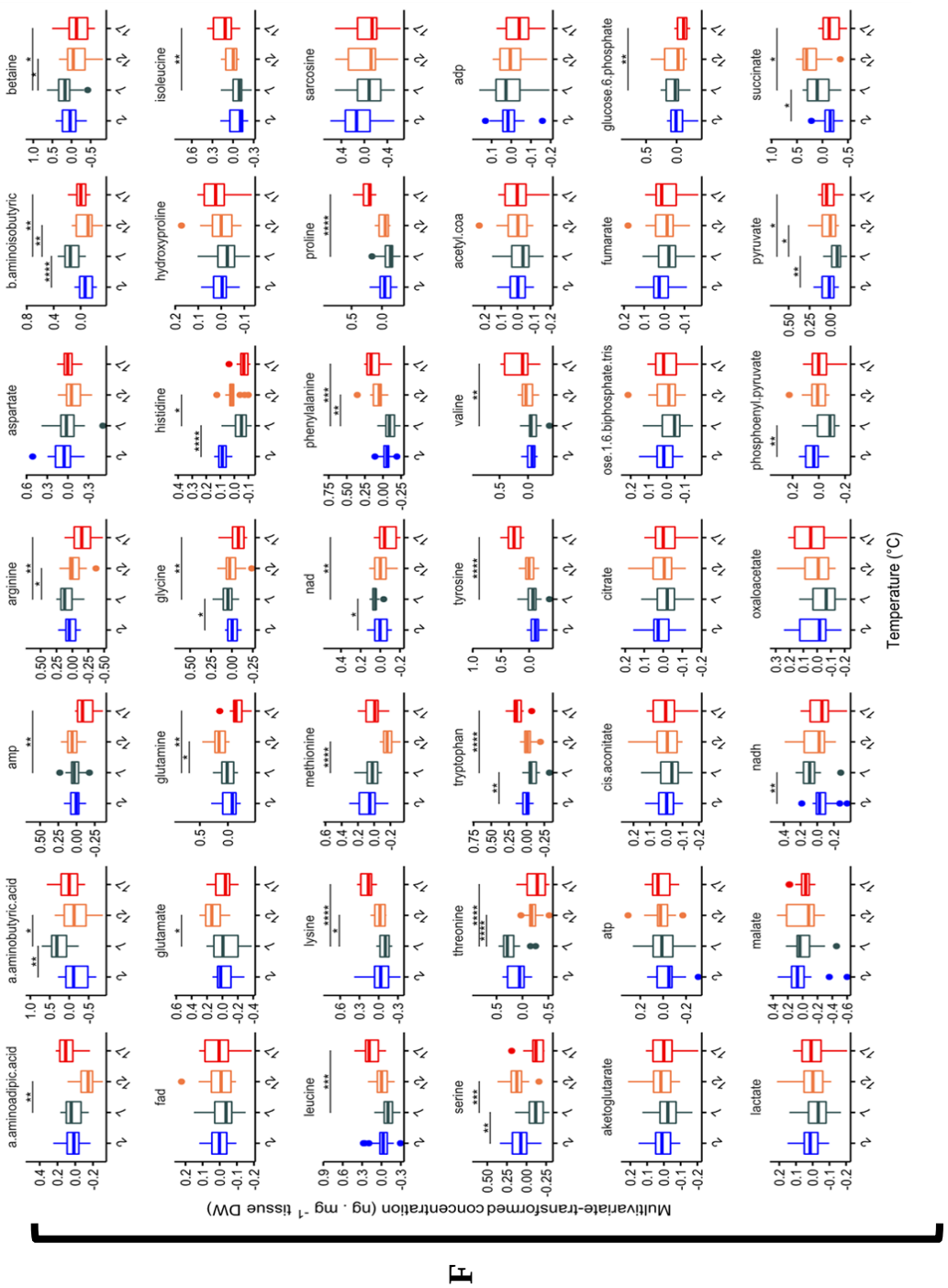
C


Multivariate-transformed concentration (ng . mg -1 tissue DW)
Temperature (°C)
a.aminoadipic.acid
a.aminobutyric.acid
amp
arginine
aspartate
b.aminoisobutyric
betaine
fad
glutamate
glutamine
glycine
histidine
hydroxyproline
isoleucine
leucine
lysine
methionine
nad
phenylalanine
proline
sarcosine
serine
threonine
tryptophan
tyrosine
valine
acetyl.coa
adp
aketoglutarate
atp
cis.aconitate
citrate
ose.1.6.biphosphate.tris
fumarate
glucose.6.phosphate
lactate
malate
nadh
oxaloacetate
phosphoenyl.pyruvate
pyruvate
succinate

D


Multivariate-transformed concentration (ng . mg -1 tissue DW)
Temperature (°C)
a.aminoadipic.acid
a.aminobutyric.acid
amp
arginine
aspartate
b.aminoisobutyric
betaine
fad
glutamate
glutamine
glycine
histidine
hydroxyproline
isoleucine
leucine
lysine
methionine
nad
phenylalanine
proline
sarcosine
serine
threonine
tryptophan
tyrosine
valine
acetyl.coa
adp
atp
cis.aconitate
citrate
ose.1.6.biphosphate.tris
fumarate
glucose.6.phosphate
ketoglutarate
malate
nadh
oxaloacetate
phosphoenyl.pyruvate
pyruvate
succinate
lactate

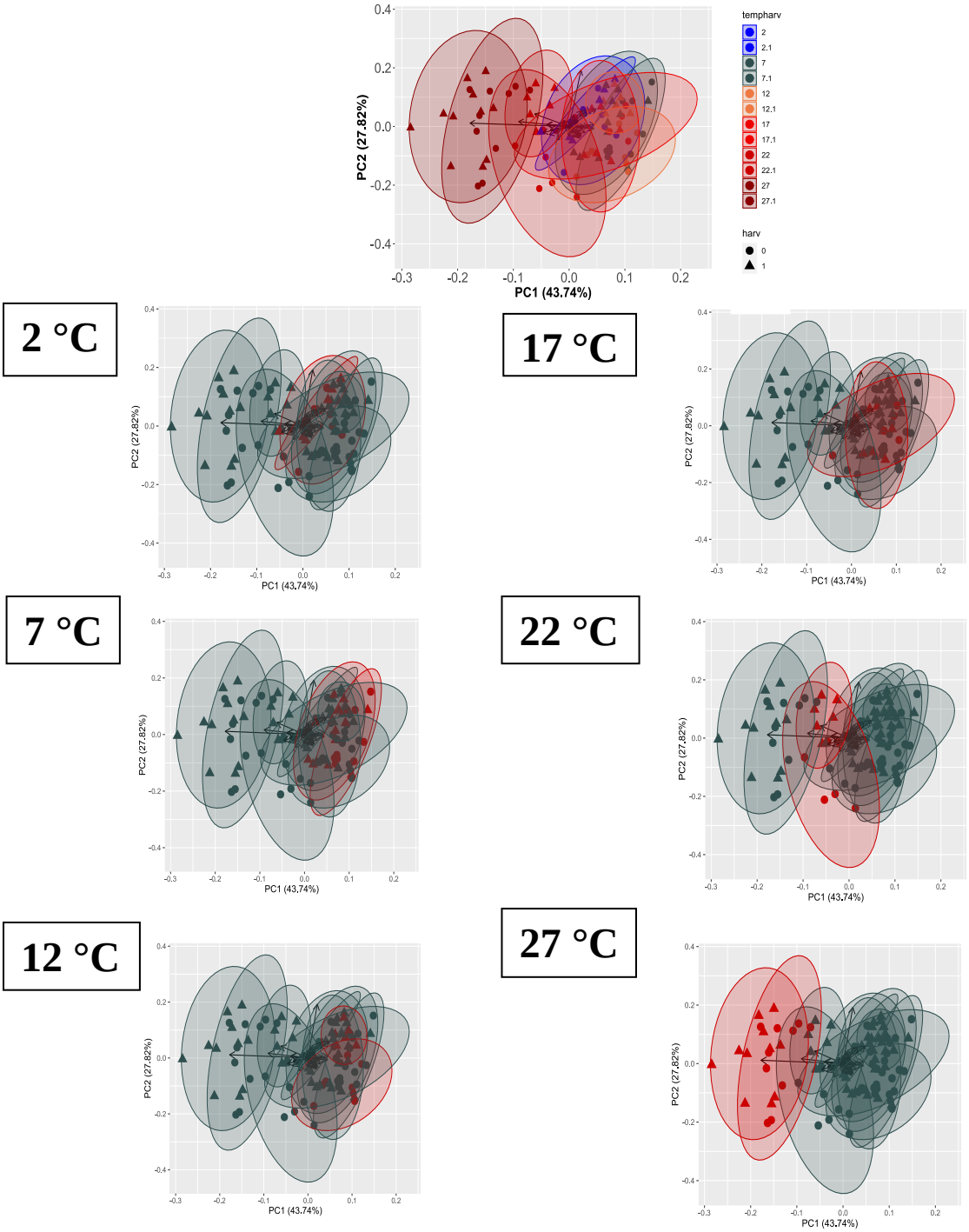
F



Supplementary Figure 3: Boxplots of log-transformed metabolite levels at every temperature treatment of *M. arenaria* tissues (2, 7, 12, 17, 22, and 27 °C) and in *M. truncata* tissues (2, 7, 12, and 17 °C). Gills, mantle, and adductor tissues are reported in panels A, B and C for *M. arenaria* and D, E, and F for *M. truncata.* Significance levels (*, **, ***, ****: < 0.05, < 0.01, < 0.001, < 0.0001) of treatments were tested against the 7 °C treatment using unpaired t-tests.

ANNEXE VIII

Supplementary Figure 4: Principal component analysis (PCA) plot displaying the principal components PC1 and PC2 of the metabolome (42 metabolites) of *gills* in *M. arenaria*. Each plot highlights one grouping variable *temperature* (2, 7, 12, 17, 22, 27 °C, if available) and *harvest* (with, without).



ANNEXE IX

Supplementary Table 5: Full VIP output results from the five first components of PLS-DA with the grouping variable temperature in all six analyses.

Mya arenaria	gills					mantle					adductor				
	comp1	comp2	comp3	comp4	comp5	comp1	comp2	comp3	comp4	comp5	comp1	comp2	comp3	comp4	comp5
α-aminoadipic.acid	**1.58**	**1.33**	**1.15**	**1.07**	0.99	**1.16**	**1.19**	**1.21**	**1.09**	**1.01**	**1.45**	**1.38**	**1.12**	0.98	**1.02**
α-aminobutyric.acid	0.80	**1.03**	**1.19**	**1.11**	**1.13**	0.58	0.95	**1.05**	0.92	0.84	**1.47**	**1.35**	**1.10**	**1.19**	**1.18**
amp	0.76	0.82	0.88	0.85	**1.02**	0.46	0.39	0.63	0.62	0.70	0.26	0.38	0.82	0.75	0.93
arginine	0.65	**1.01**	0.86	0.79	0.85	**1.03**	**1.32**	**1.29**	**1.13**	**1.15**	0.15	**1.10**	0.92	**1.06**	0.98
aspartate	0.23	0.98	**1.64**	**1.51**	**1.37**	0.59	**1.06**	0.98	**1.22**	**1.30**	**1.53**	**1.26**	**1.03**	0.89	0.96
β-aminoisobutyric	**1.60**	**1.42**	**1.34**	**1.23**	**1.21**	0.38	0.88	**1.04**	0.94	0.90	**1.65**	**1.44**	**1.18**	**1.06**	**1.07**
betaine	0.07	0.07	0.19	0.98	0.88	0.48	0.83	**1.07**	**1.02**	0.95	0.49	0.76	0.62	0.59	0.78
fad	0.95	0.81	0.64	0.79	0.77	**1.46**	**1.08**	0.91	0.80	0.73	**1.06**	**1.03**	0.89	0.89	0.83
glutamate	0.82	0.87	**1.72**	**1.58**	**1.44**	0.33	0.83	**1.31**	**1.24**	**1.21**	0.70	0.57	0.59	0.56	0.50
glutamine	**1.37**	**1.46**	**1.17**	**1.10**	**1.01**	0.76	**1.05**	**1.60**	**1.62**	**1.48**	**1.15**	0.95	0.77	**1.38**	**1.29**
glycine	0.67	**1.11**	**1.10**	**1.00**	0.92	0.59	**1.16**	0.99	0.87	0.82	0.66	0.84	0.79	0.92	0.87
histidine	**1.44**	**1.26**	0.99	0.90	0.96	**1.13**	0.98	0.83	0.84	0.96	**1.26**	**1.11**	0.89	0.78	0.75
hydroxyproline	0.09	0.10	0.08	0.79	0.71	**1.22**	0.92	0.77	0.68	0.62	**1.05**	1.00	0.82	0.87	0.84
isoleucine	0.47	0.42	0.51	0.94	0.85	0.95	**1.10**	**1.03**	0.91	0.83	0.12	0.30	0.85	0.75	**1.20**
leucine	**1.13**	0.96	**1.45**	**1.36**	**1.25**	0.23	0.36	**1.03**	**1.73**	**1.61**	0.42	0.37	**1.79**	**1.76**	**1.60**
lysine	0.54	**1.02**	0.95	0.90	0.84	**1.51**	**1.21**	**1.27**	**1.12**	**1.08**	**1.67**	**1.37**	**1.12**	**1.01**	0.94
methionine	0.85	0.72	0.63	0.63	**1.45**	0.20	0.15	**1.14**	**1.03**	**1.34**	0.19	0.54	0.75	0.67	0.64
nad	0.38	0.39	0.31	0.64	0.57	0.59	0.97	**1.03**	0.90	0.90	**1.17**	**1.13**	0.97	0.85	0.77
phenylalanine	**1.01**	0.92	0.72	0.87	0.86	0.23	**1.25**	**1.22**	**1.07**	0.98	**1.38**	**1.25**	**1.06**	0.93	0.95
proline	0.57	0.62	0.62	0.60	**1.33**	0.38	0.32	0.47	0.41	0.71	**1.31**	**1.07**	0.90	0.81	0.75
sarcosine	0.71	0.77	0.74	**1.05**	**1.30**	0.62	**1.10**	0.93	0.84	0.83	0.97	0.99	0.90	0.80	0.85
serine	**1.23**	**1.07**	0.84	0.79	0.81	**1.47**	**1.32**	**1.14**	**1.07**	**1.17**	0.93	0.95	**1.12**	0.98	0.92
threonine	**1.67**	**1.45**	**1.15**	**1.05**	**1.04**	**1.36**	**1.22**	**1.04**	**1.02**	**1.08**	**1.79**	**1.48**	**1.26**	**1.18**	**1.16**
tryptophan	**1.62**	**1.45**	**1.20**	**1.09**	**1.00**	0.79	**1.29**	**1.10**	**1.35**	**1.23**	**1.55**	**1.42**	**1.35**	**1.18**	**1.10**
tyrosine	0.07	0.49	0.85	0.85	0.77	0.32	0.54	0.77	0.92	0.91	0.92	0.90	0.74	0.67	0.62
valine	**1.36**	**1.15**	0.98	**1.01**	0.91	0.25	**1.52**	**1.29**	**1.14**	**1.04**	**1.18**	0.97	**1.21**	**1.23**	**1.40**
acetyl.coa	0.88	0.78	0.61	0.81	0.75	**1.42**	**1.05**	0.89	0.77	0.71	0.96	0.98	0.82	0.85	0.82
adp	**1.36**	**1.21**	0.97	0.90	0.81	**1.52**	**1.13**	0.98	0.86	0.88	0.54	**1.05**	**1.30**	**1.15**	**1.10**
aketoglutarate	0.57	0.73	0.57	0.61	0.72	**1.17**	0.90	0.76	0.69	0.66	0.27	0.46	0.50	0.81	0.88
atp	**1.34**	**1.30**	**1.03**	**1.00**	**1.19**	**1.49**	**1.08**	0.91	0.82	0.86	0.93	**1.03**	**1.10**	0.98	**1.39**
cis.aconitate	0.92	0.80	0.63	0.84	0.79	**1.40**	**1.04**	0.88	0.77	0.70	**1.02**	**1.02**	0.87	0.87	0.83
citrate	**1.10**	0.99	0.77	0.79	0.73	**1.42**	**1.05**	0.89	0.78	0.71	0.87	0.93	0.76	0.79	0.78
d.fructose.1.6.biphosphate.trisodium	**1.18**	**1.06**	0.84	0.81	0.76	**1.40**	**1.04**	0.90	0.79	0.75	0.74	0.84	0.68	0.80	0.80
fumarate	0.98	0.87	0.73	0.86	0.78	**1.46**	**1.08**	0.91	0.81	0.76	0.70	0.95	0.77	0.83	0.76
glucose.6.phosphate	**1.20**	**1.23**	**1.15**	**1.08**	**1.00**	**1.31**	0.95	0.96	0.86	0.80	0.29	**1.30**	**1.05**	**1.26**	**1.21**
lactate	0.22	**1.10**	0.91	0.83	0.75	0.90	**1.34**	**1.14**	**1.26**	**1.20**	**1.20**	**1.01**	0.81	**1.07**	0.97
malate	0.55	0.95	**1.19**	**1.09**	**1.08**	**1.41**	**1.16**	0.98	0.93	**1.06**	0.39	**1.11**	**1.07**	**1.16**	**1.08**
nadh	**1.47**	**1.24**	**1.06**	0.97	0.91	0.70	0.52	0.50	**1.63**	**1.59**	0.21	0.23	**1.41**	**1.34**	**1.39**
oxaloacetate	**1.01**	0.89	0.74	0.86	0.78	0.96	0.75	0.64	0.59	0.58	0.69	0.74	0.61	0.65	0.72
phosphoenyl.pyruvate	**1.23**	**1.05**	0.82	0.83	0.76	**1.09**	0.83	0.70	0.64	0.59	0.29	0.65	0.54	0.99	0.90
pyruvate	0.49	**1.10**	0.98	**1.06**	0.97	0.38	0.61	0.51	0.65	0.60	0.68	0.60	0.51	0.73	0.74
succinate	0.38	0.67	**2.03**	**1.86**	**1.71**	0.44	0.47	**1.17**	**1.03**	**1.55**	0.96	**1.04**	**1.80**	**1.61**	**1.47**

Supplementary Table 5 (cont.): Full VIP output results from the five first components of PLS-DA with the grouping variable temperature in all six analyses.

Mya truncata	gills					mantle					adductor				
	comp1	comp2	comp3	comp4	comp5	comp1	comp2	comp3	comp4	comp5	comp1	comp2	comp3	comp4	comp5
α-aminoadipic.acid	**2.10**	**1.73**	**1.43**	**1.27**	**1.24**	0.60	0.53	**1.01**	0.96	0.95	0.34	0.26	**1.37**	**1.52**	**1.48**
α-aminobutyric.acid	**1.87**	**1.63**	**1.34**	**1.22**	**1.23**	0.38	**1.46**	**1.25**	**1.26**	**1.24**	0.61	**1.14**	**1.09**	0.93	0.92
amp	0.64	0.98	0.81	**1.12**	**1.17**	0.08	0.25	**1.45**	**1.37**	**1.36**	**1.15**	0.94	**1.03**	0.92	0.89
arginine	**1.70**	**1.64**	**1.84**	**1.66**	**1.58**	**1.79**	**1.30**	**1.16**	**1.09**	**1.07**	**1.54**	**1.17**	0.93	0.82	0.80
aspartate	**1.24**	**1.19**	**1.12**	**1.00**	**1.08**	0.24	**1.44**	**1.51**	**1.35**	**1.32**	0.17	0.14	0.16	0.84	0.94
β-aminoisobutyric	0.17	0.39	0.57	0.65	0.78	0.36	**1.90**	**1.63**	**1.47**	**1.44**	0.83	**1.15**	**1.35**	**1.15**	**1.12**
betaine	0.34	0.36	0.57	0.66	**1.02**	**1.25**	0.84	0.71	0.74	0.73	**1.02**	1.00	0.78	0.76	0.73
fad	0.42	0.56	0.74	0.68	0.66	0.65	0.48	0.69	0.74	0.77	0.27	0.91	0.84	0.69	0.69
glutamate	**1.75**	**1.47**	**1.22**	**1.13**	**1.08**	0.42	0.35	0.59	0.72	0.73	0.33	0.40	0.73	0.95	0.94
glutamine	**1.18**	**1.05**	**1.50**	**1.73**	**1.65**	0.70	**1.33**	**1.23**	**1.12**	**1.09**	0.88	0.73	**1.04**	**1.31**	**1.32**
glycine	**1.06**	0.88	0.80	0.75	0.79	**1.49**	0.96	0.87	0.78	0.76	**1.13**	0.95	0.79	0.70	0.73
histidine	**1.58**	**1.28**	**1.16**	**1.04**	**1.01**	0.70	0.72	0.62	0.60	0.60	0.52	0.85	**1.69**	**1.84**	**1.79**
hydroxyproline	0.01	0.52	0.72	0.76	0.73	0.88	0.73	0.75	0.75	0.77	0.50	0.89	0.79	0.65	0.64
isoleucine	0.25	0.73	0.80	**1.11**	**1.11**	**1.48**	0.98	0.87	0.78	0.78	**1.46**	**1.10**	0.96	0.95	0.97
leucine	0.24	0.95	0.79	0.89	0.85	**1.21**	**1.82**	**1.55**	**1.40**	**1.45**	**1.61**	**1.21**	0.95	0.80	0.79
lysine	0.32	0.87	0.71	0.91	0.87	0.55	0.39	0.44	0.80	0.79	**1.60**	**1.42**	**1.23**	**1.04**	**1.01**
methionine	0.02	0.95	0.79	0.90	0.95	**1.03**	**1.12**	0.96	0.87	0.85	0.32	0.42	0.62	**1.70**	**1.66**
nad	0.69	0.87	0.73	0.77	0.91	0.63	0.45	0.38	0.59	0.58	**1.41**	**1.09**	0.86	0.73	0.72
phenylalanine	0.52	**1.05**	0.86	0.89	0.86	**1.62**	**1.15**	**1.01**	0.93	0.93	**1.72**	**1.32**	**1.04**	**1.06**	**1.06**
proline	**2.07**	**1.71**	**1.41**	**1.30**	**1.25**	**1.50**	**1.19**	1.00	**1.01**	**1.01**	**2.06**	**1.68**	**1.32**	**1.09**	**1.08**
sarcosine	0.87	0.93	0.76	0.67	0.97	**1.61**	**1.07**	0.91	0.84	0.82	0.09	0.26	0.84	0.85	0.84
serine	**2.13**	**1.90**	**1.68**	**1.47**	**1.41**	**1.13**	0.73	**1.31**	**1.22**	**1.21**	0.01	0.34	**1.96**	**1.60**	**1.55**
threonine	0.39	0.46	0.61	0.95	1.00	**2.00**	**1.34**	**1.64**	**1.48**	**1.47**	**1.85**	**1.40**	**1.23**	**1.27**	**1.24**
tryptophan	0.43	**1.10**	0.93	0.84	0.90	**1.73**	**1.41**	**1.20**	**1.31**	**1.29**	**1.84**	**1.39**	**1.09**	**1.03**	**1.05**
tyrosine	**1.48**	**1.39**	**1.15**	**1.02**	0.98	**1.77**	**1.58**	**1.32**	**1.42**	**1.38**	**1.90**	**1.89**	**1.49**	**1.29**	**1.25**
valine	0.62	0.75	0.76	**1.23**	**1.19**	**1.50**	**1.01**	0.85	0.76	0.81	**1.57**	**1.18**	0.99	0.84	0.85
acetyl.coa	0.32	0.56	0.73	0.69	0.66	0.72	0.50	0.66	0.74	0.75	0.18	0.92	0.86	0.73	0.73
adp	0.80	0.75	0.70	0.63	0.63	0.20	0.61	0.58	0.70	0.80	0.63	0.88	0.96	0.79	0.83
aketoglutarate	0.25	0.34	0.85	0.87	0.83	0.67	0.44	0.51	0.76	0.75	0.17	**1.02**	0.84	0.69	0.67
atp	**1.02**	**1.20**	**1.04**	0.93	0.94	0.24	0.97	**1.27**	**1.14**	**1.12**	0.18	0.20	0.42	0.62	0.75
cis.aconitate	0.43	0.57	0.76	0.68	0.65	0.71	0.48	0.67	0.73	0.75	0.21	0.92	0.86	0.71	0.72
citrate	0.68	0.66	0.84	0.74	0.75	0.61	0.42	0.64	0.80	0.79	0.00	**1.04**	0.87	0.73	0.71
d.fructose.1.6.biphosphate.trisodium	0.86	0.89	0.87	0.83	0.84	0.51	0.35	0.72	0.77	0.81	0.26	1.00	0.83	0.70	0.68
fumarate	0.44	0.52	0.91	0.83	0.80	0.70	0.46	0.58	0.77	0.76	0.05	0.97	0.85	0.76	0.75
glucose.6.phosphate	0.44	0.40	0.95	0.85	0.81	0.13	0.65	0.55	0.61	0.74	0.79	0.70	0.56	0.72	0.94
lactate	0.98	0.81	**1.23**	**1.26**	**1.22**	**1.11**	0.78	0.68	0.78	0.76	0.19	1.00	0.85	0.70	0.68
malate	0.66	0.53	0.78	0.92	0.88	0.20	0.23	0.19	0.40	0.68	0.05	0.32	0.25	0.27	0.67
nadh	0.47	**1.00**	0.87	0.88	0.85	0.21	**1.38**	**1.61**	**1.71**	**1.68**	0.80	0.75	**1.09**	**1.20**	**1.18**
oxaloacetate	0.03	0.27	0.86	0.87	0.83	0.62	0.41	0.34	0.76	0.77	0.68	0.93	0.75	0.62	0.65
phosphoenyl.pyruvate	0.67	0.68	0.82	0.74	0.71	0.55	0.50	0.48	0.68	0.71	0.40	**1.15**	0.98	0.94	0.92
pyruvate	0.30	0.67	0.69	0.81	0.79	0.71	0.57	0.48	0.70	0.68	0.88	**1.10**	0.88	0.75	0.72
succinate	**1.27**	**1.05**	**1.36**	**1.34**	**1.31**	0.45	**1.87**	**1.62**	**1.50**	**1.47**	0.74	0.58	0.52	**1.47**	**1.45**

ANNEXE X

Supplementary Table 6: Summary of significantly altered pathways ($P < 0.05$) at all temperature treatments in *M. arenaria* (2 - 27 °C) and *M. truncata* (2 - 17 °C) resulting from a knowledge-based network and diffusion algorithm (*FELLA*).

M. arenaria				
Gills				
2 °C	12 °C	17 °C	22 °C	27 °C
Aminoacyl-tRNA biosynthesis	Phosphatidylinositol signaling system	Taurine and hypotaurine metabolism	Aminoacyl-tRNA biosynthesis	Purine metabolism
Neuroactive ligand-receptor interaction	Endocytosis - Crassostrea gigas	Arginine biosynthesis		Ribosome biogenesis in eukaryotes
Purine metabolism	Inositol phosphate metabolism	Glutathione metabolism		Nucleocytoplasmic transport
Biosynthesis of amino acids	Autophagy - other	D-Amino acid metabolism		mRNA surveillance pathway
Lysine biosynthesis	Pyrimidine metabolism	Neuroactive ligand-receptor interaction		RNA polymerase
FoxO signaling pathway	mRNA surveillance pathway	Purine metabolism		Basal transcription factors
Alanine, aspartate and glutamate metabolism	Ubiquitin mediated proteolysis			DNA replication
mTOR signaling pathway	Ribosome biogenesis in eukaryotes			Nucleotide excision repair
Non-homologous end-joining	Basal transcription factors			Mismatch repair
Other glycan degradation	Wnt signaling pathway			Homologous recombination
Base excision repair	Homologous recombination			Non-homologous end-joining
Mismatch repair	Fanconi anemia pathway			FoxO signaling pathway
DNA replication	Mitophagy - animal			Phosphatidylinositol signaling system
Inositol phosphate metabolism	Autophagy - animal			Autophagy - other
Glutathione metabolism	Spliceosome			Mitophagy - animal
Autophagy - animal	Nucleotide excision repair			Autophagy - animal
Taurine and hypotaurine metabolism	TGF-β signaling pathway			Endocytosis
Mitophagy - animal				mTOR signaling pathway
				Wnt signaling pathway
				TGF-β signaling pathway
				Fanconi anemia pathway
				Spliceosome
				RNA degradation
				Ubiquitin mediated proteolysis

M. arenaria				
Mantle				
2 °C	12 °C	17 °C	22 °C	27 °C
Alanine, aspartate and glutamate metabolism	2-Oxocarboxylic acid metabolism	Aminoacyl-tRNA biosynthesis	Drug metabolism - other enzymes	Phosphatidylinositol signaling system
Pyrimidine metabolism	Valine, leucine and isoleucine biosynthesis	Folate biosynthesis	Neuroactive ligand-receptor	Endocytosis
Other glycan degradation		Phenylalanine, tyrosine and tryptophan biosynthesis	Base excision repair	Wnt signaling pathway
		Valine, leucine and isoleucine biosynthesis	RNA polymerase	Autophagy - other
			Mismatch repair	TGF-β signaling pathway
			DNA replication	Basal transcription factors
			Purine metabolism	Autophagy - animal
			Pyrimidine metabolism	Mitophagy - animal
			Alanine, aspartate and glutamate metabolism	Phagosome
			Arginine biosynthesis	Homologous recombination
			Phenylalanine, tyrosine and tryptophan biosynthesis	Spliceosome
			Nucleotide excision repair	Nucleocytoplasmic transport
			Thiamine metabolism	Fanconi anemia pathway

M. arenaria				
Muscle				
2 °C	12 °C	17 °C	22 °C	27 °C
Endocytosis	Inositol phosphate metabolism	Aminoacyl-tRNA biosynthesis	Biosynthesis of amino acids	D-Amino acid metabolism
Ribosome biogenesis in eukaryotes		mTOR signaling pathway	Basal transcription factors	β-Alanine metabolism
Autophagy - other			Wnt signaling pathway	Glycine, serine and threonine metabolism
Phosphatidylinositol signaling syst.			mRNA surveillance pathway	Arginine and proline metabolism
Nucleotide excision repair			Endocytosis	Neuroactive ligand-receptor interaction
Homologous recombination			TGF-β signaling pathway	
Non-homologous end-joining			Nucleocytoplasmic transport	
Mitophagy - animal			Mitophagy - animal	
Wnt signaling pathway			Homologous recombination	
mRNA surveillance pathway			Aminoacyl-tRNA biosynthesis	
Fanconi anemia pathway			RNA polymerase	
Autophagy - animal			Spliceosome	
TGF-β signaling pathway				
Basal transcription factors				
Ubiquitin mediated proteolysis				
DNA replication				
Mismatch repair				
Aminoacyl-tRNA biosynthesis				
Sulfur relay system				
Base excision repair				
Cysteine and methionine metabolism				
Purine metabolism				
Lipoic acid metabolism				
FoxO signaling pathway				
Thiamine metabolism				
Selenocompound metabolism				
Spliceosome				

M. truncata		
Gills		
2 °C	12 °C	17 °C
Aminoacyl-tRNA biosynthesis Phenylalanine, tyrosine, and tryptophan biosynthesis Valine, leucine, and isoleucine biosynthesis.	ABC transporters Valine, leucine, and isoleucine biosynthesis Aminoacyl-tRNA biosynthesis Arginine and proline metabolism	Neuroactive ligand-receptor interaction Biosynthesis of amino acids Phenylalanine, tyrosine, and tryptophan biosynthesis Thiamine metabolism Arginine biosynthesis Taurine and hypotaurine metabolism Endocytosis Phosphatidylinositol signaling system

M. truncata		
Mantle		
2 °C	12 °C	17 °C
Aminoacyl-tRNA biosynthesis mTOR signaling pathway Phagosome Non-homologous end-joining DNA replication Mismatch repair Autophagy - animal Base excision repair Nucleotide excision repair Mitophagy - animal Ribosome biogenesis in eukaryotes Homologous recombination	Cysteine and methionine metabolism Selenocompound metabolism Sulfur relay system Porphyrin and chlorophyll metabolism Ubiquinone and other terpenoid-quinone biosynthesis One carbon pool by folate Thiamine metabolism Lipoic acid metabolism Phenylalanine metabolism Phenylalanine, tyrosine, and tryptophan biosynthesis Folate biosynthesis Glycine, serine, and threonine metabolism	ABC transporters Glycine, serine, and threonine metabolism Aminoacyl-tRNA biosynthesis D-Amino acid metabolism Arginine and proline metabolism Valine, leucine, and isoleucine biosynthesis Phenylalanine, tyrosine, and tryptophan biosynthesis

M. truncata		
Muscle		
2 °C	12 °C	17 °C
Ascorbate and aldarate metabolism Sulfur relay system	Cysteine and methionine metabolism Selenocompound metabolism D-Amino acid metabolism Histidine metabolism β-Alanine metabolism Biosynthesis of amino acids Sulfur relay system Lysine biosynthesis Porphyrin and chlorophyll metabolism Arginine biosynthesis	Aminoacyl-tRNA biosynthesis ABC transporters Valine, leucine, and isoleucine biosynthesis

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