Intertidal limits shape covariation between metabolic plasticity, oxidative stress and telomere dynamics in Pacific oyster (*Crassostrea gigas*)

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Abstract :

In intertidal zones, species such as sessile shellfish exhibit extended phenotypic plasticity to face rapid environmental changes, but whether frequent exposure to intertidal limits of the distribution range impose physiological costs for the animal remains elusive. Here, we explored how phenotypic plasticity varied along foreshore range at multiple organization levels, from molecular to cellular and whole organism acclimatization, in the Pacific oyster (Crassostrea gigas). We exposed 7-month-old individuals for up to 16 months to three foreshore levels covering the vertical range for this species, representing 20, 50 and 80% of the time spent submerged monthly. Individuals at the upper range limit produced energy more efficiently, as seen by steeper metabolic reactive norms and unaltered ATP levels despite reduced mitochondrial density. By spending most of their time emerged, oysters mounted an antioxidant shielding concomitant with lower levels of pro-oxidant proteins and postponed age-related telomere attrition. Instead, individuals exposed at the lower limit range near subtidal conditions showed lower energy efficiencies, greater oxidative stress and shorter telomere length. These results unraveled the extended acclimatization strategies and the physiological costs of living too fast in subtidal conditions for an intertidal species.

Graphical abstract
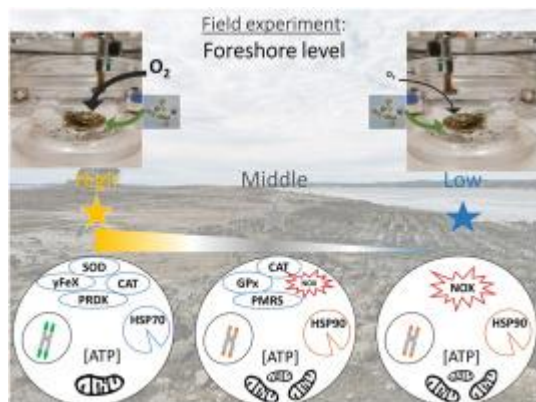
Highlights

► Growing at different foreshore levels shape acclimatization in an intertidal shellfish. ► Oysters in high shores produce antioxidant shielding and use energy more efficiently. ► These oysters have longer telomeres than those growing in middle and low shores.

Keywords : Acclimatization, Foreshore, Growth, Metabolism, Oyster, Telomeres

Introduction

Organisms inhabiting variable environments evolved with extended phenotypic plasticity,

including acclimatization capacities to endure frequent and intense environmental changes

(Burton et al., 2022; Chevin and Hoffmann, 2017; Helmuth et al., 2010). Species within the

intertidal zones experience strong variations over short period following daily successions

along tide cycles. Intertidal ectotherms are noticeably known for their physiological flexibility

to face huge thermal amplitudes and severe changes of their physiology (Clark et al., 2018; L.

Li et al., 2018). Emerged individuals should favour maintenance and physiological resistance

to multiple constraints when other functions (e.g. growth, storage, reproduction) are

hampered. Conversely, regular submersions in sea water near subtidal conditions should keep

stimulating aerobic metabolic, physiological processes and possibly accelerate pace of life

given that oysters can access and process phytoplankton (Bourlès et al., 2009). Nevertheless,

how accelerated growth rates could induce fitness costs in the long run remain to be

determined.

Bivalve molluscs constitute an emblematic clade within intertidal ecosystems (zu Ermgassen

et al., 2020). Inter-individual variation in life-history trajectories among conspecifics is

greatly explained by geographic gradients. For example, latitudinal changes in temperature

explain variation of growth rates and phenology (Fleury et al., 2020; Gourault et al., 2019;

Mazaleyrat et al., 2022; Thomas et al., 2016), which are known as major sources of selection

in these organisms (L. Li et al., 2018). Such geographic gradients are likely to shape

metabolic acclimation to optimise energy use (Gaitán-Espitia et al., 2017). For example in

ectotherms, the temperature dependence of metabolic rates may be stronger in cold climates

to help individuals performing physiological activities despite suboptimal conditions [e.g.,

metabolic cold adaptation, (Dupoué et al., 2017; White et al., 2011)]. Flexible reaction norms

62 may also occur along vertical gradient, across foreshore levels (Dong et al., 2022). Bivalves

63 then represent particularly useful and relevant models to go deeper and explore mechanisms

64 framing contrasted life-history trajectories across foreshore levels.

65

66 Among those, oxidative stress and telomere dynamics have been regularly proposed as the

67 molecular regulators of the pace of life (Andrade et al., 2021; Giraudeau et al., 2019).

68 Oxidative stress arises whenever the natural redox balance is altered due to generation of pro-

69 oxidant compounds [reactive oxygen species (ROS)] that cannot be neutralised by antioxidant

70 activity (Costantini, 2014). ROS are continuously produced by the aerobic metabolism and

71 therefore, individuals with faster pace of life might be more likely to generate oxidative stress

72 (Metcalfe and Monaghan, 2001). Enhanced ROS levels can alter the integrity and functions of

73 biomolecules, eventually framing trade-offs between accelerated life style and delayed

74 survival or reproduction costs (Costantini, 2018; Dupoué et al., 2020; Metcalfe and Alonso-

75 Alvarez, 2010). Oxidative stress also contributes to aging by accelerating telomere erosion

76 (Chatelain et al., 2020; Reichert and Stier, 2017). Telomeres, the terminal nucleo-proteins

77 complex capping linear chromosomes, protect DNA from oxidative damages and to help

78 cellular machinery during cell replication (Monaghan et al., 2009). Given that they shorten

79 over cell divisions and may integrate various environmental stresses, they represent relevant

80 biomarkers of long-term and intergenerational fitness costs (Dupoué et al., 2022; Monaghan

81 et al., 2009; Wilbourn et al., 2018). Despite this potential, studies that investigated telomere

82 dynamics in marine ectotherms such as bivalves remain anecdotal (Godwin et al., 2012;

83 Gruber et al., 2014).

84

4

85   Here, we explored environmental imprinting of metabolic plasticity, oxidative stress markers

86   and telomere dynamics in the intertidal Pacific oyster (*Crassostrea gigas*), a marine bivalve

87   originated from Japan which has spread worldwide due to aquaculture (Troost, 2010).

88   Extensive research on this ecologically and economically important shellfish (i.e., 2 million

89   tons produced each year worldwide) proves an opportunity to clarify cellular and molecular

90   factors implied in response to tide cycle and heat stress (L. Li et al., 2018; Sussarellu et al.,

91   2010; Zhang et al., 2012). This coastal species is relatively competitive in warming sea water

92   due to a hundred copies of gene coding for Heat Shock Protein (HSP) 70 (Zhang et al., 2012).

93   Interestingly, it also possesses a greater number of antioxidant genes as compared to other

94   marine species (Zhang et al., 2016). In Europe, it is now extending its distribution poleward

95   up to Scandinavia (Wrange et al., 2010), but it is facing higher mortality risks at lowest

96   latitudes (Fleury et al., 2020; Mazaleyrat et al., 2022; Pernet et al., 2019). Using the

97   temperature and food dependence of physiological norms (i.e., thermal dependence of

98   physiological processes) in this species, growth rate is forecasted to be a key determinant of

99   oyster persistence in its habitat along latitude (Thomas and Bacher, 2018). The impacts of

100   other environmental gradients, especially bathymetry, should also represent important drivers

101   of contrasted life trajectories (Corporeau et al., 2022; A. Li et al., 2018; Meng et al., 2018). In

102   a previous study, juvenile oysters placed in bags at three foreshore elevations covering

103   intertidal range limits, shaped differential growth rates, resistance to polymicrobial disease

104   and reduction of mortality risks (Corporeau et al., 2022). In the present study, we investigated

105   the molecular and cellular mechanisms of phenotypic responses associated with accelerated

106   life cycles in oysters at the lower foreshore limit compared to those mostly emerged at the

107   upper limit (Fig. 1). We were interested in deciphering the intricate relationships between

108   physiological adjustments (whole organism reaction norms, haemocyte ATP content and

109   mitochondrial density), oxidative stress markers (differential protein expression) and telomere

110 biology (molecular assays). We hypothesized that oysters should acclimatize their physiology

111 to limit thermal dependence of energy expenditure when the oysters grow closed to subtidal

112 habitats. We predict that this lower range limit should expose oysters to greater cellular costs,

113 including oxidative stress and telomere shortening.

114

115 **Material & Methods**

116 *Animals and experimental design*

117 We used diploid *C. gigas* produced by the Ifremer laboratory from standardized gene pools

118 genitors (Fig. 1A, 1B) as extensively detailed in Petton et al. (2015) from three different

119 cohorts (cohort 1: produced in 2017; cohort 2: in 2020; cohort 3: in 2021). Each year (one

120 year corresponds to one cohort, see below), we deployed 2,700 spats (7 months old) in the

121 Bay of Brest (Brittany, France, 48° 20′ 06.19″ N, 4° 19′ 06.37″ W) at three foreshore levels,

122 using 3 regular-sized mesh oyster bags per level (triplicates; 300 oysters per bag). This

123 vertical gradient, hereafter called "High" (5m above mean sea level), "Middle" (3m), and

124 "Low" (1.6m) shores differ in time spent submerged over the month, respectively with ca. 20,

125 50, and 80% (Fig. 1C). These three elevations were selected to cover the bathymetric range

126 exploited by this intertidal bivalve in the field. We used thermal probes STPS

127 (http://www.nke-instrumentation.fr) to record environmental variation in temperature at 10-

128 min frequency and assess the fluctuating situation of the oysters, either submerged in sea

129 water or emerged in the air. Associated environmental data are available online (Petton et al.,

130 2020).

131

132 We used oysters of cohort 1 (9 months old) to examine short-term physiological responses

133 (proteomic) to different foreshore levels for 2 months while oysters of cohort 2 (17 to 23

6

months old) were studied for ca. 1-year acclimatization responses to contrasted environments

(reaction norms of food ingestion and oxygen consumption, mitochondrial density, ATP

content, and telomere length). Oysters from cohort 3 (8 months old) were sampled before

field deployment and were used as reference for telomere assays (see below). Timing,

husbandry conditions, sample size and assays performed on each cohort are summarised in

Table S1.

Molecular response to foreshore level - Proteomic assays

We collected proteomes in n = 15 oysters (5 individuals per foreshore level), that were flash

frozen in liquid nitrogen immediately at collection in the field and stored at -80°C until

biochemical assays. Total proteins content was extracted from entire animals following a

standard protocol as described previously (Méar et al., 2022) and extensively in

Supplementary Information. Briefly, a complete proteome was obtained for each individual

using Liquid Chromatography Tandem-Mass Spectrometry (Girard et al., 2022) and

alignment of mass spectra on the UniProt KB *C. gigas* protein database (release 31/07/2020;

40023 sequences) and a common proteomic contaminant database from the Max Planck

Institute of Biochemistry, Martinsried (247 sequences). Functional analysis of protein was

performed with topGO R Package using the elim algorithm and a background generated in

Ensembl – Biomart (Cunningham et al., 2022) for *C. gigas*, so we could focus our analyses on

chaperone proteins and those involved in oxidative stress responses.

Cellular responses to foreshore level – Haemocyte energetic efficiency

We collected haemolymphs (ca. 100 µL) in n = 135 oysters of cohort 2 sampled in June 2022

at 22 months old to use haemocytes as tools to investigate metabolic alterations at the cellular

level. Haemolymph was collected from the pericardiac sinus with an ice-cold 1 ml syringe

(non-heparinized) and a 27-gauge needle. Haemocytes were analysed for two indexes of

energy production: ATP levels and mitochondrial density. To examine if the food limitation

during the acclimation period could interfere with the results, half animals were fed *ad libitum*

with *Tisochrysis lutea* while the other half were kept fasted a week before sampling. We used

colorimetric assays to determine ATP content in haemolymph freshly sampled (CellTiter

GLO ®, PROMEGA G7570) and mitochondrial density from haemolymph fixed in

formaldehyde (MITO-ID ®, Enzo Life Science). Samples were measured in duplicates

following a protocol detailed in Supplementary Information.

Cellular response to foreshore level - Telomere assays

Multiple tests were performed to optimise a protocol for telomere assay in oysters from the

cohorts 2 sampled in June 2022 at 22 months old (i.e., 15 months in the field) and cohort 3

sampled in May 2022 at 8 months old. DNA was extracted using DNA Isolation kit for cell

and tissue (Sigma Aldrich, Roche) from 20-30 mg oyster gills freshly sampled. We sampled

gills because this tissue has been previously used in the few telomere studies on bivalves

(Godwin et al., 2012; Gruber et al., 2014). We used a NanoDrop 2000 (Thermo Scientific) to

check that absorbance ratios of extracted DNA met the optimal criteria (mean ± SD,

A260/A280: 1.88 ± 0.05, A260/A230: 2.22 ± 0.30). As detailed in Supplementary

Information, we also performed gel electrophoresis to confirm the purification of high

molecular weight DNA. Then, we used two methods to quantify telomere length (TL) using

absolute (Telomere Restriction Fragment - TRF) and relative quantifications (quantitative

Polymerase Chain Reaction - qPCR). TRF method is based on an initial DNA digestion with

two restriction enzymes (*Hinf I* and *Rsa I*), followed by a gel electrophoresis for 4h (0.8%

agarose) and southern blot technics from the TeloTTAGG assay kit (Sigma Aldrich, Roche).

183   We followed the protocol described by the manufacturer to obtain absolute TL measures from

184   n = 10 oysters of cohort 3. We used these 8 month old individuals (range of TL: 5.06 - 9.36

185   kb) to control that TL provided reliable estimates regardless of the methods. qPCR protocol

186   for telomere assay was performed on a thermocycler using *Sybr Green Supermix* (BioRad,

187   California, USA) and optimised from the protocol proposed by (Cawthon, 2009) as detailed in

188   supplementary methods. Samples were measured in triplicates and each run included a pool of

189   DNA as a gold standard and blank control (water) for each primer pair. Samples relative

190   quantification values were normalized to a unique copy gene in *C. gigas* genome (GAPDH)

191   and relative to the gold standard as recommended by Pfafll (2001) to measure relative

192   telomere length (rTL) as follow:

193    $$rTL = \frac{2.016^{(Cq\ telomere_{Pool} - Cq\ telomere_{Sample})}}{2.071^{(Cq\ GAPDH_{Pool} - Cq\ GAPDH_{Sample})}}$$

194

Whole organism response to foreshore level - Thermal reaction norms

196   We assayed thermal reaction norms in n = 60 individuals from cohort 2 sampled in January

197   2022 at 17 months old (i.e., 10 months in the field), and maintained in laboratory at ca. 12°C

198   without food until being assayed. Oysters were acclimated in a 300 L tank with seawater

199   running at 300 L/h. We used a COSA system to determine the temperature-dependence of

200   oxygen consumption (VO_2) as index of aerobic maximal metabolic rate (MMR) and filtration

201   rates at four temperature: 13, 19, 25 and 31°C, a thermal range covering temperature naturally

202   experienced by oysters in the field (13 to 25°C) and one closed to high-temperature limit

203   (31°C). As previously explained (Pousse et al., 2018; Tallec et al., 2021) and detailed in

204   Supplementary Information, this multiplexed system allows to automatically switch between

205   9 chambers (8 occupied by individuals, 1 left empty as reference). Water temperature was

206   controlled upstream within four-water tanks using 300W resistances and thermostats and

circulated in the system using two peristaltic pumps. We designed a program to automatically

switch between chambers every 8 min using solenoid valves. Water of the selected chamber

was analysed downstream by an oxygen optical sensor (WTW Multi 3430, $O_2 \pm 0.05$ mg/L), a

fluorometer (WetLabs, SeaBird, USA, Fluorometry ± 0.1 FFU), a flow meter (Sonoflow,

Sonotec GmbH; FR ± 0.1 ml/sec), and a pH meter (WTW Multi 3430, pH ± 0.05 NBS scale).

We considered the flow rate (mean $\pm$ SD: 28.3 ± 2.8 ml/min) and the differential in

fluorometric signals (in FFU) or O_2 concentration (mg/ml) between the reference chamber

and each animal chambers to determine filtration rate and VO_2. We obtained individual

reaction norms for both filtration rate and MMR. Fitting the Arrhenius curve from oysters

reactive norms of MMR, the Arrhenius temperature T_A represents the slope of the

temperature-metabolic relationship, and provides an individual estimation of energy

activation and thermal dependence in metabolic rate (Kooijman, 2010):

$$k(T) = k_1 \cdot e^{\frac{T_A}{T_1} - \frac{T_A}{T}}$$

T_A was obtained for each oyster, where k is the mean VO_2 measure at a given temperature T,

from a reference rate (k_1, here estimated at a reference temperature $T_1 = 25°C$).

Statistical analyses

Molecular responses to foreshore level (differential protein expression) were analysed using

the R package BetaBinomial 1.2 implemented in Proline Studio 2.0.1. We compiled proteins

of interest (chaperone proteins and those involved in response to oxidative stress) identified

with differential expression between foreshore levels (significant enrichment was set using an

exact Fisher test p-value < 0.05) within R software [R Core Team (2021)] in a Principal

Component Analysis [package ade4 (Dray and Dufour, 2007)] to ease visualization of

multivariate proteomic profiles.

231

232   Cellular and physiological responses to foreshore levels were analysed with linear models and

233   linear mixed models [package *nlme* (Pinheiro et al., 2020)]. For all tested variables, we built a

234   complete model, including all possible fixed factors and their interaction, and we used the

235   *dredge* function to compare and rank all model performance, based on AICc (see Table S2)

236   [package *MuMIn* (Barton, 2019)]. We thus identified the fixed terms of interest without *a*

237   *priori* expectations. Oyster weight (shell and flesh) was primarily influenced by foreshore

238   level due to strong differences in growth rates (Corporeau et al., 2022) so we scaled body

239   weight (log transformed) within each foreshore level [package *standardize* (Eager, 2017)] to

240   control for allometry while limiting collinearity among explanatory factors. ATP production

241   and mitochondrial density were analysed with linear models, testing the effects of body

242   weight (log transformed), food access (fed or unfed), foreshore level and first order

243   interaction terms. We analysed factors explaining variations in TL using two independent

244   model sets. First, we examined the effect of age, comparing TL in oysters from cohort 3 (8-

245   month old) to those from cohort 2 (22-month old). Second, in 22-month old oysters, we built

246   a separate model to investigate the effects of body weight (log transformed), foreshore level

247   and first order interaction. Thermal reaction norms were measured on the same animals at the

248   4 temperatures so we set oyster identity as random intercept. Filtration rate and VO_2 (log

249   transformed) were treated as dependent variables and we tested the fixed effects of scaled

250   body weight, number of days fasted, linear and quadratic effect of temperature, foreshore

251   level and first order interaction.

252

253   We determined quality of cellular and physiological assays by checking repeatability among

254   samples and individuals using rptR package (Stoffel et al., 2017).

255

256 **Results**

257 *Molecular responses to foreshore levels*

258 After two months in the field, foreshore level shaped differential expression of multiple

259 proteomic markers of response to thermal stress and antioxidant defenses in High shore (all B

260 Binomial tests ; $p < 0.05$). Oysters from this upper shore limits exhibited greater production of

261 HSP 70 together with multiple antioxidant proteins, including the superoxide dismustase, 3

262 catalases, peroxidase YfeX, peroxiredoxin, non-selenium glutathione peroxidase and a

263 peptide-methionine (R)-S-oxide reductase compared to oysters from Middle and Low shores

264 (Fig. 2). On the contrary, oysters from Low shores showed higher HSP 90 and glutathione

265 peroxidase (Fig. 2). These oysters regularly submerged also experienced higher risk of

266 oxidative attacks by two NADPH oxidase (Fig. 2).

267

268 *Cellular responses to foreshore level*

269 Haemocyte energetic efficiency

270 ATP content and mitochondrial density showed high repeatability (respectively $r = 0.99$, $p <$

271 0.001 and $r = 0.92$, $p < 0.001$). ATP content did not differ between foreshore levels and was

272 only influenced by food access (fed oysters showed higher ATP content than fasted ones)

273 (Fig. 3A). Conversely, variation in mitochondrial density significantly differed between

274 foreshore levels, being lower in High compared to Middle and Low shore oysters (Table 1,

275 Table S2, Fig. 3B).

276

277 Telomere dynamics

278 Assays of individual rTL showed good repeatability among plates (r = 0.76, p < 0.001). RTL

279 decreases with age (Table 1, Table S2, Fig. 4). In addition, at 22-month old, rTL was

280 influenced by foreshore level irrespective of the interaction term (Table S2). Oysters from

281 High shores exhibited 28% longer rTL compared to Middle and Low shore individuals (Table

282 1, Fig. 4).

283

284 *Physiological acclimatization at whole organism level*

285 Filtration rate was positively influenced by oyster weight (range: 1.5 - 21.2 g), number of

286 days spent starved (range: 5 - 27 days), linear and quadratic slope along temperature and

287 foreshore level (Table 1, Table S2). Filtration was highly variable, even within individuals (r

288 = 0.38, p < 0.001) but on average, filtration rates noticeably dropped at high temperature

289 irrespective of foreshore level (Fig. 5A), and significantly more for Low shores oysters

290 (interaction term, Table 1, Fig. 5A). Maximum metabolic rate was more repeatable within

291 individuals (r = 0.91, p < 0.001) and positively influenced by oyster weight, linear slope with

292 temperature, and first order interaction term between foreshore level and temperature (Table

293 1, Table S2). Metabolic reaction norm was steeper in individuals from High compared to Low

294 shore level, while Middle ones were intermediate (Table 1, Fig. 5B). On average, this resulted

295 in Arrhenius temperatures greater in High shore oysters (T_A = 8968 ± 323K) compared to

296 Middle (T_A = 7685 ± 431K, t = -2.3, p = 0.023) and Low ones (T_A = 7017 ± 445K, t = -3.5, p

297 = 0.001).

298

299 **Discussion**

300 In this study, we investigated the physiological determinants of phenotypic plasticity in the

301 Pacific oyster along a vertical gradient of bathymetry. In line with previous examinations

13

302 highlighting contrasted life history trade-off between growth rates and mortality risk

303 (Corporeau et al., 2022), we found that foreshore level shaped differential life trajectories

304 sustained by physiological acclimatization. That is, oysters exposed to aerial conditions most

305 part of their life mounted an antioxidant shielding and experience slower growth rate. These

306 oysters also gained in metabolic efficiencies given that they appeared more efficient to

307 produce energy (steeper metabolic reaction norms and ATP concentration) despite lower

308 mitochondrial density. In the opposite range limits, at Low intertidal levels, oysters grew

309 faster at the cost of faster telomere attrition, suggesting acceleration of the aging rate. Below,

310 we discussed these results and their implications in explaining vertical range limits.

311

312 A first sign of short-term acclimatization to different foreshore levels, oysters modified their

313 proteomes related to thermal stress avoidance and oxidative stress responses after two months.

314 Proteomic profiles differed between intertidal conditions, as shown by the over-expression of

315 chaperone and antioxidant proteins in oysters that endure regular emersion. For instance, we

316 observed higher levels of HSP 70 concomitantly with adaptations of intertidal organisms to

317 limit the risk of frequent heat stress in High shores (A. Li et al., 2018; Wang et al., 2021).

318 Instead, oysters from Low shores experienced greater HSP 90 and glutathione peroxidase

319 levels, which may respond to metabolic activities in subtidal conditions. In this environment,

320 oysters may experience greater ROS production, given that they showed higher levels of

321 NADPH oxidase (Dupuy et al., 1991). In addition poorer neutralization by lower level of

322 endogenous antioxidant may exacerbate oxidative stress in oysters submerged most of their

323 time, which could ultimately trade faster growth rate against survival (Buttemer et al., 2010).

324 Instead, in oysters within High shores experiencing regular hypoxia during low tide (i.e., less

325 ROS production), it remains uncertain why they also mounted an antioxidant shielding. As

326 previously hypothesized, intertidal bivalves may boost production of endogenous antioxidant

327 during low tides in anticipation of the metabolic burst during the next submersion (Almeida

328 and Bainy, 2006; Andrade et al., 2021; Sussarellu et al., 2010). In support of this statement,

329 we also found that High shore oysters were those with enhanced metabolic reaction norm as

330 discussed hereafter.

331

332 Individuals further showed significant adjustments of energy metabolism after 4 more months

333 in different habitats. Temperature dependence of physiological rates appeared significantly

334 impacted by foreshore levels, including steeper reaction norms of filtration and maximal

335 metabolic rates in High shore oysters. These whole-organism responses provide reliable

336 measures acclimatization, in line with previous evidences that ectotherms may optimize the

337 limits imposed by fewer opportunities to initiate physiological rates by upregulating thermal

338 dependence of metabolism (Dupoué et al., 2017; White et al., 2011). In other words, oysters

339 from High shores probably showed steeper metabolic reaction norms to optimize

340 physiological processes despite fewer opportunities to generate and mobilize energy (only

341 20% of time spent submerged). This is allowed by Arrhenius temperature (inversely

342 proportional to energy activation) ca. 20% greater in High shore oysters compared to Middle

343 and Low shore levels. Growing 9 months at contrasted foreshore level, oysters also

344 experienced similar energy content and thus, despite lower mitochondrial density. Previous

345 microscopic examinations have highlighted that ultrastructure of mitochondria is modified by

346 foreshore level as a result of a metabolic reprogramming in harsh intertidal environments

347 (Corporeau et al., 2022). Altogether, these results at cellular and individual levels suggest that

348 growing in High shore elevations more efficiently generate energy. A differential in

349 mitochondrial efficiencies would imply greater ratio ATP/O (Metcalfe and Olsson, 2022),

350 which we hypothesize to be optimized in High shore oysters. Further investigations using *in*

351 *situ* assays (e.g., high-resolution respirometry, mitochondrial bioenergetics, and

352 metabolomics) will allow us to explore how foreshore level may reshape mitochondrial

353 functioning.

354

355 Eventually, acclimatizing to foreshore level had further consequences on telomere dynamics.

356 Telomere length decreases with age as regularly documented in other animals (Dunshea et al.,

357 2011), however the age-related telomere erosion additionally depended upon environment

358 conditions. That is, telomeres were shorter in oysters from Middle and Low shores compared

359 to those from High shores. This result suggests that living faster may come at the cost of

360 accelerated aging pace when a species adapted to intertidal habitats approach distribution

361 range limits closed to subtidal conditions. It is worth noting that individual rTL was not

362 correlated to oyster weight (flesh and shell), implying that telomere may not erode as direct

363 consequence of growth rate. Our results rather suggest that telomeres shorten as consequence

364 of enhanced oxidative stress, related to subtidal conditions, as in other species (Chatelain et

365 al., 2020; Reichert and Stier, 2017). Another functional explanation may involve DNA

366 hypermethylation in subtidal environments (Clark et al., 2018; Wang et al., 2021), which

367 might in turn alter telomere dynamics (Sheldon et al., 2021). Further evidence is now needed

368 to i) identify the most relevant environmental factors shaping telomere erosion (e.g., sea

369 surface temperature, food quantity and/or quality), ii) quantify the degree of telomere

370 heritability, iii) explore the role of telomere length in shaping fitness costs (mortality risks,

371 lifespan, reproductive investment and success), and iv) establish a model of environment-

372 telomere-lifespan [e.g., Weibull acceleration ageing factors; (Kooijman, 2010)]. This will

373 allow using this species as a model to understand altered aging pace when facing global

374 changes.

375

16

Conclusion

Phenotypic plasticity is essential to thrive when exposed to rapid environmental variation, and it will constitute a critical determinant of species persistence toward ongoing global changes (Somero, 2010). As reported here and in line with many studies, we confirmed the extreme plasticity of the intertidal Pacific oyster. Despite being originated from a standardized genetic pool, foreshore level strongly imprinted life trajectories through variable growth rates. It remains to clarify whether acclimatization to foreshore level would persist in time or if it is reversible after few months. Then, bringing oysters back to common garden conditions (e.g., same foreshore level) for a year should help to decipher between phenotypic flexibility and/or developmental plasticity in this intertidal bivalve. In addition, this study probably reflects at what costs plasticity arises, as unraveled by greater oxidative stress and aging pace when growing faster even though oysters downregulated temperature dependence of metabolic rates. In support of high flexibility, *C. gigas* is now colonizing elevated latitudes, thanks to warming ocean, where summer temperature allows reproductive events (Thomas et al., 2016). Conversely, this species survive less in its southern limit range, where it is enduring mass mortality events (Fleury et al., 2020). Eventually, bathymetry might select genotypes resistant to subtidal conditions (Meng et al., 2018). We hypothesize that altered telomere dynamics could constitute one of the central tenet of natural (expansion) or artificial (farming) selection. If telomere triggers fitness costs, as in most species, we predict that this cellular marker should explain foreshore range limits bearable for intertidal ectotherms.

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Data availability

The datasets generated and the code used for analyses during the current study are available in the Zenodo repository (DOI: 10.5281/zenodo.7763023).

Conflict of Interest

We declare no competing financial interest.

Table 1. Summarized outcomes of final selected models investigating the oyster responses to foreshore level. The table reports information on

sample size, oyster cohort and age, marginal and condition r^2, estimates (variance terms and fixed effects $\beta \pm SE$) together with test statistics.

Continuous explanatory variables were previously scaled by z-score transformation to optimize model convergence. See methods section for

details on statistical models and analyses.

Variable	Cohort	Age (month)	Final models	Model summary							
ATP content	2020	22	Food access	Number of individuals	R^2_m	R^2_c	Type	Term	β ($\pm$ SE)	t-stat	P value
				n = 121	0.04	-	Fixed	Intercept	66.1 $\pm$ 5.6	11.7	0.000 ***
								Food access (relative to Fed) :			
								unfed	-17.0 $\pm$ 7.9	-2.1	0.034 *
Mitochondrial density	2020	22	Foreshore level	Number of individuals	R^2_m	R^2_c	Type	Term	β ($\pm$ SE)	t-stat	P value
				n = 126	0.07	-	Fixed	Intercept	1117.9 $\pm$ 40.7	27.5	0.000 ***
								Foreshore level (relative to High) :			
								Middle	147.6 $\pm$ 55.0	2.7	0.008 **
								Low	143.1 $\pm$ 56.8	2.5	0.013 *

rTL	2020 and 2021	8 and 22	Age	Number of individuals	R^2_m	R^2_c	Type	Term	$β$ (± SE)	t-stat	P value	
				n = 83	0.12	-	Fixed	Intercept	1.316 ± 0.099	13.3	0.000	***
								Age	-0.017 ± 0.005	-3.3	0.002	**

rTL	2020	22	Foreshore level	Number of individuals	R^2_m	R^2_c	Type	Term	$β$ (± SE)	t-stat	P value	
				n = 59	0.18	-	Fixed	Foreshore level (relative to High) :				
								Middle	-0.207 ± 0.072	-2.9	0.006	**
								Low	-0.238 ± 0.073	-3.2	0.002	**

Filtration rate	2020	17	Weight + days starved + temperature + temperature^2 + Foreshore level + temperature^2 × Foreshore level + (1|Oyster ID)	Number of individuals	R^2_m	R^2_c	Type	Term	Variance			
				n = 52	0.62	0.74	Random	Oyster ID	0.16			
								Residual	0.23			
									$β$ (± SE)	t-stat	P value	
							Fixed	Intercept	-0.62 ± 0.24	-2.52	0.013	
								Weight	0.30 ± 0.03	10.9	0.000	***
								Days starved	-0.05 ± 0.03	-2.0	0.046	*

Term	β (± SE)	t-stat	P value	
Temperature	0.11 ± 0.02	4.9	0.000	***
Temperature^2	-0.002 ± 0.001	-4.8	0.000	***
Foreshore level (relative to High) :				
Middle	0.28 ± 0.11	2.6	0.014	*
Low	0.36 ± 0.11	3.3	0.002	**
Temperature^2 × Foreshore level:				
Temperature^2 × Middle	$-1.8 \cdot 10^{-4} \pm 1.5 \cdot 10^{-4}$	-1.2	0.224	
Temperature^2 × Low	$-4.2 \cdot 10^{-4} \pm 1.5 \cdot 10^{-4}$	-2.8	0.006	**

				Number of individuals	$R^2{}_m$	$R^2{}_c$	Type	Term	Variance			
Maximum metabolic rate	2020	17	Weight + temperature + Foreshore level + temperature × Foreshore level + (1|Oyster ID)									
				n = 52	0.86	0.90	Random	Oyster ID	0.09			
								Residual	0.14			
									β (± SE)	t-stat	P value	
							Fixed	Intercept	-2.2 ± 0.07	-32.5	0.000	***
								Weight	0.21 ± 0.02	13.3	0.000	***
								Temperature	0.06 ± 0.00	20.7	0.000	***
								Foreshore level (relative to High) :				

	Middle	0.34 ± 0.10	3.4	0.001	**
	Low	0.55 ± 0.10	5.5	0.000	***
Temperature × Foreshore level:					
Temperature × Middle		-0.006 ± 0.004	-1.5	0.143	
Temperature × Low		-0.013 ± 0.004	-3.3	0.001	**

638

639

Figure captions

641 Figure 1. Schematic illustration of oyster production and experimental design. A) Wild spats

642 are collected every year at the Aix Island (46°0'51N, 1°9'39W) to enrich a captive brood

643 stock of a diversified gene pool from the same population. These individuals are exposed to

644 natural conditions at the Aber Benoît (48°34'30N, 4°36'18W) for up to 10 years. B) Each

645 summer, we pool gametes of ca. 100 reproductive adults in laboratory to produce a cohort of

646 captive spats. C) At 7 month old, 2,700 captive spats are distributed in the field along

647 foreshore levels. Due to month variation of tidal range, oysters at Low shore (blue star) are

648 exposed 80% of their time to sea water, approaching subtidal conditions. Instead, oysters from

649 High shore (yellow star) are submerged only during strong tidal range, spending only 20% of

650 their time submerged. Oysters from Middle shore (grey star) are exposed half of their time to

651 sea water. We sampled oyster of different cohorts for investigating physiological adjustments

652 as described in the text. Picture copyrights fall to Andréaz Dupoué, Stéphane Pouvreau (larva

653 lifecycle) and the Ifremer.

654

655 Figure 2. Multivariate analyze of inter-individual proteomic profiles of oysters from High

656 (yellow), Middle (grey) or Low (blue) shores regarding the relative expression of chaperone

657 proteins, and those involved in oxidative status. This principal component analysis

658 discriminate the three foreshore levels (ellipse of 95% confidence interval), along the first axe

659 (horizontal) given that High shores oysters experienced enhanced antioxidant shielding

660 compared to Low shore individuals. Abbrevations : Heat Shock Protein (HSP), NAD(P)H

661 oxidase (NOX), Catalase (CAT), Non-selenium Glutathione Peroxidase (Non-Se GPx),

662 peroxidase YfeX (YfeX), peroxiredoxin 5 (PRDX5), superoxide dismutase (SOD),

663 Glutathione Peroxidase (GPx), Peptide methionine R S oxide reductase (PMRS).

664

Figure 3. Additive effects of foreshore level and food access on energetic efficiency. A) Fed oysters (open dots) showed higher ATP concentration than unfed individuals (closed dots) regardless of foreshore level, although B) haemocytes in oysters from High shore (yellow dots) had lower mitochondrial density than those from Middle (grey dots) and Low shores (blue dots). Significant effects of foreshore level on ATP concentration and mitochondrial density are symbolized: * $p < 0.05$, and n.s. (non-significant).

671

Figure 4. Effects of oyster age class and foreshore level on variation in relative telomere length. Telomeres were shorter in older oysters, in addition to an environmental effect related to foreshore level. On average, oysters from High shore (yellow dot and line) exhibited longer telomeres than individuals from Middle (grey dot and line) and Low shores (blue dot and line).

677

Figure 5. Impacts of foreshore level on the temperature dependence of A) filtration rate and B) maximum metabolic rate (estimated through $V\mathrm{O}_2$ in feeding oysters). Filtration rate did not differ between foreshore levels, while temperature dependence of metabolic rate was stronger in oysters from High shore (yellow symbols and lines), compared to those of Middle (grey symbols and lines) and Low shores (blue symbols and lines). Data are mass-adjusted values (residuals from linear relationship between log-transformed filtration or $V\mathrm{O}_2$ and oysters weight) and best linear regression fits with prediction intervals.

685

686   Figure 1.

A) Brood stock collection

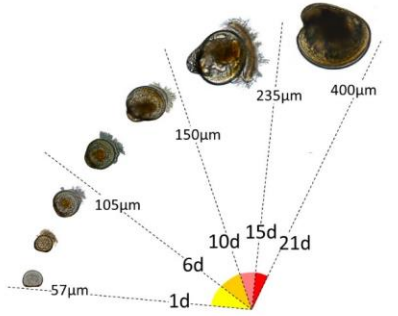Field site 1:
Spat collection

Field site 2:
Growing 1 - 10 years

Standardized gene pool   *Reproductive adults*

B) Reproduction

Laboratory (n = 100 adults/year):
Fecundation & early development

400μm

235μm

150μm

105μm

57μm

10d 15d 21d
6d
1d

(n = 1 cohort/year)

C) Field experimentation

n = 2 700 juveniles/cohort

Time spent submerged
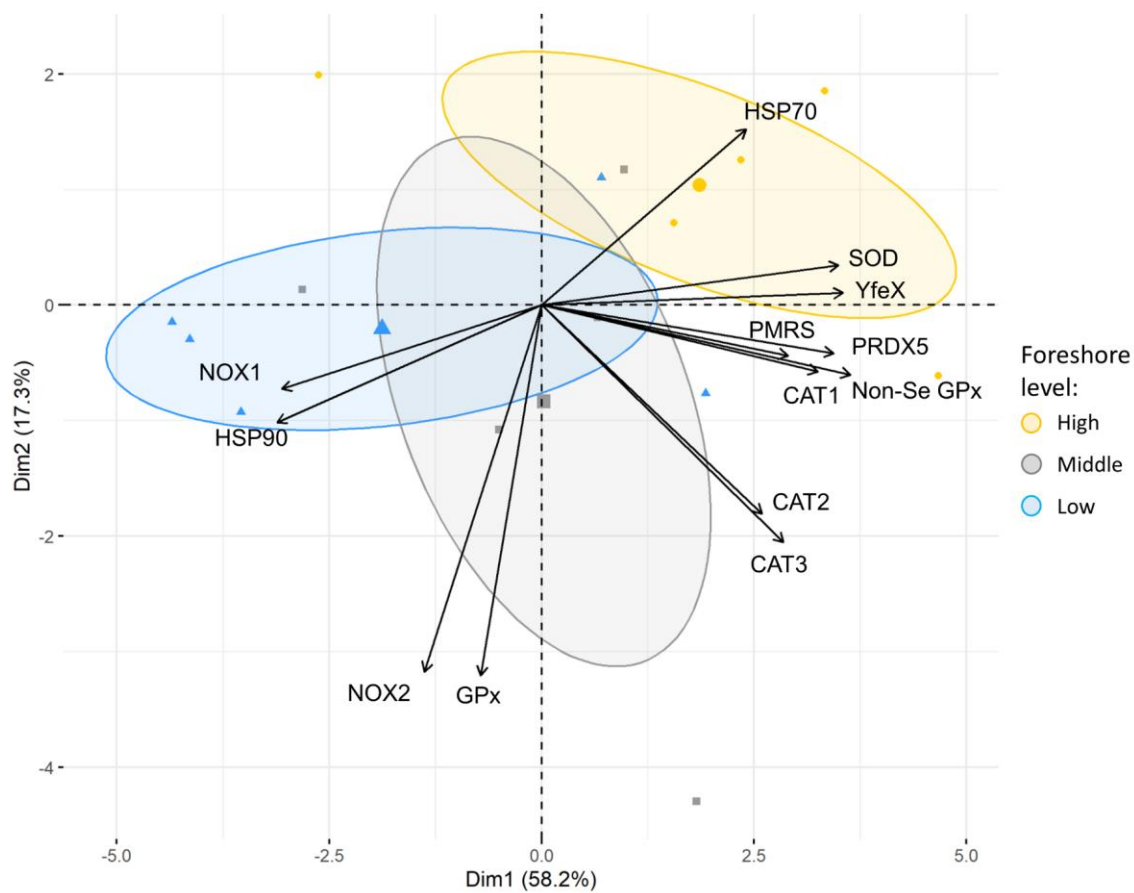
Foreshore level

High   Middle   Low

Sampling strategy:

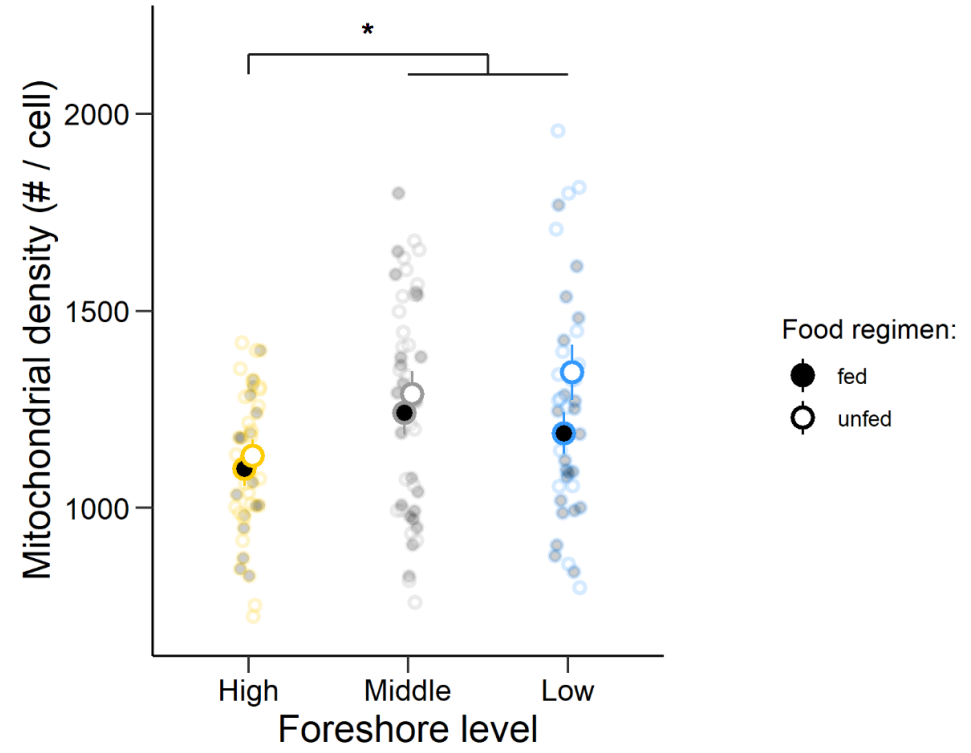| Measures	Acclimatizaton time to foreshore level (month)	Oyster age (month)
Oxidative response	2	9
Cellular energetics	15	22
Telomere length	15	22
Physiological norms	10	17

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688

30

Figure 2.

692   Figure 3.

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694   Figure 4.

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697　　Figure 5.

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