Science Advances
AAAS

Supplementary Materials for

Reproductive aging weakens offspring survival and constrains the telomerase response to herpesvirus in Pacific oysters

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Sci. Adv. **10**, eadq2311 (2024)
DOI: 10.1126/sciadv.adq2311

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Fig. S1.

Experimental oyster pairing design used to determine the impacts of reproductive aging and quantify narrow-sense heritability in offspring traits. We crossbred young, middle age and old oysters (12 females and 12 males) to create 36 full-sibling families (F1 to F36). Each mother was mate with three fathers and *vice versa* to obtain 72 half-sibling combinations.

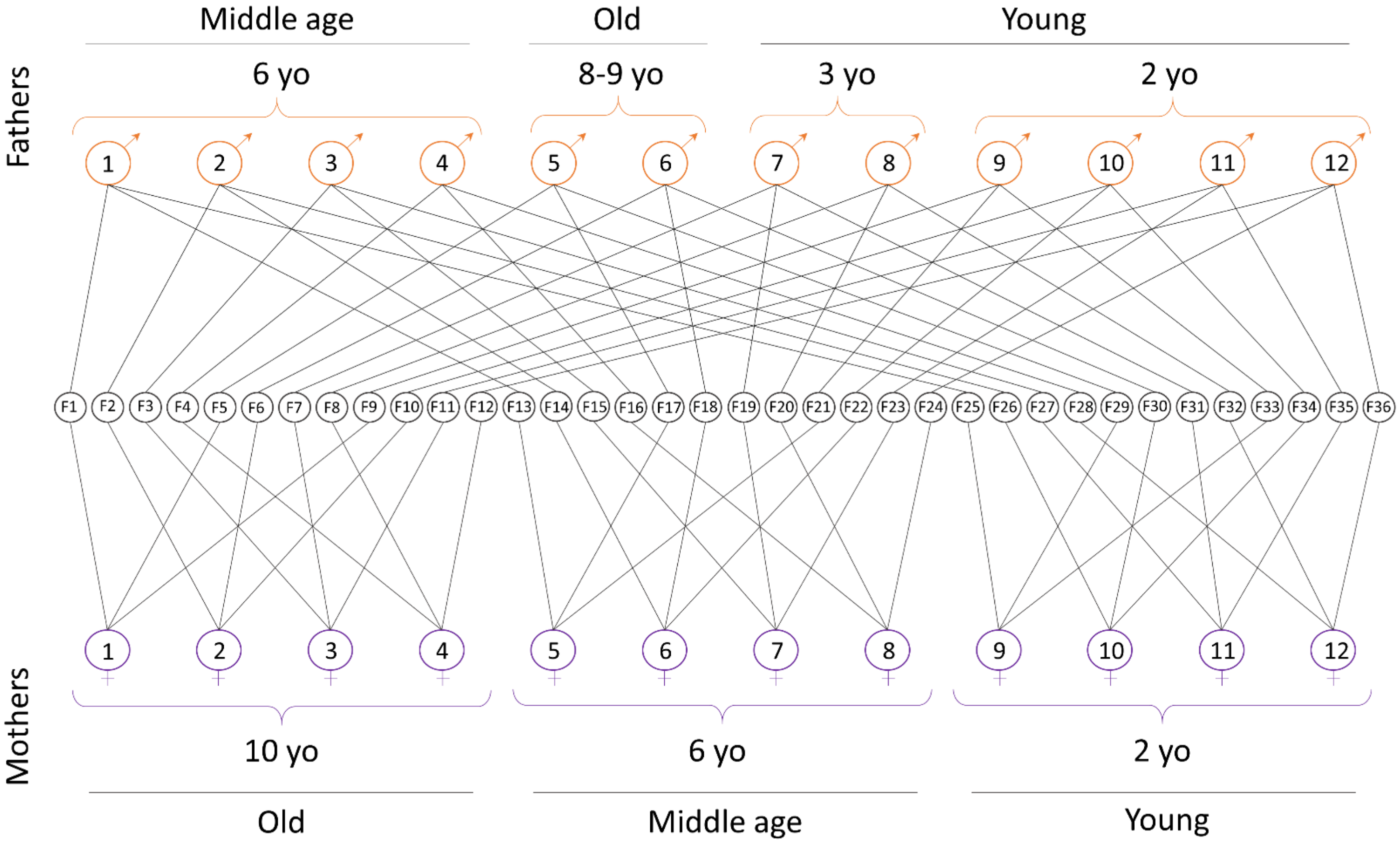


Fig. S2.

Timeline of the sampling strategy based on natural life cycle of the Pacific oyster (*Crassostrea gigas*). We monitored larval development (survival and growth between 2 and 40 days old) in laboratory followed by wintering in field conditions, with relatively few selective pressures (no mortality observed). At the end of spring, we exposed spat oysters (40 to 365 days old) to two natural environments to avoid pseudo-replication related to contrasted selective pressure between habitats. Extrinsic selection by pathogens began with a peak of OsHV1 µVar in early June and followed by bacterial infection, especially *Vibrio aestuarianus* in Early fall of the next year when oysters were adults (> 1 year old). We sampled offspring at different time to examine intrinsic and extrinsic pressures on telomere dynamics and telomerase activity.

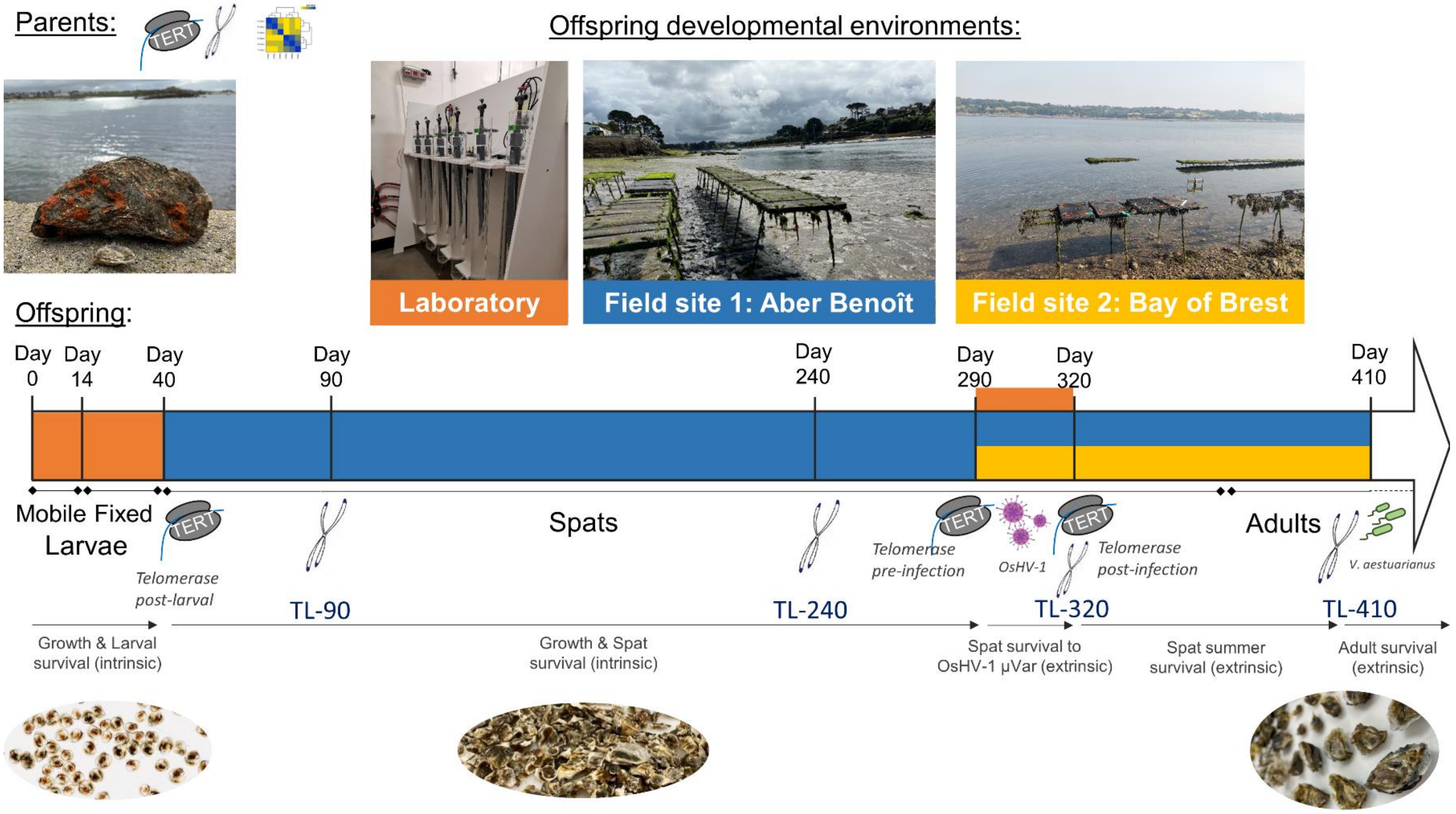
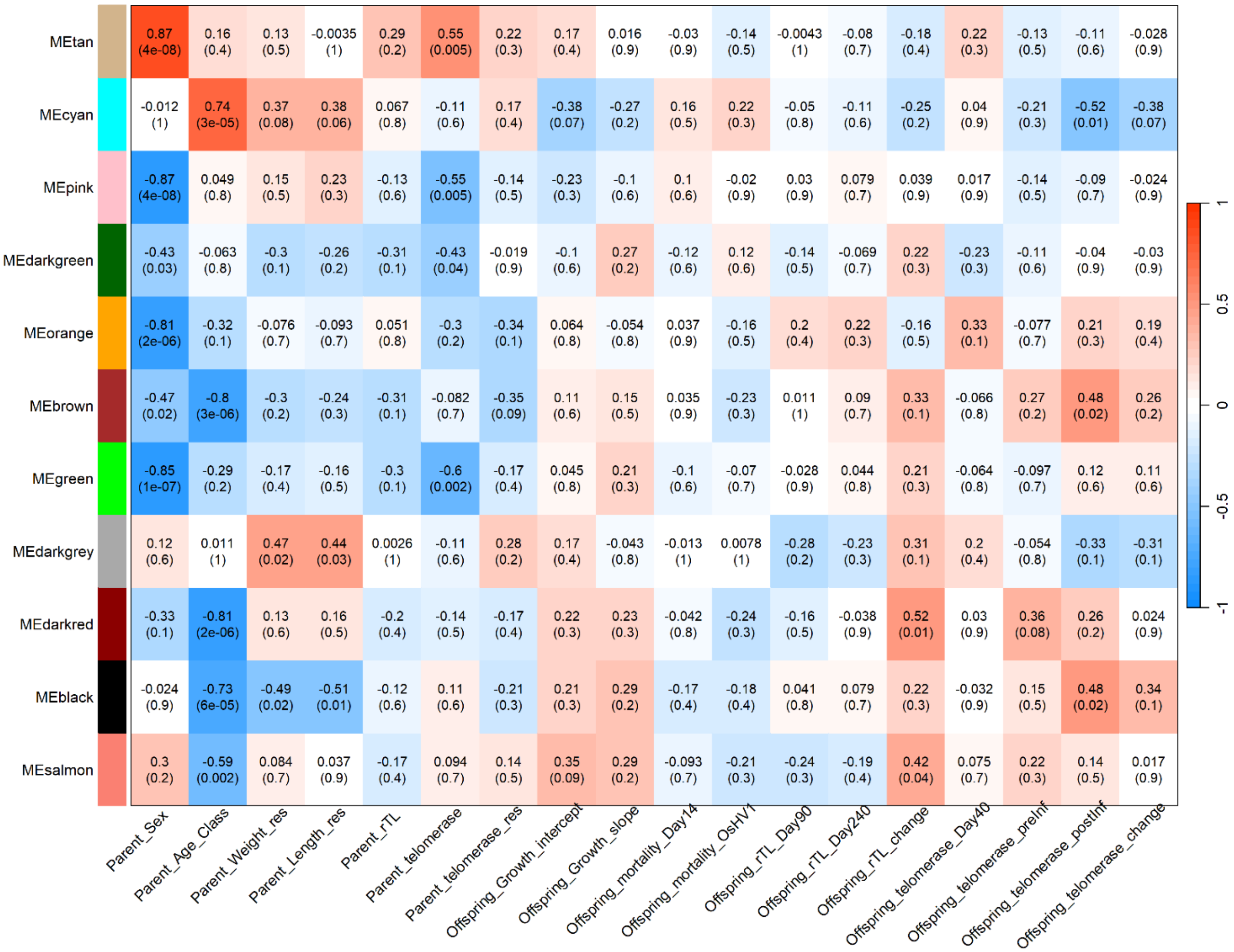


Fig. S3.

Correlation matrix between parent proteomes and their sexes, age classes, and their averaged offspring traits. The heatmap shows the Pearson r coefficient together with p-value, between protein expression modules (modules of colors obtained in WGCNA) and individual traits averaged at individual scale (parent traits) or among their offspring (offspring traits). Colors stand for positive (red nuance) or negative correlations (blue nuance) and color intensity for significance level. See methods for details.



	Parent_Sex	Parent_Age_Class	Parent_Weight_res	Parent_Length_res	Parent_rTL	Parent_telomerase	Parent_telomerase_res	Offspring_Growth_intercept	Offspring_Growth_slope	Offspring_mortality_Day14	Offspring_mortality_OsHV1	Offspring_rTL_Day90	Offspring_rTL_Day240	Offspring_rTL_change	Offspring_telomerase_Day40	Offspring_telomerase_preInf	Offspring_telomerase_postInf	Offspring_telomerase_change
MEtan	0.87 (4e-08)	0.16 (0.4)	0.13 (0.5)	-0.0035 (1)	0.29 (0.2)	0.55 (0.005)	0.22 (0.3)	0.17 (0.4)	0.016 (0.9)	-0.03 (0.9)	-0.14 (0.5)	-0.0043 (1)	-0.08 (0.7)	-0.18 (0.4)	0.22 (0.3)	-0.13 (0.5)	-0.11 (0.6)	-0.028 (0.9)
MEcyan	-0.012 (1)	0.74 (3e-05)	0.37 (0.08)	0.38 (0.06)	0.067 (0.8)	-0.11 (0.6)	0.17 (0.4)	-0.38 (0.07)	-0.27 (0.2)	0.16 (0.5)	0.22 (0.3)	-0.05 (0.8)	-0.11 (0.6)	-0.25 (0.2)	0.04 (0.9)	-0.21 (0.3)	-0.52 (0.01)	-0.38 (0.07)
MEpink	-0.87 (4e-08)	0.049 (0.8)	0.15 (0.5)	0.23 (0.3)	-0.13 (0.6)	-0.55 (0.005)	-0.14 (0.5)	-0.23 (0.3)	-0.1 (0.6)	0.1 (0.6)	-0.02 (0.9)	0.03 (0.9)	0.079 (0.7)	0.039 (0.9)	0.017 (0.9)	-0.14 (0.5)	-0.09 (0.7)	-0.024 (0.9)
MEdarkgreen	-0.43 (0.03)	-0.063 (0.8)	-0.3 (0.1)	-0.26 (0.2)	-0.31 (0.1)	-0.43 (0.04)	-0.019 (0.9)	-0.1 (0.6)	0.27 (0.2)	-0.12 (0.6)	0.12 (0.6)	-0.14 (0.5)	-0.069 (0.7)	0.22 (0.3)	-0.23 (0.3)	-0.11 (0.6)	-0.04 (0.9)	-0.03 (0.9)
MEorange	-0.81 (2e-06)	-0.32 (0.1)	-0.076 (0.7)	-0.093 (0.7)	0.051 (0.8)	-0.3 (0.2)	-0.34 (0.1)	0.064 (0.8)	-0.054 (0.8)	0.037 (0.9)	-0.16 (0.5)	0.2 (0.4)	0.22 (0.3)	-0.16 (0.5)	0.33 (0.1)	-0.077 (0.7)	0.21 (0.3)	0.19 (0.4)
MEbrown	-0.47 (0.02)	-0.8 (3e-06)	-0.3 (0.2)	-0.24 (0.3)	-0.31 (0.1)	-0.082 (0.7)	-0.35 (0.09)	0.11 (0.6)	0.15 (0.5)	0.035 (0.9)	-0.23 (0.3)	0.011 (1)	0.09 (0.7)	0.33 (0.1)	-0.066 (0.8)	0.27 (0.2)	0.48 (0.02)	0.26 (0.2)
MEgreen	-0.85 (1e-07)	-0.29 (0.2)	-0.17 (0.4)	-0.16 (0.5)	-0.3 (0.1)	-0.6 (0.002)	-0.17 (0.4)	0.045 (0.8)	0.21 (0.3)	-0.1 (0.6)	-0.07 (0.7)	-0.028 (0.9)	0.044 (0.8)	0.21 (0.3)	-0.064 (0.8)	-0.097 (0.7)	0.12 (0.6)	0.11 (0.6)
MEdarkgrey	0.12 (0.6)	0.011 (1)	0.47 (0.02)	0.44 (0.03)	0.0026 (1)	-0.11 (0.6)	0.28 (0.2)	0.17 (0.4)	-0.043 (0.8)	-0.013 (1)	0.0078 (1)	-0.28 (0.2)	-0.23 (0.3)	0.31 (0.1)	0.2 (0.4)	-0.054 (0.8)	-0.33 (0.1)	-0.31 (0.1)
MEdarkred	-0.33 (0.1)	-0.81 (2e-06)	0.13 (0.6)	0.16 (0.5)	-0.2 (0.4)	-0.14 (0.5)	-0.17 (0.4)	0.22 (0.3)	0.23 (0.3)	-0.042 (0.8)	-0.24 (0.3)	-0.16 (0.5)	-0.038 (0.9)	0.52 (0.01)	0.03 (0.9)	0.36 (0.08)	0.26 (0.2)	0.024 (0.9)
MEblack	-0.024 (0.9)	-0.73 (6e-05)	-0.49 (0.02)	-0.51 (0.01)	-0.12 (0.6)	0.11 (0.6)	-0.21 (0.3)	0.21 (0.3)	0.29 (0.2)	-0.17 (0.4)	-0.18 (0.4)	0.041 (0.8)	0.079 (0.7)	0.22 (0.3)	-0.032 (0.9)	0.15 (0.5)	0.48 (0.02)	0.34 (0.1)
MEsalmon	0.3 (0.2)	-0.59 (0.002)	0.084 (0.7)	0.037 (0.9)	-0.17 (0.4)	0.094 (0.7)	0.14 (0.5)	0.35 (0.09)	0.29 (0.2)	-0.093 (0.7)	-0.21 (0.3)	-0.24 (0.3)	-0.19 (0.4)	0.42 (0.04)	0.075 (0.7)	0.22 (0.3)	0.14 (0.5)	0.017 (0.9)

Fig. S4.

Focus on gene ontologies (GOs) related to biological processes of telomere biology. A) Telomere maintenance GOs and B) Oxidative stress and DNA damages GOs were identified within the same protein networks and increase both in females and in young oysters.

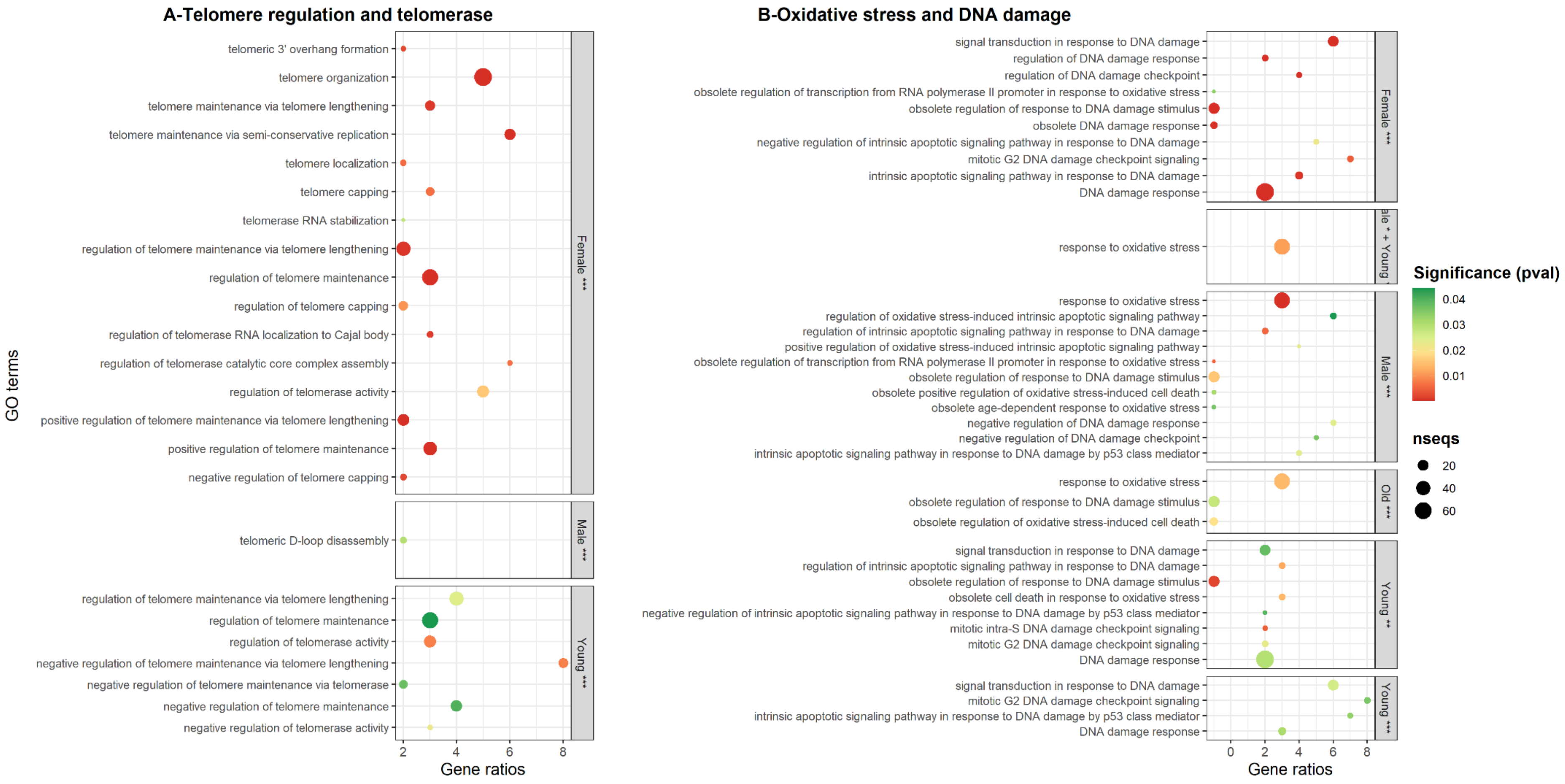


Fig. S5.

Distribution of TL by age classes in the Pacific oyster. We report the density of TL data among adults (> 2 yo, n = 24, blue ribbon) and spat oysters before exposure to pathogenic pressure (< 1 yo, n = 443, red ribbon).

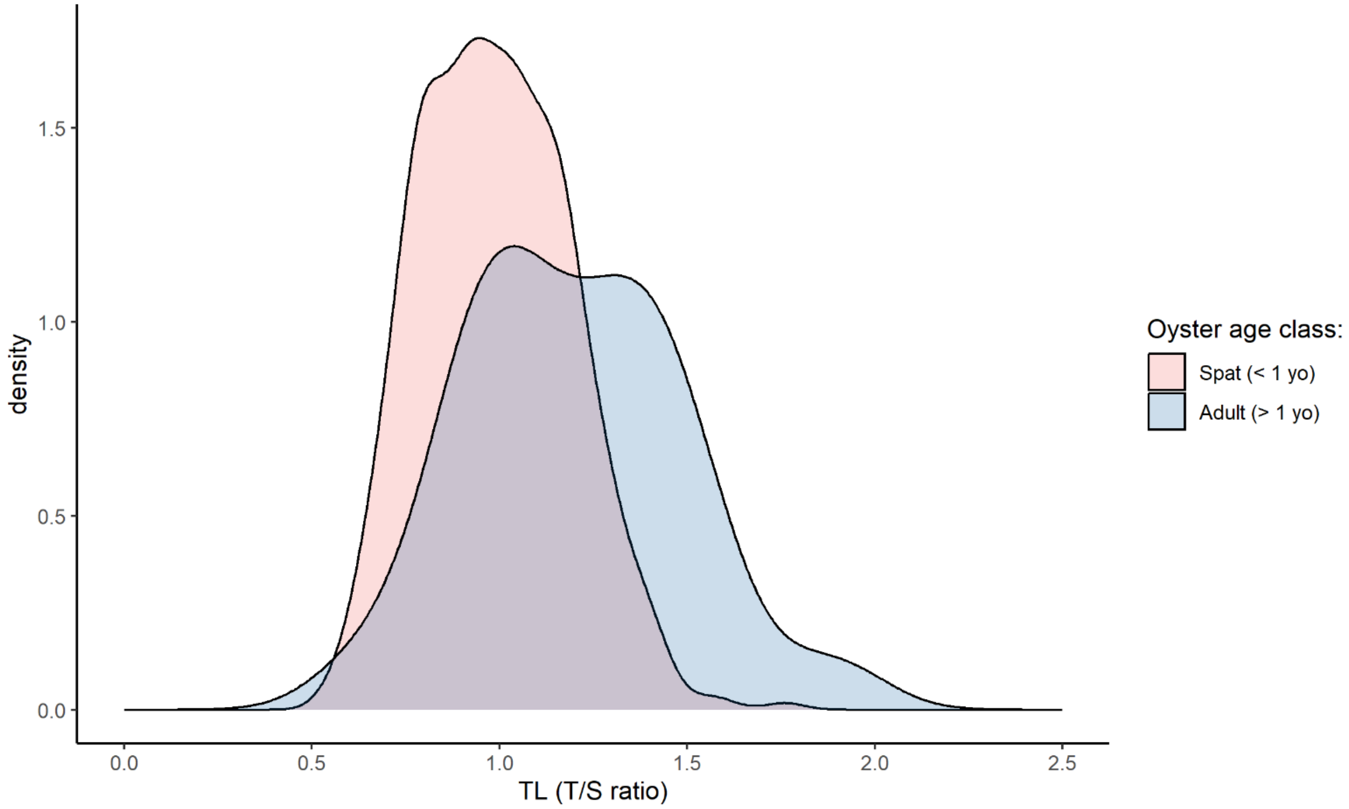


Fig. S6.

Growth and survival rates in spat oysters without selective pressure. A) Growth rates remain negatively impacted by paternal age after larval life, up to adult stage, and B) spat survival rates remain above 95% without pathogenic pressure.

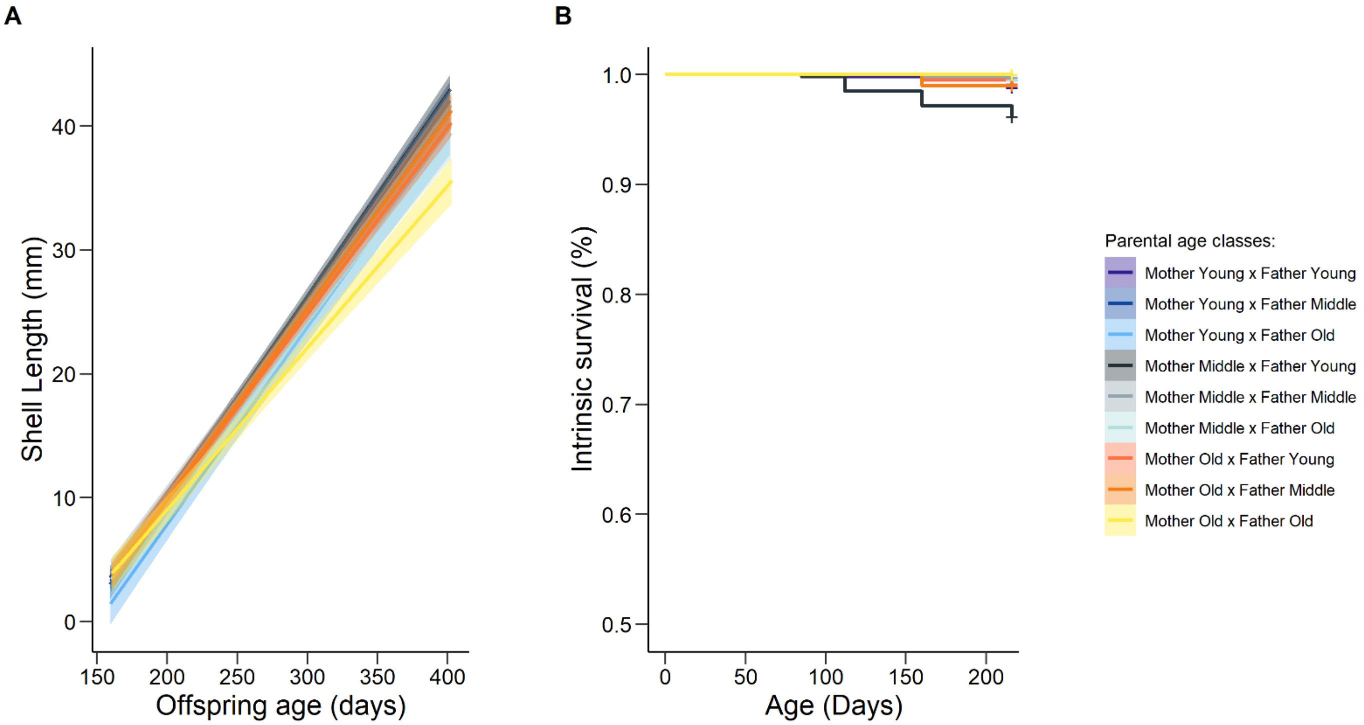


Fig. S7.

Multivariate telomerase gene expression in response to OsHV-1 challenge. RNAseq data were obtained from publicly available projects (referred in Table S1, below). Each point is a replicate (a pool of 10 oysters) at a given time post-infection. We used the difference in Log2 gene level at different time post-infection minus their basal expression at t0, before viral challenge. For statistics, we considered the integrative score along the main axis (PC1), resulting from stimulation of TRiC, HSP90, GAR1, NHP2, NOP10, and TCAB and inhibition of TERT and TIAR. TEP1 gene was uncorrelated to other telomerase genes in this analyze.

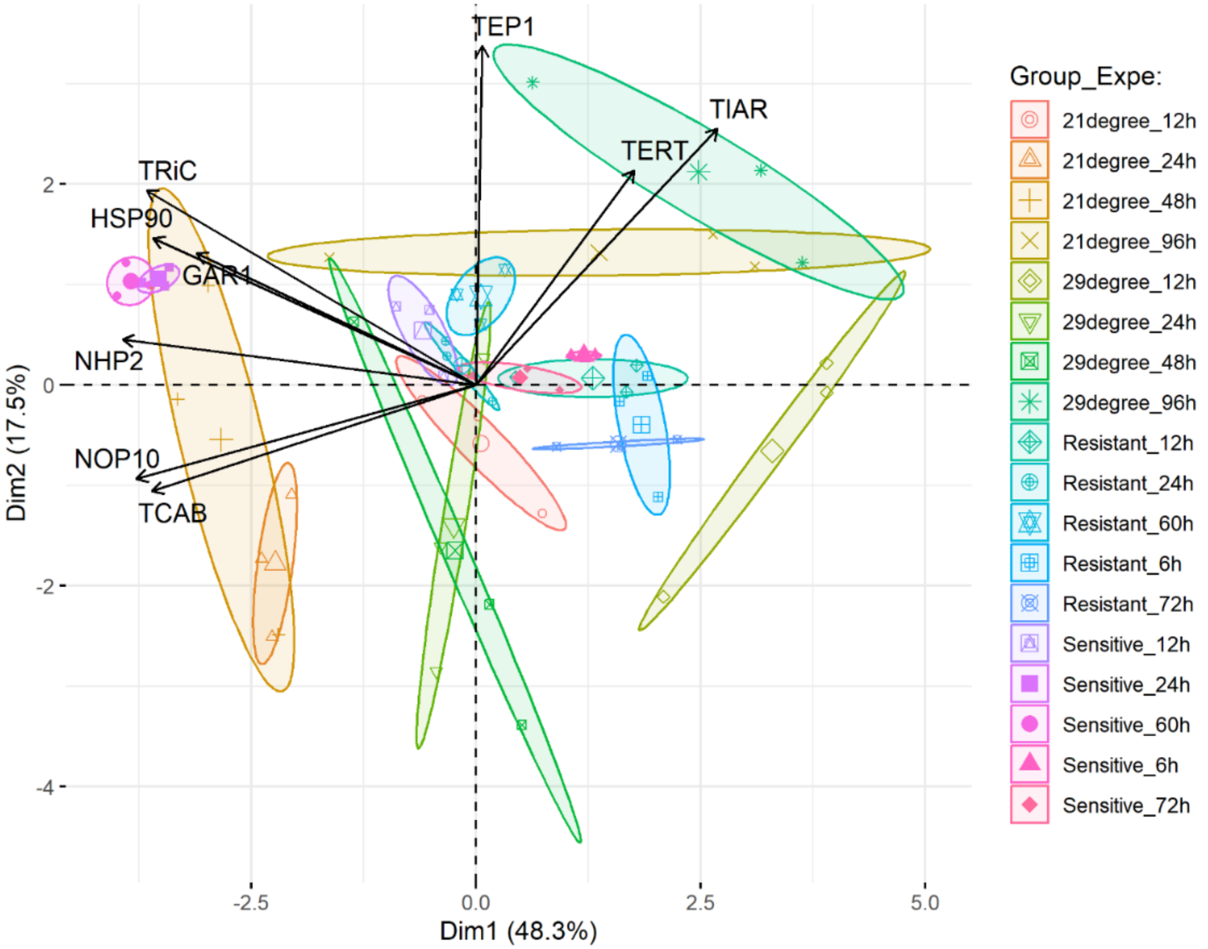


Fig. S8.

Virus replication quantified in seawater. OsHV-1 DNA copies was quantified with qPCR from 500 µL of infected seawater during the OsHV-1 µVar challenge in controlled conditions (21°C and food *ad libitum*). From 16th to 30th June, we exposed n = 4,325 spats from 36 families distributed in 3 tanks (i.e., 40 receiving oysters par family and per tanks) to donor oysters previously impregnated for 48h to field viral load. After contact (grey arrow) between donors and receivers, viral replication was perceptible in seawater. See text for details.

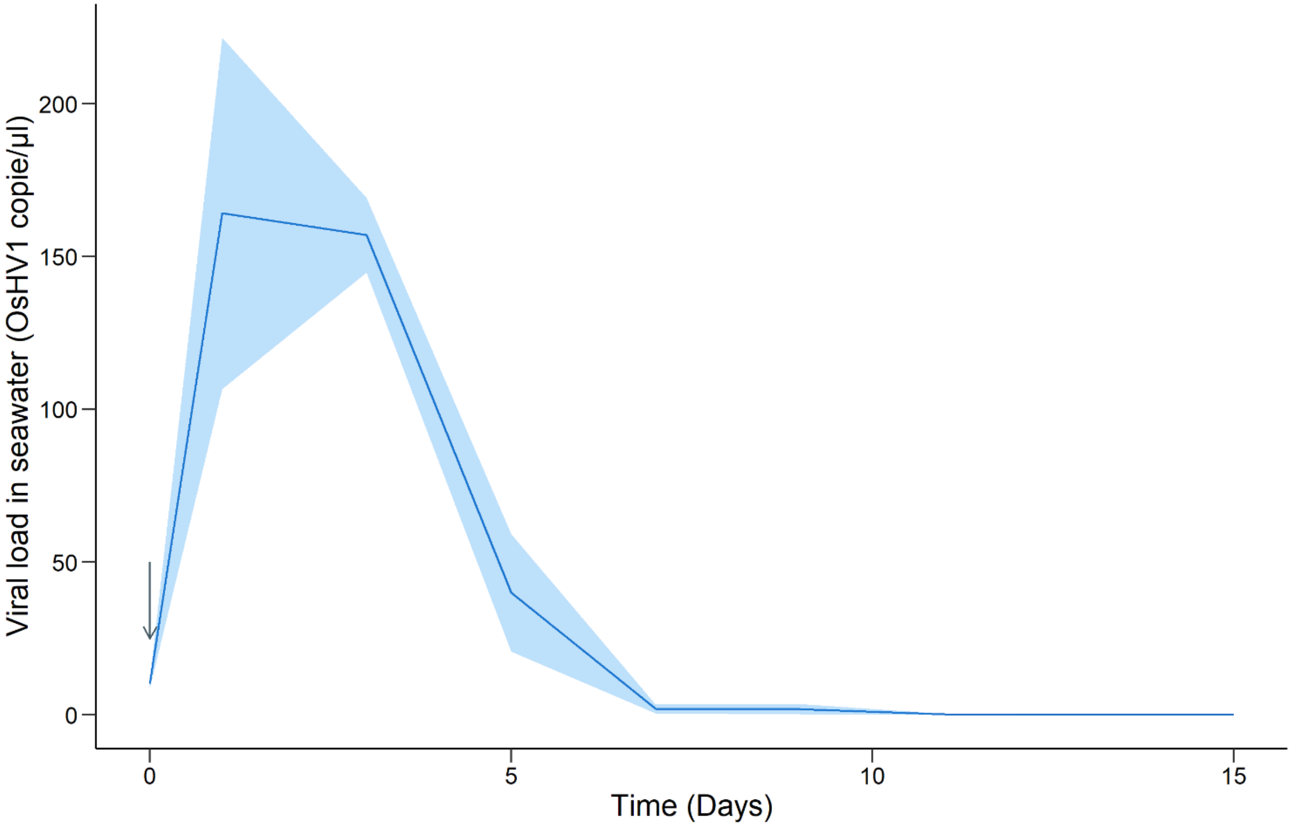


Table S1.

Description of the three datasets retained to explore the conserved telomerase genes in response to OsHV-1 μVar. Table reports the study identification and the method used for transcriptomic analyses. It also mentions the information onto sample strategy and data processing.

Reference in the main text	DOI	NCBI's Bioproject	Data	Sample size (n pools of 10 oysters)	Trimming opts.
(*30*)	10.1038/s41467-018-06659-3	PRJNA423079	Illumina HiSeq 2500 2x75bp	n = 36 pools of 10 oysters (3 replicates per family and per time post infection)	Leading:20 Trailing :20 Slidingwindow :30:30 Minlen:50
(*43*)	10.1186/s12864-020-6471-x	PRJNA423079	Illumina NextSeq 550 single-end 76bp	n = 36 pools of 10 oysters (6 replicates per family)	Leading:20 Trailing :20 Slidingwindow :30:30 Minlen:40
(*33*)	10.1242/jeb.226233	PRJNA593309	llumina HiSeq 4000 2x126bp	n = 30 pools of 9 oysters (3 replicates per temperature and per time post infection)	Leading:20 Trailing :20 Slidingwindow :30:30 Minlen: 100