Population-specific effects of ocean acidification in the Olympia oyster

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Abstract

Populations of marine species that respond differently to ocean acidification offer natural reservoirs of biodiversity that can be leveraged for conservation efforts and to sustain marine food systems. The molecular and physiological traits associated with tolerance to acidification must first be identified. This study leveraged oysters from three phenotypically distinct populations of the Olympia oyster, *Ostrea lurida*, but that were bred and reared in common conditions for four years. We assessed their growth, reproductive development, and transcriptional response to acidification within and across generations. Responses reveal energetic trade-offs that reflect unique physiotypes previously observed among populations. The population with the slowest growth but high survival rates, oysters from Dabob Bay, mounted the largest transcriptional response to acidification without effects to growth and reproduction. A moderate response was observed in the population with fastest growth rate but lowest fecundity (Fidalgo Bay). Oyster Bay, the population with highest fecundity but lowest survival rates, did not respond at the transcript level. Oyster Bay was also the only population for which acidification negatively affected growth and reproductive development. While exposure to acidification did not affect gene expression in the next generation's larval stage, it did result in larger larvae in the Oyster Bay population, which could partially alleviate negative effects of acidification in the wild for that population. Given the distinct transcriptional response of the Dabob Bay population to acidification and its high survival rates in previous studies, we then identified genes that were uniquely expressed in Dabob Bay oysters compared to the other populations. Genes involved in antibacterial and antiviral processes, metabolism, growth, and reproduction were uniquely expressed in Dabob Bay, and many similar functions were identified in both adults and larvae, which provides insight into the mechanisms behind a stress-tolerant

oyster population. The population-specific physiotypes and responses to acidification illustrate the diversity of physiological strategies in *O. lurida* that balance the energetic demands of growth, reproduction, cellular maintenance, and offspring viability. Taken together this study reveals that there are distinct physiotypes among marine invertebrate populations on small geographic scales with implications for species resilience to acidification and other environmental stressors.

Introduction

Following observations of shifting ocean conditions (IPCC, 2019) an enormous scientific effort has explored the response of marine species to ocean acidification (Riebesell & Gattuso, 2014). Empirical data has established that many species are vulnerable to ocean conditions projected for this century, particularly calcifying invertebrates, affecting a range of physiological processes over the lifetime of an organism, including development, recruitment, growth, reproduction, and survival (Gazeau et al., 2013; Kroeker et al., 2013; Lemasson et al., 2017; Melzner et al., 2019). However, these studies also indicate that biological responses are quite variable, related to an organism's genetic and environmental ancestries (Eirin-Lopez & Putnam, 2019; He & Silliman, 2019; Przeslawski et al., 2015; Sunday et al., 2014). Some species are more tolerant to the effects of acidification than others (Branch et al., 2013; Figuerola et al., 2021), as are some populations within species (Bitter et al. 2019; Swezey et al. 2020; Kelly et al. 2013; Vargas et al. 2017). There is also evidence of intergenerational (spanning one generation) and transgenerational (spanning 2+ generations) plasticity, which may buffer future populations against challenging conditions (Salinas et al., 2013; Zhao et al., 2020). Ultimately, there will be a spectrum of responses to shifting ocean chemistry, dependent on species' capacity to mitigate, acclimatize,

and adapt to shifting conditions. For effective conservation and management of marine calcifiers, it is critical to identify the genotypes, physiotypes, and molecular mechanisms that impart tolerance to acidification, as well as quantify the range of responses within taxa.

Impacts of acidification to oysters were among the earliest observations of negative biological effects. Now, in an effort to build resilient commercial and wild stocks, breeding programs and researchers are increasingly seeking to identify oyster species and populations that are tolerant to acidification and other stressors. One such group of oysters appears to be species from the genus *Ostrea*, which includes the Olympia (*O. lurida*), European flat (*O. edulis*), Chilean flat (*O. chilensis*), and Australian flat oyster (*O. angasi*). Multiple studies have reported little to no effects of acidification in *Ostrea* spp. at the adult (Lemasson et al., 2018; Lemasson et al., 2019; Lemasson & Knights, 2021), juvenile (Navarro et al., 2020), and larval stages (Cole et al., 2016; Pereira et al., 2019; Waldbusser et al., 2016). In one study *O. edulis* larval growth and survival responded positively to acidification exposure (Prado et al., 2016). Unique *Ostrea* spp. life history traits, in particular brooding of veliger larvae in the maternal pallial cavity, may contribute to the species' relative tolerance to acidification, as pH can quickly decrease to levels as low as 6.96 during periods of valve closures (Chaparro et al., 2009; Gray et al., 2019). There are, however, other studies that have observed negative effects of acidification in *Ostrea*, such as decreased larval growth in *O. lurida* that persist to the juvenile stage (Hettinger et al., 2012, 2013; Sanford et al., 2014). Contrasting effects could be explained by population-specific responses, which are commonly observed in marine invertebrate taxa (Barber et al., 1991; Macdonald & Thompson, 1988). Indeed, populations of *O. lurida* from northern California have diverged salinity tolerances, which is facilitated by distinct physiological responses at the cellular level (cell death regulation, mantle ciliary activity) (Maynard et al., 2018). Molecular

81 strategies of *Ostrea* spp. need closer examination to understand the functions that enable
82 tolerance to acidified conditions in some, but not all, populations (Melzner et al., 2009).
83 Previous studies have identified a suite of molecular functions in marine calcifiers that
84 are sensitive to ocean acidification, which has been thoroughly reviewed by Melzner et al. (2019)
85 and Strader et al. (2020). Interestingly, the directionality and magnitude of molecular responses
86 can differ by species and/or populations within the same species. Opposing changes in
87 antioxidants and molecular chaperones have been reported for acidification-tolerant
88 (upregulated) and wild-type (downregulated) Sydney rock oysters in acidified conditions, which
89 then reverse in subsequent generations (Goncalves et al., 2016, 2017). Changes to transcripts
90 associated with energy production provide evidence for both metabolic depression and increased
91 metabolic demand in response to acidification (Strader et al., 2020). In some cases the degree of
92 transcriptional plasticity varies in response to acidification (Kenkel & Matz, 2017). For instance,
93 upon exposure to acidification, transcripts for genes related to ATP production increased in two
94 populations of urchins, but the magnitude of increase was more pronounced in the population
95 that experiences more frequent periods of low pH compared to those from more stable pH
96 environments (Evans et al., 2017). These studies highlight the variety of molecular responses to
97 acidification that can occur among populations of the same species. It is vital to identify the
98 fundamental molecular signatures of acidification-tolerant oysters to inform breeding and
99 conservation efforts.

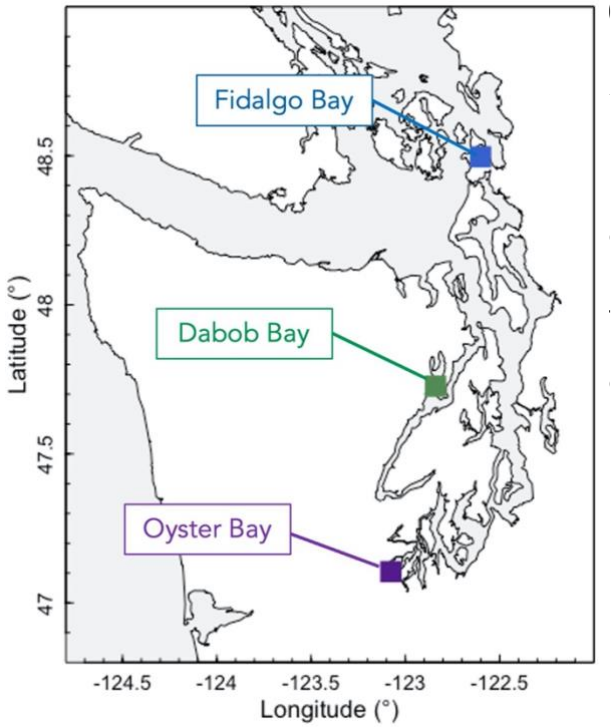


Figure 1: Location of the focal *O. lurida* populations in the greater Puget Sound estuary, Washington, USA

In the present study we examine effects of acidification on multiple populations of the Olympia oyster, *Ostrea lurida*. *O. lurida* is native to the North American Pacific Coast, inhabiting dynamic estuarine environments that are influenced by coastal upwelling and ocean acidification (Feely et al., 2010; McGraw, 2009; Reum et al., 2014). We build upon previous studies that have identified unique fitness traits in three populations from disparate regions of greater Puget Sound in Washington State (Figure 1, Table 1) (Heare et al., 2017; Heare et al., 2018; Silliman et al., 2018; Spencer et al., 2020; White et al., 2017). Oysters derived from Oyster Bay in the South Puget Sound are highly fecund, and compared to other populations require fewer degree-days to begin reproducing (Heare et al., 2017; Silliman et al., 2018; Spencer et al., 2020). Oysters from Dabob Bay in the Hood Canal basin consistently display slower growth than other populations but have higher survival rates in field testing and during hatchery rearing (Heare et al., 2017; Spencer et al., 2020, Ryan Crim, *pers. comm.*). Those from Fidalgo Bay, a

small basin in the northern reaches of greater Puget Sound, grow faster and are less fecund or have delayed reproduction (Heare et al., 2017; Silliman et al., 2018; Spencer et al., 2020).

Here, we use high-throughput sequencing to perform the first transcriptional characterization of an *Ostrea* species to ocean acidification. Expression analyses are paired with biometric data (gonad development, growth) to capture system-wide changes in energy allocation due to acidification exposure (Sokolova et al., 2012). By examining oysters from the aforementioned Olympia oyster populations (Dabob Bay, Fidalgo Bay, and Oyster Bay) we leverage a comprehensive understanding of the traits characteristic of each population (Table 1) (Heare et al., 2017; Heare et al., 2018; Silliman et al., 2018; Spencer et al., 2020), and by using individuals that were bred and grown in common conditions we control for within-generation carryover effects (Hettinger et al., 2012, 2013). Given the possible influence of intergenerational exposures on an organism's physiology (Goncalves et al., 2016, 2017), we also extend the analysis to include a second generation (larval offspring) to examine the potential impacts of parental exposures and population-of-origin on basal functions (larval size, gene expression). By fully describing its molecular and physiological response to acidification across distinct populations we show that *O. lurida* is a good candidate for aquaculture investment & conservation.

Table 1. Traits that are characteristic of the focal *O. lurida* populations from the greater Puget Sound region, summarized from previous studies of the same populations: (1) Heare et al. 2017 (2) Heare et al. 2018 (3) Silliman et al. 2018 (4) Spencer et al. 2020 (5) Ryan Crim *pers. comm*. Additionally, genetic differentiation among populations was characterized by White et al. (2017).

Location of *O. lurida* population	**Growth rate, size at maturity**	**Reproductive output, timing**	**Performance in field & hatchery trials**	**Immediate transcriptional response to stress**
Fidalgo Bay	Fast growth, large at maturity[1,3]	Low/Moderate fecundity[1,3,4]	Moderate survival[1,4]	No response[2]
Dabob Bay	Slow growth, small at maturity[1,3,4]	Moderate fecundity[1,3,4]	High survival in field, high larval survival in hatchery[1,4,5]	No response[2]
Oyster Bay	Moderate growth, medium/large at maturity[1,3,4]	Very high fecundity, early to reproduce[1,3,4]	Low/moderate survival in field[1,4]	Altered expression of targeted genes 1-hr following heat & mechanical stress[2]

Methods

Adult oyster source and history

Experimental oysters were bred in 2013 as described in Heare et al. (2017) in common conditions from three populations of wild broodstock, which were harvested from Dabob Bay in Hood Canal, Oyster Bay in South Puget Sound, and Fidalgo Bay in North Puget Sound. Oysters were then maintained in common conditions in a pearl net adjacent to the Kenneth K. Chew Center for Shellfish Research and Restoration in central Puget Sound for approximately 3.5 years. Upon entering experimental conditions, shell height for each population was on average 29.8 ± 4.6mm, 35.7 ± 4.5mm, and 35.7 ± 4.4mm for Dabob Bay, Oyster Bay, and Fidalgo Bay, respectively. Experimental oysters were therefore mature adults that had been produced and reared in common conditions, but had distinct genetic heritage.

157 ***Adult treatments and larval collection***

158 Beginning February 16, 2017 adults were exposed to two pCO_2 treatments for 52 days (control
159 pCO_2: 841 ± 85 µatm, pH 7.82 ± 0.02; high pCO_2: 3,045 ± 488 µatm, pH 7.31 ± 0.02), as
160 described in Spencer et al. (2020) and Venkataraman et al. (2019) (Supplemental Materials).
161 Following experimental exposures, adults from all populations and pCO_2 treatments were
162 returned to common conditions, reproductively conditioned, and spawned to produce offspring.
163 Because *O. lurida* are viviparous spermcasters and brood larvae to the veliger stage, larvae were
164 captured upon maternal liberation, which commenced on May 14th and persisted for 57 days.
165 Details of the experimental and spawning conditions are described in (Spencer et al., 2020).
166 Prior to the pCO_2 treatments adults were also exposed to two temperature regimes for 60 days.
167 These represent what would be considered normal or ambient temperature (6.1 ± 0.2°C) and a
168 temperature that would be considered elevated at the experimental site (10.2 ± 0.5°C) (see
169 Spencer et al. 2020). Given that temperature did not interact with pCO_2 to affect the focal
170 characteristics in adults or larvae, only effects of parental pCO_2 exposure were further examined
171 for this study.

172 ***Tissue sampling***

173 Adult ctenidia tissue was collected immediately upon terminating pCO_2 treatments from Dabob
174 Bay, Fidalgo Bay, and Oyster Bay. Nine oysters from each population and pCO_2 treatment were
175 sacrificed and ctenidia tissue was collected and flash-frozen at approximately -116°C using a
176 solution of ethanol and dry-ice then preserved at -80°C. Gonad tissue was collected from the
177 same individuals, preserved in histology cassettes using the PAXgene Tissue FIX System
178 (PreAnalytiX, Hombrechtikon, Switzerland), then processed for reproductive development
179 analysis by Diagnostic Pathology Medical Group (Sacramento, California, USA).

Larval offspring were sampled at the veliger stage upon maternal liberation. For each group of larvae that was released, a portion were reared as described in Spencer et al. (2020) and the remaining were preserved by rinsing with fresh water into microcentrifuge tubes, removing water, then placing directly into -80°C freezer. Given the variable reproductive rates, the number of larval groups preserved for downstream analysis from control pCO_2 and high pCO_2 varied, and was 7 and 10 for Fidalgo Bay, 5 and 7 for Dabob Bay, and 17 and 13 for Oyster Bay, respectively. Each larval sample contained thousands of larvae (on average 170k), and consisted of siblings that were released from the same female on the same day. However, given that adults were continuously spawning and releasing larvae, it is possible that some larval samples contained a mix of multiple families from the same population and treatment.

Adult growth and reproductive development

Adult oysters were measured for shell height before and after pCO_2 treatments (n=9 per population x pCO_2 treatment) using digital calipers (mm), defined as the maximum distance from the umbo along the dorsal/ventral axis. Shell height after pCO_2 treatments were compared among population and pCO_2 treatments using 2-way ANOVA. Since we were most interested in assessing effects of high pCO_2 exposure on growth rate for each population, population-specific differences in size between high-, control- and pre-pCO_2 treatments were tested using 1-way ANOVA, and Tukey honest significant difference tests were performed to assess pairwise comparisons.

Gonad samples (n=9 per population x pCO_2 treatment) collected before and after pCO_2 treatments were assigned sex and stage as described in Spencer et al, (2020). For each population, contingency tables were constructed for gonad sex, developmental stage of sperm, and developmental stage of eggs, and differences between pCO_2 treatments were compared using

Chi-Square or Fisher Exact Tests (depending on the number of males or females present within a population) and P-values were estimated using Monte-Carlo simulations with 1,000 permutations.

Larval offspring size

Newly liberated veliger larvae were measured using a Nikon eclipse Ni microscope and the NIS-Elements BR imaging and measuring software (version 4.60). Mean shell height (distance from hinge to margin, perpendicular to hinge), and mean shell width (longest distance parallel to hinge) were estimated from at minimum 48 larvae per collection from each tank. Mean shell height and width were compared among population and parental treatments using 2-Way ANOVA and Type II Sums of Squares with the car package (Fox & Weisberg, 2018). Then, since we were most interested in assessing population-specific responses, effects of parental pCO_2 on larval size were assessed for each population using 1-Way ANOVA with Type II Sums of Squares.

Library construction and sequencing

RNA was isolated from 52 frozen adult ctenidia samples (n=8 or 9 per population x pCO_2 treatment) and 61 pooled whole-body larvae (5-13 per population x parental pCO_2 treatment) (Table 2). Each sample was homogenized in liquid nitrogen with a stone mortar and pestle and isolated following the RNAzol® RT protocol for Total RNA Isolation (Molecular Research Center, Inc., Cincinnati, OH). RNA pellets were resuspended in DEPC-treated water, residual DNA contamination was removed using the Turbo DNase kit (Life Technologies, Carlsbad, CA), then RNA was quantified using the Qubit RNA assay with Qubit 3.0 Fluorometer (2.0ul of each sample for quantification, Life Technologies, Carlsbad, CA), and quality was assessed for a

subset of RNA isolates using the Bioanalyzer RNA 6000 Pico Chip assay (Agilent Technologies, Santa Clara, CA).

Library preparation was performed following the QuantSeq 3' mRNA-Seq protocol (v.015UG009V0251, Lexogen, Vienna, Austria), also known as TagSeq, which generates cDNA from the 3' end of mRNA strands and only generates one fragment per mRNA transcript, allowing for accurate gene expression data at a reduced cost (Meyer et al., 2011). Briefly, Total RNA (350 ng) was used to generate single-stranded DNA using reverse transcription (oligoT priming), which binds to the poly(A) tail and includes the read 1 adapter; RNA was removed, then the second DNA strand + Illumina adapter was synthesized by random priming; the double stranded cDNA + adapters were purified using magnetic beads, then an aliquot (1uL) of each library was amplified using qPCR to determine the optimal number of cycles needed for Endpoint PCR, which ranged from 14 to 17; the libraries were then amplified and indexed with unique barcodes, then re-purified. Prior to sequencing, all libraries were quantified using Qubit High Sensitivity DNA kits (Life Technologies, Carlsbad, CA), and the quality (e.g. fragment length) was assessed for a subset using a Bioanalyzer High Sensitivity DNA chip kit (Agilent Technologies, Santa Clara, CA). Single-end sequencing with 100-bp read length was conducted on a NovaSeq platform (Illumina, San Diego, CA) by the University of Washington's Northwest Genomics Center, who also demultiplexed the raw sequencing data.

Table 2. The number of QuantSeq libraries examined per population and parental pCO_2 treatment for adult and larval tissues.

	Adult ctenidia *individuals*		Larval whole-body *pooled by maternal liberation group*	
	Control pCO_2	High pCO_2	Control pCO_2	High pCO_2
Fidalgo Bay	9	8	5	10
Dabob Bay	9	8	5	5
Oyster Bay	9	9	13	11

Expression data processing & analysis

Raw reads were inspected for quality using FastQC (Andrews 2010) and MultiQC (Ewels et al., 2016), trimmed to remove Illumnina adapters, poly(A)- and poly(G)-tails, and quality filtered (≥20 read length, >15 quality score) using the Cutadapt toolkit v2.10 (Martin, 2011), then aligned to the draft *O. lurida* genome v081 (GenBank accession GCA_903981925.1) using Bowtie2 v2.4.1 (Langmead & Salzberg, 2012) and local alignment with the pre-set --sensitive-local option. Average alignment rate was 75.7±4.4% for adult libraries and 70.7±8.2% for larval libraries. From the resulting alignment files (.bam), the number of reads that uniquely mapped to each *O. lurida* gene was determined using featureCounts v2.0.0 (Liao et al., 2014) and a gene annotation file that was adjusted to extend the 3' end by 2kb, as libraries were generated at the 3' end of the original mRNA transcripts.

Differential gene expression analysis was performed using DESeq2 (Love et al., 2014) in R v4.0.4 using RStudio interface v1.3.1093 (R Core Team, 2021; RStudio Team, 2020). Read counts were first filtered to remove genes with fewer than 10 total counts across all samples (1,229 and 7,725 genes were discarded for adult ctenidia and larval pools, respectively). For adults the number of reads retained for analysis ranged from 1.18M to 3.30M per sample and averaged 1.99M ±SD442K, and for larvae total reads ranged from 653K to 2.04M and averaged

263 1.32M ±SD332K. The total number of genes and per-sample average retained for analysis was
264 30,981 and 28,110±607 for adult ctenidia, and 24,485 and 23,114 ±SD1,259 for larvae. Gene
265 counts were assessed for differential gene expression for adult ctenidia between pCO_2 exposure
266 within populations, and for larvae between parental pCO_2 exposures within and across
267 populations. DESeq2 uses raw count data to generate generalized linear models and internally
268 corrects for library size, therefore counts were not normalized prior to differential expression
269 analysis.

270 Given the high performance of the Dabob Bay population in previous lab and field trials
271 (Heare et al. 2017; Spencer et al. 2020; Ryan Crim *pers. comm.*), we examined genes that were
272 constitutively expressed at unique levels in Dabob Bay. Pairwise differences in expression
273 among Dabob Bay and the other two populations were identified in DESeq2 using
274 transcriptomes from adults held in control conditions (n=9 libraries per population), and
275 separately using transcriptomes from all larval samples (Table 2). For each life stage (adult and
276 larvae), genes from the two pairwise comparisons (Dabob vs Fidalgo, Dabob vs. Oyster) were
277 clustered into two gene sets: 1) genes that were more abundant, and 2) genes that were less
278 abundant than the other two populations. Overlapping genes uniquely abundant in both adults
279 and larvae from Dabob Bay were then interrogated for relative expression patterns (i.e. higher or
280 lower than the other populations).

281 Differentially expressed genes were merged with the *O. lurida* genome to generate lists
282 of Uniprot IDs from annotated genes. Enriched biological processes of differentially expressed
283 genes sets were determined using the Gene-Enrichment and Functional Annotation Tool from
284 DAVID v6.8, and were defined as those with modified Fisher Exact P-Values (EASE Scores)
285 <0.1.

Results

Effects of acidification on adult growth, sex ratio

Adult oyster growth rate was reduced by acidification in the Oyster Bay population only: shell size did not differ after the high pCO_2 exposure, whereas those that were exposed to control pCO_2 were larger after treatment (Figure 2, Table 3). The Dabob Bay and Fidalgo Bay populations did not differ in size after either pCO_2 treatment compared to before (Figure 2, Table 3). Shell height differed by population before ($F(2,72)=3.44$, $p=0.034$) and after ($F(2,84)=16.5$, $p=9.28e^{-7}$) pCO_2 treatments, with the Dabob Bay population significantly smaller than both Fidalgo Bay and Oyster Bay, particularly after pCO_2 treatments.

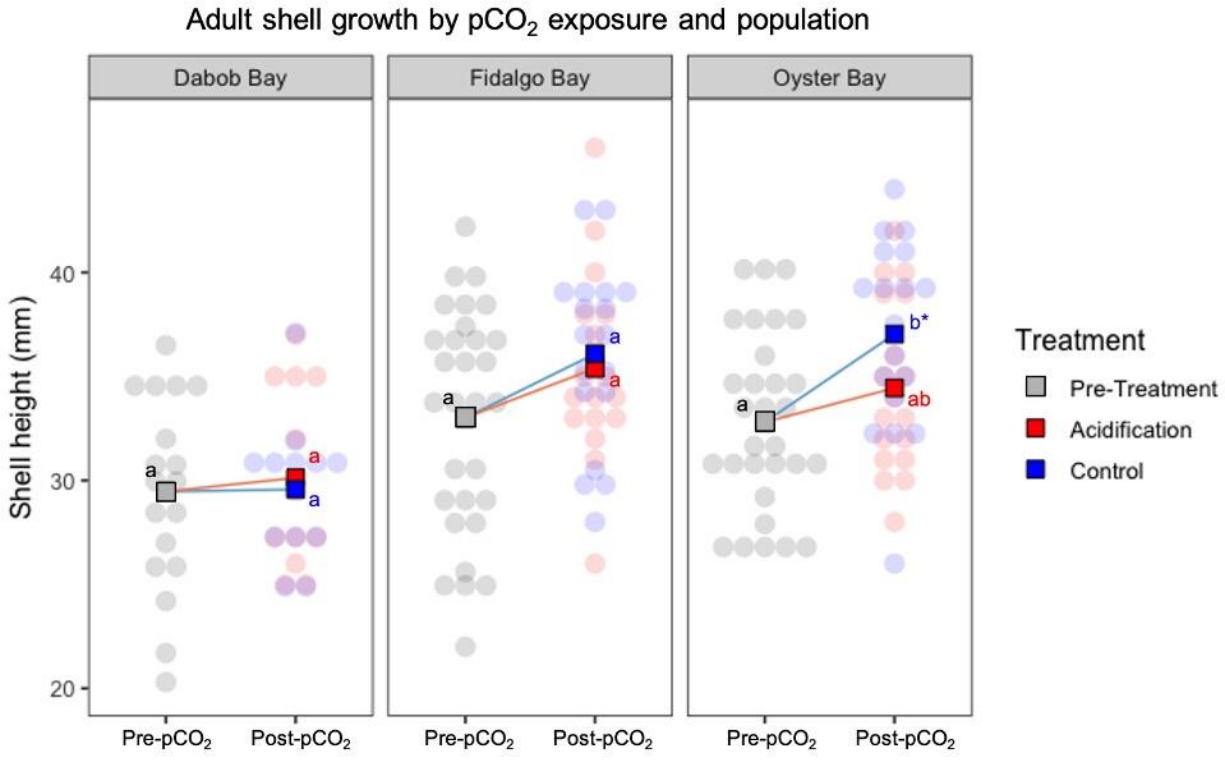


Figure 2. Adult shell height before pCO_2 treatment ("Pre-pCO_2") and after 52 days in acidification (pCO_2 = 3045 ± 488 µatm: pH = 7.31 ± 0.02) and control conditions (pCO_2 = 841 ± 85 µatm; pH = 7.82 ± 0.02) for each population. Transparent points each represent one adult

299 oyster color-coded by pCO_2 treatment, and overlaid square points indicate mean shell height.
300 Those exposed to control pCO_2 were larger than before the pCO_2 treatments in the Oyster Bay
301 population only.

302 **Table 3**: Shell size statistics for each population. 1-way ANOVA compared shell height of oysters before
303 treatment (Pre-Treatment) and after acidification and control treatments. Tukey multiple comparison of means
304 was used to test for significant growth of oysters during the 52-day exposure to control conditions and
305 acidification.

Population	ANOVA statistics	Tukey comparison of means: *Control vs. Pre-treatment*	Tukey comparison of means: *Acidification vs. Pre-treatment*
Dabob Bay	F(2,39)=0.09, p=0.91	Δ=0.10, p-adj=1.0	Δ=0.66, p-adj=0.91
Fidalgo Bay	F(2,63)=0.26, p=0.079	Δ=3.08, p-adj=0.094	Δ=2.38, p-adj=0.24
Oyster Bay	**F(2,63)=5.35, p=0.0071***	**Δ=4.22, p-adj=0.0049***	Δ=1.64, p-adj=0.42

306

307 Progression from one sex to the other in these hermaphroditic oysters was affected by
308 acidification in the Oyster Bay population only: the ratio of predominantly-
309 females:predominantly-males differed significantly between pCO_2 treatments (p-sim=0.015),
310 with fewer females present after exposure to high pCO_2 (Figure 3). The developmental stages of
311 sperm and eggs (when present) did not differ between control and high pCO2 treatment in any
312 population. Sperm development did not change during the 52-day exposure period in any
313 population. The Fidalgo Bay population exposed to control conditions contained more advanced-
314 and late-stage oocytes following the 52-day exposure compared to before (p-sim=1.0e-4), but
315 this was not the case for Fidalgo Bay oysters exposed to acidification, or for any other
316 population. Additionally, compared to before pCO_2 treatment, the sex ratio differed following
317 control pCO_2 exposure in Oyster Bay (p-sim= $8.0e^{-4}$) and Fidalgo Bay (p-sim=$1.3e^{-3}$), but did not

differ following high pCO_2 exposure in any population, or between any comparison in the Dabob Bay population.

The developmental stages of sperm and eggs (when present) did not differ between control and high pCO_2 treatment in any population. Sperm development did not change during the 52-day exposure period in any population. The Fidalgo Bay population exposed to control conditions contained more advanced- and late-stage oocytes following the 52-day exposure compared to before (p-sim=$1.0e^{-4}$), but this was not the case for Fidalgo Bay oysters exposed to acidification, or for any other population.

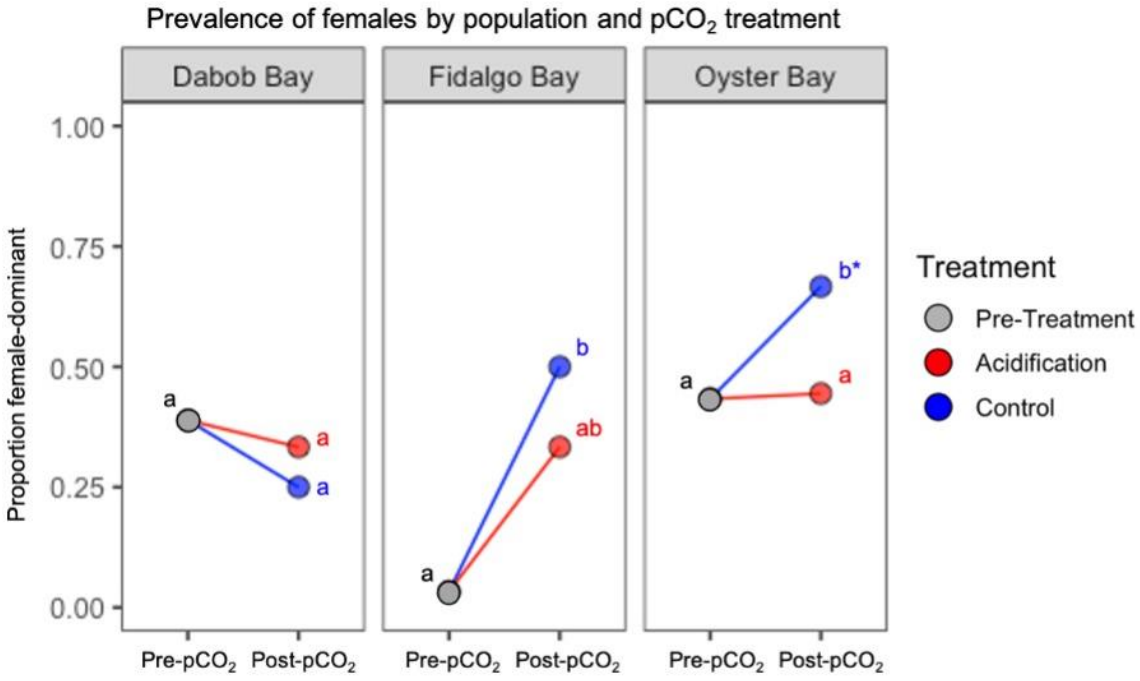
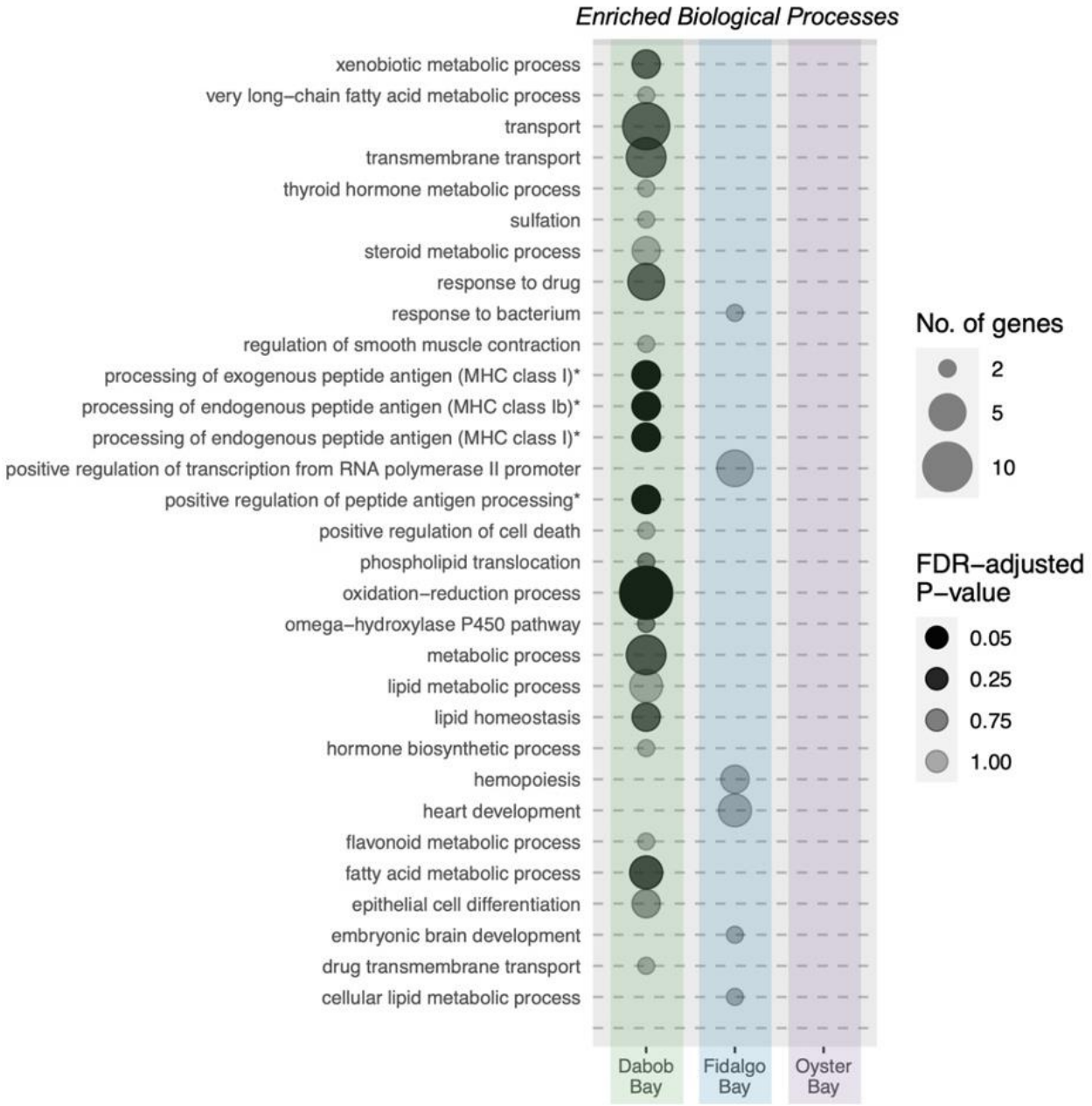


Figure 3. Proportion of oysters that were female or female-dominant before pCO_2 treatment ("Pre-pCO_2") and after 52 days in acidification (pCO_2 = 3045 ± 488 µatm: pH = 7.31 ± 0.02) and control conditions (pCO_2 = 841 ± 85 µatm; pH = 7.82 ± 0.02) for each population. The prevalence of females differed among pCO_2 treatments in the Oyster Bay population only.

Differential expression in adults upon direct exposure to acidification

Of 32,210 genes in the draft *O. lurida* genome, we detected expression in 30,981 genes in adult ctenidia tissue. Within populations, 132 and 76 genes were differentially expressed in Dabob Bay and Fidalgo Bay in response to pCO_2 treatments, respectively (Supplemental Materials). No expression differences were detected in the Oyster Bay population upon exposure to pCO_2 treatments. The annotated Dabob Bay and Fidalgo Bay DEGs were enriched for 25 and 6 biological functions (Figure 4). Four genes were differentially expressed in both Dabob Bay and Fidalgo Bay, which code for Cytochrome P450 2B4 (CYP2B4, p-adj=0.021), Cytochrome P450 2U1 (CYP2U1, p-adj=0.015), Fatty acid-binding protein (FABP4, p-adj=0.032), and Thyroxine 5-deiodinase (DIO3, p-adj=0.028), all of which were more abundant in oysters exposed to high pCO_2.



342

343 **Figure 4**. Enriched biological processes of differentially expressed genes upon exposure to high

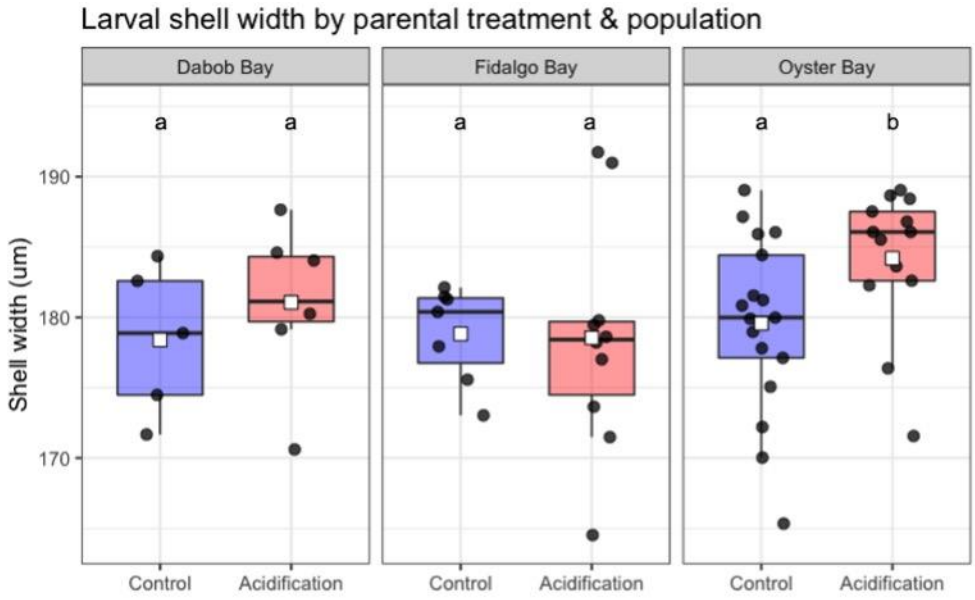
344 pCO_2 in three populations with different bays-of-origin. No genes were differentially expressed

345 in the Oyster Bay population, and thus no processes were enriched. Asterisks indicate GO terms

346 that have been edited for length.

347 ***Impacts of parental exposures on larval offspring size***

348 Within populations, larval shell width and height differed by parental pCO_2 in the Oyster Bay
349 population only (width: $F(1,29)=5.46$, $p=0.027$; height: $F(1,29)=4.56$, $p=0.041$), but did not
350 differ significantly among parental pCO_2 in Fidalgo Bay (width: $F(1,15)=0.0071$, $p=0.93$; height:
351 $F(1,159)=0.14$, $p=0.71$), or Dabob Bay (width: $F(1,10)=0.71$, $p=0.49$; height: $F(1,10)=0.10$,
352 $p=0.76$) (Figure 5).



353

354 **Figure 5**. Laval shell size upon maternal liberation was significantly affected by parental
355 exposure to high pCO_2 in the Oyster Bay population only. Shell width is shown here. Each point
356 represents the mean shell width of 40+ larvae from a release group. Treatments were terminated
357 prior to adult reproductive conditioning and spawning, so larvae were never directly exposed to
358 treatments.

359 ***Larval offspring gene expression***

360 Larvae were pooled by maternal release pulse and assessed for gene expression differences by
361 parental exposure to acidification. We detected expression of 24,485 genes in larval samples.

Within populations, one gene was differentially expressed by parental pCO_2 in the Fidalgo Bay population, "OLUR_00020618" (log2 fold change = -1.6, p-adj = 0.011), which was not annotated. Across all populations no expression differences were detected between parental pCO_2 exposures.

Constitutive expression differences in the high-performing Dabob Bay population

Of the 30,981 genes examined in adults, 280 differed between Dabob Bay and Oyster Bay (0.9% of genes), and 379 differed between Dabob Bay and Fidalgo Bay (1.2% of genes), 89 of which were identified by both comparisons. Of these genes uniquely expressed in Dabob Bay, 220 were annotated (Supplemental Materials) and were enriched for 16 biological processes. There were 31 genes that were both constitutively expressed at different levels in Dabob Bay compared to other populations and were differentially expressed in response to acidification. Of these overlapping genes, eleven were less abundant than the other populations constitutively, but in response to acidification they increased in abundance. Twenty were more abundant constitutively, but in response to acidification they became less abundant.

Of the 24,485 genes examined in larval offspring, 260 and 452 differed between Dabob Bay and Fidalgo Bay and Oyster Bay, respectively (0.86% and 0.88%), 59 of which were identified by both comparison and 25 of which were annotated (Supplemental Materials). Forty-two genes were differentially expressed in both adult and larval stages among Dabob Bay and the other populations, which largely followed the same constitutive expression patterns in both life stages: 26 genes were more abundant in Dabob Bay adults and larvae compared to the other populations, and 14 were less abundant in both stages.

Discussion

This study explored the within- and intergenerational response of *O. lurida* to acidification in three populations that had been reared in common conditions, but had distinct genetic ancestries. Each population demonstrated unique physiologies, which were evident in growth, gonad development, and transcriptional responses to pCO_2 treatments. In the Dabob Bay population there was no growth or change in sex ratio regardless of treatment. However, Dabob Bay oysters demonstrated a robust transcriptional response to high pCO_2 enriched for oxidation-reduction/detoxification, lipid metabolism, and other key processes. In stark contrast, no transcriptional response was detected in Oyster Bay oysters, but growth rate and prevalence of females were both negatively affected by acidification. There was a moderate transcriptional response in the Fidalgo Bay population, with no change in growth rate and minor effects to sex ratio as a result of acidification. This study also explored intergenerational carryover effects of adult exposure to acidification on basal gene expression and size of larvae upon maternal liberation across populations. Larvae were larger from adults exposed to high pCO_2 in the Oyster Bay population only. Counter to predictions we found no signature of parental exposure to acidification in larval transcriptomes. The unique responses to acidification support previous observations of varying stress-tolerance in the same populations.

Population-specific effects of acidification on growth and reproduction are associated with performance

Growth rate varies among Puget Sound *O. lurida* populations (Heare et al., 2017; Heare et al., 2018; Silliman et al., 2018; Spencer et al., 2020), and this study indicates that it also responds to acidification in a population-specific manner. Shell growth was stunted by acidification in the adults from Oyster Bay, as they grew in control conditions but did not in acidified conditions. In

407 contrast, adults from Dabob Bay and Fidalgo Bay did not grow during the 52-day experiment
408 regardless of pCO_2 treatment. Previous studies characterized Dabob Bay as the slowest growing
409 population, but also found that those oysters also performed best in hatchery and field trials.
410 Heare et al. (2017) and Silliman et al. (2018) both observed slowest growth in the Dabob Bay
411 population and fastest growth in the Fidalgo Bay population. Heare et al. (2017) also observed
412 highest deployment survival in Dabob Bay progeny. In Spencer et al. 2020 the Dabob Bay
413 population was slowest to reach the eyed larval stage (~18 days) (Fidalgo Bay was the fastest,
414 ~14 days), and had the highest survival during the larval stage (the lowest survival was in Oyster
415 Bay population). Together, these studies indicate that *O. lurid*a from Dabob Bay may prioritize
416 stress resilience and survival at the cost of growth rate and size at maturity.
417 Acidification affected reproductive processes in a population-specific manner. The
418 natural male-female sexual progression was significantly altered by acidification in the Oyster
419 Bay population only. Reproductive traits appear to be heritable in Puget Sound *O. lurida*, with
420 notable differences in the Oyster Bay population. In a reciprocal transplant study, Heare et al.
421 (2017) found that Oyster Bay had considerably higher incidents of brooding, and reached
422 maximum percent brooding 20-30 days earlier than Dabob Bay and Fidalgo Bay populations
423 (145-159 degree days). Silliman et al. (2018) and Spencer et al. (2020) also found the Oyster Bay
424 population to be the most reproductively active, and in Spencer et al. (2020) Oyster Bay oysters
425 began releasing larvae on average 9.9 days earlier than Fidalgo and Dabob Bays (99 degree days
426 earlier). Here, we find that Oyster Bay was the only population for which pCO_2 exposure
427 impacted reproductive processes, resulting in fewer females. A populations' reproductive
428 capacity is limited by the number of oysters spawning as females, therefore the productivity of
429 oysters from Oyster Bay may be uniquely impacted by acidification.

If we look beyond our focal populations, individuals that grow slowly and are not highly fecund may be more capable of withstanding high pCO_2 environments. Waldbusser *et al.* compared the response of *O. lurida* from Oregon with the faster growing Pacific oyster (*Crassostrea gigas*) to acidification, and suggested that slow shell secretion (a measure of growth rate) in *O. lurida* is a beneficial trait, contributing to their resilience to acidified conditions (Waldbusser et al., 2016). The growth rate of acidification-tolerant abalone is considerably lower than those sensitive to acidification, resulting in juveniles that are up to 80% smaller at 3-months old (Swezey et al., 2020). Whether growth rate is a proxy for resilience to acidification may not be applicable to all calcifiers, as faster growth is linked to ocean acidification resilience in selectively bred Sydney rock oysters (*Saccostrea glomerata*) (Parker et al., 2011; Stapp et al., 2018; Thompson et al., 2015). While no previous studies have associated reproductive investment with tolerance to acidification, a selectively bred line of Pacific oysters that are less sensitive to summer mortality and hypoxia also allocate less energy to reproductive tissue (Samain et al. 2007). Ultimately, the substantial and consistent resilience of the slow growing, moderately fecund Dabob Bay population in this study indicates that it is important to maintain a diversity of phenotypes, including those of lower fecundity and of smaller size. Oyster hatcheries routinely cull slow-growing larvae to maximize survival through metamorphosis, a practice that could be consistently removing stress-resilient genotypes. Highly fecund females and populations can also become overrepresented in cohorts of oyster seed. For long-term resilience to acidification and other stressors, commercial and restoration hatcheries should consider retaining slow-growing larvae and breed adults from a variety of sizes and productivity levels.

Population-specific upregulation of some, but not all, detoxification genes in response to acidification

The transcriptional response of *O. lurida* acclimated to ocean acidification varied considerably by population, ranging from a robust response in Dabob Bay to no significant measurable response in Oyster Bay. The pronounced response of Dabob Bay to acidification could reflect that populations' higher tolerance to stressors, and its ability to maintain altered homeostasis during the prolonged (52-day) exposure to acidification. For instance, Dabob Bay adults exposed to acidification contained a pronounced increase in transcripts from genes involved in detoxification, including several Cytochrome P450 (CYP), cytosolic sulfotransferases (SULT), and Glutathione transferases (GST). CYPs and SULTs are detoxification enzymes which metabolize both endogenous products (e.g. fatty acids, hormones) and xenobiotics (Coughtrie 2016; Snyder, 2000). Glutathiones are important cellular antioxidants which scavenge reactive metabolites, including hydrogen peroxide, and have been found at higher levels in many bivalves in response to acidification (Matozzo et al., 2013; Timmins-Schiffman et al., 2013), which may be related to their ability to protect proteins from oxidative stress (Abele et al., 2011; Sandamalika et al., 2019; Tomanek, 2015). Enhanced expression of GST and other intracellular stress mechanisms have similarly been observed in acidification-tolerant Sydney rock oysters upon intergenerational exposure to acidification (Goncalves et al., 2017), and direct exposure to thermal stress (McAfee et al., 2018). Two genes that codes for S-crystallins were also more prevalent in Dabob Bay oysters acclimated to high pCO_2. S-crystallins are known to be structural components of cephalopod eyes, however they are apparently derived from GSTs (Tomarev & Piatigorsky, 1996) as they have very similar amino acid sequences, and while they are generally thought to lack enzymatic activity they are induced by heat shock (Lang et al., 2009). This could

475 indicate that the Dabob Bay population activates additional detoxicant pathways for a more
476 robust response to environmental stressors.

477 Interestingly, none of the typical enzymatic antioxidants (catalase, superoxide dismutase,
478 peroxiredoxin) were differentially expressed in response to acidification in any population,
479 despite detection and sufficient coverage. Opposing expression patterns have previously been
480 reported for GST and enzymatic antioxidants in a transgenerationally acclimated acidification-
481 tolerant line of Sydney rock oysters (Goncalves et al., 2016), which the authors suggested
482 reflects distinct detoxification systems and/or stimuli. The glutathione scavenging system is also
483 uniquely upregulated in a heat-tolerant species of vent polychaete compared to a heat-sensitive
484 congener (Dilly et al., 2012). GST and related proteins may therefore act as the main cellular
485 antioxidants in populations of oysters and other invertebrates tolerant to acidified conditions and
486 other abiotic stressors.

487 ***Population-specific changes in metabolism and energy production***

488 Acidification altered expression of genes involved in lipid metabolism and transport in a
489 population-specific pattern similar to the detoxification genes such that they ranged from highly
490 upregulated in Dabob Bay to nearly unresponsive in Oyster Bay. These included genes that code
491 for proteins in the peroxisome, which is an organelle that produces phospholipids, a major
492 component of cell membranes, and metabolize long-chain fatty acids for energy production. The
493 peroxisome proliferator-activated receptor gene, for instance, was much more active in the
494 Dabob Bay population, which indicates the need for synthesis of peroxisome organelles. The co-
495 activation of lipid metabolic and oxidation-reduction processes, most notably in Dabob Bay,
496 could reflect enhanced mobilization of energy stores to support increased detoxicant synthesis
497 (Goncalves et al., 2016; Hochachka & Somero, 2002; Mayor et al., 2015; Sokolova et al., 2012).

There are previous reports of acidification-induced increases in intracellular energy production, particularly in organisms tolerant to acidification and following long-term exposure (reviewed by Strader et al., 2020), and coinciding with activation of antioxidant defenses (Goncalves et al., 2016).

Several other genes involved in mitochondrial energy production were altered in acidification-acclimated Dabob Bay oysters, suggesting increased energy production. This included the Acadl gene which codes for the Long-chain specific acyl-CoA dehydrogenase, a mitochondrial protein involved in production of energy from fats (specifically, fatty-acid beta-oxidation), and the si:dkey-18l1.1 gene (von Willebrand factor) which is involved in mitochondrial ATPase activity, and the alxA gene, which codes for alternative oxidase and which may increase mitochondrial respiration when the cytochrome respiratory pathway is restricted. There were also population-specific changes to a clustered mitochondria protein homolog (AAEL000794) which is involved in the distribution of mitochondria in the cytoplasm. Basal levels were fewer in Dabob Bay than other populations (not significantly), but were then substantially higher in Dabob Bay in high pCO_2. The same expression pattern was observed in atad3-a (ATPase family AAA domain-containing protein 3-A), a protein that is essential for mitochondrial organization. Taken together, the basal and induced expression patterns in Dabob Bay could reflect a unique shift in mitochondrial efficiency and density in response to high pCO_2 to produce sufficient energy to maintain cellular homeostasis in prolonged acidification exposure.

Constitutive expression differences in the Dabob Bay population

We explored constitutive expression in Dabob Bay in an effort to understand why that population is unique in its transcriptional response to acidification, and how that might relate to its track

record of high survival and stress tolerance (Heare et al., 2017; Silliman et al., 2018; Spencer et al., 2020). Many of the annotated genes that were uniquely expressed in control Dabob Bay adults were involved in immune functions (e.g. the complement system) and were enriched for antimicrobial and antiviral processes (e.g. cellular response to interferon-gamma), indicating that the Dabob Bay population is uniquely equipped to combat pathogens. Metabolic, growth, and reproduction processes were also overrepresented in Dabob Bay adult expression, and many of the same gene families were also uniquely expressed in Dabob Bay larvae. These annotated gene sets unique to Dabob Bay adults and larvae (220 and 25, respectively, Supplemental Materials) provide insight into the mechanisms behind the population's unique energy distribution across life stages, and should be validated in other stress-tolerant, high-performing marine invertebrate populations.

Given its slow growth rate and relatively low/moderate fecundity, the Dabob Bay population was theorized to allocate more resources towards stress-response processes than other populations by constitutively expressing higher levels of genes that respond to acidified conditions. In this way, Dabob Bay would be uniquely "primed" for acidification by maintaining more transcripts of beneficial genes under typical conditions, which then become even more prevalent in acidification-acclimated oysters. Contrary to this prediction, those genes that were at higher levels in Dabob Bay constitutively become less abundant in response to acidification, and conversely those that were less abundant constitutively increased in acidification. The annotated genes that were depressed constitutively but were activated in response to acidification were largely involved in lipid transport and energy production, in addition to protein stabilization and cell migration. The annotated genes that were more active constitutively but then decreased in response to acidification were involved in immune function, cell cycle, and reproduction. Our

544 findings suggest that populations of marine invertebrates tolerant to long-term acidification
545 exposure are capable not necessarily because they are more prepared at the transcript level, but
546 rather because they can mount then sustain a shift in resources to maintain lipid metabolic
547 function, while down-regulating functions that are not critical to the acidification response, such
548 as reproduction, immune function, and cell cycle.

549 ***Population-specific carryover effect of parental exposure to acidification***

550 Parental exposure to acidification resulted in larger larval offspring in the Oyster Bay population
551 only. Oyster Bay larvae from parents exposed to acidification were on average 5µm (3%) larger
552 than those from control parents. Larval size is positively associated with lipid content, growth
553 rate, and feeding ability in many bivalves, and can reduce predation risk (Bailey, 1984; Gonzalez
554 Araya et al., 2012; Helm et al., 1973; Marshall & Keough, 2007; Wilson et al., 1996). Increased
555 larval size following intergenerational exposure to acidification and warming could therefore
556 benefit some *O. lurida* populations in the wild, particularly those that also encounter
557 environmental stressors (Gibbs et al., 2021). Our results align with previous studies showing that
558 parental exposure to acidification can influence the physiology of invertebrate larval offspring.
559 This was first detected in the Sydney rock oyster (Parker et al., 2012), as parental exposure to
560 acidification resulted in higher larval survival and growth rates. Subsequently, some studies also
561 reported positive intergenerational and transgenerational carryover effects (reviewed in Zhao et
562 al., 2020), while others found negative effects (Parker et al., 2017; Venkataraman et al., 2019), or
563 no signal of parental exposure (Clements et al., 2021). In the present study, carryover effects
564 were only detected in one of three populations, indicating that parental priming may only be
565 triggered by acidification in some genotypes or epigenotypes. Furthermore, populations unable

566 to acclimatize directly to acidification (i.e. Oyster Bay in this study) may instead invest in
567 parental priming.

568 The variety of intergenerational responses observed here and in previous studies could
569 also stem from the mechanisms by which offspring are affected, which are theorized as changes
570 to maternal provisioning, gamete mRNAs, and epigenetic changes (Eirin-Lopez & Putnam,
571 2019). If so, one would expect associated signals in larval transcriptomes, reflecting either
572 energetic differences, remnant maternal mRNA transcripts, or regulatory shifts due to epigenetic
573 changes (Gavery & Roberts, 2010, 2013). Curiously, despite size differences associated with
574 parental treatments in the Oyster Bay population, there were no differences in gene expression.
575 Differing sizes may therefore not be due to variable growth rate, as that would likely have been
576 reflected in expression profiles (Meyer & Manahan, 2010; Pace et al., 2006). It is possible that
577 cryptic intergenerational transcriptome plasticity could be induced by high pCO_2 or other
578 environmental stressors, but that was not revealed under ambient conditions in which larvae were
579 reared. It remains unclear why intergenerational acidification exposure increases larval size in
580 some populations and species.

581 ***Puget Sound O. lurida populations have unique physiotypes which may be adaptive***

582 To date, this and five other studies have characterized Puget Sound *O. lurida* with Fidalgo Bay,
583 Dabob Bay, and Oyster Bay heritage (Heare et al., 2017; Heare et al., 2018; Silliman et al., 2018;
584 Spencer et al., 2020; White et al. 2017). The three populations are close proximally (all are
585 within the greater Puget Sound estuary), yet they represent distinct physiotypes in how they
586 prioritize energy allocation constitutively, and when responding to an environmental stressor.
587 Remarkably, in contrast to the other populations, high pCO_2 elicited no transcriptional response
588 in Oyster Bay, which raises the question as to where energy typically allocated towards

589 reproduction and growth was utilized in Oyster Bay oysters. A previous study on the same
590 populations provides insight into Oyster Bay population's response. Heare et al. (2018) measured
591 expression of targeted genes involved in the immediate (1-hr) stress-response following acute
592 heat and mechanical shock, and reported that Oyster Bay was the only population for which a
593 transcriptional response was detected. The discrepancy between Heare et al. (2018) and the
594 present study could reflect unique responses to short-term vs. long-term abiotic stress.
595 Specifically, those populations that are not capable of homeostatic stress-response over long
596 periods could display the most pronounced short-term, acute response (i.e. Oyster Bay). We
597 therefore suggest that the Oyster Bay population's aerobic scope shifted nearer to or into the
598 pessimus range compared to the other populations (Sokolova et al. 2012), and was not capable of
599 maintaining its regulatory response for the full 52-day exposure to acidification. Time-series
600 expression analysis would improve our understanding of populations' varying abilities to
601 respond to and maintain cellular functions over the course of prolonged exposure to acidification.
602 As suggested by Heare et al. (2017) and Silliman et al. (2018), the bay of origins' distinct
603 environments may explain varying physiotypes observed in Puget Sound *O. lurida*. Dabob Bay
604 is located within the Hood Canal, which is a notoriously challenging environment for marine
605 organisms. As a highly stratified, silled fjord with long residence times, it experiences slow turn-
606 over, periods of hypoxia, and elevated temperature (Babson, Kawase, & MacCready, 2006;
607 Banas et al., 2015; Khangaonkar et al., 2018; Newton et al., 2007). Thus, the slow-growing,
608 transcriptionally responsive physiotype observed in Dabob Bay oysters may have arisen due to
609 selection for genotypes that allocate a high proportion of energy to cellular maintenance and
610 chronic stress response. Fidalgo Bay is located in the Puget Sound's North Basin, and is more
611 heavily influenced by tidal exchange and coastal oceanographic conditions. Fidalgo Bay

612 characteristics (faster growth, larger mature size, low/moderate reproduction and transcriptional
613 response) could reflect an ancestral population that has not experienced extreme selection events,
614 and therefore represents a less specialized physiotype. Oyster Bay is located in Southern Puget
615 Sound, which is a system of shallower finger-like basins that are highly productive, mixed, and
616 experience large seasonal temperature swings (Moore et al., 2008). South Puget Sound is well
617 suited for both wild and farmed shellfish, and may have preferentially selected for individuals
618 that are highly fecund but lack the ability to acclimatize to acidification. Given that population-
619 of-origin was also a dominant factor influencing gene expression in larvae, the genetic or
620 epigenetic contributions to diverse physiologies should not be underestimated for populations of
621 *O. lurida* and related species, even in small geographic scales.
622

623 ## Conclusion

624 This is the first study to assess the transcriptional response of an oyster from the genus *Ostrea* to
625 ocean acidification. In doing so, we greatly expand our understanding of how different oyster
626 species will respond to shifting ocean conditions. There is increasing evidence that *Ostrea* spp.
627 may be more tolerant than other oysters to acidification (Cole et al., 2016; Gray et al., 2019;
628 Spencer et al., 2020; Waldbusser et al., 2016). Exploring cellular strategies in stress-tested
629 larvae, a highly vulnerable stage, is an important avenue for future research. Furthermore, a
630 physiological response spectrum was observed in three *O. lurida* populations exposed to ocean
631 acidification. Given previous observations of stress tolerance in oysters from Dabob Bay we
632 suggest that the robust transcriptomic changes in acclimated oysters and slow growth rate
633 represent the more acidification-tolerant physiotype. However, observations of positive

634 carryover in Oyster Bay indicates that intergenerational plasticity could improve the outlook for

635 future generations of less tolerant physiotypes.

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Supplemental Materials

Supplemental Table 1: Carbonate chemistry parameters for three time points during the pCO_2 treatments, which are averages (± SE) from three replicate tanks per treatment. All parameters except for total alkalinity differed significantly between control/ambient (Amb.) and experimental/high (High.) tanks (One-way ANOVA). More details are available in *Venkataraman et al., 2019.*

Day	pH*** F(1,16) = 5838, p = 6.12e-22		Total Alkalinity (µmol/kg) F(1,16) = 1.38, p = 0.257		pCO_2 (µatm)*** F(1,16) = 235, p = 5.44e-11		DIC (µmol/kg)* F(1,16) = 7.12, p = 0.0168		$\Omega_{calcite}$*** F(1,16) = 529, p = 1.10e-13		$\Omega_{aragonite}$*** F(1,16) =527, p = 1.14e-13	
	Amb.	**High**	**Amb.**	**High**	**Amb.**	**High**	**Amb.**	**High.**	**Amb.**	**High**	**Amb.**	**High**
5	7.82 ± 0.004	7.33 ± 0.002	2307.41 ± 25.45	2332.36 ± 31.05	747.51 ± 13.94	2481.23 ± 29.83	2233.41 ± 25.29	2408.51 ± 31.76	1.86 ± 0.02	0.62 ± 0.01	1.16 ± 0.012	0.58 ± 0.007
33	7.81 ± 0.005	7.31 ± 0.004	2747.00 ± 21.13	2917.60 ± 18.36	912.22 ± 12.69	3309.52 ± 7.22	2664.57 ± 19.99	3020.99 ± 17.99	2.23 ± 0.03	0.77 ± 0.02	1.40 ± 0.020	0.48 ± 0.014
48	7.82 ± 0.015	7.29 ± 0.004	2611.40 ± 31.01	2808.39 ± 12.24	863.47 ± 42.42	3343.89 ± 49.49	2533.28 ± 35.45	2920.52 ± 15.11	2.13 ± 0.06	0.68 ± 0.01	1.32 ± 0.035	0.42 ± 0.004

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936 *Ostrea lurida* gene sets that were differentially expressed in response to ocean acidification in
937 some populations (Supplemental Table 2), and were uniquely expressed in the a stress-tolerant
938 population (Dabob Bay) at the adult stage (Supplemental Table 3) and veliger larval stage
939 (Supplemental Table 4).

940

941 **Supplemental Table 2**. Genes that were differentially expressed in response to ocean acidification in *Ostrea*
942 *lurida* populations, Dabob Bay and/or Fidalgo Bay. Positive Log2-FC values indicate abundances were higher
943 in oysters exposed to acidification.

O. lurida Gene ID	Log2 Fold Change	p-adj	Uniprot SPID	Similar Annotated Gene	Population with DEG
OLUR_00030878	0.87	2.41E-02	Q17N71	AAEL000794: Clustered mitochondria protein homolog	Dabob Bay
OLUR_00016317	0.78	6.54E-03	Q99758	ABCA3: ATP-binding cassette sub-family A member 3	Dabob Bay
OLUR_00013619	1.93	6.82E-03	P08183	ABCB1: Multidrug resistance protein 1	Dabob Bay
OLUR_00016960	1.59	4.24E-02	P06795	Abcb1b: Multidrug resistance protein 1B	Dabob Bay
OLUR_00011049	2.7	1.48E-03	P21440	Abcb4: Phosphatidylcholine translocator ABCB4	Dabob Bay
OLUR_00000769	1.05	2.10E-02	Q93YS4	ABCG22: ABC transporter G family member 22	Fidalgo Bay
OLUR_00022318	0.73	5.56E-03	P51174	Acadl: Long-chain specific acyl-CoA dehydrogenase%2C mitochondrial	Dabob Bay
OLUR_00024728	2.03	2.89E-02	Q9R0H0	Acox1: Peroxisomal acyl-coenzyme A oxidase 1	Dabob Bay
OLUR_00012845	2.27	4.20E-02	Q5R4M8	ALDH18A1: Delta-1-pyrroline-5-carboxylate synthase	Fidalgo Bay
OLUR_00011263	1.85	9.64E-04	Q9P959	alxA: Alternative oxidase, C mitochondrial	Dabob Bay
OLUR_00000533	-0.9	3.40E-03	Q8N264	ARHGAP24: Rho GTPase-activating protein 24	Fidalgo Bay
OLUR_00022323	1.44	4.15E-02	Q58E76	atad3-a: ATPase family AAA domain-containing protein 3-A	Dabob Bay
OLUR_00004651	0.47	4.31E-02	Q99933	BAG1: BAG family molecular chaperone regulator 1	Dabob Bay
OLUR_00001152	-1.3	2.80E-02	A8Y1P7	bre-4: Beta-1%2C4-N-acetylgalactosaminyltransferase bre-4	Fidalgo Bay
OLUR_00029448	-0.8	3.31E-02	Q24157	brn: Beta-1%2C3-galactosyltransferase brn	Dabob Bay
OLUR_00021130	-2.94	8.88E-03	Q8CFR0	C1ql2: Complement C1q-like protein 2	Dabob Bay
OLUR_00026389	2.94	2.30E-02	Q4ZJM9	C1ql4: Complement C1q-like protein 4	Fidalgo Bay
OLUR_00013332	0.88	2.80E-02	Q62717	Cadps: Calcium-dependent secretion activator 1	Fidalgo Bay
OLUR_00015091	-0.54	4.80E-02	P97864	Casp7: Caspase-7	Fidalgo Bay
OLUR_00017184	-2.36	5.56E-03	P49817	Cav1: Caveolin-1	Dabob Bay
OLUR_00001710	-2.25	5.56E-03	P32320	CDA: Cytidine deaminase	Dabob Bay
OLUR_00008459	-0.68	4.72E-02	B7Z0W9	CG42265: Proton channel OtopLc	Dabob Bay

OLUR_00005263	-1.35	2.97E-02	Q8R4G9	Chrna3: Neuronal acetylcholine receptor subunit alpha-3	Dabob Bay
OLUR_00002411	-2.08	2.21E-05	A2AX52	Col6a4: Collagen alpha-4	Dabob Bay
OLUR_00019549	0.61	4.82E-02	P17886	crn: Protein crooked neck	Dabob Bay
OLUR_00014282	0.73	3.62E-02	Q01406	CTTN1: Src substrate protein p85	Dabob Bay
OLUR_00025713	1.57	3.61E-02	Q64583	Cyp2b15: Cytochrome P450 2B15	Dabob Bay
OLUR_00012237	1.74	2.10E-02	P00178	CYP2B4: Cytochrome P450 2B4	Dabob Bay, Fidalgo Bay
OLUR_00016688	3.34	9.20E-03	P24470	Cyp2c23: Cytochrome P450 2C23	Fidalgo Bay
OLUR_00008469	1.9	3.91E-03	P10632	CYP2C8: Cytochrome P450 2C8	Dabob Bay
OLUR_00024274	2.08	4.97E-02	P11714	Cyp2d9: Cytochrome P450 2D9	Dabob Bay
OLUR_00026774	1.69	6.54E-03	P24461	CYP2G1: Cytochrome P450 2G1	Dabob Bay
OLUR_00020244	1.41	4.97E-02	P52786	CYP2J1: Cytochrome P450 2J1	Dabob Bay
OLUR_00018748	2.88	1.24E-03	Q6VVW9	Cyp2r1: Vitamin D 25-hydroxylase	Dabob Bay
OLUR_00027367	2.8	1.50E-02	Q0IIF9	CYP2U1: Cytochrome P450 2U1	Dabob Bay, Fidalgo Bay
OLUR_00003331	1.49	3.62E-02	Q7Z449	CYP2U1: Cytochrome P450 2U1	Dabob Bay
OLUR_00008711	1.03	4.80E-02	Q6NT55	CYP4F22: Cytochrome P450 4F22	Fidalgo Bay
OLUR_00014014	-0.49	4.80E-02	Q80T85	Dcaf5: DDB1- and CUL4-associated factor 5	Fidalgo Bay
OLUR_00002100	2.44	1.65E-03	P49894	DIO1: Type I iodothyronine deiodinase	Dabob Bay
OLUR_00002101	2.02	2.80E-02	O42412	DIO3: Thyroxine 5-deiodinase	Dabob Bay, Fidalgo Bay
OLUR_00021120	-2.33	1.43E-02	Q9UGM3	DMBT1: Deleted in malignant brain tumors 1 protein	Dabob Bay
OLUR_00005690	-0.71	4.80E-02	A3R064	DOK3: Docking protein 3	Fidalgo Bay
OLUR_00012281	0.96	4.31E-02	P47823	EIF2B5: Translation initiation factor eIF-2B subunit epsilon	Dabob Bay
OLUR_00009872	-0.86	1.10E-02	P10160	EIF5A: Eukaryotic translation initiation factor 5A-1	Fidalgo Bay
OLUR_00012745	2.52	3.20E-02	P48035	FABP4: Fatty acid-binding protein%2C adipocyte	Dabob Bay, Fidalgo Bay
OLUR_00031779	-2.38	3.30E-02	Q8R508	Fat3: Protocadherin Fat 3	Fidalgo Bay
OLUR_00028383	-1.62	1.10E-02	Q3UMF9	Faxc: Failed axon connections homolog	Fidalgo Bay
OLUR_00025242	-1.16	3.40E-02	O42127	fgfr3: Fibroblast growth factor receptor 3	Fidalgo Bay
OLUR_00021504	-1.23	2.10E-02	O61491	Flo1: Flotillin-1	Fidalgo Bay
OLUR_00003007	-2.12	3.70E-02	Q6AYS4	Fuca2: Plasma alpha-L-fucosidase	Fidalgo Bay
OLUR_00031928	-3.91	2.64E-02	Q9NUV9	GIMAP4: GTPase IMAP family member 4	Dabob Bay

OLUR_00014547	1.86	3.61E-02	P34277	gsto-2: Probable glutathione transferase omega-2	Dabob Bay
OLUR_00013606	1.9	2.43E-02	Q9Z339	Gsto1: Glutathione S-transferase omega-1	Dabob Bay
OLUR_00000433	-1.21	3.42E-02	Q9WVI4	Gucy1a2: Guanylate cyclase soluble subunit alpha-2	Dabob Bay
OLUR_00018610	4.14	6.60E-03	E9QAM5	Helz2: Helicase with zinc finger domain 2	Fidalgo Bay
OLUR_00013228	3.36	1.80E-02	Q9BYK8	HELZ2: Helicase with zinc finger domain 2	Fidalgo Bay
OLUR_00014040	2.7	2.25E-02	D3YXG0	Hmcn1: Hemicentin-1	Dabob Bay
OLUR_00018477	2.24	2.70E-02	A2AJ76	Hmcn2: Hemicentin-2	Fidalgo Bay
OLUR_00001007	-1.61	1.12E-02	Q9D4G2	Hsf2bp: Heat shock factor 2-binding protein	Dabob Bay
OLUR_00011628	-1.65	2.70E-03	O43301	HSPA12A: Heat shock 70 kDa protein 12A	Dabob Bay
OLUR_00015765	-1.65	4.97E-02	C8VK14	hxnY: 2-oxoglutarate-Fe	Dabob Bay
OLUR_00018945	-0.99	4.70E-02	E9PY46	Ift140: Intraflagellar transport protein 140 homolog	Fidalgo Bay
OLUR_00026123	1.3	4.72E-02	O95069	KCNK2: Potassium channel subfamily K member 2	Dabob Bay
OLUR_00010601	0.83	2.64E-02	Q9U518	L-asparaginase	Dabob Bay
OLUR_00002203	0.78	2.70E-02	Q61805	Lbp: Lipopolysaccharide-binding protein	Fidalgo Bay
OLUR_00002201	-2.04	4.80E-02	Q96WM9	lcc2: Laccase-2	Fidalgo Bay
OLUR_00007747	-2.02	1.32E-02	P09849	LCT: Lactase-phlorizin hydrolase	Dabob Bay
OLUR_00004194	1.6	4.70E-02	B3EWZ5	MAM and LDL-receptor class A domain-containing protein 1	Dabob Bay
OLUR_00019130	1.59	4.15E-02	B3EWZ6	MAM and LDL-receptor class A domain-containing protein 2	Dabob Bay
OLUR_00016827	2.65	6.22E-04	B3EWZ6	MAM and LDL-receptor class A domain-containing protein 2	Dabob Bay
OLUR_00005789	-1.12	3.31E-02	Q16820	MEP1B: Meprin A subunit beta	Dabob Bay
OLUR_00001931	-1.67	4.97E-02	P08472	Mesenchyme-specific cell surface glycoprotein	Dabob Bay
OLUR_00013617	-1.82	2.48E-03	P08472	Mesenchyme-specific cell surface glycoprotein	Dabob Bay
OLUR_00030728	-2.28	5.00E-02	Q24400	Mlp84B: Muscle LIM protein Mlp84B	Fidalgo Bay
OLUR_00011332	0.98	3.20E-02	Q95NT6	mth2: G-protein coupled receptor Mth2	Fidalgo Bay
OLUR_00009450	-0.63	5.00E-02	Q62725	Nfyc: Nuclear transcription factor Y subunit gamma	Fidalgo Bay
OLUR_00014514	-1.07	2.43E-02	O07552	nhaX: Stress response protein NhaX	Dabob Bay
OLUR_00012374	1.55	4.80E-02	Q04721	NOTCH2: Neurogenic locus notch homolog protein 2	Fidalgo Bay
OLUR_00016842	2.03	2.43E-02	Q8CJ26	Nradd: Death domain-containing membrane protein NRADD	Dabob Bay
OLUR_00000214	0.83	2.80E-02	Q6KEQ9	PCDH11X: Protocadherin-11 X-linked	Fidalgo Bay
OLUR_00005074	0.71	2.25E-02	Q9Y5K3	PCYT1B: Choline-phosphate cytidylyltransferase B	Dabob Bay
OLUR_00004258	-1.54	4.80E-02	P86854	Perlucin-like protein	Fidalgo Bay

OLUR_00000551	-2.14	6.22E-04	Q96UX3	pkaR: cAMP-dependent protein kinase regulatory subunit	Dabob Bay
OLUR_00007563	1.19	7.87E-03	P47713	Pla2g4a: Cytosolic phospholipase A2	Dabob Bay
OLUR_00010581	0.93	1.80E-02	Q08BB2	pm20d1.2: N-fatty-acyl-amino acid synthase/hydrolase PM20D1.2	Fidalgo Bay
OLUR_00030589	-1.71	7.66E-04	A6NIZ1	Ras-related protein Rap-1b-like protein	Dabob Bay
OLUR_00025558	1.4	4.22E-02	P18426	S-crystallin SL11	Dabob Bay
OLUR_00027159	1.44	4.07E-02	P18426	S-crystallin SL11	Dabob Bay
OLUR_00006587	-0.97	3.20E-02	Q54KA7	secG: Ankyrin repeat%2C PH and SEC7 domain containing protein secG	Fidalgo Bay
OLUR_00012491	-0.99	2.39E-02	Q92008	shha: Sonic hedgehog protein A	Dabob Bay
OLUR_00011339	0.98	3.54E-02	B0R0T1	si:dkey-18l1.1: von Willebrand factor A domain-containing protein 8	Dabob Bay
OLUR_00022480	-1.04	4.40E-02	Q7ZVK3	sirt2: NAD-dependent protein deacetylase sirtuin-2	Fidalgo Bay
OLUR_00016903	-1.07	5.56E-03	Q7TM99	Slc16a9: Monocarboxylate transporter 9	Dabob Bay
OLUR_00020381	1.89	5.56E-03	P43006	Slc1a2: Excitatory amino acid transporter 2	Dabob Bay
OLUR_00022102	1.21	4.30E-02	Q9Z2J0	Slc23a1: Solute carrier family 23 member 1	Fidalgo Bay
OLUR_00010175	-2.11	2.65E-02	Q9Z2J0	Slc23a1: Solute carrier family 23 member 1	Dabob Bay
OLUR_00028935	-1.83	4.15E-02	O35488	Slc27a2: Very long-chain acyl-CoA synthetase	Dabob Bay
OLUR_00004448	0.99	2.43E-02	A0JPN2	Slc39a4: Zinc transporter ZIP4	Dabob Bay
OLUR_00012891	0.58	3.62E-02	Q07837	SLC3A1: Neutral and basic amino acid transport protein rBAT	Dabob Bay
OLUR_00024710	-1.02	2.10E-02	Q24524	sn: Protein singed	Fidalgo Bay
OLUR_00027313	0.91	3.08E-02	Q91Z69	Srgap1: SLIT-ROBO Rho GTPase-activating protein 1	Dabob Bay
OLUR_00020487	0.57	2.85E-02	Q90ZB9	stard3: StAR-related lipid transfer protein 3	Dabob Bay
OLUR_00010835	1.36	2.80E-02	P08842	STS: Steryl-sulfatase	Fidalgo Bay
OLUR_00025850	-1.5	3.67E-02	Q9HAC7	SUGCT: Succinate--hydroxymethylglutarate CoA-transferase	Dabob Bay
OLUR_00002962	4.12	1.72E-03	O43704	SULT1B1: Sulfotransferase family cytosolic 1B member 1	Dabob Bay
OLUR_00005785	3	2.94E-04	Q95JD5	SULT1B1: Sulfotransferase family cytosolic 1B member 1	Dabob Bay
OLUR_00000570	2.22	2.28E-04	F1QYJ6	sult6b1: Sulfotransferase 6B1	Dabob Bay
OLUR_00026074	3.23	6.20E-05	Q02858	Tek: Angiopoietin-1 receptor	Fidalgo Bay
OLUR_00000288	1.55	4.64E-02	Q7Z0T3	Temptin	Dabob Bay
OLUR_00002071	-1.87	3.20E-02	P08953	Tl: Protein toll	Fidalgo Bay
OLUR_00001769	-1.93	4.19E-02	Q9JIQ8	Tmprss2: Transmembrane protease serine 2	Dabob Bay
OLUR_00020170	-1.13	2.80E-02	V5NAL9	Toll-like receptor 4	Fidalgo Bay
OLUR_00013623	0.37	3.20E-02	P62997	Tra2b: Transformer-2 protein homolog beta	Fidalgo Bay
OLUR_00000822	-1.2	2.66E-02	E1BD59	TRIM56: E3 ubiquitin-protein ligase TRIM56	Dabob Bay
OLUR_00028050	2.05	4.97E-02	O74549	ubc12: NEDD8-conjugating enzyme ubc12	Dabob Bay

OLUR_00000751	0.74	2.10E-02	P62840	ube2d2: Ubiquitin-conjugating enzyme E2 D2	Fidalgo Bay
OLUR_00007224	-1.43	1.47E-02	P74897	Universal stress protein in QAH/OAS sulfhydrylase 3'region	Dabob Bay
OLUR_00020423	1.32	2.64E-02	P41824	Y-box factor homolog	Dabob Bay

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946 **Supplemental Table 3:** Genes that were constitutively expressed in Dabob Bay adults at different levels compared
947 to other populations.

***O. lurida* gene ID**	**Gene**	**Uniprot SPID**
OLUR_00032068	AADAC: Arylacetamide deacetylase	Q0P5B7
OLUR_00017044	ABCA1: ATP-binding cassette sub-family A member 1	O95477
OLUR_00016317	ABCA3: ATP-binding cassette sub-family A member 3	Q99758
OLUR_00022745	ABCC3: Canalicular multispecific organic anion transporter 2	O15438
OLUR_00001814	Abcf3: ATP-binding cassette sub-family F member 3	Q8K268
OLUR_00022318	Acadl: Long-chain specific acyl-CoA dehydrogenase, C mitochondrial	P51174
OLUR_00024957	ADAMTS13: A disintegrin and metalloproteinase with thrombospondin motifs 13	Q76LX8
OLUR_00000647	adat1: tRNA-specific adenosine deaminase 1	Q28FE8
OLUR_00022969	Adgrb1: Adhesion G protein-coupled receptor B1	Q3UHD1
OLUR_00031677	AHCY: Adenosylhomocysteinase	Q3MHL4
OLUR_00031199	AHCY: Adenosylhomocysteinase	Q3MHL4
OLUR_00011263	alxA: Alternative oxidase, C mitochondrial	Q9P959
OLUR_00002441	amy: Alpha-amylase	P29957
OLUR_00002241	Anapc4: Anaphase-promoting complex subunit 4	Q91W96
OLUR_00029061	ANGPT2: Angiopoietin-2	A0A8J8
OLUR_00018984	ANK1: Ankyrin-1	P16157
OLUR_00011963	Ank1: Ankyrin-1	Q02357
OLUR_00002563	ANKRD50: Ankyrin repeat domain-containing protein 50	Q9ULJ7
OLUR_00002438	Aoah: Acyloxyacyl hydrolase	O35298
OLUR_00006201	Aopep: Aminopeptidase O	P69527
OLUR_00019355	ARI1: Probable E3 ubiquitin-protein ligase ARI1	Q949V6
OLUR_00022769	ARSJ: Arylsulfatase J	Q5FYB0
OLUR_00002790	Aspartate aminotransferase	P23034
OLUR_00005128	Atad5: ATPase family AAA domain-containing protein 5	Q4QY64
OLUR_00010015	ATPsyngamma: ATP synthase subunit gamma, C mitochondrial	O01666
OLUR_00004651	BAG1: BAG family molecular chaperone regulator 1	Q99933
OLUR_00025267	BCO1: Beta, Cbeta-carotene 15, C15'-dioxygenase	Q9I993
OLUR_00007641	Beta-1, C3-glucan-binding protein	Q8N0N3
OLUR_00021318	Beta-1, C3-glucan-binding protein	Q8N0N3
OLUR_00014400	Bsx: Brain-specific homeobox protein homolog	Q810B3
OLUR_00021824	BTBD2: BTB/POZ domain-containing protein 2	Q9BX70

OLUR_00008482	C1qc: Complement C1q subcomponent subunit C	Q02105
OLUR_00023445	C1ql2: Complement C1q-like protein 2	Q8CFR0
OLUR_00021130	C1ql2: Complement C1q-like protein 2	Q8CFR0
OLUR_00023682	C1ql4: Complement C1q-like protein 4	Q4ZJM9
OLUR_00016044	C1ql4: Complement C1q-like protein 4	Q4ZJM9
OLUR_00002843	C1qtnf4: Complement C1q tumor necrosis factor-related protein 4	Q8R066
OLUR_00029319	C38H2.2: Glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1	Q18515
OLUR_00017184	Cav1: Caveolin-1	P49817
OLUR_00006811	ccdc169: Coiled-coil domain-containing protein 169	Q3KPT0
OLUR_00015839	CD151: CD151 antigen	P48509
OLUR_00022562	Cd209e: CD209 antigen-like protein E	Q91ZW7
OLUR_00001710	CDA: Cytidine deaminase	P32320
OLUR_00030502	CEMIP: Cell migration-inducing and hyaluronan-binding protein	Q8WUJ3
OLUR_00002764	CHDH: Choline dehydrogenase, C mitochondrial	Q8NE62
OLUR_00023309	Chrna6: Neuronal acetylcholine receptor subunit alpha-6	P43143
OLUR_00005622	CHRNA7: Neuronal acetylcholine receptor subunit alpha-7	P54131
OLUR_00009646	CHRNA9: Neuronal acetylcholine receptor subunit alpha-9	Q9PTS8
OLUR_00030281	chs-2: Chitin synthase chs-2	G5EBQ8
OLUR_00005393	CLEC17A: C-type lectin domain family 17, C member A	Q6ZS10
OLUR_00002411	Col6a4: Collagen alpha-4	A2AX52
OLUR_00027711	COL8A2: Collagen alpha-2	P25067
OLUR_00027741	COLEC12: Collectin-12	Q2LK54
OLUR_00004033	Collagen alpha-2	P27393
OLUR_00022598	Cradd: Death domain-containing protein CRADD	O88843
OLUR_00005907	CYP17A1: Steroid 17-alpha-hydroxylase/17, C20 lyase	Q92113
OLUR_00007286	CYP17A1: Steroid 17-alpha-hydroxylase/17, C20 lyase	Q29497
OLUR_00010095	CYP17A1: Steroid 17-alpha-hydroxylase/17, C20 lyase	Q9GMC7
OLUR_00010564	Ddx39b: Spliceosome RNA helicase Ddx39b	Q63413
OLUR_00009085	DDX58: Probable ATP-dependent RNA helicase DDX58	O95786
OLUR_00021120	DMBT1: Deleted in malignant brain tumors 1 protein	Q9UGM3
OLUR_00012599	drpr: Protein draper	Q9W0A0
OLUR_00004620	drpr: Protein draper	Q9W0A0
OLUR_00009894	DUR3: Urea-proton symporter DUR3	F4KD71
OLUR_00008003	DYNC1LI1: Cytoplasmic dynein 1 light intermediate chain 1	Q90828
OLUR_00008906	DZIP3: E3 ubiquitin-protein ligase DZIP3	Q86Y13
OLUR_00007341	EGFL8: Epidermal growth factor-like protein 8	A5A8Y8
OLUR_00006099	Eprs: Bifunctional glutamate/proline--tRNA ligase	Q8CGC7
OLUR_00021382	Ercc8: DNA excision repair protein ERCC-8	Q8CFD5
OLUR_00004310	exog: Nuclease EXOG, C mitochondrial	Q502K1
OLUR_00014579	F54H12.2: Uncharacterized protein F54H12.2	P34456
OLUR_00017820	FAM111B: Protein FAM111B	Q6SJ93
OLUR_00006320	FAT1: Protocadherin Fat 1	Q14517

OLUR_00029612	FBN1: Fibrillin-1	P98133
OLUR_00028174	fbxl15: F-box/LRR-repeat protein 15	E6ZHJ8
OLUR_00002961	FBXL4: F-box/LRR-repeat protein 4	Q9UKA2
OLUR_00025242	fgfr3: Fibroblast growth factor receptor 3	O42127
OLUR_00001763	G-protein coupled receptor GRL101	P46023
OLUR_00017370	G-protein coupled receptor GRL101	P46023
OLUR_00000777	Gal3st1: Galactosylceramide sulfotransferase	Q9JHE4
OLUR_00006985	GALNT13: Polypeptide N-acetylgalactosaminyltransferase 13	Q8IUC8
OLUR_00009688	gbpC: Cyclic GMP-binding protein C	Q8MVR1
OLUR_00001930	Gigasin-3a	P86786
OLUR_00031928	GIMAP4: GTPase IMAP family member 4	Q9NUV9
OLUR_00031158	GIMAP7: GTPase IMAP family member 7	Q8NHV1
OLUR_00010854	Gld: Glucose dehydrogenase [FAD, C quinone]	P18172
OLUR_00022856	GLRA2: Glycine receptor subunit alpha-2	P23416
OLUR_00011563	gltX: Glutamate--tRNA ligase	A4XA62
OLUR_00030833	GPx: Glutathione peroxidase	G9JJU2
OLUR_00002127	Heavy metal-binding protein HIP	P83425
OLUR_00025178	Heavy metal-binding protein HIP	P83425
OLUR_00011069	Hebp2: Heme-binding protein 2	Q9WU63
OLUR_00011115	HELZ2: Helicase with zinc finger domain 2	Q9BYK8
OLUR_00013228	HELZ2: Helicase with zinc finger domain 2	Q9BYK8
OLUR_00019943	Helz2: Helicase with zinc finger domain 2	E9QAM5
OLUR_00018610	Helz2: Helicase with zinc finger domain 2	E9QAM5
OLUR_00002821	Hemagglutinin/amebocyte aggregation factor	Q01528
OLUR_00000188	Hmcn1: Hemicentin-1	D3YXG0
OLUR_00014040	Hmcn1: Hemicentin-1	D3YXG0
OLUR_00024505	HMCN2: Hemicentin-2	Q8NDA2
OLUR_00001007	Hsf2bp: Heat shock factor 2-binding protein	Q9D4G2
OLUR_00006095	HSPA12A: Heat shock 70 kDa protein 12A	O43301
OLUR_00030423	IARS: Isoleucine--tRNA ligase, C cytoplasmic	P41252
OLUR_00013653	IFI44: Interferon-induced protein 44	Q8TCB0
OLUR_00025548	IFI44L: Interferon-induced protein 44-like	Q53G44
OLUR_00009305	Ift88: Intraflagellar transport protein 88 homolog	Q61371
OLUR_00006016	inlI: Internalin I	Q8YA32
OLUR_00020497	KALRN: Kalirin	O60229
OLUR_00018338	Kif9: Kinesin-like protein KIF9	Q9WV04
OLUR_00003139	Ky: Kyphoscoliosis peptidase	Q8C8H8
OLUR_00015061	Lectin BRA-3	P07439
OLUR_00022461	Lectin BRA-3	P07439
OLUR_00022071	LGMN: Legumain	Q95M12
OLUR_00019596	LHFPL3: LHFPL tetraspan subfamily member 3 protein	Q86UP9
OLUR_00022033	LRP8: Low-density lipoprotein receptor-related protein 8	Q98931

OLUR_00003482	lrrc58: Leucine-rich repeat-containing protein 58	Q32NT4
OLUR_00015101	Lrrc69: Leucine-rich repeat-containing protein 69	Q9D9Q0
OLUR_00018351	LRRK2: Leucine-rich repeat serine/threonine-protein kinase 2	Q5S007
OLUR_00005454	MAM and LDL-receptor class A domain-containing protein 1	B3EWZ5
OLUR_00005238	Mbtps1: Membrane-bound transcription factor site-1 protease	Q9WTZ2
OLUR_00027937	MDGA2: MAM domain-containing glycosylphosphatidylinositol anchor protein 2	Q7Z553
OLUR_00009020	ME1: NADP-dependent malic enzyme	P40927
OLUR_00010931	MED34: Mediator of RNA polymerase II transcription subunit 34	Q9FT73
OLUR_00006121	MEGF10: Multiple epidermal growth factor-like domains protein 10	Q96KG7
OLUR_00019978	Megf11: Multiple epidermal growth factor-like domains protein 11	Q80T91
OLUR_00024832	Megf6: Multiple epidermal growth factor-like domains protein 6	O88281
OLUR_00021337	MEGF6: Multiple epidermal growth factor-like domains protein 6	O75095
OLUR_00001931	Mesenchyme-specific cell surface glycoprotein	P08472
OLUR_00019627	METTL27: Methyltransferase-like protein 27	Q8N6F8
OLUR_00005473	mfsd1: Major facilitator superfamily domain-containing protein 1	Q32LQ6
OLUR_00006189	mfsd4b: Sodium-dependent glucose transporter 1	A4QN56
OLUR_00030728	Mlp84B: Muscle LIM protein Mlp84B	Q24400
OLUR_00014585	Mme: Neprilysin	Q61391
OLUR_00019226	MPPED1: Metallophosphoesterase domain-containing protein 1	O15442
OLUR_00015401	mpv17: Protein Mpv17	Q5TZ51
OLUR_00016396	Mrc1: Macrophage mannose receptor 1	Q61830
OLUR_00005132	MRPL30: 39S ribosomal protein L30, C mitochondrial	Q4R6U7
OLUR_00000053	Msed_2001: 3-hydroxypropionyl-coenzyme A dehydratase	A4YI89
OLUR_00019682	MTR: Methionine synthase	Q4JIJ3
OLUR_00006430	MYORG: Myogenesis-regulating glycosidase	Q6NSJ0
OLUR_00020139	NOTCH2: Neurogenic locus notch homolog protein 2	Q04721
OLUR_00001182	Nrg: Neuroglian	P20241
OLUR_00007852	ntr-2: Nematocin receptor 2	O62169
OLUR_00023494	Nup98: Nuclear pore complex protein Nup98-Nup96	P49793
OLUR_00012950	OIH: Ovoinhibitor	P10184
OLUR_00009826	Orct: Organic cation transporter protein	Q9VCA2
OLUR_00014246	Osbpl8: Oxysterol-binding protein-related protein 8	B9EJ86
OLUR_00007085	pats1: Probable serine/threonine-protein kinase pats1	Q55E58
OLUR_00010618	pats1: Probable serine/threonine-protein kinase pats1	Q55E58
OLUR_00015409	PDIA4: Protein disulfide-isomerase A4	P13667
OLUR_00010297	PELP1: Proline-, C glutamic acid- and leucine-rich protein 1	Q1W1Y5
OLUR_00004258	Perlucin-like protein	P86854
OLUR_00024841	Perlucin-like protein	P86854
OLUR_00031580	pes1: Pescadillo homolog	Q7ZY69
OLUR_00020391	PHF24: PHD finger protein 24	Q9UPV7
OLUR_00006733	PHKA1: Phosphorylase b kinase regulatory subunit alpha, C skeletal muscle isoform	P18688
OLUR_00006923	PI4KA: Phosphatidylinositol 4-kinase alpha	O02811

OLUR_00007722	PIF1: ATP-dependent DNA helicase PIF1	Q9H611
OLUR_00000551	pkaR: cAMP-dependent protein kinase regulatory subunit	Q96UX3
OLUR_00011428	PLG: Plasminogen	P06868
OLUR_00000837	PLG: Plasminogen	Q5R8X6
OLUR_00010581	pm20d1.2: N-fatty-acyl-amino acid synthase/hydrolase PM20D1.2	Q08BB2
OLUR_00008613	Pm20d2: Peptidase M20 domain-containing protein 2	A3KG59
OLUR_00006738	PNLIPRP2: Pancreatic lipase-related protein 2	A5PK46
OLUR_00003156	pnr: GATA-binding factor A	P52168
OLUR_00026215	Polyenoic fatty acid isomerase	Q8W257
OLUR_00012063	Ppfibp1: Liprin-beta-1	Q8C8U0
OLUR_00003429	Prokineticin Bm8-f	Q8JFX8
OLUR_00006792	PSMD2: 26S proteasome non-ATPase regulatory subunit 2	P56701
OLUR_00003910	Ptchd3: Patched domain-containing protein 3	Q0EEE2
OLUR_00000063	Rab35: Ras-related protein Rab-35	Q5U316
OLUR_00030952	RF_0381: Putative ankyrin repeat protein RF_0381	Q4UMH6
OLUR_00021095	RF_0381: Putative ankyrin repeat protein RF_0381	Q4UMH6
OLUR_00016450	RF_0381: Putative ankyrin repeat protein RF_0381	Q4UMH6
OLUR_00005336	RHBDF1: Inactive rhomboid protein 1	A9L8T6
OLUR_00006945	Rilpl1: RILP-like protein 1	D3ZUQ0
OLUR_00006249	Rnf213: E3 ubiquitin-protein ligase RNF213	E9Q555
OLUR_00000762	rnf8: E3 ubiquitin-protein ligase rnf8	Q803C1
OLUR_00017233	roco5: Probable serine/threonine-protein kinase roco5	Q1ZXD6
OLUR_00017405	RpL10: 60S ribosomal protein L10	O61231
OLUR_00031369	RPL17: 60S ribosomal protein L17	P37380
OLUR_00015500	RPS10: 40S ribosomal protein S10	O77302
OLUR_00032073	RpS15Aa: 40S ribosomal protein S15a	Q6XIM8
OLUR_00011247	rsp-7: Probable splicing factor, C arginine/serine-rich 7	O01159
OLUR_00016425	ruvbl1: RuvB-like 1	Q9DE26
OLUR_00013331	SACS: Sacsin	Q9NZJ4
OLUR_00019326	Sacs: Sacsin	Q9JLC8
OLUR_00018676	Scarf1: Scavenger receptor class F member 1	Q5ND28
OLUR_00024690	Sephs2: Selenide, C water dikinase 2	P97364
OLUR_00015749	SKIV2L: Helicase SKI2W	Q15477
OLUR_00010108	slc16a12: Monocarboxylate transporter 12	Q6P2X9
OLUR_00004850	Slc17a9: Solute carrier family 17 member 9	Q8VCL5
OLUR_00007946	slc22a6-a: Solute carrier family 22 member 6-A	Q66J54
OLUR_00022102	Slc23a1: Solute carrier family 23 member 1	Q9Z2J0
OLUR_00031094	Slc4a7: Sodium bicarbonate cotransporter 3	Q9R1N3
OLUR_00020573	Slc7a11: Cystine/glutamate transporter	Q9WTR6
OLUR_00006436	SLC7A5: Large neutral amino acids transporter small subunit 1	Q01650
OLUR_00000291	SLC7A9: b	Q9N1R6
OLUR_00010465	SMOX: Spermine oxidase	Q9NWM0

OLUR_00024710	sn: Protein singed	Q24524
OLUR_00029494	SPBC16D10.01c: Probable assembly chaperone of rpl4	Q1MTN8
OLUR_00009680	SPOCK3: Testican-3	Q9BQ16
OLUR_00027313	Srgap1: SLIT-ROBO Rho GTPase-activating protein 1	Q91Z69
OLUR_00020487	stard3: StAR-related lipid transfer protein 3	Q90ZB9
OLUR_00016331	Stx7: Syntaxin-7	O70439
OLUR_00025850	SUGCT: Succinate--hydroxymethylglutarate CoA-transferase	Q9HAC7
OLUR_00007158	SUPV3L1: ATP-dependent RNA helicase SUPV3L1, C mitochondrial	Q5ZJT0
OLUR_00001624	Syt11: Synaptotagmin-11	Q9R0N3
OLUR_00011931	TBK1: Serine/threonine-protein kinase TBK1	Q9UHD2
OLUR_00006736	Thap1: THAP domain-containing protein 1	Q5U208
OLUR_00017777	THAP9: DNA transposase THAP9	Q9H5L6
OLUR_00006555	Thyrostimulin beta-5 subunit	A0A0F7YZI5
OLUR_00018271	tmem97: Sigma intracellular receptor 2	Q6DFQ5
OLUR_00026489	TNKS: Tankyrase-1	O95271
OLUR_00008939	TPST2: Protein-tyrosine sulfotransferase 2	Q5ZJI0
OLUR_00012411	TRAF3IP2: Adapter protein CIKS	O43734
OLUR_00004783	trim71: E3 ubiquitin-protein ligase TRIM71	E7FAM5
OLUR_00006456	TRPM3: Transient receptor potential cation channel subfamily M member 3	Q9HCF6
OLUR_00007164	TRPM6: Transient receptor potential cation channel subfamily M member 6	Q9BX84
OLUR_00002194	TSNAXIP1: Translin-associated factor X-interacting protein 1	Q2TAA8
OLUR_00020564	Tspan33: Tetraspanin-33	Q8R3S2
OLUR_00017521	TT10: Laccase-15	Q84J37
OLUR_00022795	Tubulin alpha-1 chain	P02552
OLUR_00017099	ubiG: Ubiquinone biosynthesis O-methyltransferase	Q820B5
OLUR_00012780	UCP2: Mitochondrial uncoupling protein 2	O97562
OLUR_00027608	unc93a: Protein unc-93 homolog A	Q6DDL7
OLUR_00005827	VASH2: Tubulinyl-Tyr carboxypeptidase 2	Q86V25
OLUR_00019157	vps29: Vacuolar protein sorting-associated protein 29	Q7ZV68
OLUR_00027063	VWDE: von Willebrand factor D and EGF domain-containing protein	Q8N2E2
OLUR_00025976	Vwde: von Willebrand factor D and EGF domain-containing protein	Q6DFV8
OLUR_00028052	WRN: Werner syndrome ATP-dependent helicase	Q14191
OLUR_00014688	xpnpep1: Xaa-Pro aminopeptidase 1	Q54G06
OLUR_00002918	Xpnpep2: Xaa-Pro aminopeptidase 2	B1AVD1
OLUR_00026221	ZNF862: Zinc finger protein 862	O60290
OLUR_00021017	ZNF862: Zinc finger protein 862	O60290
OLUR_00015491	ZNF878: Zinc finger protein 878	C9JN71

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950 **Supplemental Table 4:** Genes that were constitutively expressed in Dabob Bay larvae at different levels compared
951 to other populations.

***O. lurida* gene ID**	**Gene**	**Uniprot SPID**
OLUR_00010887	FMO5: Dimethylaniline monooxygenase [N-oxide-forming] 5	P49326
OLUR_00013658	At5g10370: ATP-dependent RNA helicase DEAH1, 2C chloroplastic	F4KGU4
OLUR_00013228	HELZ2: Helicase with zinc finger domain 2	Q9BYK8
OLUR_00004343	FAT2: Protocadherin Fat 2	Q9NYQ8
OLUR_00016966	DDB_G0292642: Uncharacterized protein DDB_G0292642	Q54CX4
OLUR_00022057	MSMEG_3950: Universal stress protein MSMEG_3950/MSMEI_3859	A0QZA1
OLUR_00025230	ghrA: Glyoxylate/hydroxypyruvate reductase A	Q8FIT1
OLUR_00000611	L-proline trans-4-hydroxylase	R9UTQ8
OLUR_00029178	ucpB: Mitochondrial substrate carrier family protein ucpB	B0G143
OLUR_00004362	bmp2: Bone morphogenetic protein 2	Q804S2
OLUR_00019157	vps29: Vacuolar protein sorting-associated protein 29	Q7ZV68
OLUR_00031354	DDB_G0287015: TM2 domain-containing protein DDB_G0287015	Q54KZ0
OLUR_00003900	AAO: L-ascorbate oxidase	Q40588
OLUR_00004324	UNC93A: Protein unc-93 homolog A	A2VE54
OLUR_00003482	lrrc58: Leucine-rich repeat-containing protein 58	Q32NT4
OLUR_00013735	cep290: Centrosomal protein of 290 kDa	P85001
OLUR_00018610	Helz2: Helicase with zinc finger domain 2	E9QAM5
OLUR_00019426	K02F3.12: Putative ATP-dependent DNA helicase Q1	Q9TXJ8
OLUR_00022209	Ectin (Fragment)	B3EWZ8
OLUR_00020882	UBA6: Ubiquitin-like modifier-activating enzyme 6	A0AVT1
OLUR_00005664	ABCC1: Multidrug resistance-associated protein 1	Q5F364
OLUR_00001519	HET-E1: Vegetative incompatibility protein HET-E-1	Q00808
OLUR_00025496	HSPA12B: Heat shock 70 kDa protein 12B	Q96MM6
OLUR_00019143	NADSYN1: Glutamine-dependent NAD(+) synthetase	Q5ZMA6
OLUR_00010983	CLEC3A: C-type lectin domain family 3 member A	O75596

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