**The implications of 2016 El Niño related thermal stress coupled with ocean acidification on pteropods in the California Current System: linking cellular to population level effects through bioenergetics**

Nina Bednaršek1, Richard A.Feely 2, Marcus W. Beck1, Olivier Glippa3, Mirella Kanerva 4, Jonna Engström-Öst 3

Corresponding author; email: [ninab@sccwrp.org](mailto:ninabb@sccwrp.org)

1Southern California Coastal Water Research Project, 3535 Harbor Blvd #110, Costa Mesa, CA U.S.A.

2Pacific Marine EnvironmentalLaboratory, NOAA, Seattle, WA, USA

3Novia University of Applied Sciences, FI-10600 Ekenäs, Finland

4Ehime University, Center for Marine Environmental Studies, Laboratory of Environmental Toxicology, 790-8577, Bunkyo-cho 2-5, Matsuyama, Japan

**Running Title (100 words):**

Pteropods are pelagic calcifiers with high sensitivity to OA but unknown thermal stress response.

We documented negative effects of the 2016 El Niño on indicators of cellular, physiological and population level stress in the California Current System. When thermal, OA stress and low oxygen stressors are coupled, synergistic or additive effects on pteropod population are expected, underling the importance to understand the interactive effect among *in situ* stressors. Using oxidative stress biomarkers in pteropods appears to integrate the effects of multiple stressors. Our results prove that cellular to population level effects are likely linked through bioenergetics.

**Abstract (max 350 words) – this needs to be improved**

Understanding the interactive effects of multiple stressors on pelagic associated with global climate change is especially important in the highly productive upwelling regimes. Due to temporal overlap between the 2014-15 El Niño and springtime intensification of the upwelling, the organisms in the coastal waters in the California Current System were exposed to co-occurring thermal and ocean acidification (OA) stress, and deoxygenation. This provided a unique opportunity to investigate the effects of multiple stressors and their interactions *in situ* across cellular, physiological, and population level. This is the first study in which oxidative stress biomarkers were used to assess cellular stress levels and antioxidant defenses in pteropods. We show that OA stress induced significant oxidative stress, as indicated by the increased levels of lipid peroxidation (LPX). While thermal stress elicits the antioxidative activity of several biomarkers, OA stress is a poor antioxidative inducer, demonstrating that pteropods do not have sufficient strategies against cellular stress, carrying it over to the physiological level. The combination of OA and thermal stress indicated negative additive effect on LPX, with low temperature reducing LPX toxicity. Similar negative additive effect was demonstrated in physiological responses, such as shell dissolution and growth impairment, with low temperature, food abundance and sufficient oxygen concentration reducing the negative effect of OA. Thermal stress was the most significant negative driver of abundances, especially in combination with OA stress. Oxidative stress results in lipid peroxidation, loss of lipid reserves, and structural damage of cell membranes. Because pteropods contain high quantities of polyunsaturated fatty acids, these results corroborate pteropod extreme sensitivity to OA. Accumulation of oxidative damage requires metabolic compensation to cover for the energetic costs, implying energetic trade-offs under combined multiple stressors. Pteropods are strong candidate indicator for multiple climate change stressors, with oxidative stress biomarkers offering insights into bioenergetic requirements.

**Key words:** El Niño, coastal ecosystem, pelagic calcifiers, thermal stress, ocean acidification, deoxygenation, interaction effects, oxidative stress, antioxidant defense strategies, indicator.

**Introduction**

The consequence of global climate change include increasing of *ocean acidification* (OA)*,* *warming*, and *deoxygenation*. Independently or synergistically, once they exceed the natural envelope of physiological tolerance, suitable habitat for the organisms becomes limiting, ultimately controlling the distribution and interaction among species, and ecosystem productivity (Garcia-Reyes et al., 2013). These combined impacts are dependent on how the stressors reinforce (i.e. *synergistic*) or counter (i.e. *antagonistic*) one another. The multiple stressor interactions have a potential to trigger biological impairments that can carry over across different levels of biological organization. Interaction among multiple stressors, where the effect of one stressor is co-dependent on the other stressors, can lead to nonlinear outcomes, such that the combined effects can have outcomes more severe than expected. As demonstrated across wide range of ecosystems, nonlinear relationships between environmental variables can result in a loss of ecosystem services and reduction in biodiversity (Ryan et al., 2015; Boyd and Banzhaf 2007).

Understanding the interactive effects of global environmental stressors is especially important across the Eastern Boundary Upwelling Systems (EBUS) that are one of the most productive ecosystems in the world, providing significant economic, cultural and recreational services to large populations living along the coast (Halpern et al., 2008). California Current System (CCS) is one of the EBUS for which some of the highest rates of climate-related changes have already been demonstrated (Gruber, 2016; Turi et al., 2016, Garcia-Reyes et al., 2016; Hauri et al., 2013). Yet, the predictive abilities associated to accurately assess biological responses to interactive effects of multiple stressors are still inadequate. The challenge remains with establishing the linkages from cellular to physiological up to population level because they occur across different time scales. These are not easily replicated within the experimental conditions, failing to reflect realistic *in situ* exposure of multiple stressors. In this study, we use the conditions of El Niño with preceding heat wave and subsequent upwelling event that in combination provided multifaceted settings with multiple stressors and their in situ interactions.

The convergence of the 2013-2015 ocean heat wave coupled with the strong 2015-2016 El Niño (Bond et al., 2015; Lorenzo and Mantua, 2016; Jacox et al., 2016), causing one of the lowest phytoplankton abundance since 2013 (Gomez-Ocampo et al., 2017) and complete restructuring of the pelagic populations (Peterson et al., 2017). Prior to the WCOA16 cruise in May-June 2016, the Northeast Pacific was under the influence of a marine heatwave that lasted from late 2013 through the spring of 2016, with surface temperature anomalies ranging from 1.5 to 6.2°C (Bond et al., 2015; Di Lorenzo and Mantua, 2016;). The marine heatwave overlapped with the 2015-2016 El Niño (Gentemann et al., 2017; Hu et al., 2017). By the spring and early summer of 2016, the extremely high SST anomalies had dissipated but the surface waters generally remained warmer than normal by 1–1.5°C (Gentemann et al., 2017). Upwelling of low oxygen, low aragonite saturation waters were strong just prior to the 2016 cruise and corrosive waters approached the surface near the coast (Figures X). This confluence of oceanographic phenomena led to lower overall productivity of plankton and higher abundances of warm-water zooplankton species relative to cold-water species (Peterson et al., 2017). When El Niño came abruptly to an end around May 2016 (Jacox et al., 2016; Frischknect et al., 2017), spring transition had already induced an upwelling simultaneously creating a temporal window where El-Niño and increased upwelling overlapped. Although milder in the start, wind stress quickly intensified and resulted in intense upwelling at the end of May 2016, bringing to the shallow subsurface high CO2 waters with low aragonite saturation state (Ωar) in the near coastal region where upwelling is closest to the surface. Coupled with the increasing acidification due to anthropogenic CO2 uptake, the temporal dynamics of co-occurring El Niño-related warm waters and OA exposed pelagic communities along the US West Coast to *in situ* multiple stressors, providing unique insights into stressor effects ranging from molecular, sub-cellular and cellular, physiological and population level effects.

Pteropods are one of the most sensitive and best studied indicators for ocean acidification, with demonstrated increased OA susceptibility that is further exacerbated under thermal stress (Lischka et al., 2011; Lischka and Riebesell, 2012; Gardner et al., 2017). This includes increased shell dissolution, reduced metabolic activity, calcification, growth, and survival (Garner et al., 2017; Maas et al., 2015; Moya et al., 2016; Lischka and Ribesell, 2012; Bednaršek et al., 2016; 2017). Given the importance of pteropods as food resource for a variety of CCS ecologically and commercially important marine species (Aydin et al., 2005; Armstrong et al., 2008; Karpenko et al., 2007), it is an imperative to understand their sustainability under global environmental change scenarios.

The commonality behind the multiple stressors effects is the production of reactive oxygen species (ROS), and cellular stress that results in increased energy expenditure and compensatory energetic trade-offs on physiological level, and ultimately affect population structure. To capture the cross-level linkage, cellular and physiological biomarkers need to reflect the changes on the population level. Oxidative stress occurs as a consequence of an imbalance between ROS production and the available antioxidant (AO) defense which enables scavenging and neutralization of generated radicals (Table 1), such as superoxide anions (O2-), hydrogen peroxide (H2O2), and hydroxyl radical. While thermal stress seems to be one of the most potent stressors of ROS production, OA and deoxygenation also result in elevated oxidative stress (Murphy, 2009; Zenebe et al., 2007), especially under the interactive effects of combined stressors (Sokolova et al., 2012). ROS are capable of unrestricted oxidation of various cellular components that lead to non-specific modifications of lipids, proteins, and nucleid acids, causing disruption of the cellular homeostasis and cellular stress. From bioenergetic perspective, the results of oxidative stress can increased energy expenditure and negatively affect the efficiency of generating cellular energy, resulting in compensatory energetic trade-offs, bioenergetic dysfunction and compromised health (Wood et al., 2008; Slimen et al., 2014).

To our knowledge, there is no other study to date using oxidative stress to assess cellular stress levels and antioxidant defenses in these pelagic mollusks under combined OA and thermal stress. In addition, there is a complete lack of knowledge linking cellular to physiological and population responses in pteropods in the natural environment characterized by multiple stressors. The objective of this study is to assess and link the effects of multiple stressors and their interactions on cellular, physiological and population level of the pelagic calcifiers. By aiming at better understanding of the relative roles of drivers and their magnitude behind the stress responses, we delineated the interaction effects that determine the non-additive responses. The understanding of cellular responses of the oxidative stress can provide important new insights into the stress effects and factors that set limits to species’ tolerance of ocean acidification and warming in the natural environment, especially when mechanistically linked though energetic implications. Ultimately, this will improve the predictive capabilities of pelagic mollusks as indicators for multiple stressors.

**Methods**

During the 2016 West Coast Ocean Acidification cruise (WCOA2016), conductivity, temperature, depth and oxygen sensor profile data were collected along 17 cross-shelf transects accompanied by biological stations with accompanied vertical sections of temperature (T), salinity, nutrients, oxygen, chlorophyll-a (chla), calculated pCO2, pH, and calculated Ωar. At each station, water samples were collected in modified Niskin-type bottles, poisoned with HgCl2 and analyzed onboard the ship for dissolved inorganic carbon and total alkalinity (TA). Pteropods were collected in 100% ethanol at the biological subset of 37 stations using 200 µm mesh Bongo nets, with integrated sampling over the upper 100 m and combined with counts of *Limacina helicina* for overall abundance. Complete physical-chemical characterization of pteropod habitat was provided to link the environmental conditions with the biological responses, ranging from cellular, physiological and pteropod population level responses assessed through oxidative biomarkers, shell dissolution and growth, and abundance counts, respectively.

**Growth impairment**

To assess the impact of stressors on growth, we haveperformed length measurements on the same pteropod individual as determine shell dissolution, we eliminated organisms with lengths smaller than 0.5 mm. However, we minimized potential length bias by taking large sample size at each station where measurements were taken (N>25-35). Immediately after the sample collection, pteropods were flash frozen with liquid nitrogen before they were stored on dry ice (for transportation) and in -80°C until we resume with the biomarker analysis.

**Shell Dissolution assessment:**

To measure shell dissolution, all the pteropods were examined for mechanical damage; only intact and actively swimming individuals were considered for the analyses (N=15-20). Following Bednaršek et al. (2012) protocol, % shell dissolution across shell was determined and Type II and III damages were differentiated using image recognition interface in Matlab (Bednaršek at al., 2012). Type II shell damage is related to the dissolution of the upper prismatic layer, while Type III is related to deeper-protruding dissolution of the crossed-lamellar layer. We also examined of scarring on the shell, the pattern that visually resembles shell etchings and relates to the dissolution of the upper prismatic layer (Bednarsek et al., 2012).

**Determination of oxidative stress biomarkers**

For the determination of antioxidant defense and oxidative stress biomarkers pteropod samples were homogenized in 0.1 M K2HPO4 + 0.15 M KCl buffer (pH 7.4) using a Tissue Lyser II bead mill (Qiagen). An aliquot of 25 µL of raw homogenate was immediately frozen in liquid nitrogen and stored at -80°C for analyses of lipid peroxidation (LPX). The rest of the sample homogenate was centrifuged for 15 min at 4°C and the supernatant was divided into aliquots for Glutathione S-transferase (GST), Glutathione reductase (GR), Catalase (CAT) and Superoxide dismutase (SOD) enzyme activity determination, Oxygen Radical Absorbance Capacity (ORAC) assay.. For the latter, the sample was deproteinized by adding 5% sulfosalicylic acid, incubated on ice for 10 min and centrifuged for 10 min at 4°C. The supernatant was divided into two different tubes for reduced (GSH) and oxidized glutathione (GSSG) and 33 mM M2VP (1-methyl-2-vinylpyridinium trifluoromethanesulfonate, Sigma Chemicals) in 0.1M HCl (a scavenger of GSH), was added to the GSSG sample, in the proportion to 1:10. The sample homogenate aliquots and glutathione samples were frozen in liquid nitrogen and stored at -80°C until further analysis.

The lipid hydroperoxides were measured by using the FOXII method described in Eymard and Genot (2003), and Bou et al. with slight modifications (cf. Vuori et al. 2015). The raw homogenates were mixed with methanol and centrifuged for 5 min at room temperature. 450 μL of the Reaction mix (ferrous oxidation-xylenol orange) reagent was added to the samples (50 μL) and absorbance measured after two hours of incubation. GST and GR activities were determined as described in Vuori et al. (2015). The SOD inhibition rate was measured using SOD determination kit (Sigma Chemicals). The CAT activity was modified to microplate from the Catalase Assay kits’ colorimetric assay (Sigma Chemicals) (Vuori et al. 2012). The amount of GSH and GSSG was analyzed with Glutathione 384-well plate Fluorescent Detection Kit (Arbor Assays) and intracellular soluble antioxidant capacity with OxiSelectTM Oxygen Radical Antioxidant Capacity (ORAC) Activity Assay (Cell Biolabs) following the manufacturers’ instructions, except for adjusting the reaction volumes for 384-well plate when needed. The enzyme activities, lipid hydroperoxides and total GSH were normalized to the protein content of the samples, which was determined with PierceTM BCA Protein Assay (Thermo Scientific) with bovine serum albumin (Sigma) as the standard.

All samples, standards and blanks were analyzed in triplicate. For all assays performed in this study, the mean coefficient of variation percentage (CV%) of technical replicates ranged between 2.91 and 5.48%. Plate to plate variation was normalized with control samples and it ranged between 3.97 and 14.73%. Out of all measured biomarkers, the use of four biomarkers seem to be applicable for determining oxidative stress in pteropods, thus we focused on them in the subsequent analyses.

**Statistical analyses**

The analysis approach was to identify relationships among variables as potentially additive (positive or negative) or synergistic (interactions) by evaluating pairwise effects of stressors on each response measure. Pearson correlations of environmental parameters (OA parameters, temperature, and chlorophyll) with cellular, physiological, and population response were first evaluated to identify potential associations with individual variables. Correlations between cellular and physiological responses were also evaluated to identify links between the two levels of biological organization. Non-continuous or skewed variables were transformed to better satisfy assumptions of parametric tests (e.g., abundances were logarithmically transformed, proportions were arcsine transformed). All analyses were performed with the R statistical programming language (R Core Team 2017).

Multivariate comparisons of all cellular response measures and environmental variables were assessed using redundancy analysis (RDA) to jointly characterize relationships between sampling stations. This analysis is conceptually similar to principal components analysis with an additional constraint on the environmental matrix, where the relationships are further partitioned based on covariance among response measures at each site in addition to the covariance between environmental variables. The final triplot (two biplots of environmental and response matrices) can be used to evaluate which environmental variables are correlated, as well as their relationships to the cellular response measures at each site. The environmental and response matrices were standardized to range from 0-1 prior to RDA. The vegan package for R was used for standardization and RDA (Oksanen et al. 2017).

Covariance of the environmental parameters with latitude was checked as a potential confounding effect using correlation analysis and RDA.  Although some variables were correlated with latitude, comparison of the cellular and physiological response of pteropods to environmental gradients showed that latitude and longitude were generally orthogonal to the environmental parameters in multivariate space, particularly for temperature and latitude. As such, sampling station location was not included in any of the models.

Linear models were then developed for pairwise combinations of environmental variables to evaluate additive and interactive effects on stressor response. To reduce the likelihood of false positive results from multiple comparisons, variables were chosen a priori that were considered most relevant for describing pteropod response to stressors. Only LPX, ORAC, ORACvLPX, and SOD biomarkers were evaluated for cellular response and only abundance, dissolution, and length were evaluated for physiological and population responses. Environmental variables were selected for analysis that were orthogonal in multivariate space to reduce collinearity and included Ω saturation, fluorescence, pCO2, and temperature. Oxygen and Ω saturation were evaluated for relationships with physiological and population response measures. Variance Inflation Factors (VIF) were quantified for all pairwise combinations of environmental variables to estimate potential collinearity in each model, such that:

VIFj = 1 / ( 1 − R2j) (1)

where VIF is the reciprocal of the unexplained variance (1 − R2) of the linear regression of variable j against all other explanatory variables. Zuur et al. (2007) suggest that VIF values less than fifty may be appropriate for analysis, but we chose a value of ten for excluding combinations of environmental variables. We chose this conservative value to further reduce the potential for false positive results by reducing the number of combinations that were evaluated, in addition to reducing the likelihood of spurious results from collinear explanatory variables.

Linear models for the selected pairwise combinations of environmental variables and response measures included separate terms for the main effects of individual variables and a third term for the interaction of the pair. A model selection procedure was then used to compare every smaller subset of the global model (main and interactive effects) to identify the most parsimonious solution for each pair. The final model for each pairwise combination was chosen based on a minimization of corrected Akaike Information Criterion (Burnham and Anderson 2002, Barton 2018). Further, models with probability values greater than alpha of 0.05 for the overall model fit were excluded. These p-values were not adjusted for multiple comparisons due to the relatively small sample sizes of each model (n = 11 for all, except n = 35 for abundance).

Evidence for additive effects were based solely on the magnitude of the estimated parameter of the main effect for each variable, whereas synergistic or antagonistic effects were evaluated from the estimated parameter for the interaction, if demonstrated (Figures 4a-c, 5a-b; Tables 2 and 3). A positive interaction was evidence of a synergistic effect and a negative interaction was evidence of an antagonistic effect. Results of the linear models were further evaluated using effects plots to characterize the relationship of a pteropod response measure to continuous values for one stressor given two different values for the second stressor (constant at the minimum and maximum observed values).

**Results**

**El Niño-Induced Thermal and OA Gradients Define Pteropod Habitat**

The marine heat wave and the El Niño left a characteristic signature in the investigated region, with uniform increase in temperature across the large-scale region stretching from the Southern Oregon to the Prince William Sound in Canada. The increase in temperature of approximately 1 - 1.5 ͦC from the usual baseline was observed in the upper 80-100 m (Figure 1). Pteropods were collected across strong T and OA gradients with differential degrees of interaction depending on the location. Near the end of the El Niño, the onset of increased upwelling added a spatial complexity with respect to carbonate chemistry. The region off northern California and southern Oregon was under the strong influence of the combined stressors of warming, deoxygenation and acidification (Figure 1). Associated with these events were higher than normal aragonite saturation values in Washington, Oregon British Columbia near-surface waters and normal to lower-than-normal aragonite saturation and low oxygen values in the subsurface waters near the coast off Washington and Oregon but less so off British Columbia. Omega saturation horizon (Ωar =1) was positioned at shallow waters approximately 40-80 m at mid-shelf in Oregon and Washington coastal regions and gradually deepened northward to about 120 to 150 m in Canadian waters (Figure 1). Although to a much lesser extent, upwelling induced regionally-specific decline in oxygen, with oxygen concentrations < 100 μmol/kg occurring but not as low further north. With the general increase in T across the entire regions, there were unique regional OA gradients with most severe in the south (OR) and gradually decreasing towards north. These gradients were strong from deep to shallow water depths within the upper 100 m that is a characteristic pteropod habitat.

The multiple stressor interactions in these environmental stresses are defined by high collinearity, as depicted on the RDA plot of the upper 100m (Figure 2a, b). The two main components explained in total around >75% of the observed variance in the RDA, i.e., 53.6% of Component 1 and 29.2% of Component 2 for the cellular RDA. While OA parameters (pH, Ωar, pCO2) and oxygen are all highly correlated, as demonstrated by their loadings along the RDA1 axis, temperature shows higher loading along the RDA2 taxis, and thus less correlation with OA parameters (Figure 2a).  Food availability (chla measured as fluorescence) was not correlated with any of the environmental parameters.

**Pteropod Responses to Multiple Stressors Interacting Across Large Spatial Gradients**

Biomarkers of pteropod organismal stress showed the highest cellular stress present in the south and gradually decreased northwards. LPX (lipid peroxidation) and GSH:GSSG (glutathione ratio), both biomarkers related to cellular damage, showed significant southwards increase (Table 1; Figure 3). Also, the overall cumulative stress marker (ORAC/LPX; i.e. cellular toxicity (LPX) compensated by AO marker, with low ORAC/LPX indicating increased oxidative stress) was the highest under co-occurring conditions of thermal and OA stress in the south, while its significance decreased under single stressor in the north. Correspondingly, the highest AO defense was observed through increased SOD (sodium dismutase) levels, with values significantly higher in the south and negative correlations with LPX and ORAC/LPX. Although food availability was not significantly correlated to any oxidative markers, it positively related to AO activity, observed in GST (glutathione S-transferase) and CAT (catalase; Figure 3), and negatively to the overall stress GSH:GSSG.

**Pteropod Cellular Response to OA, Deoxygenation and Thermal Stress**

OA stress induced significant activation of oxidative stress biomarkers, as indicated by the increased levels of LPX (Figure 3), the main agent of cellular damage and representative of the first line evidence of the oxidative stress. The cumulative cellular stress level, as indicated by low ORAC/LPX ratio, is significantly correlated with OA stress. The significant induction of the stress was triggered independently by pCO2/pH and Ωar , or in combination with both OA stressor (Figures 3). The only AO pathway activated under OA was increased expression of SOD biomarker. Low oxygen also strongly increases oxidative stress and activates cellular damage pathways (Figure 3), as indicated by negative correlation between LPX and ORAC/LPX with oxygen concentration. Oxygen reactive absorbance capacity (ORAC) was not correlated with OA, unless the joint effects of pCO2 and Ωar were evaluated together in their association with ORAC, causing positive additive effects (Table 2).

Thermal stress strongly induces metabolic pathways related to increased AO activity. Data demonstrated the highest positive correlation of ORAC to thermal stress (Figure 3), while the other AO biomarkers showed positive, yet insignificant correlation. Based on the measured cellular toxicity parameters (i.e. LPX, OPAC/LPX, GSH:GSSG), temperature did not contribute to significant oxidative stress when considered by itself. Given that ORAC was not significantly correlated with latitude confirmed a uniform warming signal along the entire region related to the El Niño. To examine the effect between low oxygen and OA, the focus was on SOD biomarker, which expression was negatively correlated with declining oxygen, and LPX and ORAC/LPX as two parameters significantly involved in cellular damage at low oxygen.

The combination between OA and thermal stress for the LPX indicated negative additive effect, with high Ωar negatively and thermal stress positively related to LPX (R2=0.5; Table 2; Figure 4a). Although the individual correlations of LPX with Ωar and temperature showed that LPX decreases with an increase in both, temperature has an inverse effect when modelled together with OA, with lower temperature causing lower LPX. At a co-occurring Ωar, low temperature demonstrated mitigated effect of the cellular stress, even at the lowering of temperature of 2-3 degrees. In addition, food availability also demonstrated negative additive effect of OA (R2=0.72; Table 2; Figure 4b).

The same significant negative additive effects with temperature and food availability as two mitigating parameters was demonstrated for ORAC/LPX ratio under combined OA and thermal stress (R2=0.58 and R2=087; respectively; Table 2). Basically, while high Ωar had a positive effect on this ration, the response was magnified at lower temperature. SOD activation under combination of Ωar and temperature demonstrated positive additive effect, indicating that both stressors in combination intensify AO production (R2=0.54; Table 2; Figure X). For example, increased SOD activities were higher at combination of thermal stress and OA stress compared to increase in only one stressor (Table 2).

**The interactive effect of OA, thermal stress and deoxygenation on the physiological and population level**

Physiological level effects, measured as the shell dissolution (dissolution extent of Type II and III damage), showed strong negative correlation with OA parameters, including Ωar that is the main drivers of shell dissolution (Table 3). Shell etching was positively but not significantly correlated with thermal stress (Figure 3). The combination of different stressors can either increase or decrease physiological effects. Increased food availability, low temperature and increased level of oxygen all showed mitigating effects (R2=0.71; R2=0.87; and R2=0.85; respectively) with the co-occurrence of OA as a stressor. It also holds true the contrary, insufficient food availability, oxygen and high temperature in combination with OA can increase physiological stress. We found three significant interaction between deoxygenation and thermal stress and Ωa (O2: Ωar; and Ωar :T; Table 3) indicating synergistic effect on dissolution at the co-occurrence of any of the two stressors. With respect to temperature, the interaction effect on shell dissolution is magnified at lower Ωar while the mitigation effect is more pronounced at higher Ωar (Figure 5a). On the other hand, the mitigation effect for oxygen is most pronounced at lower Ωar. In terms of correlation between the cellular and physiological endpoints, shell dissolution corresponded negatively with the overall cellular stress (ORAC/LPX), and showed negative correlation with AO activity of CAT and GST biomarkers or no interaction with SOD (Figure 3).

Growth impairment did not show significant correlation to any environmental parameters, but was negatively correlated with shell dissolution and scarring, indicating decreased growth co-inciding at the presence of shell dissolution (Figure3). Food availability did not have any significant effect on growth (Figure 3). While single interactions were insignificant, we found additive effect between ???? The text will be added

Pteropod abundance, which exemplified population level effects, showed significant negative correlation with temperature as a single parameter (Figure 3). However, when thermal stress was interacting with OA (either Ωar or pCO2; Table 3) a negative additive effect was observed. The oxygen in combination with Ωar or temperature also demonstrated negative additive effects, with high oxygen at low Ωar  increasing the abundances, while low oxygen concentration decreasing them (Figure 5c, d; Table 3). In combination with OA stress or low O2, the co-occurrence of low temperature can have mitigating effect across the entire Ωar range. The same abundance effect was observed when combining oxygen and temperature (Table 3). Food availability did not show negative additive effect with any stressors.

**Discussion**

El Niño has been known to leave a characteristic imprint of thermal stress on the coastal and near-shore habitats in the CCS, with implications for the coastal ecosystems and fisheries management (Chavez et al., 2017, and the reference therein). However, less is known about the ecological responses when El Niño overlaps with the springtime intensification of upwelling event, characterizing habitats across wider spatial scales and lasting for several months. During this period, exposing coastal and near-shore habitats to multiple stressors compromised the suitability of habitats especially in the coastal ecosystems with potentially deleterious ecosystem consequences. This is the first synoptic study of pteropod responses under in situ multiple stressor conditions, as created during overlapping El Niño and OA events in the CCS. Because it left the imprint on the intensity of the following El Niño warming, the implications of this study can be also extrapolated to the biological effects of the heat waves. The overlap of El-Niño and upwelling also impacts oxygen distribution, with regionally low oxygen concentrations acting as a third stressor impacting the biological responses in interaction with thermal and OA stress. Above all, this study holds the predictive capacity of the effects in the EBUS systems under intensifying climate-change effects. Not only it demonstrates the wide range of conditions that EBUS systems will be experiencing in the near-future, but it delineates their interactions among the multiple stressors that will often have the most significant ecological implications. During these events, the species are affected by multiple adverse pathways acutely affecting cellular and physiological pathways, including internal acidosis, hypercapnia, metabolic depression, reduced oxygen supply and anaerobiosis, and thermal stress.

On the population level, we observed remarkable shift from OA-driven cellular and physiological responses to temperature being the main driver of abundance distribution, with OA being strong driver, but of secondary importance. Additive effects of OA and thermal stress on abundance clearly suggest that El Niño to have negative effect on the population level, while oxygen becomes more important for abundance when individuals are more stressed at high OA levels.

Pteropods are significantly more affected by these stressors in comparison with copepod, pelagic non-calcifiers, which show much lower cellular stress levels than calcifying molluscks (Engström-Öst et al., submitted). The upwelling-induced OA conditions elicit high levels of cellular stress and damage, similarly as preliminary demonstrated by Koh et al (2015), and can resonate also on the physiological levels. OA is a main driver of cellular toxicity, likely because various OA parameters (pH, pCO2 and Ωar) independently activate different cellular pathways simultaneously and amplify negative stress effect. Given that thermal stress elicits different AO biomarkers than OA stress (ORAC and SOD, respectively), this supports the notion that thermal and OA stress activate different cellular pathways. Based on the cellular and population responses, OA and deoxygenation activate similar oxidative damage pathways and AO deference. Moreover, synergistic interactions with low oxygen and thermal and OA stress were particularly important in inducing additional physiological stress, requiring more in-depth study in the future. One of the important observations relates to the fact that individual correlation of one stressor with biological end point can show inverse effect when modeled together with two stressors, such as the case of thermal effect on LPX and Ωar. In general, individual correlations are rarely descriptive (i.e. correlation does not mean causation), examining two effects in a combined manner supports mechanistic underpinning under realistic in situ conditions.

In this study, we found the magnitude of overall stress conditions to be reflected through various cellular and physiological stress, suggesting that OA stress probably remained mainly uncompensated in the south. This is likely due to deficiency of AO activity results from either insufficient expression or inefficiency of AO defenses (Matozzo et al., 2013), potentially linked to shutting down of energy-consuming processes under severe OA stress. The question thus remains how can pteropods thrive in their high abundances in various upwelling regimes (Bednaršek et al., 2012) is OA conditions are so detrimental for them. Based on our results, we infer that pelagic calcifiers have developed several approaches to mitigate against the OA effects at least partially. First, we found evidence of continuous activation of AO activity indicative of scavenging mechanisms to detoxify the excess of ROIs to decrease overall oxidative damage along the strongest OA hotspots. Secondly, pteropods likely shut down more energetically expensive processes to conserve the energy under most severe OA exposures. Thirdly, upwelled waters are cold and nutrient-rich, both of these reducing the OA stress and having mitigation effects on overall cellular toxicity. While food availability was demonstrated before to partially offset negative effects (Seibel et al., 2012), low temperature effect was demonstrated for the first time. The compensatory effects of OA and thermal stress likely occur also because of negative additive effects stimulate AO production. We recommend the future studies to focus on the mechanisms that governs the linkage between low temperature and OA, and determine the magnitude of mitigation effects in the context of multiple environmental drivers and OA intensification. This effect might be very useful for predicting future adaptation strategies to climate change across most sensitive organisms across lower trophic levels. Overall, the negative additive or antagonistic effects can be in general viewed as alleviating circumstance under multiple stressors in the upwelling regime, but are absent during El Niño years and heat waves, and intensifying effect of climate change, resulting with more severe biological implications.

**Mechanisms of oxidative stress implications on energetic status and overall pteropod vulnerability**

Various studies agree that extreme sensitivity to OA in pteropods is related to their energetics metabolism, pointing towards the trade-off processes underlined by the insufficient energy budget (e.g. Seibel et al., 2012; Lischka et al., 2017; Bednaršek et al., 2016; Peck et al., 2018). Assessing oxidative stress to multiple stressors offers an opportunity to link early warning stress effects and bioenergetics, and predict the effects beyond the cellular level. Here, we attempt to demonstrate how increased oxidative stress on the cellular levels imposes energetic trade-offs, with the implication for organismal fitness.

On the cellular level, the action mode of OA parameters elicits excess in ROS production.  This subsequently activates different oxidative damage pathways that results in negative capacity for aerobic ATP production (Abele et al., 2002) and significant damage to lipids (Monaghan et al., 2009). These mechanisms are conservative across a diverse group of organisms, including mammals, plants and fungi (Pinti et al., 2015; Quirós et al., 2014, 2015). ROS targeted-lipid peroxidation is mainly induced in the lipids with poly-unsaturated bonds (PUFA) that are more susceptible to lipid peroxidation in comparison with monounsaturated or saturated fatty acids. The amount of PUFA can impact the extent of oxidative damage (Hulbert et al., 2007). Given that across different pteropod life stages up to 60-80% of structural lipids and TAGs are predominantly composed of PUFAs (Gannefors et al., 2005), they are easily targeted by ROS. Lipid peroxidation has double negative implications for organisms: first, PUFA’s sensitivity to lipid peroxidation results in their decrease (Yin and Porter, 2003), leaving organisms without the essential lipid structure and reserves. Secondly, lipid peroxidation increases ROS-induced structural damage of cell membranes (Bou et al., 2008), as demonstrated by activation of genes involved in membrane processes (Koh et al., 2015). In the perpetual process of cellular damage, even more ROS are generated that are in the next step aiming at proteins as their next targets. Accumulation of the oxidative damage under stress exposure thus requires metabolic compensation to cover for the energetic costs of the AO activity, stress protein expression and activation of protein degradation pathways (Tomanek et al., 2011; Sokolova, 2012), which are one of the most expensive ATP sinks in the cells (Hochacka and Somero, 2002). In mollusks, for example, energy costs of protein synthesis can increase from 10% during the control conditions to up to 40% during extreme stress (Ivanina et al., 2008), or 10-25% of total ATP consumption (Cherkasov et al., 2006).

Cumulatively, this evidence suggests potential for oxidative stress impacting life history trade-offs that can manifest over different time scales. On short timescales, altered energetic demand due to increased maintenance costs (Wood et al., 2008; Moya et al., 2016) and down-regulation of aerobic metabolism (Maas et al., 2015) implies lower energy allocations and carry-over energetic trade-offs under combined OA and thermal stress. This presumption indeed agrees with our observations of increased shell dissolution as related to reduced AO activity and growth. Regarding the former, shell dissolution positively correlated with the cellular stress biomarkers (ORAC/LPX), thus we expected an increase in AO activity. Instead, we observed reduced levels of AO biomarkers (e.g. CAT, GST, ORAC), which most likely implies insufficient energetic budget unable to provide support for energetically expensive AO production (Monaghan et al., 2009). This is additionally supported by the negative correlation between shell dissolution and growth, as previously demonstrated by Lischka et al. (2011). Either growth was slowed down because of the extensive dissolution, or reduced energy budget did not support continuation of growth or AO production, along also compromising biologically important processes, such as acid-base regulation (Moya et al., 2016), up-regulation of biomineralization (Maas et al., 2015), and shell repair (Peck et al., 2018). Although the shell processes in the latter study might appeare to have been activated, they are most likely short-lived under limited available energy and they very likely not relevant towards maintaining overall calcification rates (Moya et al., 2016; Bednaršek et al., 2017). We conclude that by measuring oxidative stress on the cellular level this contributes to interpretation of the responses on the physiological level. Overall, the present study demonstrated that ORAC/LPX ratio may represent a suitable biomarker of pteropod exposure to ocean acidification and warming conditions. Understanding such subcellular processes will help identify the habitats that are or are not capable of supporting viable physiological responses (Sokolova et al., 2012).

On the longer, i.e. population level time scales, there might be longer term trade-offs in biological functions. Combined El Niño induced thermal and OA stress in the southward regions indicated that pteropods reallocated more energy to compensate against the stress compared to the northern pteropods. Our supposition from the physiological level predicts that it is bioenergetic limitations that determine the population level responses, without there being an immediate knock on effects on abundance structure. None of the cellular biomarkers or physiological endpoints were significantly correlated with abundances, although negative correlation in LPX abundance (R2= 0.25) indicates potential trend, in linking cellular and population level. However, the lack of correlation does not mean that other life history components are not already compromised (e.g. growth, fitness). Although cellular or physiological effects cannot be immediately extrapolated to the population level, the costs of energetic requirements is potentially paid by reduced reproductive potential, and will be demonstrated over longer timescales. Advancing from physiological to population level effect is obviously marked by complexities that require further understanding and method development. Nevertheless, strong correlation of thermal and OA stress on pteropod population responses, make pteropods strong candidate indicators for cumulative effects of increased temperature concurrent with OA in marine ecosystem (Bednaršek et al., 2016). This is supported by the observed pteropod population declines under cumulative stressors of global climate changes (Beaugrand et al., 2013), and also in the North Sea (Beare et al., 2013), recognizing thermal stress to be the driver of long-term pteropod decline.

In summary, this study delineates for the first time, some of the energetic implications behind pteropod vulnerability in high-temperature, low oxygen, OA stressed coastal waters. This involves the link between exceptionally high PUFA levels, pteropod cellular stress and intense energy consumption to provide a main reason behind pteropod extreme sensitivity to OA. Contrary to the previous studies, we found their sensitivity to be primarily driven by increased oxidative stress from OA, and only secondarily to shell dissolution that stems from the lack of energy resources previously utilized to mitigate against intense oxidative stress on the cellular level. As a consequence, oxidative stress biomarkers can be used as an early-warning signal of OA effects. Furthermore, based on the numerous lines of evidence, oxidative stress biomarkers offer important insights into bioenergetic requirements under multiple stress exposure for pteropods and as previously demonstrated for numerous other taxa (Ivanina et al., 2016; Ivanina and Sokolova, 2016; Lesser et al., 2006).

**Acknowledgements:** We thank the crew at NOAA WCOA 2016 for invaluable assistance with pteropod collection. Thanks to Lisette Meckes for sharing length data from pteropods that we collected.

**Funding support:** This project has been funded by NOAA OAP West Coast Cruise.

**References:**

Abele, D., Heise, K., Pörtner, H.O. and Puntarulo, S. (2002). Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam Mya arenaria. J. Exp. Biol. 205:13, 1831-1841.

Armstrong, J. L., Myers, K. W., Beauchamp, D. A., Davis, N. D., Walker, R. V., Boldt, J. L., and Moss, J. H. (2008). Interannual and spatial feeding patterns of hatchery and wild juvenile pink salmon in the Gulf of Alaska in years of low and high survival. T. Am. Fish. Soc. 137:5, 1299-1316.

Aydin, K. Y., McFarlane, G. A., King, J. R., Megrey, B. A., and Myers, K. W. (2005). Linking oceanic food webs to coastal production and growth rates of Pacific salmon (Oncorhynchus spp.), using models on three scales. Deep Sea Research Part II: Topical Studies in Oceanography. 52:5-6, 757-780.

Barton, K. 2018. MuMIn: Multi-Model Inference. R package version 1.40.4. [https://CRAN.R-project.org/package=MuMIn](https://cran.r-project.org/package=MuMIn)

Beare, D., McQuatters-Gollop, A., van der Hammen, T., Machiels, M., Teoh, S. J., and Hall-Spencer, J. M. (2013). Long-term trends in calcifying plankton and pH in the North Sea. PLoS One. 8:5, e61175.

Beaugrand, G., McQuatters-Gollop, A., Edwards, M., and Goberville, E. (2013). Long-term responses of North Atlantic calcifying plankton to climate change. Nat. Clim. Change. 3:3, 263.

Bednaršek, N., Tarling, G.A., Bakker, D.C., Fielding, S., Cohen, A., Kuzirian, A., McCorkle, D., Lézé, B. and Montagna, R., 2012. Description and quantification of pteropod shell dissolution: a sensitive bioindicator of ocean acidification. *Global change biology*, *18*(7), pp.2378-2388.

Bednaršek, N., Harvey, C.J., Kaplan, I.C., Feely, R.A. and Možina, J., 2016. Pteropods on the edge: Cumulative effects of ocean acidification, warming, and deoxygenation. *Progress in Oceanography*, *145*, pp.1-24.

Bednaršek, N., Klinger, T., Harvey, C.J., Weisberg, S., McCabe, R.M., Feely, R.A., Newton, J. and Tolimieri, N., 2017. New ocean, new needs: Application of pteropod shell dissolution as a biological indicator for marine resource management. *Ecological Indicators*, *76*, pp.240-244.

Bednarsek, N., J. Johnson, and R. A. Feely. "Comment on Peck et al: Vulnerability of pteropod (Limacina helicina) to ocean acidification: shell dissolution occurs despite an intact organic layer." *Deep Sea Research Part II: Topical Studies in Oceanography* 127 (2016): 53-56.

Bednaršek, N., Feely, R.A., Tolimieri, N., Hermann, A.J., Siedlecki, S.A., Waldbusser, G.G., McElhany, P., Alin, S.R., Klinger, T., Moore-Maley, B. and Pörtner, H.O., 2017. Exposure history determines pteropod vulnerability to ocean acidification along the US West Coast. *Scientific reports*, *7*(1), p.4526.

Belhadj Slimen, I., Najar, T., Ghram, A., Dabbebi, H., Ben Mrad, M., and Abdrabbah, M. (2014). Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. Int. J. Hyperther. 30:7, 513-523.

Bond, N. A., Cronin, M. F., Freeland, H., and Mantua, N. (2015). Causes and impacts of the 2014 warm anomaly in the NE Pacific. Geophys. Res. Lett. 42:9, 3414-3420.

Bou, Ricard, Rafael Codony, Alba Tres, Eric A. Decker, and Francesc Guardiola. (2008). Determination of hydroperoxides in foods and biological samples by the ferrous oxidation–xylenol orange method: A review of the factors that influence the method’s performance. Anal. Biochem. 377:1. 1-15.

Burnham, K.P., Anderson, D.R. 2002. Model selection and multimodel inference: a practical information-theoretic approach. 2nd ed. New York, Springer-Verlag.

Chavez, F. P., Sevadjian, J., Wahl, C., Friederich, J., and Friederich, G. E. (2017). Measurements of pCO2 and pH from an autonomous surface vehicle in a coastal upwelling system. Deep Sea Research Part II: Topical Studies in Oceanography.

Cherkasov, A. S., Biswas, P. K., Ridings, D. M., Ringwood, A. H., and Sokolova, I. M. (2006). Effects of acclimation temperature and cadmium exposure on cellular energy budgets in the marine mollusk Crassostrea virginica: linking cellular and mitochondrial responses. J. Exp. Biol. 209:7, 1274-1284.

Di Lorenzo, E., and Mantua, N. (2016). Multi-year persistence of the 2014/15 North Pacific marine heatwave. Nat. Clim. Change. 6:11, 1042-1047.

Engström-Öst, J., Glippa, O., Feely, R. A., Alin, S. R., Carter, B. R., Kanerva, M., Bednaršek, N. Comparing calcifiers and non-calcifiers responses to ocean warming and acidification from an ecophysiological perspective. (in prep.)

Eymard, S., and Genot, C. (2003). A modified xylenol orange method to evaluate formation of lipid hydroperoxides during storage and processing of small pelagic fish. Eur. J. Lipid Sci. Tech. 105:9, 497-501.

Frischknecht, M., Münnich, M., and Gruber, N. (2017). Local atmospheric forcing driving an unexpected California Current System response during the 2015–2016 El Niño. Geophys. Res. Lett. 44:1, 304-311.

Gannefors, C., Böer, M., Kattner, G., Graeve, M., Eiane, K., Gulliksen, B., ... and Falk-Petersen, S. (2005). The Arctic sea butterfly Limacina helicina: lipids and life strategy. Mar. Biol. 147:1, 169-177.

Gardner, J., Manno, C., Bakker, D. C., Peck, V. L., and Tarling, G. A. (2018). Southern Ocean pteropods at risk from ocean warming and acidification. Mar. Biol. 165:1, 8.

Gentemann, C. L., Fewings, M. R., and García‐Reyes, M. (2017). Satellite sea surface temperatures along the West Coast of the United States during the 2014–2016 northeast Pacific marine heat wave. Geophys. Res. Lett. 44:1, 312-319.

Gómez-Ocampo, E., Gaxiola-Castro, G., Durazo, R., and Beier, E. (2017). Effects of the 2013-2016 warm anomalies on the California Current phytoplankton. Deep Sea Research Part II: Topical Studies in Oceanography.

Halpern, B. S., Kappel, C. V., Selkoe, K. A., Micheli, F., Ebert, C. M., Kontgis, C., ... and Teck, S. J. (2009). Mapping cumulative human impacts to California Current marine ecosystems. Conserv. Lett. 2:3, 138-148.

Hochochka, P.W. and Somero, G.N. (2002). Biochemical adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press.

Hu, Z. Z., Kumar, A., Jha, B., Zhu, J., and Huang, B. (2017). Persistence and predictions of the remarkable warm anomaly in the northeastern Pacific Ocean during 2014–16. J. Climate. 30:2, 689-702.

Hulbert, A. J., Pamplona, R., Buffenstein, R., and Buttemer, W. A. (2007). Life and death: metabolic rate, membrane composition, and life span of animals. Physiol. Rev. 87:4, 1175-1213.

Ivanina, A. V., Cherkasov, A. S., and Sokolova, I. M. (2008). Effects of cadmium on cellular protein and glutathione synthesis and expression of stress proteins in eastern oysters, Crassostrea virginica Gmelin. J. Exp. Biol. 211:4, 577-586.

Ivanina, A.V., Nesmelova, I., Leamy, L., Sokolov, E.P. and Sokolova, I.M. (2016). Intermittent hypoxia leads to functional reorganization of mitochondria and affects cellular bioenergetics in marine molluscs. J. Exp. Biol. 219:11, pp.1659-1674.

Ivanina, A. V., and Sokolova, I. M. (2016). Effects of intermittent hypoxia on oxidative stress and protein degradation in molluscan mitochondria. J. Exp. Biol. 219:23, 3794-3802.

Jacox, M. G., Hazen, E. L., Zaba, K. D., Rudnick, D. L., Edwards, C. A., Moore, A. M., and Bograd, S. J. (2016). Impacts of the 2015–2016 El Niño on the California Current System: Early assessment and comparison to past events. Geophys. Res. Lett. 43:13, 7072-7080.

Karpenko, V. I., Volkov, A. F., and Koval, M. V. (2007). Diets of Pacific salmon in the Sea of Okhotsk, Bering sea, and northwest Pacific Ocean. N. Pac. Anadr. Fish Comm. Bull. 4, 105-116.

Koh, H. Y., Lee, J. H., Han, S. J., Park, H., Shin, S. C., and Lee, S. G. (2015). A transcriptomic analysis of the response of the arctic pteropod Limacina helicina to carbon dioxide-driven seawater acidification. Polar Biol. 38:10, 1727-1740.

Lesser, M.P. (2006). Oxidative stress in marine environments: biochemistry and physiological ecology. Annu. Rev. Physiol., 68, 253-278.

Lischka, S., Büdenbender, J., Boxhammer, T., and Riebesell, U. (2011). Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod Limacina helicina: mortality, shell degradation, and shell growth. Biogeosciences. 8:4, 919.

Lischka, S., and Riebesell, U. (2012). Synergistic effects of ocean acidification and warming on overwintering pteropods in the Arctic. Glob. Change Biol. 18:12, 3517-3528.

Lischka, S., and Riebesell, U. (2017). Metabolic response of Arctic pteropods to ocean acidification and warming during the polar night/twilight phase in Kongsfjord (Spitsbergen). Polar Biol. 40:6, 1211-1227.

Maas, A. E., Lawson, G. L., and Tarrant, A. M. (2015). Transcriptome-wide analysis of the response of the thecosome pteropod Clio pyramidata to short-term CO 2 exposure. Comp. Biochem. Phys. D. 16, 1-9.

Monaghan, P., Metcalfe, N. B., and Torres, R. (2009). Oxidative stress as a mediator of life history trade‐offs: mechanisms, measurements and interpretation. Ecol. Lett. 12:1, 75-92.

Montgomery, D.C., Peck, E.A. 1992. Introduction to Linear Regression Analysis. Wiley, New York, NY.

Moya, A., Howes, E. L., Lacoue‐Labarthe, T., Forêt, S., Hanna, B., Medina, M., ... and Watson, S. A. (2016). Near‐future pH conditions severely impact calcification, metabolism and the nervous system in the pteropod Heliconoides inflatus. Glob. Change Biol. 22:12, 3888-3900.

Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. Biochem. J.. 417:1, 1-13.

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O’Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H. 2017. vegan: Community Ecology Package. R package version 2.4-5. [https://CRAN.R-project.org/package=vegan](https://cran.r-project.org/package=vegan)

Peck, V. L., Oakes, R. L., Harper, E. M., Manno, C., and Tarling, G. A. (2018). Pteropods counter mechanical damage and dissolution through extensive shell repair. Nat. Commun. 9:1, 264.

Peterson, W. T., Fisher, J. L., Strub, P. T., Du, X., Risien, C., Peterson, J., and Shaw, C. T. (2017). The pelagic ecosystem in the Northern California Current off Oregon during the 2014–2016 warm anomalies within the context of the past 20 years. J. Geophys. Res.-Oceans. 122:9, 7267-7290.

Pinti, M., Gibellini, L., Liu, Y., Xu, S., Lu, B., and Cossarizza, A. (2015). Mitochondrial Lon protease at the crossroads of oxidative stress, ageing and cancer. Cell. Mol. Life Sci. 72:24, 4807-4824.

Quirós, P. M., Español, Y., Acín-Pérez, R., Rodríguez, F., Bárcena, C., Watanabe, K., ... and Vázquez, J. (2014). ATP-dependent Lon protease controls tumor bioenergetics by reprogramming mitochondrial activity. Cell Rep. 8:2, 542-556.

Quirós, P. M., Langer, T., and López-Otín, C. (2015). New roles for mitochondrial proteases in health, ageing and disease. Nat. Rev. Mol. Cell Biol. 16:6, 345.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Seibel, B.A., Maas, A.E. and Dierssen, H.M. (2012). Energetic plasticity underlies a variable response to ocean acidification in the pteropod, Limacina helicina antarctica. PLoS One. 7:4, p.e30464.

Sokolova, I. M., Frederich, M., Bagwe, R., Lannig, G., and Sukhotin, A. A. (2012). Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar. Environ. Res. 79, 1-15.

Todgham, A. E., and Stillman, J. H. (2013). Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. Integr. Comp. Biol. 53:4, 539-544.

Tomanek, L. (2011). Environmental proteomics: changes in the proteome of marine organisms in response to environmental stress, pollutants, infection, symbiosis, and development. Annu. Rev. Mar. Sci. 3, 373-399.

Vuori, K., Kiljunen, M., Kanerva, M., Koljonen, M.L. and Nikinmaa, M. (2012). Stock‐specific variation of trophic position, diet and environmental stress markers in Atlantic salmon Salmo salar during feeding migrations in the Baltic Sea. J. Fish Biol. 81:6, 1815-1833.

Vuori, K. A., Lehtonen, K. K., Kanerva, M., Peltonen, H., Nikinmaa, M., Berezina, N. A., and Boikova, E. (2015). Oxidative stress biomarkers in the copepod Limnocalanus macrurus from the northern Baltic Sea: effects of hydrographic factors and chemical contamination. Mar. Ecol. Prog. Ser. 538, 131-144.

Wood, H. L., Spicer, J. I., and Widdicombe, S. (2008). Ocean acidification may increase calcification rates, but at a cost. P. Roy. Soc. Lond. B Bio. 275:1644, 1767-1773.

Yin, H., and Porter, N. A. (2003). Specificity of the ferrous oxidation of xylenol orange assay: analysis of autoxidation products of cholesteryl arachidonate. Anal. Biochem. 313:2, 319-326.

Zenebe, W.J., Nazarewicz, R.R., Parihar, M.S. and Ghafourifar, P. (2007). Hypoxia/reoxygenation of isolated rat heart mitochondria causes cytochrome c release and oxidative stress; evidence for involvement of mitochondrial nitric oxide synthase. J. Mol. Cell. Cardiol. 43:4, 411-419.

Zuur, A.F., Ieno, E.N., Smith, G.M. (2007). Analysing Ecological Data. Springer, New York, NY.