Universidad de Concepción
Postgraduate School
Faculty of Natural an Oceanographic Sciences
PhD program in Oceanography

Physiological responses to upwelling-induced hypoxia in epipelagic copepods inhabiting the coastal zone in central-southern Chile.

Respuestas fisiológicas a la hipoxia inducida por la surgencia en copépodos epipelágicos que habitan la zona costera del centro-sur de Chile.

Thesis to qualify for the degree of PhD in Oceanography

Leissing Frederick Figueroa

Concepción- Chile

2024

Supervisor: Rubén Escribano Veloso
Co-supervisor: Mauricio Urbina Foneron
Oceanography Department, Faculty of Natural and Oceanographic Sciences.
University of Concepción.

University of Concepción

Postgraduate Direction

The Doctoral Thesis in Oceanography entitled "Physiological responses to hypoxia induced by upwelling in epipelagic copepods that inhabit the coastal zone of central-southern Chile", by Ms. Leissing Frederick Figueroa and carried out under the Faculty of Natural and Oceanographic Sciences, University of Concepción, has been approved by the following Evaluation Commission:

PhD. Rubén Escribano
Guide Professor
Universidad de Concepción ______________________________

PhD. Mauricio Urbina
Co-Guide Professor
Universidad de Concepción ______________________________

PhD. Osvaldo Ulloa
Thesis Committee Member
Universidad de Concepción ______________________________

PhD. Ángel Urzúa Osorio
Thesis Committee Member
Universidad Catolica de la Santisima Concepción ______________________________

PhD. Kurt Paschke La Manna
External Evaluator
Universidad Austral de Chile ______________________________

PhD. Pamela Hidalgo
Director
PhD Program in Oceanography
Universidad de Concepción ______________________________

ACKNOWLEDGEMENT

I would like to express my gratitude to my supervisors Dr. Ruben Escribano and Dr. Mauricio Urbina, for their guidance and support throughout my PhD and for believing in my work.

I would like to thank the "Agencia Nacional de Investigación y Desarrollo", ANID, for funding my PhD study. Also, I would like to thanks to "Instituto Milenio de Oceanografía-IMO" and Grant FONDECYT Nº 1210071 and Grant FONDECYT 1181682 for funding this study.

Many thanks to ECOP and the Animal Physiology laboratory for their support and company during the long days of experiments in the laboratory: Daniel, Susana, Erika, Javier and Rocio.

I also want to thank my friends Camila, Cristina and Carcamin, for being a support every day and always being close despite the time and distance and refuge in the gray days. To my "partners", with whom we survived this doctorate, Carlos, Macarena and Reynier. And of course thank my friends for laughter, lunches and unconditional support Valentina, Daniel, Susana and Katty.

"Agradecer a quienes son el pilar fundamental en mi vida, mi amada familia. Me enseñaron el valor de trabajo duro y la perseverancia. A nunca rendirme sin intentar y dar lo mejor de mí pese a la adversidad. Por su comprensión e infinito amor, esta tesis también es fruto de su trabajo".

And lastly, thank my "adventure buddy" Mario, for you love and being my safe place. The journey has just begun, there is much to discover and stories to write.

Curriculum vitae

Leissing Frederick Figueroa

Born on April 2, 1986, Concepción, Chile

2011: College degree in Marine Biology, Universidad de Concepción. Chile

2017: Master´s in Science degree whit a minor in Oceanography, Universidad de Concepción. Chile.

2018-2024: Candidate of PhD in Oceanography, Universidad de Concepción. Chile.

RESEARCH AREAS

Principal: Biological Oceanography

Secondary: Plankton Ecology

Others: Physiology

PUBLICATIONS

Frederick, L., Urbina-Foneron, M., and Escribano, R. 2024. Adjusting metabolic rates and critical oxygen tension in planktonic copepods under increasing hypoxia in highly productive coastal upwelling zones, Limnology and Oceanography, 9999:1-14.

Frederick, L., Escribano, R., Morales, C.E., Hormazabal, S., Medellín-Mora, J. (2018). Mesozooplankton respiration and community structure in a seamount region of the eastern South Pacific. Deep Sea Research Part I: Oceanographic Research Papers, 135: 74-87.

Escribano, R; Hidalgo, P; Valdés, V & **Frederick**, L. (2013). Temperature effects on development and reproduction of copepods in the Humboldt Current: the advantage of rapid growth. Journal of Plankton Research, 36(1): 104-116.

LABORAL EXPERIENCE

- 2017- 2018. Research assistant in Laboratory of Pelagic Ecology (ECOP) in Marine Biology Station, Dichato, of the University of Concepción.

- 2017- 2018. Research assistant in project ATACAMEX- LOWPHOX II, at the Millennium Institute of Oceanography (IMO) of Universidad of Concepcion.
- 2016. Research assistant in project LOWPHOX I, at the Millennium Institute of Oceanography (IMO) of Universidad of Concepcion.
- 2016. Research assistant in FONDECYT project 1130511 “Oceanographic-climatic processes modulating ecosystem responses in the Humboldt Current System: plankton indicators

TEACHING EXPERIENCE

- Instructor of the curricular course “Comparative Animal Physiology”, for the Biology and Marine Biology degree, University of Concepción (2022 and 2023)
- Research assistant Laboratory of Pelagic Ecology (ECOP) at the Marine Biology Station, Dichato, of the University of Concepción (2017-2019)
- Research assistant in the ATACAMEX- LOWPHOX II project, at the Millennium Institute of Oceanography (IMO) of the University of Concepción (2017-2018)
- Research assistant in the LOWPHOX I project, at the Millennium Institute of Oceanography (IMO) of the University of Concepción (2016)
- Research assistant in the FONDECYT project 1130511 “Oceanographic-climatic processes that modulate the responses of ecosystems in the Humboldt Current System: plankton indicators” (2016)
- Instructor of the curricular course “Pelagic Systems” for the Marine Biology degree, University of Concepción (2014 to 2017)

RESEARCH PROJECTS EXPERIENCE

- Thesis student FONDECYT 1210071 “Metabolic and molecular responses to environmentally relevant multiple stressors in the oxygen minimum zone (OMZ): hypoxia and elevated carbon dioxide, by Dr. Mauricio Urbina, Universidad de Concepción, Chile (2021-2023)

- Thesis student FONDECYT project N°. 1181682 "Zooplankton Ecology in Upwelling Systems" (ZEUS), by Dr. Rubén Escribano, Universidad de Concepción, Chile (2018-2021)
- Researcher in IAI-ANTARES project and as Research Assistant at the Millennium Institute of Oceanography (2017)
- Research assistant, FONDECYT Project 1130511 "Oceanographic-climatic processes modulating ecosystem responses in the Humboldt Current System: plankton indicators" by Dr. Rubén Escribano, University of Concepción. (2013-2014)
- Collaborator in Oceanographic Concepción Time Series. FONDAP COPAS Center, University of Concepción (2013)
- Research assistant: FONDECYT Project 1110539 "Ocean stratification and its impact on the metazooplanktonic system: An experimental approach" (2012)

SCHOLARSHIP AND FINANCING

- 2022-2024. Financial support granted by Instituto Milenio de Oceanografía, IMO-Chile.
- 2021. Financial support granted by FONDECYT 1210071
- 2020. Financial support granted by FONDECYT 1181682
- 2018. National PhD scholarship ANID 20181372.
- 2016. Financial support granted by FONDECYT 1120478.
- 2015. Financial support by Millennium Institute of Oceanography (IMO).
- 2014. DPG Scholarship University of Concepción.

OCEANOGRAPHIC CAMPAIGNS

- ATACAMEX-LOWPHOX Oceanographic Cruise aboard Cabo de Hornos Oceanographic Vessel. Project funded by CONICYT (2017)
- CIMAR Oceanographic Cruise 22- Desventuradas Islands aboard the Cape Horn Oceanographic Vessel. Project funded by CONA (2016)

- MOPEX Oceanographic Cruise aboard the Cabo de Hornos Oceanographic Vessel. Project funded by CONICYT AUB160003 (2016)
- LOWPHOX Oceanographic Cruise aboard the Cape Horn Oceanographic Vessel. Project funded by CONICYT (2015)
- FIP-Seamounts Oceanographic Cruise aboard the Abate Molina Oceanographic Vessel (2015)

PARTICIPATION IN COURSES AND CONFERENCES

- **Frederick, L.**, M. Urbina, E. Jorquera & R. Escribano. 2023. Ocean deoxygenation may drastically constraint planktonic assemblies in highly productive coastal upwelling zones. ASLO- Aquatic Sciences Meeting 2023, which took place from June 4 to 9 in Palmas de Mallorca, Spain.
- **Frederick, L**., Urbina, M. & Escribano R. 2022. Metabolic rate and critical partial pressure of oxygen (P_{crit}) in three planktonic copepods inhabiting the coastal upwelling zone off Chile. EBUS. Lima Peru.
- Course "Strengthening technical-scientific capabilities of new methods for the study of marine biodiversity in Colombia and Chile, BIOMACC." Project coordinator for Chile. Presidential Agency for International Cooperation of Colombia, APC-Colombia and Chilean Agency for International Development Cooperation, AGCID-Chile (2020)
- Course "The 1st Global Ocean Oxygen Network (GO2NE) International Summer School" held. September 2-8, 2019, Xiamen, China, within the framework of the study of ocean deoxygenation.
- **Frederick, L**., R. Escribano & J. Medellín-Mora. 2017. Community respiration of mesozooplankton in northern and south-central Chile. XVII COLACMAR. Camboriu Brazil.
- **Frederick, L**., Escribano, R. 2017. The interplay between zooplankton diversity and metabolism in an Eastern boundary upwelling system. IMBIZO 5: Marine biosphere research for a sustainable ocean: Linking ecosystems, future states and resource management. Woods Hole Oceanographic Institution, Woods Hole, MA, USA.

- **Frederick, L**., R. Escribano. 2017. Metabolic rates of zooplankton associated with their community structure in three Seamounts in the oligotrophic region of the eastern South Pacific. XXXVIII Congress of Marine Sciences. Valparaíso Chile.
- **Frederick, L**., R. Escribano. 2016. Metabolic response of the dominant zooplankton off the Chilean coast and its relationship with the zone of minimum oxygen. XXXVII Congress of Marine Sciences. Concepción Chile.
- **Frederick, L.**, R. Escribano. 2015. Community respiration of zooplankton in the coastal upwelling zone of Chile. XVI COLACMAR-SENALMAR. Santa Marta. Colombia

OUTREACH EXPERIENCE

- Speaker in the project "Strengthening technical-scientific capabilities of new methods for the study of marine biodiversity in Colombia and Chile, BIOMACC". Project coordinator for Chile. Presidential Agency for International Cooperation of Colombia, APC-Colombia and Chilean Agency for International Development Cooperation, AGCID-Chile.
- Monitor at "Nuestro Oceano" scientific camp within the framework of the 4th International Marine Protected Areas Congress, IMAPCT4. Coquimbo, Chile (2017)
- CHILE-VA project monitor. Project funded by EXPLORA CONICYT. Tome, Chile (2016)
- CHILE-VA project monitor. Project funded by EXPLORA CONICYT. Chillán, Chile (2016)

TABLE OF CONTENTS

RESUMEN………………………………………………………………….………… i

ABSTRACT…………..…………………………………………………………… ii

1. INTRODUCTION..1

1.1 Ocean Deoxygenation ……………………………………………………….. 2

1.2 Deoxygenation in Eastern Boundary Upwelling Systems (EBUS)…...…………5

1.3 Impact of Hypoxia in Zooplankton……………………………………………...9

1.4 Zooplankton Mechanisms to deal with Hypoxia………………………13

1.4.1 Ecological Effects……………………………………………………13

1.4.1.1Vertical Distribution………………………………………...13

1.4.1.2 Reproduction………………………………………………..16

1.4.1.3 Body Size…………………………………………………….. 17

1.4.2 Physiological Effects…………………………………………………17

1.4.2.1 Anaerobic Metabolism…………………………………….. 17

1.4.2.2 Depressed Metabolic Rate…………………………………18

1.4.2.3 Preparation to Oxidative Stress (POS)…………………….. 21

1.4.2.4 Antioxidant Defense………………………………………..22

2. HYPOTHESES AND SPECIFIC GOALS...24

2.1 General goal……………………………………………………………………24

2.2 Hypotheses…………………………………………………………………..24

2.3 Specific goals…………………………………………………………………..25

3. MATERIAL Y METHODS…………………………………………………………...25

3.1 Study area…………………………………………………………………..25

3.2 Oceanographic data……………………………………………………...27

3.3 Biological sampling…………………………………………………………..28

3.4 Experimental design………………………………………………………….29

3.4.1 Metabolic Rate and P_{crit} Estimation……………………………29

3.4.2 Data Analysis and Statistics……………………………………….32

4. RESULTS..33

4.1 Chapter 1: Adjusting metabolic rates and critical oxygen tension in planktonic copepods under increasing hypoxia in highly productive coastal upwelling zones ………...33

4.2 Chapter 2: Reviews and synthesis on increasing hypoxia in eastern boundary upwelling systems: a major stressor for zooplankton……………………………………….50

5. DISCUSSION...77

6. CONCLUSIONS...84

7. REFERENCES...85

8. ANNEXES…………………………………………………………………………..99

INDEX OF FIGURES

Figure 1: Global dissolved oxygen changes (mmol m^{-3} per year) from 1920 to 2100 under the RCP8.5 scenario. The linear trends of oceanic oxygen changes are estimated in the (a) epipelagic, (b) mesopelagic, and (c) bathypelagic zones. The blue and red color of color represent the deoxygenation and oxygenation, respectively……………………………………2

Figure 2: Processes affecting the O_2 dynamics in the ocean and mechanisms of oxygen minimum zones (OMZs) formation……………………………………………………….4

Figure 3: Schematic view of thermocline ventilation patterns with, as an example, a focus on the eastern subtropical North Pacific OMZ. The wind-stress curl in the subtropics drives subduction of oxygenated waters that, via ocean interior circulation or western boundary currents, reaches biotically productive upwelling regions at the eastern boundary. High oxygen demand, together with weak oxygen supply due to sluggish circulation, results in the occurrence of an OMZ. The inset shows processes particularly relevant to OMZs connected to eastern boundary upwelling…………………………………………………………….6

Figure 4: Zooplankton abundances, biomass, and oxygen. For oxygen (top row), purple shading indicates low oxygen (≤5 µM, 0.11 mL/L), and gray shading indicates high oxygen (≥8 µM, 0.18 mL/L). For copepods (second row), *P. abdominalis* is shown for shallower tows and *L. hulsemannae* is shown for the deep tow. The next rows show total euphausiids, the fish *Cyclothone* spp., and zooplankton biomass. All graphed taxa show significant abundance differences between samples in high versus low oxygen categories, except 800m euphausiids and 800-m total biomass…………………………...…………10

Figure 5: Daily vertical migration of dominant species of zooplankton in and out of the main OMZ core (shaded area)………………………………………………………………15

Figure 6: Oxygen consumption rate decreases with decreasing environmental oxygen tension (ρO2) when ρO2 < P_{crit}. On the other hand, oxygen consumption rate is unaffected by the changes in oxygen tension when ρO2 > P_{crit} ……………………………………………20

Figure 7: Total energy (ATP) demand is influenced by seawater oxygen partial pressure (ρO2) below critical values. The solid blue line represents the effect of increasing ATP demand on the critical oxygen partial pressure at a basal rate of metabolism (P_{crit}). The P_{crit} is indicated by the horizontal line. The white region indicates that anaerobic metabolism contributes to maintain total ATP flux. In the gray region anaerobic metabolism is insufficient to maintain ATP flux. Metabolism must be suppressed below that point to avoid impaired function. As pO_2 approaches P_{crit}, respiratory adjustments may be made to maintain the rate of oxygen provision to sites of consumption…………………………………..21

Figure 8: Map illustrating Surface ocean currents in the eastern South Pacific along the Chilean coast, northward: the Humboldt Current and southward, the Cape Horn Current, and the subsurface and intermediate water masses…………………………………………...26

Figure 9: Map illustrating the sampling area (red box) in Coliumo Bay. Additionally, the offshore stations corresponding to the ZEUS series sampling are shown…………………28

FIGURES FROM THE ARTICLE "ADJUSTING METABOLIC RATES AND CRITICAL OXYGEN TENSION IN PLANKTONIC COPEPODS UNDER INCREASING HYPOXIA IN HIGHLY PRODUCTIVE COASTAL UPWELLING ZONES"

Figure 1: Metabolic rate as a function of environmental oxygen and estimated critical partial pressure of oxygen (P_{crit}) in three copepod species, *Calanoides patagoniensis*, *Paracalanus* cf. *indicus,* and *Acartia tonsa*. These species inhabit the coastal upwelling of central-southern Chile and were captured in two seasonal periods (spring/summer and autumn-winter). Different colors represent replicated experiments within seasonal upwelling conditions. The vertical arrows signal P_{crit} values…………………………………………………………40

Figure 2: Variability in Temperature, Chlorophyll-a, and Dissolved Oxygen. The seasonal cycle of A: temperature, B: Chlorophyll-a, C: Dissolved Oxygen in the water column at Station 18 in central-southern Chile. The seasonal cycle was obtained from monthly means from the period 2002-2016. D: Vertical distribution of species-specific depth of P_{crit} in relation to the annual variability of oxygen in the column water at station 18 off central/southern Chile. Continuous line is P_{crit} estimated during the spring-summer and the dotted line represents P_{crit} depth for an autumn-winter condition…………………………..41

Figure 3: Vertical distribution of three copepods in the coastal upwelling zone of central-southern Chile during the austral winter 2008. A) *Calanoides patagoniensis,* B) *Paracalanus* cf. *indicus,* C) *Acartia tonsa*. Stations are over a cross-shelf transect from the coast (St1, 50 m depth) westward to most oceanic station (St5, 500 m depth) (see Fig. S1, Supp. Mat.). Only St5 included a daytime vs a nighttime sampling, while the other stations were sampled in daylight conditions. The gray bars in St5 showed nighttime abundances………………...43

Figure 4: The expected metabolic rate as a function of environmental oxygen partial pressure. The metabolic rate is theorized for two seasons in a coastal upwelling zone based on two situations: A: lack of adaptive response, where P_{crit} will move following changes in MR, B: showing an adaptive response 1 in which P_{crit} increases upon a changing oxyconforming slope to maintain MR. In response 2, P_{crit} is maintained by increasing the slope of the oxyconforming segment during the upwelling season. The green shaded area represents the possible metabolic scope, which only remains unaffected in the adaptive response depicted in B………………………………………………………………...45

FIGURES FROM THE ARTICLE "REVIEW AND SYNTHESIS ON INCREASING HYPOXIA IN EASTERN BOUNDARY UPWELLING SYSTEMS: A MAJOR STRESSOR FOR ZOOPLANKTON"

Figure 1: Projected effects of increasing upwelling in eastern boundary upwelling systems (EBUS). A) Under present (initial) conditions, wind-driven upwelling rises the OMZ system and brings cold-water into shallow depths at the inshore as illustrated by the 12°C isotherm, and so fertilizes the photic zone and promotes plankton aggregation. B) Ocean warming effects manifest mainly at surface in the offshore region, while increased upwelling may instead cool down the coastal zone (B), also vertically expanding and further shoaling the OMZ at the inshore area, causing more hypoxia and reducing the oxygenated habitat………………………………………………………………………………………55

Figure 2: Two adaptive modes in marine zooplankton as a response to hypoxia (modified from Rogers et at. (2016))……………………………………………………………………57

Figure 3: The expected metabolic rate as a function of environmental oxygen partial pressure. The metabolic rate is theorized for two seasons: Autumn-winter (blue line) and spring-summer (red line) in a coastal upwelling zone, where P_{crit} will move following changes in MR (A), an adaptive response 1 in which P_{crit} increases upon a changing oxyconforming slope to maintain MR (B) and adaptive response 2, where P_{crit} is maintained by increasing the slope of the oxyconforming segment during the upwelling season (C) (modified from Frederick et al, 2024)……………………………………………………………………….58

Figure 4: Changes in the daily vertical migration and short-term response to oxidative stress in zooplankton. Illuminated area shows the daytime……………………………………...64

Figure 5: Oxidative stress (ROS) and the potential adaptive response (POS) of zooplankton subjected to seasonal upwelling-hypoxia conditions……………………………………….66

TABLE INDEX

Table 1: Tolerance to hypoxia in pelagic communities. Arrows in column 6 indicate the direction of impact when oxygen drops below the critical value (Extracted by Ekau et al, 2010)……………………………………………………………………………………….12

Table 2: Campaigns carried out to obtain plankton samples during spring-summer and autumn-winter 2021……………………………………………………………………..29

Table 3: Summary of respiration rate experiments for three planktonic copepods, which were carried out between January 2021 and October 2021. n=number of individuals per vial and Inc. time is the incubation time. Temperature was kept constant at 13°C for all experiments in a cold room and dark conditions……………………………………………………….30

Table 4: Mean and standard deviation of size of copepods to experimental periods. N is number of organisms measured. For testing differences in size, T-test was applied to *C. patagoniensis* and *A. tonsa*, while for *P.* cf. *indicus* we applied an ANOVA……………………………………………………………………….………...31

TABLES FROM THE ARTICLE "ADJUSTING METABOLIC RATES AND CRITICAL OXYGEN TENSION IN PLANKTONIC COPEPODS UNDER INCREASING HYPOXIA IN HIGHLY PRODUCTIVE COASTAL UPWELLING ZONES"

Table 1: Metabolic rate (MR) and critical oxygen partial pressure (P_{crit}). MR and P_{crit} were estimated for three planktonic copepods (mean ± standard deviation), *C. patagoniensis, P.*

cf. *indicus* and *A. tonsa* in two seasons; A-W: autumn-winter, S-S: spring-summer. Bold values indicate significant differences (P<0.05) between species…………………………39

TABLES FROM THE ARTICLE "REVIEW AND SYNTHESIS ON INCREASING HYPOXIA IN EASTERN BOUNDARY UPWELLING SYSTEMS: A MAJOR STRESSOR FOR ZOOPLANKTON"

Table 1: Zooplankton species exposed to different stress leading to ROS. DVM (daily vertical migration) can be "Y" (yes migration) or "N" (non-migration) and "NI" (no information). Arrows in column 5 indicate the increase or decrease in the biomarker signal in relation at the stressor agent. * indicate that the samples were obtained from areas having an Oxygen Minimum Zone……………………………………………………………63

ANNEX 1: SUPPLEMENTAL MATERIAL FOR THE ARTICLE "ADJUSTING METABOLIC RATES AND CRITICAL OXYGEN TENSION IN PLANKTONIC COPEPODS UNDER INCREASING HYPOXIA IN HIGHLY PRODUCTIVE COASTAL UPWELLING ZONES"

Figure S1: The coastal upwelling zone of central-southern Chile in the Southeast Pacific, illustrating the sampling stations where oceanographic variables were measured and zooplankton samples were obtained. The square shows the area where copepods were captured for the experiments. The time series for monthly observations (2002-2020) on temperature, oxygen and chlorophyll-a was carried out at Station 18…………………….99

Figure S2: Annual P_{crit} for three species of planktonic copepods inhabiting the coastal upwelling of central-southern Chile. A: *C. patagoniensis*, B: *P.* cf. *indicus* and C: *A. tonsa*. Colors represent experiments conducted at different seasons. Vertical arrows signal average P_{crit} values, and its standard deviation is signaled by the light-yellow shading……………100

Figure S3: Variation of the oxygen content in the water column obtained from the time series at Station 18 (A) and Zeus monitoring (B) for the study area. Oxygen profiles were obtained with a SeaBird SBE Conductivity Temperature Depth (CTD) device, equipped with a SeaBird SBE-43 oxygen sensor…………………………………………………………101

Figure S4: Monthly variability in calculated vertical position of P_{crit}. Historical monthly variation in the depth at which P_{crit} values of three copepods species are encountered in the water column during the time series study (2002-2019) at Station 18 of central/southern Chile. A: *Calanoides patagoniensis,* B: *Paracalanus* cf. *indicus,* C: *Acartia tonsa*. P_{crit} were empirically estimated in two seasons: spring-summer and autumn-winter. Dotted-lines are smoothed series (3-months moving average)……………………………………………..102

Figure S5: Vertical oxygen profiles obtained during plankton sampling to carry out experiments on respiration rate of copepods. Profiles were obtained with a SeaBird SBE Conductivity Temperature Depth (CTD) device, equipped with a SeaBird SBE-43 oxygen sensor…………………………………………………………………………………...103

Figure S6: Monthly variation in the oxygen concentration in the first 50 m of depth, obtained from the time series at Station 18 (shown in Fig. S1). Oxygen profiles were obtained with a SeaBird SBE Conductivity Temperature Depth (CTD) device, equipped with a SeaBird SBE-43 oxygen sensor…………………………………………………………………………104

Fig. S7: Cross-shelf section of oxygen concentration in the coastal upwelling zone of central-southern Chile during the spring 2008 and the winter 2009 at the latitude of 36°30 S, illustrating the presence of the oxygen minimum zone (OMZ) in the subsurface layer. The dotted red line shows the upper limit of the OMZ (1 mL O_2 L^{-1})…………………………105

Table S1: Summary of respiration rate experiments for three planktonic copepods carried out between January 2021 and October 2021 in central-southern Chile. n=number of individuals per vial and Inc. time is the incubation time. Temperature was kept constant at 13°C for all experiments in a cold room and dark condition……………………………106

Table S2: Two-way ANOVA to test the effects of season and species on the respiratory rate (MR) and P_{crit} of three copepod species, *Calanoides patagoniensis*, *Paracalanus* cf. *indicus* and *Acartia tonsa*..………………………………………………………………………106

Table S3: One-way ANOVA to test the effect of season on the respiratory rate (MR) and P_{crit} of three copepods from the upwelling zone of central-southern Chile. *=Significant effect (P<0.05), **=Highly significant effects (P<0.01), ns=non-significant……………107

Table S4: Mean ± standard deviation of copepod sizes from variable experimental periods. (n) is the number of organisms measured. t-test was applied to *C. patagoniensis* and *A. tonsa*, while for *P.* cf. *indicus* we applied a one-way ANOVA. ns=non-significant differences (P>0.05)……………………………………………………………………………...107

Table S5: Mean and standard deviation of P_{crit} (kPa) to 10, 13 and 15°C to copepods estimated from slope of curve (P_{crit} vs MR)……………………………………………108

Table S6: The slopes ± Standard deviation of the oxyconforming regression after fitting the two-step regression model of metabolic rate as a function of oxygen pressure (kPa) to estimate P_{crit} in three copepods species. Seasonal effects on the slopes were tested by two-tailed t-test. *Significant differences (P<0.05), ns=non-significant differences (P>0.05)……………………………………………………………………………...108

Table S7: Time to reaching P_{crit} (T) and time to survival (TS) after reaching P_{crit}, and percentage of survivors (NS) after reaching P_{crit}…………………………………………109

Table S8: Location and depth strata used for zooplankton sampling performed in August 2008 in the coastal upwelling zone of central-southern Chile. Sampling was carried out with a midi-type Hydrobios Multinet horizontally towed. Stations location is also illustrated in Fig. S1………………………………………………………………………………109

RESUMEN

Los sistemas de surgencia de borde oriental (EBUS) son regiones marinas del océano mundial de importancia ecológica y económica. En estos sistemas, el zooplancton desempeña un papel clave en la transferencia de energía en las tramas tróficas. Estudios recientes muestran que el calentamiento global está provocando una desoxigenación gradual de los océanos del mundo, mientras que en EBUS una expansión vertical de la zona mínima de oxígeno subsuperficial (OMZ) lo que exacerba aún más las condiciones hipóxicas para el zooplancton que habita en la zona de surgencia costera. La hipoxia puede afectar al zooplancton al alterar sus tasas metabólicas, migración, reproducción y desarrollo. Sin embargo, estos efectos dependen de algunas adaptaciones específicas de los organismos que han evolucionado en hábitats, permanente o episódicamente, sujetos a aguas con poco oxígeno.

En el capítulo 1 se analizó las respuestas diferenciales en las tasas metabólicas de tres especies de copépodos, *Calanoides patagoniensis*, *Paracalanus* cf. *indicus* y *Acartia tonsa* expuestos a condiciones experimentales de hipoxia. Estas condiciones de bajo oxígeno, fueron asociadas a dos periodos del año: surgencia activa (primavera-verano) y no surgencia (otoño-invierno). Los resultados muestran que *Calanoides patagoniensis* duplicó su tasa metabólica durante la temporada de surgencia, indicando que aprovecha mejor la floración de fitoplancton de primavera-verano para alimentarse y reproducirse, manteniendo su presión parcial de oxígeno crítica sin cambios entre estaciones. Por el contrario, *Paracalanus* cf. *indicus* y *Acartia tonsa*, mantuvieron sus tasas metabólicas a lo largo de las estaciones, pero aumentaron significativamente su presión parcial crítica de oxígeno durante el período de

surgencia activa, volviéndose menos tolerantes a la hipoxia en primavera-verano. Al contrastar estos hallazgos con observaciones de series de tiempo, podemos ver que los niveles de oxígeno igual o inferior a la presión parcial crítica de oxígeno es una condición común (aproximadamente el 70% de la probabilidad de ocurrencia) para los copépodos durante un ciclo anual mientras habitan la capa superior de los 50 m. Estos resultados sugieren la existencia de un balance dependiente de la especie entre la tasa metabólica y la presión parcial crítica de oxígeno. Estas respuestas adaptativas dependientes de las especies, sugieren que la hipoxia exacerbada, impulsada por la desoxigenación del océano y el aumento de la surgencia, conducirá a un cambio en la distribución vertical de los copépodos como consecuencia de una compresión del hábitat, y aumentando su mortalidad, con consecuencias potencialmente drásticas para las tramas tróficas marinas.

En el capítulo 2 se realizó una síntesis bibliográfica de las respuestas adaptativas del zooplancton para resistir a la hipoxia leve o grave y el eventual estrés oxidativo derivado de condiciones de oxígeno altamente fluctuantes, en sistemas de surgencia de borde oriental (EBUS). Estudios recientes dan cuenta de una expansión vertical de la zona de mínimo oxígeno junto con una intensificación de la surgencia costera impulsada por el viento, exacerbando las condiciones hipóxicas para el zooplancton que habita la zona de surgencia costera. La presencia o ausencia de respuestas adaptativas puede desempeñar un papel crucial en la dinámica del zooplancton en EBUS con importantes consecuencias para su red alimentaria y su productividad biológica.

ABSTRACT

Eastern boundary upwelling systems (EBUS) are marine regions of the global ocean of ecological and economical importance. In these systems, zooplankton play a key role in the energy transfer through the food webs. Recent studies show that global warming is causing a gradual deoxygenation of the world's oceans, while in EBUS a vertical expansion of the subsurface oxygen minimum zone (OMZ) which further exacerbates hypoxic conditions for zooplankton living in the coastal upwelling zone. Hypoxia can affect zooplankton by altering their metabolic rates, migration, reproduction and development. However, these effects depend on some specific adaptations of organisms that have evolved in habitats, permanently or episodically, subjected to low-oxygen waters.

In chapter 1, the differential responses in the metabolic rates of three species of copepods, *Calanoides patagoniensis*, *Paracalanus* cf. *indicus* and *Acartia tonsa* exposed to hypoxic experimental conditions area assessed. These low oxygen conditions were associated with two periods of the year: active upwelling (spring-summer) and non-upwelling (autumn-winter). The results show that *Calanoides patagoniensis* doubled its metabolic rate during the upwelling season, indicating that it better takes advantage of the spring-summer phytoplankton bloom to feed and reproduce, maintaining its critical oxygen partial pressure unchanged between seasons. On the contrary, *Paracalanus* cf. *indicus* and *Acartia tonsa*, maintained their metabolic rates throughout the seasons, but significantly increased their critical partial pressure of oxygen during the period of active upwelling, becoming less tolerant to hypoxia in spring-summer. By contrasting these findings with time series observations, we found that oxygen levels equal to or less than the critical partial

pressure of oxygen is a common condition (approximately 70% probability of occurrence) for copepods during an annual cycle, while they inhabit the upper layer of 50 m. These results suggest the existence of a species-dependent balance between metabolic rate and critical oxygen partial pressure. These species-dependent adaptive responses, under oxygen levels ≤ the critical partial pressure of oxygen, suggest that exacerbated hypoxia, driven by ocean deoxygenation and increased upwelling, will lead to a change in the vertical distribution of copepods, as a consequence of habitat compression, and increasing their mortality, with potentially drastic consequences for marine food webs.

In chapter 2, a bibliographic synthesis of the adaptive responses of zooplankton to resist mild or severe hypoxia and the eventual oxidative stress derived from highly fluctuating oxygen conditions, in eastern edge upwelling systems (EBUS), was carried out. EBUS systems are of great ecological and economic importance, where zooplankton plays a fundamental role in carbon transfer in food webs. Recent studies show a vertical expansion of the oxygen minimum zone along with an intensification of wind-driven coastal upwelling as a result of climate change, further exacerbating hypoxic conditions for zooplankton inhabiting the coastal upwelling zone. The presence or absence of adaptive responses may play a crucial role in zooplankton dynamics in EBUS with important consequences for its food web and biological productivity.

1. INTRODUCTION

1.1 OCEAN DEOXYGENATION

Ocean deoxygenation is one of the primary marine environmental issues related to climate change (Keeling et al. 2010). Observational data have suggested that deoxygenation has occurred in most global oceans over the past decades (Helm et al. 2011; Schmidtko et al. 2017). In addition, ocean models predict a decline in the dissolved oxygen inventory of the global ocean of one to seven per cent by the year 2100 (Fig 1.), caused by a combination of a warming-induced decline in oxygen solubility and reduced ventilation of the deep ocean (Gong et al. 2021; Bopp et al. 2013; Froelicher et al. 2009; Oschlies et al. 2008).

Oxygen decline in ocean models is linked to warming-induced declines in oxygen solubility, and reduced ventilation of deeper waters from enhanced upper-ocean stratification (Keeling et al. 2010; Long et al. 2016). Changes detected over the past few decades have been attributed in part to these factors, but with additional complexities.

In time series, long-term oxygen trends are superimposed with variations at interannual to multi-decadal timescales consistent with natural climate variability, including thermal and wind forcing changes from the North Atlantic Oscillation (NAO) in the Atlantic Ocean (Stendardo et al. 2016), the Southern Annular Mode (SAM) in the Southern Ocean, or the El Niño/Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (PDO) (Deutsch et al. 2011). In the low-latitude, oxygen decline is attributed to a shoaling of the tropical and subtropical thermocline depth (Deutsch et al. 2011). For other regions, explanations include warming-induced changes in gases solubility, wind forcing and large-scale ocean circulation.

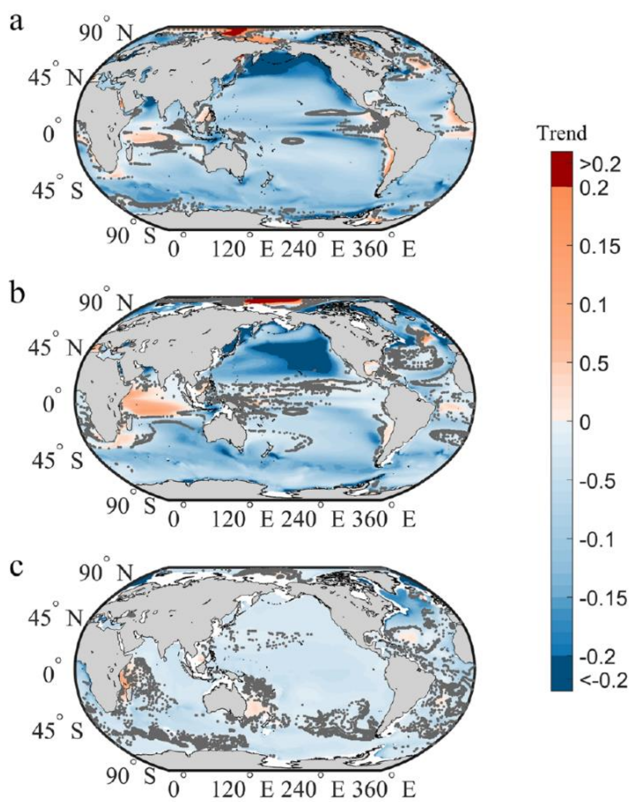

Figure 1: Global dissolved oxygen changes (mmol m−3 per year) from 1920 to 2100 under the RCP8.5 scenario. The linear trends of oceanic oxygen changes are estimated in the (a) epipelagic, (b) mesopelagic, and (c) bathypelagic zones. The blue and red color of color represent the deoxygenation and oxygenation, respectively (Gong et al. 2021)

Many oceanic regions exhibit a loss of oxygen surpassing 4% per decade in isolated areas near oxygen minimum zone (OMZ) (Schmidtko et al. 2017). The largest decrease, 373 ± 165 Tmol, is found in the tropical and North Pacific Ocean. Both areas already have low oxygen concentrations below the thermocline. The largest absolute losses of oxygen, up to

30 mol m−2 per decade, are found in the Equatorial and North Pacific Ocean, the Southern Ocean and the South Atlantic Ocean (Schmidtko et al. 2017).

In the open ocean the oxygen inventory has decreased (0.5–3%) and the OMZs are expanding. The dynamics of O_2 in the ocean is governed by both physical, biogeochemical and biological processes (Fig. 2). The ocean gains O_2 in the upper layer (0–100 m) due to photosynthesis and dissolution from the atmosphere in undersaturated waters near the surface. Conversely, O_2 is lost due to the diffusion of O_2 to the atmosphere in over-saturated surface waters, and from the respiration of aerobic organisms and oxidation of chemical species in the water column and sediments. In fact, in the coastal ocean, O_2 deficient zones are mostly in the benthic boundary layer where the O_2 consumption by respiration outpaces the O_2 supply by ventilation and bottom photosynthesis (Pitcher et al. 2021).

The surface mixed layer is well oxygenated in most of the ocean, but below the sunlit surface layer, there is no photosynthesis and, the renewal of the oxygen consumed requires a physical mechanism that transports relatively well-oxygenated waters to regions of lower oxygen. This mechanism, called ocean ventilation, is responsible for the oxygenation of the ocean interior and modifies the distribution of oxygen within the ocean. The distribution of oxygen in the ocean interior thus offers information on physical and biogeochemical mechanisms in the ocean.

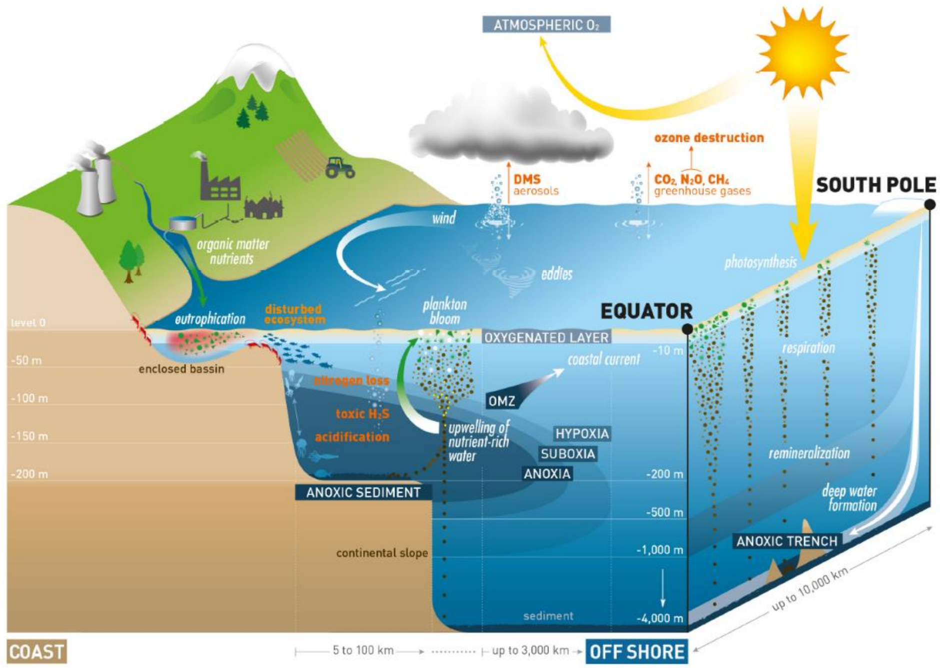

Figure 2: Processes affecting the O_2 dynamics in the ocean and mechanisms of oxygen minimum zones (OMZs) formation (Paulmier 2017).

In some regions where the renewal of oxygen by physical transport is low, the oxygen concentration can reach levels that are too low to support the survival of many aerobically respiring organisms. Organisms that are found in these areas can exhibit reduced growth and reproduction and, in extreme cases and for non-mobile species, experience low-O2 induced mortality (Levin et al. 2009). In the open ocean, OMZs are mainly found in the Eastern boundary upwelling systems (EBUS). In these OMZs, the degradation of the organic material sinking from the surface takes place through denitrification leading to a consumption of fixed nitrogen and production of N_2 and potentially N_2O. Observational analysis and model results indicate that OMZs have expanded during recent decades (Stramma et al. 2012; Schmidtko et al. 2017).

1.2 DEOXIGENATION IN EASTERN BOUNDARY UPWELLING SYSTEMS

Eastern Boundary Upwelling Systems (EBUS) are biologically productive marine regions of the world ocean covering only about 1% of the ocean surface area, although they are widely recognized as very important for the world's fisheries (Pauly & Christensen. 1995). The high productivity of these systems results from the ocean-atmosphere interaction, modulated by equatorward winds in combination with the Coriolis force, and so inducing wind-driven coastal upwelling near the coast (Strub et al. 1998). This advective process causes the ascent of deeper, cold, and nutrient-rich waters with high CO_2 concentrations, low pH, and low dissolved oxygen concentrations into the coastal photic zone (Huyer 1983), thus promoting new primary production (Fig. 3). In EBUS, the key underlying physical process is forced by the cross-shore atmospheric pressure gradients leading to alongshore, equatorward winds that drive coastal upwelling (Garcia-Reyes et al. 2015).

During the twentieth century, global ocean temperatures have increased and are expected to continue to increase with global warming (Stocker et al. 2013). However, it is known that in EBUS, sea surface temperatures in the coastal zone are exhibiting negative trends compared to other coastal regions at similar latitudes, due to increasing wind-driven upwelling (Bakun et al. 2010; Xiu et al. 2018). Therefore, trends in coastal temperature in EBUS are expected to differ substantially with regional and global patterns of global warming. With the surface heating of the sea and the increase in stratification of the water column, a reduction in the ventilation of deep-water masses is also foreseen (Oschlies 2019). As these water masses continue to be forced to the surface by the upwelling process, a future decrease in oxygen concentration and pH and an increase in nutrient concentration have been projected (Xiu et al. 2018). Persistent changes in pH or oxygen can have important repercussions in the biotic environment, such as the displacement of suitable habitats (Grantham et al. 2004; Breitburg et al. 2018).

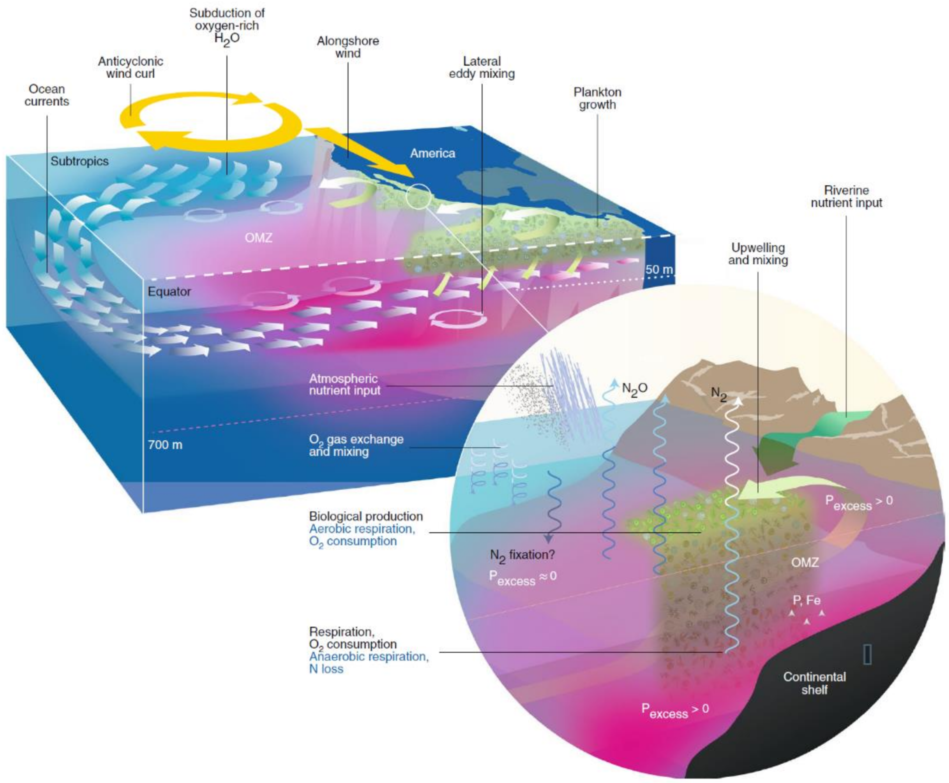

Figure 3: Schematic view of thermocline ventilation patterns with, as an example, a focus on the eastern subtropical North Pacific OMZ. The wind-stress curl in the subtropics drives subduction of oxygenated waters that, via ocean interior circulation or western boundary currents, reaches biotically productive upwelling regions at the eastern boundary. High oxygen demand, together with weak oxygen supply due to sluggish circulation, results in the occurrence of an OMZ. The inset shows processes particularly relevant to OMZs connected to eastern boundary upwelling (Oschlies 2018)

The decrease in oxygen concentration, which is evident in many areas of the ocean, becomes more critical in regions with oxygen minimum zones (OMZ). These OMZ´s are defined by their extremely low oxygen concentrations (<20-45 μmol kg $^{-1}$), covering large areas of the ocean and associated with very productive coastal and oceanic regions (Gilly et al. 2018). An expansion of OMZs is thus suggested and it is related to a shoaling of its upper boundary (and descent of the lower boundary), increasing the volume in a given area. In some cases, the minimum oxygen concentrations in the OMZ cores have also decreased, intensifying the OMZ (Chan et al. 2008; Stramma et al. 2008). Oxygen plays a key role in the structuring and functioning of marine ecosystems and it may control the spatial-temporal distribution of many marine organisms, because low oxygen levels, considered as hypoxic, can be harmful for most of the biota. The effects of a decrease in oxygen can affect organisms in many ways, including acute physiological impairment, changes in growth and reproductive success, and altered behavior of mobile forms searching for more favorable oxygen regimes (Stramma et al. 2010). At the ecosystem level, the tolerances to low oxygen of several species to a changing environment will result in a differential success among species, and consequently alterations in community structure and trophic webs, inducing changes in the predator-prey dynamics and changes in the abundance and accessibility of commercial species. While many of the species will be negatively affected by these effects, others more tolerant to hypoxia may expand their range of distribution or exploit new niches (Stramma et al. 2010; Breitburg et al. 2018).

The adaptation of animals to low oxygen is triggered by a strong selective pressure to maintain aerobic metabolism by optimizing and enhancing oxygen uptake from hypoxic water (Childress & Seibel 1998). The physiological adaptations that permit occupation of the

OMZ have been studied in some species. For example, one of the mechanisms for the incursion of organisms in the OMZ during daytime migration is the metabolic suppression, which is an adaptive response of cells to minimize damage caused by oxidative stress (Seibel 2011). However, little is known on the molecular changes taking place upon these adaptations. At the molecular level, plankton responses to changes in oxygen levels are not known nor understood. For instance, the generation of reactive oxygen species (ROS) that can increase under hypoxia has been described as causing harmful effects on proteins, lipids and DNA, such that if the ROS levels exceed the cell defense systems, as an antioxidant to maintain a redox balance, the fate of the cell with irreversible lesions can be death (Kowaltowski et al. 2009).

Given the high variability in physical-chemical parameters of the water column, planktonic organisms need to regulate their physiology to ensure survival. All the changes in the physiology of organisms are a result of gene expression that allows them to cope with these variations. In this context, the present thesis proposes to establish a relationship between the level of expression of genes coding for certain antioxidant enzymes, the concentration of dissolved oxygen and the critical exposure time to carry out the expression of genes. All these processed as required to ensure the survival of planktonic copepods inhabiting the OMZ in coastal upwelling areas of the eastern South Pacific off Chile.

1.3 IMPACT OF HYPOXIA IN ZOOPLANKTON

Oxygen is essential for organisms with aerobic metabolism. For all of them, lack of oxygen is highly deleterious. The ability of an organism to tolerate environmental stress is directly related to its ability to maintain an internally balanced physiological state (i.e. homeostasis) to counteract stressor-induced physiological perturbations.

Oxygen loss in subsurface waters of the world's oceans is now recognized as a critical environmental and ecological issue associated with ongoing climate change. In OMZs, are projected to expand in intensity and extent, based on recent data and models (Stramma et al. 2008; Keeling et al. 2010). Zooplankton are critical components of midwater food webs and biogeochemical cycles, serving as a major trophic link between primary producers (phytoplankton) and larger animals, including marine mammals and commercially important fishes and squids. Their feeding, defecation, respiration, and vertical migration in the water column affect the vertical transport of carbon and particles to depth as part of the biological pump (Robinson et al. 2010; Steinberg et al. 2017). Zooplankton distributions, diel vertical migration, and ecological functions are strongly affected by the vertical oxygen gradients of the OMZ (Wishner et al. 2018, 2013). Vertical oxygen gradients in the OMZ strongly affect zooplankton distributions, daily vertical migration, and ecological functions (Wishner et al. 2013). For example, it has been observed that there are subsurface maxima of zooplankton biomass, certain abundances of species and trophic activities that are associated with the upper and lower edges of the OMZ (Wishner et al. 2018) (Fig. 4). The depth, abundance, and composition of these unique communities in the water column and thus their effects on food webs and particle fluxes vary with the thickness of the OMZ.

Many animals living in the OMZ also have unique physiological adaptations for tolerating extreme hypoxia and maintaining metabolic function at very low oxygen concentrations (Childress et al. 1998; Seibel et al. 2016). However, species that occur in the most pronounced OMZs appear to be living at oxygen levels lower than previously measured tolerance and must be near their physiological limits (Seibel 2011). Thus, small changes in oxygen concentration may have important consequences for mesopelagic and deep-sea communities.

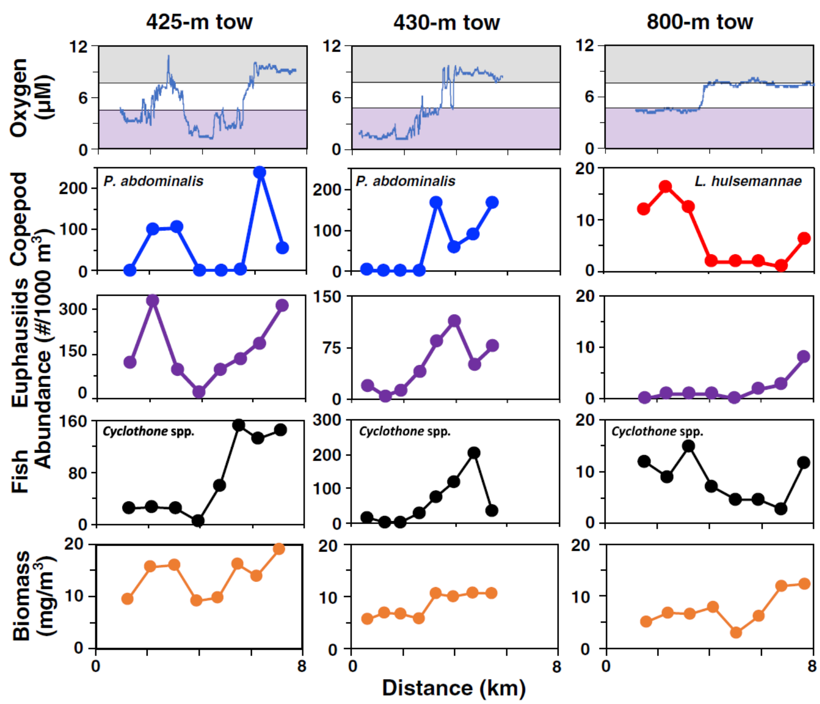

Figure 4: Zooplankton abundances, biomass, and oxygen. For oxygen (top row), purple shading indicates low oxygen (≤5 µM, 0.11 mL/L), and gray shading indicates high oxygen (≥8 µM, 0.18 mL/L). For copepods (second row), *Pleuromamma abdominalis* is shown for shallower tows and *Lucicutia hulsemannae* is shown for the deep tow. The next rows show total euphausiids, the fish *Cyclothone* spp., and zooplankton biomass. All graphed taxa show

significant abundance differences between samples in high versus low oxygen categories, except 800m euphausiids and 800-m total biomass (Wishner et al. 2018).

Impacts of hypoxia on the structure and processes in pelagic communities has been review by Ekau et al. (2010) (Table 1). Some organism show a tolerance to hypoxia, example small crustacean such as copepods and euphausiids can tolerate much lower dissolved oxygen levels than larger zooplankton such as scyphozoans and ctenophores. In most cases, hypoxia negatively affects egg production (Sedlacek & Marcus 2005), survival (Roman et al. 1993; Auel & Verheye 2007), and egg hatching (Lutz et al. 1994). Therefore, hypoxia can be a selective force for zooplankton.

In the work of Marcus (2011), she shows an indirect evidence of hypoxia adaptation in that zooplankton from OMZs are less sensitive to hypoxia than zooplankton from coastal waters, where hypoxia is more seasonal. Moreover, crustaceans inhabiting the OMZ have evolved a variety of physiological adaptations to enhance oxygen uptake: enhanced ventilator capability, large gill surfaces, short diffusion distances, and respiratory proteins with very high oxygen affinity (Childress & Seibel 1998). However, the strategy of zooplankton inhabiting the OMZ appears to be a combination of short-term anaerobic metabolism coupled with vertical migration into well-oxygenated zones to repay the oxygen debt generated while in the OMZ (Marcus 2011).

Table 1: Tolerance to hypoxia in pelagic communities. Arrows in column 6 indicate the direction of impact when oxygen drops below the critical value (Extracted by Ekau et al. (2010)).

Group	Species	Respiration rate µmol O_2 g^{-1} h^{-1}	Anoxia tolerance h	Critical values mL O_2 L^{-1} % saturation	Impact	Source
Scypho-/Hydrozoa		0.18–0.78				Rutherford and Thuesen, 2005
Hydrozoa	*Muggiaea atlantica*			3%	Occurrence ↓	Rutherford and Thuesen, 2005
Hydrozoa	*Euphysa flammea*		<2	19%	Occurrence ↓	Rutherford and Thuesen, 2005
Hydrozoa	*Aequorea victoria*		>10			Rutherford and Thuesen, 2005
Scyphozoa	*Aurelia aurita*			0.7	Feeding ↑	Shoji et al., 2005a
Ctenophora	*Mnemiopsis leidyi*			0.7–1	Occurrence ↓	Keister et al., 2000
Ctenophora				1.1–1.8	Growth ↓	Grove and Breitburg, 2005
Crustacea	*Themisto gaudichaudi*	3.93			Respiration g^{-1} DW	Auel and Ekau, 2009
Crustacea	Copepods and euphausiids			<0.1	Avoidance	Sameoto et al., 1987
Copepoda	*Acartia tonsa*			0.04–0.08	Egg development ↓	Invidia et al., 2004
Copepoda	*Acartia tonsa*			0.07	Hatching ↓	Lutz et al., 1994
Crustacea	zooplankton			0.2	Abundance ↓	Böttger-Schnack, 1996
Crustacea	*spp. juveniles*			0.5–0.8	LC_{50}	Miller et al., 2002
Cladocera	*Moina micrura*			0.5–0.6	Swimming ↑, Filtering ↓	Svetlichny and Hubareva, 2002
Crustacea	*spp. larvae*			1.1–2.6	LC_{50}	Miller et al., 2002
Crustacea	*Oratosquilla oratoria*			2.78	Survival	Kodama et al., 2006
Copepoda	*Acartia tonsa*		20–32 days		Eggs	Invidia et al., 2004
Copepoda	*Acartia bifilosa*		10 months		Eggs	Katajisto, 2004
Cephalopoda	*spp.*	0.3–8.8		2–12%	pelagic spp	Seibel et al.. 1997
Cephalopoda	*Dosidicus gigas*	1.4			hypoxic condition	Rosa and Seibel, 2008
Cephalopoda	*Dosidicus gigas*	7.0			normoxic condition	Rosa and Seibel, 2008
Cephalopoda	*Gonatus onyx*			0.5	Occurrence ↓, adults	Hunt and Seibel, 2000
Cephalopoda	*Gonatus onyx*			1	Occurrence ↓, juveniles	Hunt and Seibel, 2000
Cephalopoda				1.4	Occurrence ↓	Rabalais et al., 2001
Cephalopoda	*Lodig pealei*			2.1	Catch ↓	Howell and Simpson, 1994
Cephalopoda	*Loligo vulgaris*			3	Spawning	Roberts, 2005
Cephalopoda				3	Avoidance	Roberts and Sauer, 1994
Cephalopoda				19%		Hunt and Seibel, 2000
Cephalopoda	*Lolliguncula brevis*			41–49%	Onset anaerobic metabol.	Zielinski et al., 2000
Pisces	*Pogonophryne scotti*	0.91			at −1 °C	Saint-Paul et al., 1988
Pisces	*Gadus morhua*	1.1–2.4			5–15 °C	Schurmann and Steffensen, 1997
Pisces	*Ammodytes tobianus*	2.16				Behrens and Steffensen, 2007
Pisces	*Gobius cobitis*	1.27–3.62			at 12.5–25 °C	Berschik et al., 1987
Pisces	*Sardinops sagax*	4.31			Lowest swimming resp.	van der Lingen, 1995
Pisces	*Sarda chiliensis*	7.81				Sepulveda et al., 2003
Pisces	*Katsuwonus pelamis*	16.3				Gooding et al., 1981
Pisces	*Sprattus sprattus*			0.5/7%	Distribution	Kaardvedt et al., 2009
Pisces	*Gobiodon, Paragobiodon*			3%	Occurrence, at night	Nilsson et al., 2007a
Pisces	*Trachurus capensis*			10%		A. Kunzmann, personal commmunication, 2008
Pisces	*spp. juveniles*			0.4–1.1	LC_{50}	Miller et al., 2002
Pisces	*Fundulus grandis*			1	Gonad size ↓	Landry et al., 2007
Pisces	*spp. larvae*			1–1.7	LC_{50}	Miller et al., 2002
Pisces	*Gadus morhua*			21%	LC_{50}	Plante et al., 1998
Pisces	*Pseudopleuronectes americanus*			<1.4	Growth ↓	Bejda et al., 1992
Pisces	*Cynoscion regalis*			1.4	Distribution	Tyler et al., 2007
Pisces	*Gobiosoma bosc*			1.4	Distribution	Keister et al., 2000
Pisces	*Anchoa mitchilli* eggs			1.4	Distribution	MacGregor and Houde, 1996
Pisces	*spp.*			1.2–1.5	Abundance/catch ↓	Dethlefsen and Westernhagen, 1983
Pisces	*spp.*			1.7	Abundance ↑	Burleson et al., 2001
Pisces	*Menidia beryllina*			<2.1	Avoidance	Weltzien et al., 1999
Pisces	*Clupea harengus*			10–25%	School disruption	Domenici et al., 2000
Pisces	*Gobiosoma bosc*			<1.5	Larval mortality ↑	Breitburg, 1994
Pisces	*Anchoa spp.*			1.75	Distribution	Taylor et al., 2007
Pisces	*Gadus morhua*			16.5–30%	Temperature dependance	Schurmann and Steffensen, 1997
Pisces	*Gadus morhua*			2	Vertical larval distrib. ↓	Grønkjaer and Wieland, 1997
Pisces	*Anchoa mitchelli*			<2.1	Larval mortality ↑	Breitburg, 1994
Pisces	*Electrophorus electricus*			2.1–2.8	Avoidance	Crampton, 1998
Pisces	*spp.*			2–2.9	Abundance/catch ↑	Howell and Simpson, 1994
Pisces	*Acipenser transmontanus*			50%	Activity ↓	Crocker and Cech, 1997
Pisces	*Sardinops sagax*			2.5–3	Vertical larval distrib. ↓	Ekau and Verheye, 2005
Pisces	*Engraulis capensis*			2.5–3	Vertical larval distrib. ↓	Ekau and Verheye, 2005
Pisces	*Clupea harengus*			50%	Hatching length ↓	Braum, 1973
Pisces	*Katsuwonus pelamis*			3–3.5	Distribution	Barkley et al., 1978
Pisces	*Gadus morhua*			70%	Abundance ↑	Chabot and Claireaux, 2008
Pisces	*Gadus morhua*			70%	Growth ↓	Chabot and Claireaux, 2008

In nature, many animals survive long periods under oxygen deprivation that cause stress. Increasing in duration of environmental stressors leads to elevated costs of the basal maintenance of an organism reflected in an increased basal metabolic rate and in elevated energy demands to physiological mechanisms and energy costs to the cellular stress protection and damage repair (Breitburg et al. 2018). Under such condition, some physiological and ecological processes have been studied, and several mechanisms have been suggested.

1.4 ZOOPLANKTON MECHANISMS TO DEAL WITH HYPOXIA

Some organisms to inhabiting the OMZ, have evolved a variety of physiological adaptations to enhance oxygen uptake: enhanced ventilatory capability, large gill surfaces, short diffusion distances, and respiratory proteins with very high oxygen affinity (Childress & Seibel 1998). In other cases, ontogenic vertical zonation in the OMZ is shown that correlates to physiological (low oxygen tolerance) and ecological (predation and food availability) constraints (Wishner et al. 2000), thus hypoxia can be a selective force for zooplankton.

1.4.1 ECOLOGICAL EFFECTS

1.4.1.1 VERTICAL DISTRIBUTION

Oxygen minimum zones have an important impact on the vertical distribution and migratory patterns of pelagic organisms (Saltzman & Wishner 1997; Wishner et al. 2008). Some species are virtually excluded from the OMZ, while others are able to stay or cross it

(Herring et al. 1998; Gonzalez & Quiñones. 2002). Most zooplankton taxa show minimum abundances in the core of the OMZ, with higher abundances at the lower OMZ interface (Auel & Verheye 2007; Wishner et al. 1995). A local maximum in zooplankton abundance or biomass at the lower OMZ interface seems to be a unique feature of OMZ regions. Different species-specific tolerances to hypoxic conditions influence zooplankton community composition in areas affected by OMZ (Saltzman & Wishner 1997). Zooplankton abundance and biomass have been studied to decrease dramatically when oxygen concentration drops below 0.2 mL O_2 L^{-1} (Longhurst 1967; Saltzman and Wishner 1997). Deep-sea oceanic species are observed to avoid the OMZ, while only a few species of copepods and euphausiids are regularly found within the OMZ (Fig. 5). Often, individuals found in the core of the OMZ are early stages of species with ontogenetic or daily vertical migrations (Longhurst 1967). Most euphausiids and some copepods avoid the core of the OMZ with less than 0.1 mL O_2 L^{-1} (Sameoto et al. 1987; Saltzman & Wishner 1997). Increasing abundances of copepods and euphausiids below the OMZ indicate that the OMZ acts as a barrier for some species, although specifically adapted.

The evolution of hypoxia-driven zooplankton habitat selection could be related to the seasonality of coastal hypoxia. It appears that in coastal waters the adaptation costs of anaerobic metabolism are high for zooplankton, and adaptation is behavioral rather than physiological. Roman et al. (1993) studied the effect of severe summer hypoxia on the bottom waters of the Chesapeake Bay. During most of this period, copepods are not found in bottom waters, they migrate to surface waters, where oxygen conditions are favorable to develop their vital functions. In contrast, during episodic mixing events, when hypoxia relaxes, maximum copepod abundance often occurs in bottom waters. The degree of adaptation to hypoxic conditions will then be mediated by the duration of the hypoxic episodes, therefore in conditions of sporadic hypoxia it is unlikely that avoidance behavior will persist in the long term (Dam 2013).

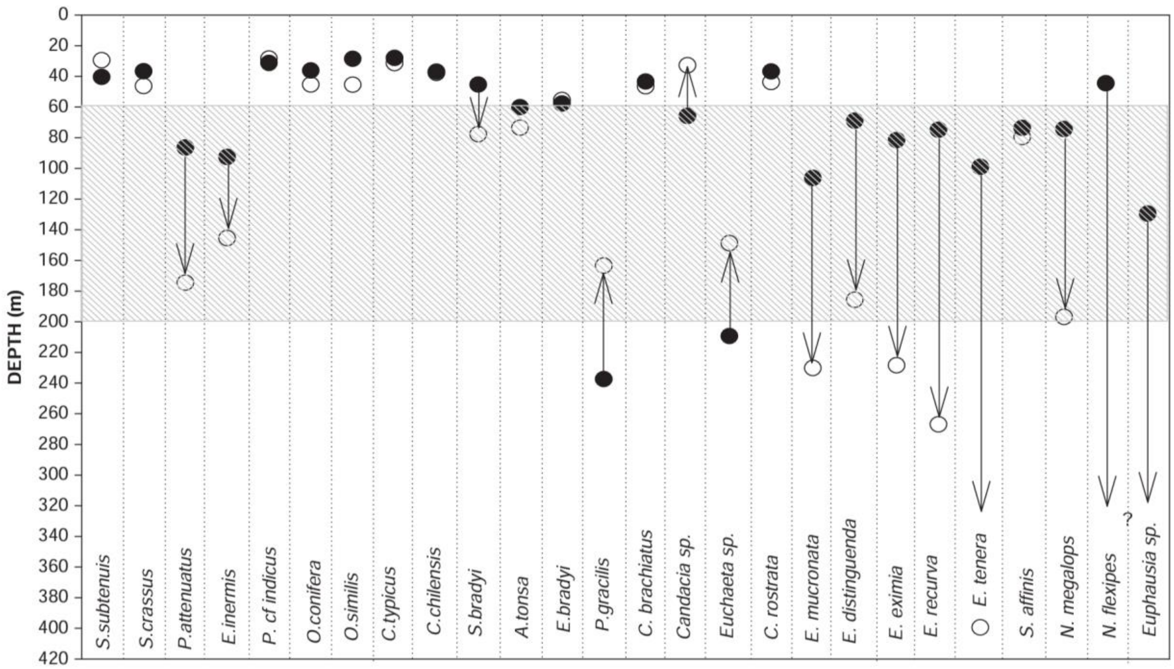

Figure 5: Daily vertical migration of dominant species of zooplankton in and out of the main OMZ core (shaded area) (Escribano et al. 2009)

An increase in hypoxic conditions and the expansion of the OMZ could lead to cascading effects on benthic and pelagic ecosystems, including habitat compression and community reorganization (Bograd et al. 2008). Mesopelagic organisms such as crustaceans and myctophid fishes that live in the upper boundary region of the OMZ or perform diel vertical migrations to feed in the surface layer at night may be strongly affected (Childress & Seibel 1998), with consequent impacts on their epipelagic prey.

1.4.1.2 REPRODUCTION

Studies on the effects of hypoxia on the reproduction of some crustaceans have focused on egg production, hatching success, and copepod viability. Invidia et al. (2004) performed experiments of short exposure to hypoxia and reoxygenation in copepod *A. tonsa* eggs. The results showed inactivity in the eggs, but there was no effect on egg viability and subsequent growth and survival after reoxygenation. However, prolonged exposure times (>15 days) caused significant decreases in hatching and strong reduction in life expectancy.

In *Acartia bifilosa*, a copepod species abundant in the Baltic Sea, low oxygen concentrations <0.17 mL O_2 L^{-1} induced dormancy in eggs. Anoxic conditions prevented the eggs from hatching, but they survived these conditions for 10 to 12 months, depending on temperature. After 10 months of being subjected to anoxic conditions under a temperature of 4°C, approximately 40% of the eggs hatched when returned to normoxic conditions (Katajisto 2004). Since copepod recruitment from benthic eggs is prevented during anoxic events, the duration, area and timing of anoxic events, as well as the specific tolerance of eggs to low oxygen concentrations, will affect the dynamics of the copepod population and the composition of the zooplankton community in general. (Roman et al. 1993). Conversely, temporarily anoxic conditions could even protect benthic eggs and resting stages from predation (McQuoid et al. 2002; Katajisto 2004).

1.4.1.3 BODY SIZE

Lower oxygen availability may favor organism with better oxygen uptake capacity, which could relate to both cell size and body size. For example, smaller cell size in smaller copepods within a given species and stage may lead to an increased capacity, because oxygen needs to be transported across the cell membrane to the mitochondria; and for smaller cells there is greater surface area and shorter diffusion distances (Verberk et al. 2021). Smaller body size also translates into a greater surface area-to-volume ratio to favor more efficient oxygen transport. Allometric models have used to predict environmental effects on copepod metabolism and their impact on community structure and function (Ward et al. 2012).

1.4.2 PHISIOLOGICAL EFFECTS

1.4.2.1 ANAEROBIC METABOLISM

The ability of certain zooplankton species to cross or even live in oxygen minimum zones is apparently linked to the presence and activity of enzymes of the anaerobic metabolism. Enzymatic analysis in some dominant zooplankton species reveal a strong activity of both aerobic and anaerobic (LDH) metabolic components within the OMZ (Escribano 2006). In a comparative study on the specific lactic dehydrogenase (LDH) activity in *Euphausia mucronata* and *Calanus chilensis* from the Humboldt Current upwelling system, the specific LDH activity in *E. mucronata* was two orders of magnitude higher than that of *C. chilensis*, consistent with *E. mucronata* ability to conduct daily vertical migrations through the oxygen minimum zone, whereas *C. chilensis* is restricted to oxygenated waters above the OMZ (Gonzalez & Quiñones 2002).

1.4.2.2 DEPRESSED METABOLIC RATE

A primary mechanism facilitating the tolerance to low oxygen in species inhabiting de OMZ, is an ability to dramatically reduce energy expenditure during daytime forays into low oxygen waters. One well-known survival mechanism is the depression of the metabolic rate, in association with lower rates of ATP production *via* fermentative pathways. The ability to slow down many energy-consuming pathways is a key strategy for survival. This includes reduction of key metabolic enzymes activities *via* post-translational modifications, or *via* decreased transcription and/or translation. Changes in enzymes expression, transcription factors, and micro-RNAs participate in such responses (Moreira et al. 2016).

In the OMZ regions, oxygen concentrations drop within intermediate water depths to a threshold level beyond which further adaptation for oxygen provision appears constrained (Seibel 2011). Below this 'adaptation threshold', vertical migrators often reduce their oxygen consumption rates by 40–80% (Maas et al. 2012; Seibel et al. 2014; Elder & Seibel 2015; Svetlichny et al. 2000; Kiko et al. 2015; Auel et al. 2005). Such a strategy limits the consumption, and excretion of carbon and nutrients by vertical migrators while at depth in pronounced OMZs, thereby impacting biogeochemical cycling in OMZs.

Based on metabolism and hypoxia tolerance experiments, Seibel et al. (2016), evaluated the effects of hypoxia on migrant euphausids from the Eastern Tropical Pacific, *Euphausia eximia* and *Nematoscelis gracilis*, capable of tolerating a partial pressure of oxygen of 0.8 kPa for at least 12 h without mortality. In contrast, the California Current species, *Nematoscelis difficilis*, is unable to survive a partial pressure of 2.4 kPa for more than 3 h. In tolerant species, metabolic suppression during daily depth migrations reduces the metabolic expenditure of these species and decreases their contribution to the vertical flux of carbon and nitrogen (biological pump) by an equivalent amount.

To predict the biological response to changing oxygen levels it is necessary to identify the oxygen thresholds critical (P_{crit}) for organism survival and performance, recognizing interspecific variation in oxygen tolerance and the capacity of organisms to adjust oxygen provision and demand to compensate for oxygen limitation.

Critical Oxygen Pressure (P_{crit}) can be defined as the ρO_2 at which the rate of oxygen consumption (metabolic rate) is no longer independent of ρO_2 (Fig. 1.6). P_{crit} can also be defined as the ρO_2 beyond which anaerobic metabolic end products (e.g., lactate) accumulate and which can indicate the degree of adaptation of an organism to hypoxic conditions (Seibel et al. 2016). P_{crit} describes the lower oxygen limit to an organism's ability to make physiological adjustments that regulate its metabolic rate at a constant level independent of the ambient ρO_2 (oxyregulation) (Fig. 6). Possible adjustments include increased heart rate, enhanced gill surface area (Mitrovic et al. 2009), increased blood pigment levels, adjustment of enzyme systems, and elevated rates of ventilation. Some of these adjustments are triggered by hypoxia-mediated upregulation of gene expression patterns (Flück et al. 2007) while others are regulated physiologically. By contrast, animals whose metabolic rate drops with declining ρO_2 are termed 'oxyconformers'. Practically, oxyconformation merely describes the metabolic response of organisms to oxygen levels below their P_{crit}. This is because there must always be an upper limit to metabolism beyond which additional O_2 will have no influence.

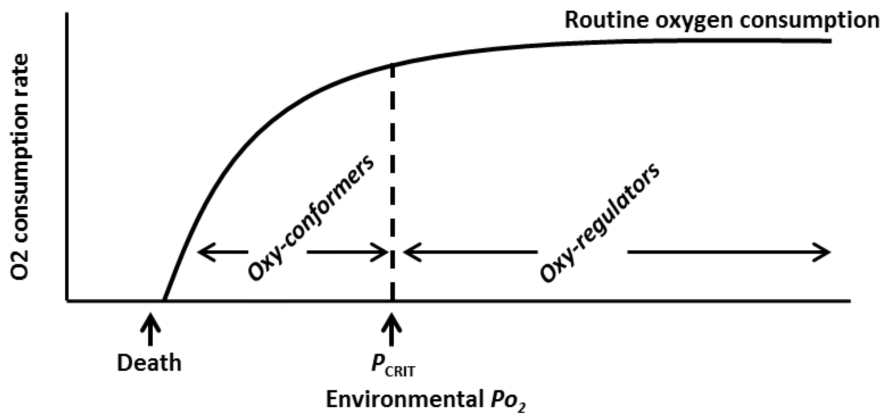

Figure 6: The plotted curve shows an oxyregulator organism, where the oxygen consumption rate is unaffected by the changes in oxygen tension ($\rho O_2 > P_{crit}$). This oxygen consumption rate can be decreases with decreasing environmental oxygen tension ($\rho O_2 < P_{crit}$) in oxyconformers organisms (Rogers et al. 2016).

In their work, Connett et al. (1990) provided a framework for understanding the influence of ρO2 on metabolic rate and the need for anaerobic metabolism and metabolic suppression (Fig. 7). In their model they proposed the need to actively regulate metabolism (physiological adjustments) as ρO_2 decreases, this measured by the total energy demand (ATP), as well as the critical ρO_2 of a species. As energy demand increases, P_{crit} also increases and anaerobic metabolism may be required to contribute to the total flow of ATP. Some adjustments include increased heart rate, enhanced gill surface area, increased blood pigment levels and elevated ventilation.

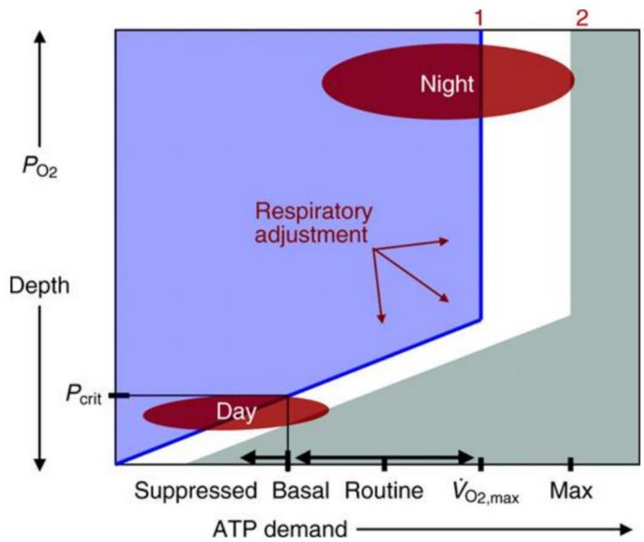

Figure 7: Total energy (ATP) demand is influenced by seawater oxygen partial pressure (ρO_2) below critical values. The solid blue line represents the effect of increasing ATP demand on the critical oxygen partial pressure at a basal rate of metabolism (P_{crit}). The P_{crit} is indicated by the horizontal line. The white region indicates that anaerobic metabolism contributes to maintain total ATP flux. In the gray region anaerobic metabolism is insufficient to maintain ATP flux. Metabolism must be suppressed below that point to avoid impaired function. As ρO_2 approaches P_{crit}, respiratory adjustments may be made to maintain the rate of oxygen provision to sites of consumption (Connett et al. 1990).

1.4.2.3 PREPARATION TO OXYDATIVE STRESS (POS)

In the early 1990s, it was established that decreased blood circulation and the passage of fluid in the mammalian organs elicited intense formation of radical oxygen species (ROS) during the recirculation of oxygenated blood. There was evidence that increased formation of ROS during the re-oxygenation is one of the key factors involved in cell damage under these conditions. Organisms must therefore produce antioxidant enzymes to prevent cell damage.

Many studies have reported that exposure to anoxia or hypoxia can induce gene expression for antioxidant enzymes. In conditions where the animals are exposed to hypoxia and then re-oxygenated, the enhancement of antioxidant defenses is regarded as an important adaptation to cope with these conditions, and as a mechanism of preparation for battling oxidative stress (POS). However, not all animals respond to oxygen restriction by increasing expression of endogenous antioxidants, thus the process of POS is not a universal adaptive mechanism to fight-back the stress produced under low oxygenation, although POS is present in a large number of species evolved under the selective pressure of low oxygen stress (Gyraud et al. 2019).

1.4.2.4 ANTIOXIDANT DEFENSE

Molecular oxygen (O_2) is the primer biological electron acceptor that serves vital roles in fundamental cell functions. However, it is also the precursor of ROS formation. ROS are produced from the molecules of oxygen as a result of normal cellular metabolism. These ROS can be divided into 2 groups: free radicals and non-radicals. Molecules containing one or more unpaired electrons and thus giving reactivity to the molecule are called free radicals. When 2 free radicals share their unpaired electrons, non-radical forms are created. The 3 major ROS of physiological significance are superoxide anion (O_2^-.), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) (Guérin et al. 2001). Linked to the presence of these radicals, a defense mechanism is the synthesis of antioxidant molecules capable of neutralizing the oxidative action of these free radicals. When the balance between antioxidants and ROS becomes disrupted, because of either depletion of antioxidants or accumulation of ROS, oxidative stress occurs (Birben et al. 2012). The term oxidative stress is thus related to a state of respiratory imbalance in which animals cannot maintain a constant tissue oxygenation and, instead, undergo rapid changes between over-oxygenation and hyper-oxygenation (Tremblay et al. 2010). The mechanisms of cellular protection against ROS include several antioxidant enzymes and non-enzymatic proteins, such as Superoxide dismutase (SOD), Catalase (CAT), Peroxidases, Glutation S-transferase (GST) and metallothionein (MT), among other, where their participation becomes relevant in ROS detoxification processes in organisms subjected to oxidative stress.

Variation in oxygen levels in the marine environment ranges from normoxia to hypoxia and causes diverse environments where planktonic copepods and other crustaceans live. Because of changes in the levels of oxygen available in the aquatic environment, the generation of reactive oxygen species (ROS) has been extensively studied (Welker et al. 2013). When animals are re-oxygenated after hypoxic exposure, ROS formation occurs, which, if not neutralized by the body's antioxidant defenses, this may cause oxidative damage and eventually cellular disorder and death. Tremblay et al. (2010) showed that krill species adapted to hypoxia have a sufficiently high antioxidant protection whereas less adapted species suffered a strong oxidative stress measurable as lipid peroxidation. Thus, antioxidants play an important role in neutralizing the oxidative action of free radicals.

Higher production of ROS in the body may change DNA structure, result in modification of proteins and lipids, and activation of several stress-induced transcription factors (Birben et al. 2012). DNA modifications which involves degradation of bases, singleor double stranded DNA breaks, purine, pyrimidine or sugar-bound modifications, mutations, deletions or translocations, and cross-linking with proteins. Most of these DNA modifications are highly relevant for carcinogenesis, aging, and neurodegenerative, cardiovascular, and autoimmune diseases (Birben et al. 2012).

2. HYPOTHESES AND SPECIFIC GOALS

2.1 GENERAL GOAL

This thesis aims at enhancing our understanding on how processes that promote hypoxic conditions in the water column can affect the physiology of planktonic organisms and their responses in coastal upwelling systems.

2.2 HYPOTHESES

Considering the antecedents described above and within the context of intensification of hypoxia, associated with the presence of a shallow oxygen minimum zone in upwelling systems, the following working hypotheses are proposed:

H1: In coastal upwelling zones, the exposure of epipelagic copepods to hypoxia, upon presence of a shallow and intense oxygen minimum zone, can induce species-dependent metabolic responses.

H2: Species-dependent metabolic responses of epipelagic copepods to upwelling-induced hypoxia are associated with the seasonal cycle of upwelling.

2.3. SPECIFIC GOALS

1) Evaluate the existence of metabolic adaptations in dominant planktonic copepods to cope with hypoxic conditions in the water column caused by presence of a shallow oxygen minimum zone.

2) Understanding the role that upwelling variation over a seasonal scale can have in modulating the metabolic responses to hypoxia in planktonic copepods.

3) Assessing the presence of antioxidant response to oxidative stress in planktonic copepods subjected to short-term variation in hypoxic conditions induced by upwelling variability.

3. MATERIAL AND METHODS

3.1 STUDY AREA

The Humboldt Current Systems (HCS) is a highly productive and large marine ecosystem (Fig. 8). In this ecosystem off the coast of Chile there are two major upwelling regions controlled by the tropical South Pacific anticyclone, with seasonality of the winds defined by the displacement of their center between 27ºS in summer and 32ºS in winter (Strub et al. 1998; Blanco et al. 2001; Schneider et al. 2016), where high rates of primary production have been estimated (> 10 g C m^{-2} d^{-1}) (Montero et al. 2007). In the central zone, upwelling events are predominantly seasonal, prevailing during the austral spring-summer (Sobarzo et al. 2007). The high seasonal primary production observed in the central zone supports important microzooplankton, mesozooplankton and ichthyoplankton communities (Morales et al. 2007; Landaeta et al. 2008; Escribano et al. 2016), which can reach their highest growth rates in spring- summer (Anabalón et al. 2007). The area off Concepción (36° S) corresponds to the widest section of the continental shelf along the south-central HCS (Sobarzo & Djurfeldt 2004), characterized by south-westerly (SW) winds prevailing in spring-summer (September-March) (Sobarzo et al. 2007). The latter favor the presence of strong seasonal coastal upwelling events that intensify during spring-summer (Strub et al. 1998; Sobarzo et al. 2007). The highest primary production rates reported for this region were observed in October when winds favorable to upwelling intensify (Daneri et al. 2000; Montero et al. 2007).

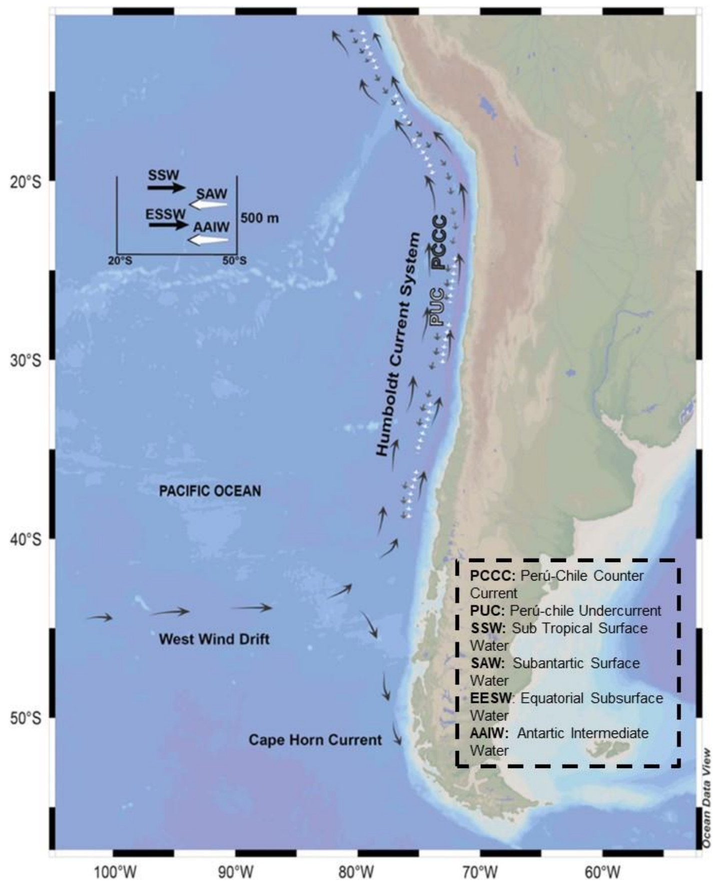

Figure 8: Map illustrating Surface ocean currents in the eastern South Pacific along the Chilean coast, northward: the Humboldt Current and southward, the Cape Horn Current, and the subsurface and intermediate water masses (modified Merino et al. 2018)

3.2. OCEANOGRAPHIC DATA

To interpret the experimental results in relation to environmental oxygen conditions, in this work the oceanographic time series that has been carried out since 2002 at Station 18 (Fig. 9) was used, located at 36°. 30'S and 73°08' W at 90 m depth on the continental shelf off Concepción. In this time series, hydrographic measurements were obtained with a SeaBird-SBE-25 CTD, along with water samples at 9 depths (0-80 m), to parameters such as oxygen concentration, temperature and salinity and biological measurements, such as chlorophyll-a and zooplankton biomass (Escribano et al. 2012). In our study, we used time series data from 2002 through 2016 data to assess intra-seasonal, seasonal and interannual variability in oxygen and temperature throughout the water column (Sobarzo et al. 2007) to evaluate the hypoxic conditions to which copepods are exposed.

The time series data were complemented with additional monthly samplings at St. 18 carried out by ZEUS project, with a SeaBird-19 plus CTD equipped with an oxygen sensor for the period July 2019 and March 2020. No data were available for the period between April 2016 and July 2019.

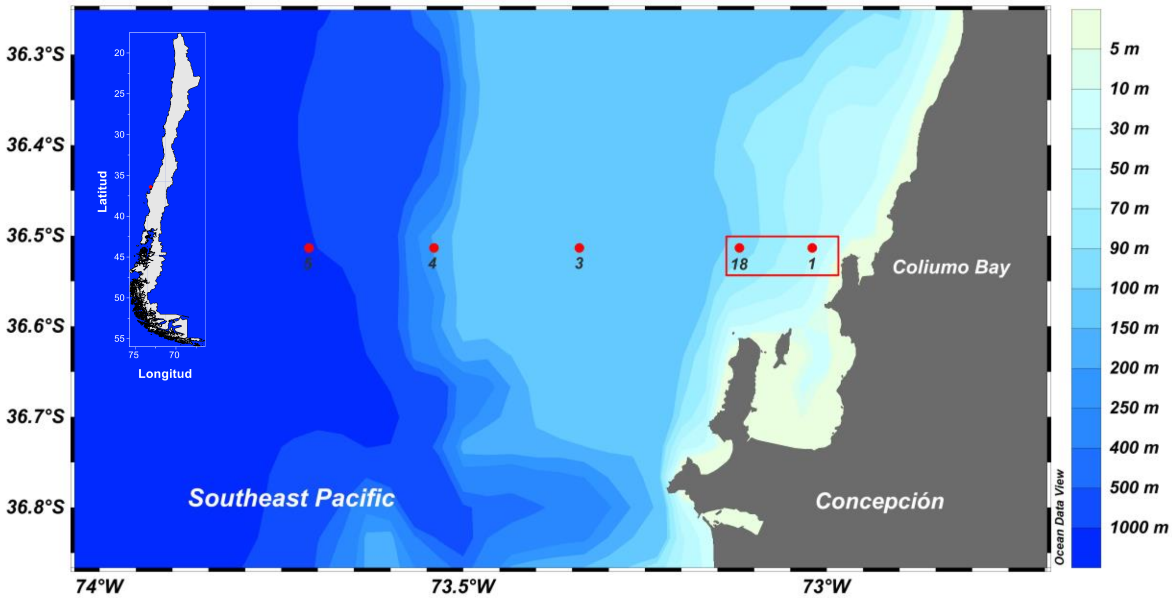

Figure 9: Map illustrating the sampling area (red box) in Coliumo Bay. Additionally, the offshore stations corresponding to the ZEUS series sampling are shown.

3.3. BIOLOGICAL SAMPLING

The zooplankton samples were collected during 2021 (Table 2), from the upper layer of the water column (approximately 50 m) using vertical trawls. A WP2 plankton net was used with a mesh size of 200 microns and 0.5 m opening diameter, equipped with a 2 L non-filtering cod. Once the sample was collected, it was placed in a cooler and diluted with water of sea and taken to a cold chamber where it was maintained at a controlled temperature (12-14°C) while the incubation medium was prepared. Additionally, water samples for incubations were taken using a 10 L Niskin bottle at a depth of 10 m.

Table 2: Campaigns carried out to obtain plankton samples during spring-summer and autumn-winter 2021.

Season	Sample Date	Station	Depth (m)
Spring-Summer	Jan 2021	1	0-40
	Sept 2021	18	0-50
	Sept 2021	18	0-50
	Oct 2021	1	0-30
Autumn-Winter	March 2021	1	0-40
	March 2021	1	0-50
	May 2021	1	0-50
	May 2021	1	0-50
	May 2021	1	0-50

3.4 EXPERIMENTAL DESIGN

3.4.1 METABOLIC RATE AND P_{crit} ESTIMATIONS

The metabolic activity and the critical oxygen concentration (P_{crit}) in copepods were measured estimating by closed respirometry in spring-summer (upwelling conditions) and autumn-winter (non-upwelling conditions) season, during 2021. The incubations were performed in 4 mL glass vials with 0.2 µm filtered seawater obtained from the field and kept at the cold room temperature at dark. Live individual copepods were placed in the vials with a pipette in variable numbers: 13- 60 individuals, according to their sizes and using an optic oxygen sensor (Robust probe OXROV10), connected to a FireSting-O2 four channel meter (PyroScience) were measured as pO_2 declines over time. All the experiments were carried out at a constant temperature of 13 °C, as representing the average condition at about 10 m depth at St. 18 throughout the year. The incubation period varied between 8 and 12 h depending on species size, but a dissolved oxygen reading was taken every fifteen minute during the whole incubation period. At the end of each incubation, the mortality of individuals in hypoxic condition were determined by counting the number of dead individuals. The number of experiments for seasonal period, species, and replicates are summarized in Table 3.

Table 3: Summary of respiration rate experiments for three planktonic copepods, which were carried out between January 2021 and October 2021. n=number of individuals per vial and Inc. time is the incubation time. Temperature was kept constant at 13°C for all experiments in a cold room and dark conditions.

Season	Date	Species	Replicates	N° of vials	n	Inc. time (hours)
Spring-Summer	Jan 2021	*C. patagoniensis*	2	8	10-15	8
	Sept 2021	*P.* cf. *indicus*	2	12	40-50	8
	Sept 2021	*A. tonsa*	1	5	30-40	10
	Oct 2021	*A. tonsa*	1	5	30-40	11
Autumn-Winter	March 2021	*C. patagoniensis*	2	8	10-15	9
	March 2021	*P.* cf. *indicus*	2	4	50	9
	May 2021	*C. patagoniensis*	1	4	10-15	8
	May 2021	*P.* cf. *indicus*	1	4	40-50	9
	May 2021	*A. tonsa*	1	3	35-40	10

The critical oxygen level (P_{crit}), defined as the lower partial oxygen pressure down to routine metabolic rate could no longer be maintained independently of the oxygen partial pressure (Birk 2021) was estimated from oxygen consumption as a function of oxygen pressure by fitting two-steps segmented linear regression using the R-package "Respirometry" (Birk 2021). P_{crit} was assumed as the oxygen pressure corresponding to the interception point between these two regressions. The respiration rate (MR) of each species was estimated as the consumption of oxygen (µmol) during a time interval (15 min) of two subsequent measurements of oxygen decay when the oxygen pressure was reaching the P_{crit} value. MR was thus calculated by the coefficient of oxygen dissolution at that given temperature and salinity, accounting for the incubation volume, standardized by individual body mass (µg C), and unit of time (h). For body mass, the average dry weight of adults of each species was obtained from regression of size (Table 4).

$$MR: ((O_f - O_i) * \text{Conversion Factor} * \text{Volume}) / (\text{Time} * \text{Weight})$$

Where, O_f-O_i is the final and initial oxygen concentration of each incubation. To estimate the body mass (weight) of copepods, the size of each species was measured and a weight-size relationship was used.

Table 4: Mean and standard deviation of size of copepods to experiments periods. N is number of organisms measured. T-test was applied to *C. patagoniensis* and *A. tonsa*, while for *P.* cf. *indicus* we applied an ANOVA.

Species	**Month**	**Size (µm) (N)**	**t-test/ANOVA**
C. patagoniensis	January	2019 ± 168.9 (13)	0.177
	August	1925 ± 153.2 (10)	
P. cf. *indicus*	April	746 ±21.61 (14)	0.472
	July	753 ± 28.68 (16)	
	August	766 ± 20.41 (6)	
A. tonsa	May	965 ±83.27 (19)	0.618
	October	971 ±75.23 (13)	

The MR experiments to estimate the metabolic rate (MR) and the critical partial pressure of oxygen (P_{crit}) were carried out at a temperature of 13°C. As the temperature of the upper 50 m of the water column ranged between 10 and 15°C, both values (MR and P_{crit}) were recalculated under these temperature conditions. For this, the relationship reported by Heine et al. 2019 of the increase/decrease of MR by 7% per degree Celsius was used. These values were compared to the fitted values of MR and P_{crit} using Q10 = 2 (Hochachka & Somero 1984). P_{crit} values were adjusted to in situ temperature to represent its vertical distribution in the water column throughout the time series.

3.4.2 DATA ANALYSIS AND STATISTICS

Two-way ANOVA was used to test seasonal effects and species differences in MR and P_{crit}. Furthermore, with one-way ANOVA the seasonal effect within species was tested. The normality of the data was previously tested with the Shapiro-Wilks test and the homogeneity of variances using a Levene test. Both tests were not significant ($P>0.05$) in all cases, except the normality test for P_{crit} (Shapiro-Wilk=0.729, $P<0.05$). Therefore, P_{crit} data were log-transformed before ANOVA to meet parametric assumptions ($P>0.05$). All of these analyzes were performed using the Systat v.13 package (Wilkinson 1990).

Monthly time series data for temperature and dissolved oxygen allowed the construction of interpolated contours using nonlinear kriging, and the depth of P_{crit} values across the time series were compared between species using one-way ANOVA, separately by upwelling (September to March) and no. -upwelling conditions (April to August). Significance levels were assumed at P = <5%.

4. RESULTS

4.1 Chapter 1: "Adjusting metabolic rates and critical oxygen tension in planktonic copepods under increasing hypoxia in highly productive coastal upwelling zones". Scientific article accepted in Limnology and Oceanography, https://doi.org/10.1002/lno.12556

Summary:

Ongoing ocean deoxygenation is threatening marine organisms and food webs globally. In eastern boundary upwelling systems, planktonic copepods dominate the zooplankton biomass, being crucial in the marine food web. Yet, they must cope with severe hypoxia caused by shoaling of the oxygen minimum zone (OMZ). Based on laboratory experiments conducted during 2021, we found differential responses in the metabolic rate of three abundant copepod species. *Calanoides patagoniensis* doubled its metabolic rate during the upwelling season, so better exploiting the spring phytoplankton bloom for feeding and reproduction while maintaining their critical oxygen partial pressure unchanged between seasons. Contrastingly, *Paracalanus* cf. *indicus and Acartia tonsa*, maintained their metabolic rates throughout seasons, but they significantly increased their critical oxygen partial pressure during the upwelling period, and so becoming less tolerant to the spring-summer hypoxia. Time series observations showed that oxygen levels equal or lower than the critical oxygen partial pressure is a common condition (ca. 70% of probability of occurrence) that copepods encounter during a year cycle while inhabiting the upper 50 m layer. These findings suggest the existence of a species-dependent trade-off between the metabolic rate and the critical oxygen partial pressure where a species is able to maintain their latter despite fluctuations in their metabolic rate (improved hypoxia tolerance) or maintain their metabolic rate at expenses of a larger critical oxygen partial pressure (improved energy expending). These species-dependent adaptive responses, under oxygen levels ≤ the critical oxygen partial pressure suggest that exacerbated hypoxia, driven by ongoing ocean deoxygenation and increased upwelling, will lead to a vertical compression of the habitat, changing copepod distribution and higher copepod mortality, with potentially drastic consequences for marine food webs.

Resumen

La actual desoxigenación de los océanos está amenazando a los organismos marinos y las redes alimentarias en todo el mundo. En los sistemas de surgencia de borde oriental, los copépodos planctónicos dominan la biomasa del zooplancton, siendo cruciales en la trama trófica marina. Sin embargo, deben hacer frente a la hipoxia severa causada por la zona mínima de oxígeno (OMZ). Con base en experimentos de laboratorio realizados durante 2021, encontramos respuestas diferenciales en la tasa metabólica de tres especies abundantes de copépodos. *Calanoides patagoniensis* duplicó su tasa metabólica durante la temporada de afloramiento, por lo que aprovecha mejor la floración de fitoplancton de primavera para alimentarse y reproducirse, manteniendo su presión parcial de oxígeno crítica sin cambios entre estaciones. Por el contrario, *Paracalanus* cf. *indicus* y *Acartia tonsa*, mantuvieron sus tasas metabólicas a lo largo de las estaciones, pero aumentaron significativamente su presión parcial crítica de oxígeno durante el período de afloramiento, por lo que se volvieron menos tolerantes a la hipoxia primavera-verano. Las observaciones de series de tiempo mostraron que los niveles de oxígeno iguales o inferiores a la presión parcial crítica de oxígeno es una

condición común (aproximadamente el 70% de la probabilidad de ocurrencia) que los copépodos encuentran durante un ciclo anual mientras habitan la capa superior de 50 m. Estos resultados sugieren la existencia de un balance dependiente de la especie entre la tasa metabólica y la presión parcial crítica de oxígeno, donde una especie es capaz de mantener esta última a pesar de las fluctuaciones en su tasa metabólica (mejor tolerancia a la hipoxia) o mantener su tasa metabólica a expensas de una mayor presión parcial crítica de oxígeno (mejor gasto de energía). Estas respuestas adaptativas dependientes de las especies, bajo niveles de oxígeno ≤ la presión parcial crítica de oxígeno, sugieren que la hipoxia exacerbada, impulsada por la desoxigenación oceánica y el aumento de la surgencia, conducirá a una compresión vertical del hábitat, cambiando la distribución de los copépodos y aumentando su mortalidad, con consecuencias potencialmente drásticas para las tramas tróficas marinas.

LIMNOLOGY and OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 9999, 2024, 1–14

doi: 10.1002/lno.12556

Adjusting metabolic rates and critical oxygen tension in planktonic copepods under increasing hypoxia in highly productive coastal upwelling zones

Leissing Frederick,[1] **Mauricio A. Urbina**,[2,3*] **Erika Jorquera**,[4] **Ruben Escribano**[3,5]

[1]University of Concepción, Concepción, Chile
[2]Department of Zoology, Faculty of Natural Sciences and Oceanography, University of Concepción, Concepción, Chile
[3]Millennium Institute of Oceanography, Chile
[4]Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Concepción, Chile
[5]Department of Oceanography, Faculty of Natural Sciences and Oceanography, University of Concepción, Concepción, Chile

Abstract

Ongoing ocean deoxygenation is threatening marine organisms globally. In eastern boundary upwelling systems, planktonic copepods dominate the epipelagic zooplankton, being crucial in the marine food web. Yet, they must cope with severe hypoxia caused by shoaling of the oxygen minimum zone. Based on laboratory experiments during 2021, we found differential responses in the metabolic rate (MR) and critical oxygen partial pressure of three abundant copepods. *Calanoides patagoniensis* doubled its MR during the upwelling season, so better exploiting the spring phytoplankton bloom for feeding and reproduction while maintaining their critical oxygen partial pressure unchanged between seasons. Contrastingly, *Paracalanus* cf. *indicus* and *Acartia tonsa*, maintained their MRs throughout seasons, but significantly increased their critical oxygen partial pressure during the upwelling period, becoming less tolerant to hypoxia. Field observations showed that oxygen levels equal to or lower than the critical oxygen partial pressure is a common condition (70% of occurrence) that copepods encounter during the year in the upper 50 m layer. These findings suggest a species-dependent trade-off between the MR and the critical oxygen partial pressure, where some species can maintain the latter despite fluctuations in their MR (improved hypoxia tolerance) or maintain their MR at the expenses of a larger critical oxygen partial pressure (improved energy expenditures). These adaptive responses, under oxygen levels equal to or lower than the critical oxygen partial pressure, suggest that exacerbated hypoxia, driven by ocean deoxygenation and increased upwelling, will alter copepod distribution and cause higher copepod mortality, with potentially drastic consequences for marine food webs.

Evidence for gradual and steady ocean deoxygenation has been rapidly growing (Keeling et al. 2010; Schmidtko et al. 2017; Breitburg et al. 2018), rising major concerns about the predicted impacts on marine biota (Limburg et al. 2020; Sampaio et al. 2021). Decreasing oxygen levels become more evident and severe in regions subjected to subsurface oxygen minimum zones (OMZs), characterized by extremely low-oxygen levels (< 20–45 μmol kg^{-1}), covering large coastal areas, but also regarded as highly productive marine regions (Gilly et al. 2018). As a consequence of the ocean deoxygenation, an expansion of the OMZs has been predicted to occur due to the shoaling of their upper and the descent of the lower boundaries, thus increasing OMZ volume and its influence on marine organisms (Stramma et al. 2012; Zhou et al. 2022). The oxygen concentrations in the OMZ core have also been reported to decrease, thus further increasing the severity of hypoxia (Chan et al. 2008; Stramma et al. 2008).

Dissolved oxygen shapes the structure and functioning of coastal upwelling ecosystems and the spatial–temporal distribution of many marine organisms (Auel and Verheye 2007; Escribano et al. 2009; Wishner et al. 2013). This is because hypoxic conditions can be harmful for most of the biota (Roman et al. 2019; Sampaio et al. 2021), as many organisms reside near the upper oxycline and face daily changes in oxygen levels. Shoaling of the OMZ may, therefore, reduce the available habitat for pelagic fishes and zooplankton

*Correspondence: mauriciourbina@udec.cl

Additional Supporting Information may be found in the online version of this article.

Author Contribution: Study design and execution: LF, RE, MU. Conceptualization: LF, RE, MU and EJ. Data adquistion: LF and MU. Data processing and analysis: LF, RE, EJ. Writing—original draft: LF, RE, MU. Writing—review & editing: LF, RE, MU, EJ.

(Wishner et al. 2013) as they move above, below, or between hypoxic waters. This not only changes the volume of the habitat they can exploit, but also predator–prey interactions, which can increase as the habitat becomes strongly compressed (Stramma et al. 2010).

Hypoxia can affect organisms in many ways, first altering the behavior of mobile organisms seeking more favorable oxygen conditions or sessile animals trying to isolate themselves from the medium. When behavioral responses do not suffice, physiological, and biochemical responses are triggered, likely leading to lower growth and reproductive success in the long term, assuming they survived acute hypoxia (Stramma et al. 2010). At the ecosystem level, species-specific tolerances to low oxygen will result in a differential success under a changing environment, likely leading to alterations in community structure and trophic webs (Diaz and Rosenberg 2008). While some species will be negatively affected by these effects, others that are more hypoxia-tolerant may expand their distribution range or have access to more resources (Stramma et al. 2010; Wishner et al. 2018).

The adaptations of organisms to low oxygen are driven by a selective pressure to optimize and enhance oxygen uptake from hypoxic waters to maintain aerobic metabolism in most animals (Childress and Seibel 1998), while others would just lower their metabolic demands. ATP requirements may be satisfied by two modes: as an oxyconformer organism, decreasing its aerobic metabolic rate (MR) as environmental oxygen decreases, mainly relying on anerobic metabolism or metabolic depression, or as an oxyregulator one, able to maintain aerobic metabolism at least down to a certain oxygen level (critical oxygen partial pressure: P_{crit}) (Hochachka and Somero 2002). For an oxyconformer, the MR decreases proportionally with oxygen concentration, leaving them with less energy to meet their demands. These oxygen-conformer species may prevail under stable-oxic conditions, as they are hypoxia-sensitive organisms (Rogers et al. 2016). For an oxygen-regulator, instead, a lower P_{crit} is under positive selection for hypoxia tolerance (Rogers et al. 2016; Cobbs and Alexander 2018). However, under severe hypoxia, oxygen levels lower than P_{crit} lead to metabolic failure unless they perform metabolic suppression (Seibel 2011).

Coastal upwelling zones with shallow OMZ's, can exhibit severe hypoxic conditions near the surface, although they are highly unstable environments both in space and time in terms of oxygen levels (Paulmier et al. 2006). Upwelling causes the intermittent but strongly seasonal (summer months) ascent of cold, low-oxygen waters to the photic zone where the highest biomass of organisms concentrates (Grantham et al. 2004; Escribano et al. 2009; Seibel 2011). Many organisms occupying the photic zone are thus subjected to occasional hypoxia during their entire life cycle (Ekau et al. 2010; Kiko et al. 2016). Among them, the zooplankton may even experience more frequent hypoxic conditions because many perform daily incursions into hypoxic subsurface layers to avoid predators (Wishner et al. 2013; Riquelme Bugueño et al. 2020; Tutasi and Escribano 2020). Zooplankton must then have evolved some adaptations to cope with the events of hypoxia coupled with upwelling pulses. These events can be more periodical over seasonal scales at temperate latitudes (Sobarzo et al. 2007; Escribano et al. 2012) and thus a predictable driver of evolution.

In the coastal upwelling zone of central-southern Chile (Supporting Information Fig. S1), copepods are the dominant component of the zooplankton (Hidalgo et al. 2010), but also key in terms of zooplankton biomass and production (Escribano and Schneider 2007; Medellín-Mora et al. 2020). Copepods are considered suitable models to assess the behavior and the metabolic responses to short-term, unpredictable hypoxia, but also to examine their responses to more predictable seasonal hypoxia. Copepods are multigenerational, producing several cohorts during an annual cycle, such that different selective pressures may be acting over different cohorts throughout the year. For instance, in spring–summer, strong upwelling promotes primary production and thus increases copepod feeding and reproduction (Vargas et al. 2006). This condition may demand more energy; thus, an increased MR. By contrast, during suppressed upwelling (autumn–winter), reduced feeding and reproduction may result in lower MRs, as shown (Donoso and Escribano 2014). Therefore, there might be a trade-off between MRs and the critical oxygen partial pressure, resulting from a simultaneous rise in the energy demands for feeding, growth, and reproduction and the need for minimizing the critical oxygen partial pressure (P_{crit}) to cope with low-oxygen conditions during spring–summer months. Given the several copepod cohorts during the year and the strongly predictable and seasonal hypoxia in this upwelling system, we hypothesize a hypoxia-driven decrease in P_{crit} during summer months (if hypoxia-driven adaptation exists). If not, it would signal a strategy for better exploiting the more favorable summer conditions at the expenses of hypoxia tolerance. To test this hypothesis, we measured the MR and P_{crit} on different seasonal cohorts of three abundant planktonic copepod species, *Acartia tonsa*, *Paracalanus* cf. *indicus*, and *Calanoides patagoniensis*, inhabiting the coastal upwelling zone off Chile. With the determination of their MR and P_{crit}, we aimed to test the adaptive capacity of these planktonic copepods to adjust their metabolism to confront variations in the availability of oxygen over the seasonal and intra-seasonal time scales of upwelling.

Materials

Study area

The upwelling zone of central-southern Chile is in the eastern South Pacific (33°S–38°S) (shown in Supporting Information Fig. S1). In this zone, upwelling is controlled by the South Pacific subtropical anticyclone (Strub et al. 1998; Schneider et al. 2016), causing seasonal upwelling events prevailing during the austral spring–summer (Sobarzo et al. 2007). The high primary production (> 10 g C m^{-2} d^{-1}) (Daneri et al. 2000) observed in the spring–summer supports important planktonic communities (Morales et al. 2007; Landaeta et al. 2008; Escribano et al. 2016), which reach their highest growth rates in spring–summer (Vargas et al. 2010; Escribano et al. 2016).

The area off Concepción (36°S) corresponds to the widest section of the continental shelf along the central-south of Chile (Sobarzo and Djurfeldt 2004), characterized by northern winds prevailing in winter, whereas southern Westerly Winds (predominate in the spring–summer (September–March) (Sobarzo et al. 2007).

In this region, an oceanographic time series was initiated in August 2002 at Sta. 18 (indicated in Supporting Information Fig. S1). The Sta. 18 is located at 36°30′S and 73°08′W at 90 m depth over the continental shelf off Concepción. Since 2002, monthly CTD casts, oxygen concentration, and biological measurements, such as chlorophyll *a* (Chl *a*) and zooplankton biomass, have been made at Sta. 18 (Escribano and Morales 2012). In our study, oxygen data were used to assess hypoxia conditions to which copepods are exposed.

Copepod sampling and treatment

Live copepods for experiments were obtained in the area surrounding Sta. 18 (Supporting Information Fig. S1). Zooplankton samples were collected from the upper 50 m layer through vertical tows of a zooplankton WP2 net with a mesh size of 200 μm and 0.5 m of opening diameter, equipped with a non-filtering cod-end. Live samples were placed in coolers and diluted with surface seawater and taken to the Dichato Marine Laboratory within 1 h. In addition, seawater for the incubations was collected with 10 L Niskin bottles from 20 to 30 m and maintained in carboy containers. At the laboratory, samples were maintained in cold rooms at a controlled temperature of 13°C, which is the average in situ temperature at Sta. 18 during the year cycle. In situ temperature and oxygen conditions at each sampling were assessed by the deployment of a conductivity–temperature–depth (CTD) profiler, SeaBird-19 plus, equipped with an oxygen sensor. The CTD was operated from the surface down to 80 m depth.

In the laboratory, live copepods were sorted and identified under a microscope. Adult stages, females, and males of the target species in apparently good physical condition were selected when present in the samples and kept in the cold room at dark in filtered seawater (0.2 μm) for an acclimatation period of 2 h. Depending on their abundance in the samples, a total amount of 50–200 individuals were sorted for each target species: *C. patagoniensis*, *P.* cf. *indicus*, and *A. tonsa*.

Experimental design

The MR and the critical oxygen partial pressure (P_{crit}) of copepods were estimated for each species by closed respirometry conducted in a temperature-controlled cold room at different times of the year, including spring–summer (upwelling conditions) and autumn–winter (non-upwelling conditions) during 2021. A summary of experiment metadata is provided in Supporting Information Table S1. The incubations were performed in 4 mL glass vials filled in about 80% with 0.2 μm filtered seawater obtained from the field and kept at the cold room temperature at dark. Live individual copepods were placed in the vials with a pipette in variable numbers: 13–60 individuals, according to their sizes. Vials were then filled to the top with seawater and hermetically closed, avoiding air bubbles, with a top equipped with a silicone seal and a steel-protected optic oxygen sensor (Robust probe OXROV10) connected to a FireSting®-O_2 four-channel meter (PyroScience). When many copepods in good condition were available, up to four of the above-described O_2 meters were used in parallel. Therefore, oxygen consumption was measured as the decline of pO_2 over time in each vial. For each run, a vial lacking copepods was run in parallel to account for any potential bacterial metabolism. All the experiments were carried out at a constant temperature of 13°C, representing the average condition at about 10 m depth at Sta. 18 throughout the year. The incubation period varied between 8 and 12 h depending on species size (*see* Supporting Information Table S1), but a dissolved oxygen reading was automatically recorded every minute during the whole incubation period. At the end of each incubation, the mortality of individuals in hypoxic conditions was determined by counting the number of dead individuals. The number of experiments for seasonal periods, species, and replicates are summarized in Supporting Information Table S1.

The critical oxygen partial pressure (P_{crit}) was estimated from oxygen consumption as a function of oxygen pressure by fitting two-step segmented linear regressions using the R-package "Respirometry" (Birk 2021). P_{crit} was assumed as the oxygen pressure corresponding to the intersection point between these two regressions. The MR of each species was estimated as the consumption of oxygen (μmol) during a time interval (15 min) of two subsequent measurements of oxygen decay well above reaching the P_{crit} value. MR was thus calculated by the coefficient of oxygen dissolution at that given temperature and salinity, accounting for the incubation volume, standardized by individual body mass (μg C), and time (h). For body mass, the individual C content of each species was obtained from C-length regressions after measuring the prosome lengths (μm) of copepods incubated for each experiment (data shown in Supporting Information Table S4). C-length regressions (*see* Supporting Information Table S4) were derived from direct measurements of individual C contents and prosome lengths for each species obtained in previous work in the same study area (unpublished data).

Oceanographic and complementary data

Aiming at interpreting our experimental results in the context of environmental conditions and oxygen variation to which copepods are exposed in nature, we used field data from Sta. 18 previously obtained during the time series study. During the oceanographic time series of Sta. 18, bimonthly sampling was undertaken during 2002 and 2003, and monthly sampling thereafter. Hydrographic measurements were obtained with a SeaBird-SBE-25 CTD, along with water samples at nine depths (0–80 m). In this study, we used time series data from 2002 to 2016 data to assess intra-seasonal, seasonal, and interannual variability in oxygen and temperature throughout the water column (Sobarzo et al. 2007). These data were complemented with additional monthly samplings at Sta. 18 carried out with a SeaBird-19 plus CTD equipped with an oxygen sensor for the period July 2019 and March 2020. No data were available for the period between April 2016 and July 2019.

In addition to the time series data, we used data on cross-shelf vertical distribution of copepod species from a seasonal sampling carried out in 2008 to assess the actual oxygen conditions copepods experience. The sampling included Sta. 18, but also deeper stations located nearby to assess whether vertical distribution at Sta. 18 could be more widely replicated. Zooplankton sampling was carried out with a Hydrobios Multinet Midi type (200 μm mesh size) to obtain vertical stratified samples from the surface to a maximum depth of 500 m at the deepest station. The Multinet was towed horizontally to sample 4 and 5 strata in 5 cross-shelf stations (*see* Supporting Information Table S8).

Data analysis and statistics

Both MR and critical oxygen partial pressure (P_{crit}) were estimated at the incubation temperature (13°C), but since the upper 50 m of the water column temperature ranged between 10°C and 15°C, both values (MR and P_{crit}) were also recalculated at these temperature conditions following the reported increasing/decreasing MR in 7% per °C (Heine et al. 2019). These values were further compared with the adjusted MR and P_{crit} values using a Q10 = 2 (Hochachka and Somero 1984). P_{crit} values were so adjusted to the in situ temperature to represent their vertical distribution in the water column throughout the time series.

Table 1. Metabolic rate (MR) and critical oxygen partial pressure (P_{crit}). MR and P_{crit} were estimated for three planktonic copepods (mean ± standard deviation), *Calanoides patagoniensis*, *Paracalanus* cf. *indicus*, and *Acartia tonsa* in two seasons; A-W: autumn–winter, S-S: spring–summer. Bold values indicate significant differences ($p < 0.05$) between species.

Species	Season	MR (μmol O_2 μg^{-1} h^{-1})	P_{crit} (kPa)
C. patagoniensis	S-S	**19.39±10.22**	3.83±1.39
	A-W	10.22±2.80	3.18±0.79
P. cf. *indicus*	S-S	2.23±0.57	**4.64±0.61**
	A-W	2.42±0.64	2.77±0.89
A. tonsa	S-S	3.92±2.03	**4.90±0.59**
	A-W	3.63±2.66	3.06±1.71

Seasonal effects and species differences in MR and P_{crit} were tested by two-way analysis of variance (ANOVA) and further with one-way ANOVA for testing seasonal effects within species. Data were previously tested for normality with the Shapiro–Wilk test and the homogeneity of variances by a Levene test. Both tests were non-significant ($p > 0.05$) in all cases, except the test of normality for P_{crit} (Shapiro–Wilk = 0.729, $p < 0.05$). Therefore, data for P_{crit} were log-transformed prior to ANOVA to meet parametric assumptions ($p > 0.05$). All these analyses were performed using the Systat v.13 package (Wilkinson 1990). Monthly time series data of temperature and dissolved oxygen allowed the construction of contours interpolated by nonlinear kriging and the depth of P_{crit} values over the time series were compared between species by one-way ANOVA, separately by upwelling (September–March) and non-upwelling (April–August) conditions. Significance levels were assumed at $p = <5\%$.

Results

MR and critical oxygen partial pressure

The three copepod species were subjected to incubation experiments in different seasons, divided into autumn–winter (non-upwelling) and spring–summer (upwelling). There were at least two replicated experiments per season for each species (Supporting Information Table S1). From these experiments, the estimates of MR and critical oxygen partial pressure (P_{crit}) are summarized in Table 1.

Mean values of MR during the spring–summer are higher than those of the autumn–winter in *C. patagoniensis* but did not considerably change in the other two species between seasons (Table 1). Among species, *C. patagoniensis* exhibited the highest MR in both seasons. P_{crit} also exhibited considerable variation between species and seasons (Table 1). Two-way ANOVA showed significant seasonal effects and significant differences between species in MR. There were also significant seasonal effects on P_{crit}, but with no significant differences between species (Supporting Information Table S2). When further testing seasonal effects separately by species, one-way ANOVA revealed that MR significantly increased in the spring–summer in *C. patagoniensis*, but seasonal changes of P_{crit} in this species were non-significant (Supporting Information Table S3). By contrast, *P.* cf. *indicus* and *A. tonsa* had non-significant differences in MR between seasons, but their P_{crit} differed significantly between seasons (Supporting Information Table S3), increasing more than 60% during the spring–summer compared to autumn–winter (Table 1).

The strong variability in MR and similarity in P_{crit} across species, and the seasonal effects on both MR and P_{crit}, became evident when integrating data for the three species, independently of seasonal effects (Supporting Information Fig. S2). When separating by seasonal conditions, it was found that MR also exhibited strong intra-seasonal variability throughout the experimental range of pO_2, although data scattering within season and within species seems greater above the estimated P_{crit} values (Fig. 1). The estimated slopes of the linear regressions from each data set are provided in Supporting Information Table S6.

Coupling environmental conditions and copepods' metabolism

A time series of temperature and dissolved oxygen at a fixed station (Sta. 18, shown in Supporting Information Fig. S1) was available from August 2002 to March 2020, with missing data during half of 2016 through June 2018 (all data plotted Supporting Information Fig. S3). From this series, monthly climatology was derived for temperature, Chl *a*, and oxygen. There is a strong seasonal pattern during the annual cycle (Fig. 2). Most variability occurs in the upper 20 m with a surface warming between December and March and colder conditions during July–August, with a clear upwelling signal from mid-September to March when colder water (< 11°C) ascents into the upper 50 m (Fig. 2A). Chl *a* pattern reveals the strong phytoplankton bloom occurring during the spring–summer (Fig. 2B). The annual cycle of dissolved oxygen also has a strong seasonal signal. During spring–summer (upwelling), only the upper 30 m had O_2 above 1 mL L^{-1}, and below the OMZ prevails associated with cold-upwelled waters (Fig. 2C).

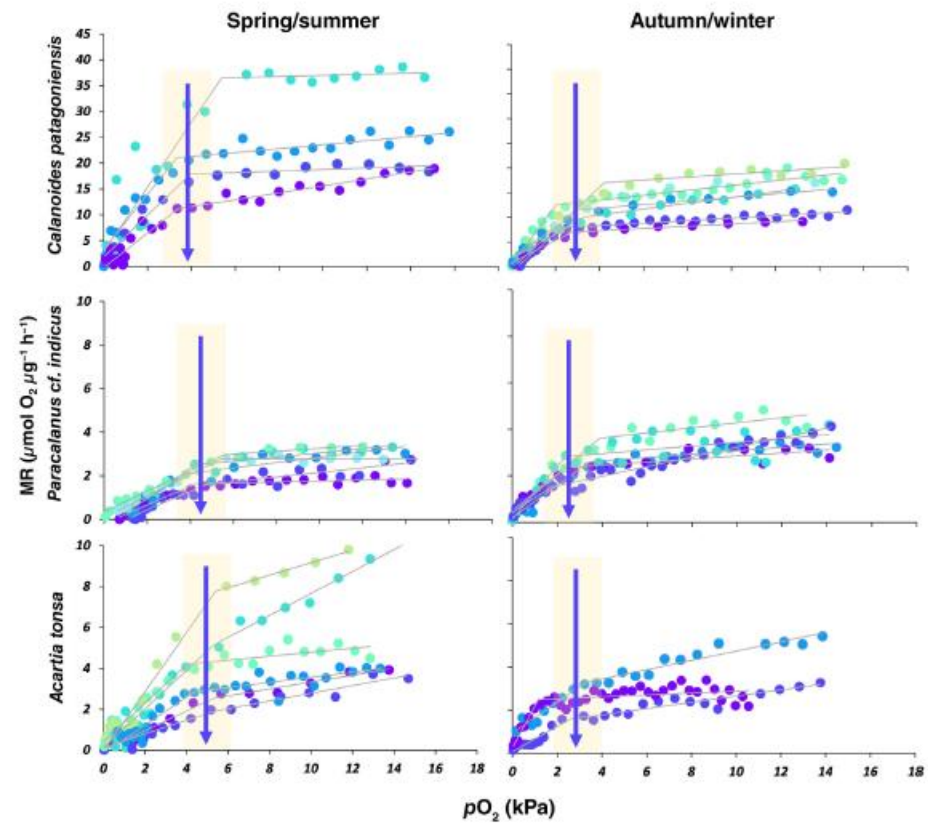

Fig. 1. Metabolic rate as a function of environmental oxygen and estimated critical partial pressure of oxygen (P_{crit}) in three copepod species, *Calanoides patagoniensis*, *Paracalanus* cf. *indicus*, and *Acartia tonsa*. These species inhabited the coastal upwelling of central-southern Chile and were captured in two seasonal periods (spring/summer and autumn–winter). Different colors represent replicated experiments within seasonal upwelling conditions. The vertical arrows signal P_{crit} values.

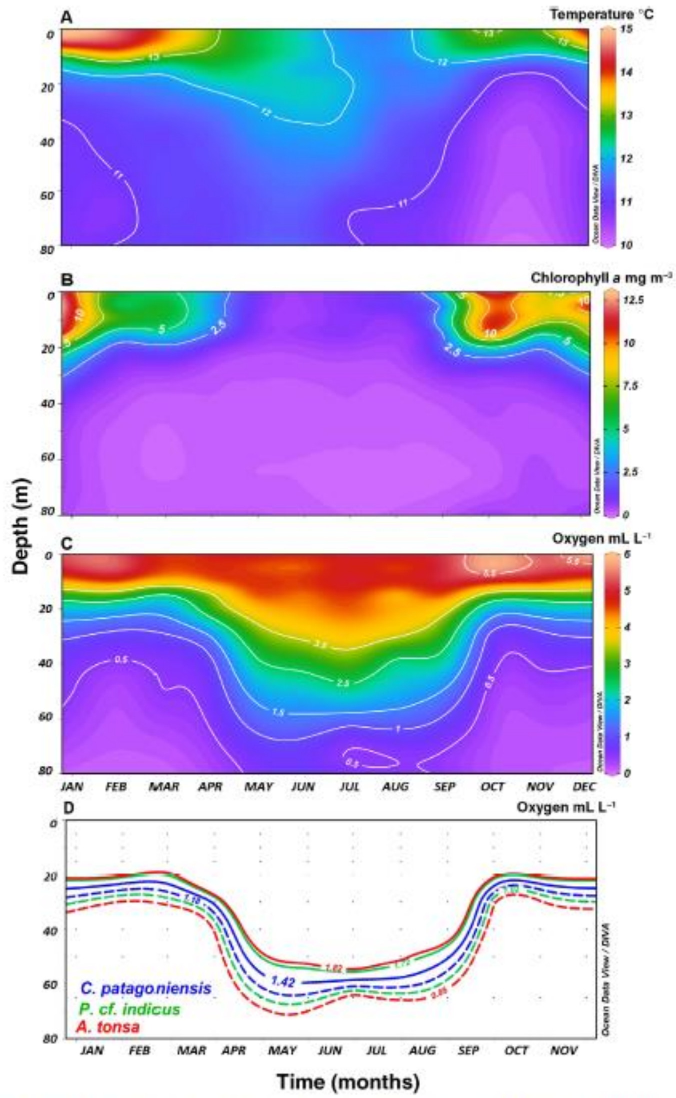

Fig. 2. Variability in temperature, chlorophyll *a*, and dissolved oxygen. The seasonal cycle of (**A**) temperature, (**B**) chlorophyll *a*, (**C**) dissolved oxygen in the water column at Sta. 18 in central-southern Chile. The seasonal cycle was obtained from monthly means from the period 2002 to 2016. (D) Vertical distribution of species-specific depth of P_{crit}. in relation to the annual variability of oxygen in the column water at Sta. 18 off central/southern Chile. The continuous line is P_{crit} estimated during the spring–summer and the dotted line represents P_{crit} depth for an autumn–winter condition.

Contrastingly, in autumn–winter the oxic layer deepens down to about 55 m while the OMZ seems to disappear at the Sta. 18 (Fig. 2C). We also used the oxygen time series to assess the depth at which copepod species could encounter oxygen conditions equal to or below their P_{crit} values during the year. We estimated the depth of the P_{crit}'s values for the spring–summer and autumn–winter conditions, both values converted to oxygen concentration (mL L^{-1}) for the entire year cycle (Fig. 2D). The seasonal pattern of upwelling and oxygenation at Sta. 18 becomes clearly reflected in the annual cycle of the "depth at P_{crit}" values. This cycle shows that the three species can encounter oxygen levels equal to or lower than their P_{crit} during most of the year within the upper 50 m. This condition becomes more critical during the spring–summer (September–March), when P_{crit} conditions are found in the upper 30 m layer (Fig. 2D).

Since the annual cycle may not represent the full range of oxygen conditions nor the eventual long-term fluctuations in water column oxygenation, we estimated the changes in depth of P_{crit} values for the entire time series by adjusting its values according to the mean temperature of the upper 50 m layer (Supporting Information Fig. S4). The mean depth of P_{crit} in *C. patagoniensis* was 34.3 ± 16.95 (mean ± SD) in the spring–summer, deepening down to 55.3 ± 16.81 m in the autumn–winter. In *P.* cf. *indicus*, the depth of P_{crit} was 27.2 ± 15.23 m in the spring–summer months and 55.4 ± 19.96 m during autumn–winter, whereas in *A. tonsa*, depth of P_{crit} ranged between 29.5 ± 15.52 m in spring–summer and 56.1 ± 16.56 in autumn–winter months. When separating both seasonal conditions, one-way ANOVA showed highly significant differences ($F_{2,327} = 7.51$, $p < 0.01$) in the depth of P_{crit} between species during the upwelling season (spring–summer), but with no significant differences ($F_{2,216} = 0.05$, $p > 0.05$) between species during the non-upwelling season (autumn–winter).

For the whole period, the depth of P_{crit} falls within the upper 50 m during most of the year, except for the months of July and August, when it deepens down to about 70 m. A similar pattern was observed for the three species (Supporting Information Fig. S4), also reflecting the strong differences in depth of P_{crit}'s between spring–summer vs. autumn–winter months.

Over the long term, based on these oxygen data, the "depth of P_{crit}" values exhibit strong interannual fluctuations, characterized by years when low oxygen prevails even during a non-upwelling condition (autumn–winter), such as during the winter of 2003, 2009, and partly 2012 (Supporting Information Fig. S4). There was also a weak low-frequency (3–4 yr) variation revealed by the smoothed series and the lack of a positive or negative trend during the entire period (Supporting Information Fig. S4).

Vertical distribution of copepods

During winter 2008, the vertical distribution of the three species over a cross-shelf transect is shown in Fig. 3. Copepods appeared mostly concentrated in the shallow stations close to the coast (Sta. 1, Sta. 18, and Sta. 3), and *P.* cf *indicus* was the most abundant species with the highest abundance of ca. 200 individuals m^{-2} in the upper layer (0–20 m) at Sta. 1. *C. patagoniensis* and *A. tonsa* also showed their maximum in the upper 20 m of Sta. 1 with 80 and 4 individuals × m^2, respectively. The abundance of *C. patagoniensis* and *P.* cf. *indicus* remained relatively high (> 10 individuals m^{-2}) in Sta. 18 and Sta. 3, whereas the abundance of *A. tonsa* decreased drastically westward down to less than two orders of magnitude, compared to the shallow Sta. 1 and Sta. 18. At the coastal stations (Sta. 1, Sta. 18, and Sta. 3), copepods were found at all sampled strata down to 100 m, except for *A. tonsa* which appeared mostly restricted to the upper 50 m (Fig. 3). Although, the abundance decreased with depth, copepods in the deeper strata (> 50 m depth) still exhibited densities comparable to those in the upper 20 m layer in the coastal stations (Fig. 3).

At the offshore stations (Sta. 4 and Sta. 5), the three copepod species were found in layers deeper than 50 m, although with 2–3 orders of magnitude less abundant than in the other coastal stations. At Sta. 5, the three species coincided in the same deep layers (> 100 m) with very low densities (< 2 individuals m^{-2}). Also, at this station, a nighttime sampling showed some variation in the vertical distribution of the three species between day and night abundances, although these changes did not clearly reveal a vertical migration across strata.

Discussion

Copepods inhabiting in the coastal upwelling zone exhibit a high ecological flexibility to withstand this physical and chemically unstable environment (Peterson 1998). They can also couple their annual life cycle to seasonal upwelling variation to optimize their temperature-dependent growth rates (Escribano et al. 2014) and their feeding behavior upon seasonal changes in the quality and quantity of food resources (Vargas et al. 2006). During the autumn–winter, under depressed upwelling and low phytoplankton biomass (*see* Fig. 2B), copepods favor an omnivorous diet, in contrast with the diatom-based diet prevailing during the spring–summer (Vargas et al. 2006). From this study, it seems copepods can also prime their oxygen uptake and transport in some cohorts. Copepod reproduction may also be coupled to seasonal upwelling (Hidalgo and Escribano 2007), such that even though reproduction can occur year-round, it increases during the spring–summer active upwelling, coinciding with a major phytoplankton bloom. Under these conditions, a greater feeding rate may induce increased MRs of copepods, as suggested by previous studies (Lampert 1984; Ikeda et al. 2000; Donoso and Escribano 2014), allowing them to maximize the utilization of food resources, thus fueling the intensified reproductive activity. This may be the case for *C. patagoniensis*, which can almost double its MR during the spring–summer.

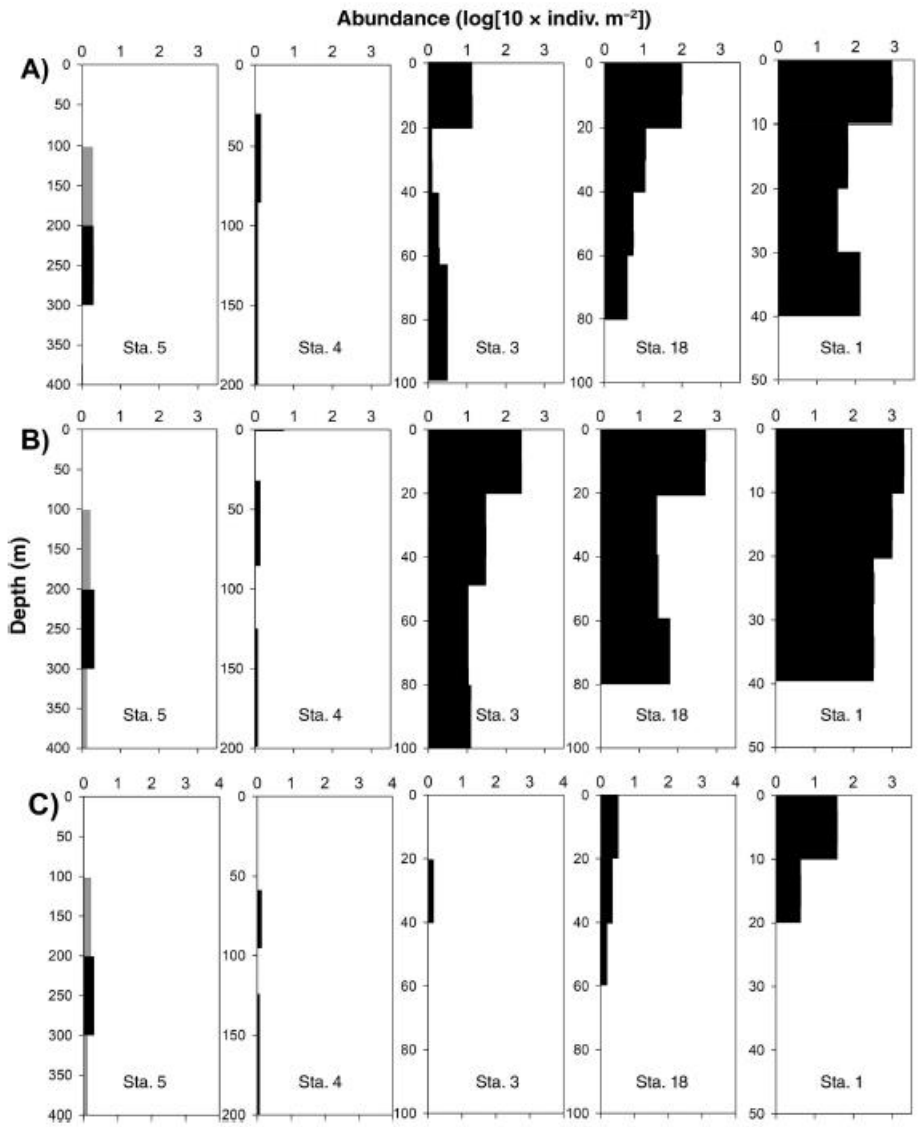

Fig. 3. Vertical distribution of three copepods in the coastal upwelling zone of central-southern Chile during the austral winter 2008. (**A**) *Calanoides patagoniensis*, (**B**) *Paracalanus* cf. *indicus*, (**C**) *Acartia tonsa*. Stations are over a cross-shelf transect from the coast (Sta. 1, 50 m depth) westward to most oceanic station (Sta. 5, 500 m depth) (*see* Supporting Information Fig. S1). Only Sta. 5 included a daytime vs. a nighttime sampling, while the other stations were sampled in daylight conditions. The gray bars in Sta. 5 showed nighttime abundance.

Contrastingly, *P.* cf. *indicus* and *A. tonsa* can maintain their MR throughout the year, allowing them to exploit alternate seasonal sources of food for year-round growth and reproduction (Hidalgo and Escribano 2007). During our respirometry experiments, incubations did not contain any food, and therefore, copepods may have fasted during the 6–8 h experiments. Studies on the gut content of copepods indicate that small species may respond rapidly (within hours) to changing food conditions (e.g., Kiørboe et al. 1985) and, so, affecting their MRs. The larger-sized *C. patagoniensis* may perhaps not respond so rapidly to starving conditions compared to the smaller *A. tonsa* and *P.* cf. *indicus*, providing more stored energy to satisfy metabolic demands. In this respect, Heine et al. (2019) found no significant differences in the response of respiration rate to increasing temperature when comparing fed and fasted copepods, and it is, therefore, possible that fasting conditions did not considerably affect the estimates of MR in our experiments. In fact, our results show that the MR is regulated for several hours down to the critical oxygen partial pressure, not showing any signs of changes in metabolic substrate usage or shortage. It is also important to note that *C. patagoniensis* had a much higher MR (10 times greater) than the other two species despite their larger body mass (Supporting Information Table S4). Respiration has been found to decrease with copepod size (allometric effect) (Hirst and Sheader 1997; Roman and Pierson 2022). However, some species-dependent adaptations may cause allometric deviations in animals inhabiting OMZs (Childress and Seibel 1998), and it has been suggested that in various animals, the oxygen supply capacity is adjusted to meet metabolic demands regardless of their size (Seibel and Deutsch 2020).

A higher MR in *C. patagoniensis* during the spring–summer was associated with warmer water in these seasons. A higher temperature can increase the MR (Roman et al. 2019), as expected for any ectotherm. The temperature at Sta. 18 was in the range of 11.8–13.6°C at 10 m depth (mean = 12.5°C), with an average of 12.0°C for the whole 50 m layer. During a year cycle, copepods are exposed to an entire range of 10.0–15.0°C in this layer (0–50 m). Our experiments were all performed at a constant temperature of 13°C, such that the temperature effect on the MR was controlled in the laboratory. In the field, however, the temperature varied by 5°C between winter and summer in the upper 50 m layer where most copepods reside, and therefore, when extrapolating our results to the field, both MR and critical oxygen partial pressure needed to be adjusted to in situ temperature (Supporting Information Table S5). This adjustment altered the estimates of the critical oxygen partial pressure, increasing/decreasing its value by about 5% per °C, suggesting that warmer conditions may reduce copepod tolerance to hypoxia by increasing their critical oxygen partial pressure and so encountering critical oxygen levels in shallower water.

When interpreting and understanding the effects of seasonal upwelling on critical oxygen partial pressure it must be considered that copepods are multigenerational organisms with a life span in the range of 10–40 d for most species. The small *P.* cf. *indicus* may have > 30 cohorts a year (Escribano et al. 2014), and although we lack information on the number of cohorts per year on *A. tonsa*, based on its size, it may have a similar number of generations per year to that of *P.* cf. *indicus*. This means that different cohorts are exposed to different oxygen regimes, suggesting the possibility of seasonal adaptation to the prevailing oxygen levels. However, during the spring–summer, when hypoxic conditions prevail in the water column, we observed greater critical oxygen partial pressure values, which will be encountered at shallower depths in the water column. In contrast, during winter, with a more oxygenated water column, lower values of critical oxygen partial pressure were found, allowing individuals to distribute deeper under oxygen levels higher than their P_{crit}. This response is not accompanied by seasonal changes in MR in *P.* cf. *indicus* and *A. tonsa*. Earlier, Fry and Hart (1984), based on the slope of the conforming segment, where the MR is directly proportional to ambient pO_2, realized that an increase in MR will mathematically result in a greater critical oxygen partial pressure if the slope of the conforming segment remains constant. This slope, so-called alpha (α) and recently subject to debate, is the line of respiratory dependence. In fact, it has been proposed that critical oxygen partial pressure is the result of reaching the maximum oxygen supply capacity (Seibel et al. 2021) on an organism, this is "the maximum amount of oxygen that can be supplied per unit time and oxygen pressure" and is a species-specific and temperature-specific constant. Based on our initial hypothesis, we would expect a shift in the α signaling hypoxia-driven adaptations. To verify this possibility, the slopes of the regressions and their corresponding standard errors were derived from the fitted equations, and they were found to be in the range of 0.07–0.60 (Supporting Information Table S6). A Student's *t*-test comparison between seasons, within species, revealed significant differences ($p < 0.05$) in *C. patagoniensis* and *P.* cf. *indicus*, but not in *A. tonsa* ($p > 0.05$). These findings suggest the existence of differential responses to hypoxia in the species under study, as illustrated in Fig. 4. *P.* cf. *indicus* and *A. tonsa* are able to maintain their MR throughout season, possibly to sustain year-round reproduction and growth, but at cost of increasing the critical oxygen partial pressure (P_{crit}) and so becoming less tolerant to upwelling-driven hypoxia (adaptive response 1 in Fig. 4). *C. patagoniensis* on the other hand appear optimizing their MR during the upwelling season to better exploit the spring–summer phytoplankton bloom, being able to maintain critical oxygen partial pressure and thus with a greater capacity to cope with seasonal hypoxia (adaptive response 2 in Fig. 4). Both types of adaptive responses imply a change in the slope (shown as α in Fig. 4), that is, in the oxygen supply capacity (or "conductance," Piiper et al. 1971), which should remain constant upon non-adaptive response (Fig. 4). To our knowledge, the present study is the first

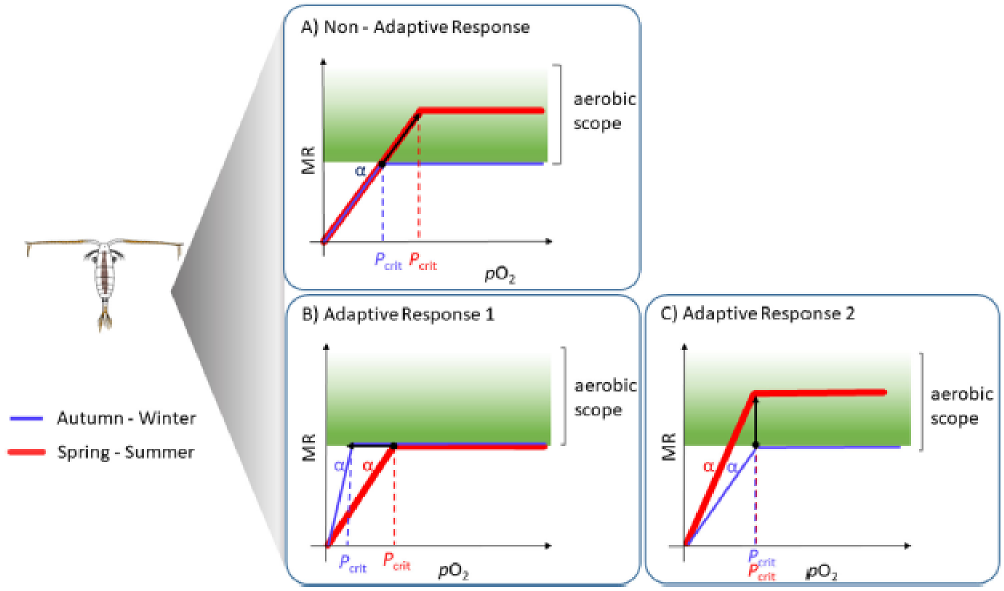

Fig. 4. The expected metabolic rate as a function of environmental oxygen partial pressure. The metabolic rate is theorized for two seasons in a coastal upwelling zone based on two situations: (**A**) lack of adaptive response, where P_{crit} will move following changes in MR, (**B**) showing an adaptive response 1 in which P_{crit} increases upon a changing oxyconforming slope to maintain MR. In response 2, P_{crit} is maintained by increasing the slope of the oxyconforming segment during the upwelling season. The green shaded area represents the possible metabolic scope, which only remains unaffected in the adaptive response depicted in **B**.

showing seasonal changes oxygen supply capacity (α), likely possible by the existence of different cohorts between contrasting seasons and thus, selecting for an elevated metabolic capacity when hypoxia intensifies. This appearance of more "athletic" individuals has been hypothesized to occur under persistent hypoxia (Seibel et al. 2021), such as an upwelling system, and mediated by improved conductance along the oxygen cascade (Farrell et al. 2021). Modifying the oxygen supply capacity should thus be considered as a species-dependent physiological adaptation in seasonal cohorts of copepods, to optimize metabolic outcomes under seasonally varying food sources, temperature, and dissolved oxygen.

The adaptive response 1 to hypoxia (Fig. 4B) can have major ecological consequences for copepods under a scenario of increasing upwelling in eastern boundary upwelling systems (Schneider et al. 2016; Xiu et al. 2018). The shoaling of the OMZ upon increased upwelling will lead to an increased occurrence of conditions with oxygen levels equal to or lower than critical oxygen partial pressure, implying an effective vertical reduction of the normoxic environment. Our monthly time series of dissolved oxygen in the water column shows that out of 163 monthly observations, there were 111 occasions (i.e., ca. 70%) having oxygen levels lower than the critical oxygen partial pressure of all species tested within the upper 50 m layer. This implies strong limitations on the aerobic metabolism of copepods, which, given the frequency of occurrence, may have drastic implications for the whole planktonic community and food webs. Studies show negative effects of hypoxia on the occurrence and abundance of zooplankton (Auel and Verheye 2007; Ekau et al. 2010; Roman et al. 2019), with unpredictable consequences for the whole pelagic food web due to alterations at basal levels.

The vertical distribution and potential migration of copepods, becomes crucial to maximize aerobic metabolism in the water column. Our zooplankton sampling in 2008 shows that copepods can be found at any depth within the upper 50 m during winter conditions (Supporting Information Fig. S4) in the coastal area. Although we do not have this data for spring–summer conditions, a previous study performed in a nearby area during January 1985 (austral summer) (Castro et al. 2007) showed that *A. tonsa* concentrate in the upper 20 m, *P.* cf. *indicus* (named as *Paracalanus parvus*) in the upper 30 m, and *C. patagoniensis* seem more widely spread in the upper 50 m. Yet more remarkably, this summer vertical distribution fits with the species-specific measured critical oxygen partial pressure, thus with their physiological limit to sustain aerobic metabolism matching its ability to exploit different habitat volumes. There are also some differences in vertical distribution, depending on the cross-shelf position. For instance, *A. tonsa* seem to aggregate in shallow water nearshore (Sta. 1 in Supporting Information Fig. S1), whereas the other two species appear more widely distributed at all coastal stations. The presence of the three species at the offshore stations (Sta. 4 and Sta. 5) in deep strata, in very low abundances, may not necessarily reflect the actual habitat of these copepods. Copepods found at such depths may be part of their populations being advected offshore and further subducted at depth by physical processes taking place at the upwelling zone, as described in Gonzalez et al. (2023). We do not have information regarding diel vertical migration (DVM) on these species in this area, but for these small and mid-size copepods the vertical amplitude of DVM may not be greater than 30 m (Tutasi and Escribano 2020), and so even performing DVM they can hardly avoid being exposed to hypoxic water within the upper 50 m.

Differential distribution may influence the impact of the hypoxia on the species population but also on the whole community. For instance, *A. tonsa* may be less affected by residing in the nearshore and shallow water, which is usually highly oxygenated, contrasting with *C. patagoniensis* and *P.* cf. *indicus*, both inhabiting deeper under the influence of the OMZ waters. Differences in vertical distribution in relation to the cross-shelf dimension may also relate to the spatial distribution of the OMZ, which tends to become much shallower in the nearshore, especially during the summer, by entering the upper 50 m (*see* Supporting Information Fig. S7).

Despite the potential differential impact of hypoxia, depending on vertical distribution and on the adaptive responses shown (Fig. 4), the three species are indeed frequently exposed to hypoxic events, which become greatly augmented during the spring–summer condition. In our experiments, copepod mortality was greater than 70% (Supporting Information Table S7) after reaching critical oxygen partial pressure, suggesting that copepods in the field are experiencing severe episodes of mortality. Previous studies in this region have already shown that increased upwelling negatively impacts copepod abundances (Escribano et al. 2012; Pino-Pinuer et al. 2014), and this impact may relate to increased hypoxia upon upwelling intensification. Such effects can be greater in early life stages, which tend to increase in number during the spring–summer when hypoxia also increases, thus with major consequences for copepods population dynamics, but also for the whole food web.

Ocean deoxygenation appears as a gradual global process triggered by global warming (Breitburg et al. 2018). However, observations and modeling regarding the responses of highly productive coastal eastern boundary upwelling systems to global warming also indicate and predict the intensification of wind-driven upwelling (Bakun et al. 2010; Schneider et al. 2016; Xiu et al. 2018). More upwelling implies a shoaling of the OMZ at the upwelling zone and a consequent shoaling of the "depth of critical oxygen partial pressure," so further reducing the normoxic habitat of copepods.

The present results highlight the vulnerability of planktonic copepods to climate change, even in highly variable environments such as the upwelling zone, where the OMZ waters intrude seasonally to shallower waters. Further analysis on species-specific physiological tolerances may allow better and more environmentally relevant predictions on what species are more likely to prevail or to be less constrained under the currently seen and future predicted OMZ expansion.

DATA AVAILABILITY STATEMENT

Data on experimental work are available atDOI: 10.5281/zenodo.10668060.

References

Auel, H., and H. Verheye. 2007. Hypoxia tolerance in the copepod *Calanoides carinatus* and the effect of an intermediate oxygen minimum layer on copepod vertical distribution in the northern Benguela Current upwelling system and the Angola–Benguela Front. J. Exp. Mar. Biol. Ecol. **352**: 234–243. doi:10.1016/j.jembe.2007.07.020

Bakun, A., D. B. Field, A. Redondo-Rodriguez, and S. J. Weeks. 2010. Greenhouse gas, upwelling-favorable winds, and the future of coastal ocean upwelling ecosystems. Glob. Change Biol. **16**: 1213–1228. doi:10.1111/j.1365-2486.2009.02094.x

Birk, M. A. 2021. Respirometry: Tools for conducting and analyzing respirometry experiments. R package version 1.3.0. https://CRAN.R-project.org/package=respirometry

Breitburg, D., and others. 2018. Declining oxygen in the global ocean and coastal waters. Science **359**: eaam7240. doi:10.1126/science.aam7240

Castro, L. R., V. A. Troncoso, and D. R. Figueroa. 2007. Fine-scale vertical distribution of coastal and offshore copepods in the Golfo de Arauco, central Chile, during the upwelling season. Prog. Oceanogr. **75**: 486–500. doi:10.1016/j.pocean.2007.08.012

Chan, F., J. A. Barth, J. Lubchenco, A. Kirincich, H. Weeks, W. T. Peterson, and B. A. Menge. 2008. Emergence of anoxia in the California Current large marine ecosystem. Science **319**: 920. doi:10.1126/science.1149016

Childress, J., and B. Seibel. 1998. Life at stable low oxygen levels: Adaptations of animals to oceanic oxygen minimum layers. J. Exp. Biol. **201**: 1223–1232. doi:10.1242/jeb.201.8.1223

Cobbs, G. A., and J. E. Alexander Jr. 2018. Assessment of oxygen consumption in response to progressive hypoxia. PloS One **13**: e0208836. doi:10.1371/journal.pone.0208836

Daneri, G., V. Dellarossa, R. Quiñones, B. Jacob, P. Montero, and O. Ulloa. 2000. Primary production and community respiration in the Humboldt Current System off Chile and associated oceanic areas. Mar. Ecol. Prog. Ser. **197**: 41–49. doi:10.3354/meps197041

Diaz, R. J., and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science **321**: 926–929. doi:10.1126/science.1156401

Donoso, K., and R. Escribano. 2014. Mass-specific respiration of mesozooplankton and its role in the maintenance of an oxygen-deficient ecological barrier (BEDOX) in the upwelling zone off Chile upon presence of a shallow oxygen minimum zone. J. Mar. Syst. **129**: 166–171. doi:10.1016/j.jmarsys.2013.05.011

Ekau, W., H. Auel, H. O. Portner, and D. Gilbert. 2010. Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish). Biogeosciences **7**: 1669–1699. doi:10.5194/bg-7-1669-2010

Escribano, R., and W. Schneider. 2007. The structure and functioning of the coastal upwelling system off central/southern Chile. Prog. Oceanogr. **75**: 343–346. doi:10.1016/j.pocean.2007.08.020

Escribano, R., P. Hidalgo, and C. Krautz. 2009. Zooplankton associated with the oxygen minimum zone system in the northern upwelling region of Chile during March 2000. Deep-Sea Res. II: Top. Stud. Oceanogr. **56**: 1083–1094. doi:10.1016/j.dsr2.2008.09.009

Escribano, R., and C. E. Morales. 2012. Spatial and temporal scales of variability in the coastal upwelling and coastal transition zone off central-southern Chile (35–40°S). Prog. Oceanogr. **92–95**: 1–7. doi:10.1016/j.pocean.2011.07.019

Escribano, R., P. Hidalgo, M. Fuentes, and K. Donoso. 2012. Zooplankton time series in the coastal zone off Chile: variation in upwelling and responses of the copepod community. Prog. Oceanogr. **97–100**: 174–186. doi:10.1016/j.pocean.2011.11.006

Escribano, R., P. Hidalgo, V. Valdés, and L. Frederick. 2014. Temperature effects on development and reproduction of copepods in the Humboldt Current: the advantage of rapid growth. J. Plankton Res. **36**: 104–116. doi:10.1093/plankt/fbt095

Escribano, R., E. Bustos-Ríos, P. Hidalgo, and C. E. Morales. 2016. Non-limiting food conditions for growth and production of the copepod community in a highly productive upwelling zone. Cont. Shelf. Res. **126**: 1–14. doi:10.1016/j.csr.2016.07.018

Farrell, A. P., C. A. Mueller, and R. S. Seymour. 2021. Coming up for air. J. Exp. Biol **224**: jeb243101. doi:10.1242/jeb.243101

Fry, F. E. J., and J. S. Hart. 1984. The relation of temperature to oxygen consumption in the goldfish. Biol. Bull. **94**: 66–77. doi:10.2307/1538211

Gilly, W. G., M. Beman, S. Y. Litvin, and B. H. Robison. 2018. Oceanographic and biological effects of shoaling of the oxygen minimum zone. Ann. Rev. Mar. Sci. **5**: 393–420. doi:10.1146/annurev-marine-120710-100849

Gonzalez, C. E., A. Bode, I. Fernández-Urruzola, P. Hidalgo, V. Oerder, and R. Escribano. 2023. The lateral transport of zooplankton explains trophic and taxonomic similarities over the zonal gradient of central Chile. J. Mar. Syst. **238**: 103840. doi:10.1016/j.jmarsys.2022.103840

Grantham, B. A., F. Chan, K. J. Nielsen, D. S. Fox, J. A. Barth, A. Huyer, J. Lubchenco, and B. A. Menge. 2004. Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes in the northeast Pacific. Nature **429**: 749–754. doi:10.1038/nature02605

Heine, K. B., A. Abebe, A. E. Wilson, and W. R. Hood. 2019. Copepod respiration increases by 7% per °C increase in temperature: A meta-analysis. Limnol. Oceanogr.: Lett. **4**: 53–61. doi:10.1002/lol2.10106

Hidalgo, P., and R. Escribano. 2007. The coupling of life cycles of the copepods *Calanus chilensis* and *Centropages brachiatus* to upwelling variability in the central-south upwelling region off Chile. Prog. Oceanogr. **75**: 501–517. doi:10.1016/j.pocean.2007.08.028

Hidalgo, P., R. Escribano, O. Vergara, E. Jorquera, K. Donoso, and P. Mendoza. 2010. Patterns of copepod diversity in the Chilean coastal upwelling system. Deep-Sea Res. II: Top. Stud. Oceanogr. **57**: 2089–2097. doi:10.1016/j.dsr2.2010.09.012

Hirst, A. G., and M. Sheader. 1997. Are in situ weight specific growth rates body-size independent in marine planktonic copepods? A re-analysis of the global syntheses and a new empirical model. Mar. Ecol. Prog. Ser. **154**: 155–165. doi:10.3354/meps154155

Hochachka, P. W., and G. N. Somero. 1984. Biochemical adaptation. Princeton Univ. Press.

Hochachka, P. W., and G. N. G. Somero. 2002. Biochemical adaptation: Mechanism and process in physiological evolution. Oxford Univ. Press.

Ikeda, T., J. J. Torres, S. Hernández-León, and S. P. Geiger. 2000. Metabolism, p. 455–532. *In* R. Harris, P. H. Wiebe, J. Lenz, H. R. Skjoldal, and M. Huntley [eds.], ICES zooplankton methodology manual. Academic Press.

Keeling, R. E., A. Körtzinger, and N. Gruber. 2010. Ocean deoxygenation in a warming world. Ann. Rev. Mar. Sci. **2**: 199–229. doi:10.1146/annurev.marine.010908.163855

Kiko, R., H. Hauss, F. Buchholz, and F. Melzner. 2016. Ammonium excretion and oxygen respiration of tropical copepods and euphausiids exposed to oxygen minimum zone conditions. Biogeosciences **13**: 2241–2255. doi:10.5194/bg-13-2241-2016

Kiørboe, T., F. Møhlenberg, and K. Hamburger. 1985. Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. Mar. Ecol. Prog. Ser. **26**: 85–97. doi:10.3354/meps026085

Lampert, W. 1984. The measurement of respiration, p. 413–468. *In* J. A. Downing and F. H. Rigler [eds.], A manual on methods for the assessment of secondary productivity in fresh waters, 2nd ed. Blackwell.

Landaeta, M. F., R. Veas, L. Letelier, and L. R. Castro. 2008. Larval fish assemblages off central Chile upwelling ecosystem. Rev. Biol. Mar. Oceanogr. **43**: 569–584. doi:10.4067/S0718-19572008000300016

Limburg, K. E., D. Breitburg, D. P. Swaney, and G. Jacinto. 2020. Ocean deoxygenation: A primer. One Earth **2**: 24–29. doi:10.1016/j.oneear.2020.01.001

Medellín-Mora, J., A. Atkinson, and R. Escribano. 2020. Community structured production of zooplankton in the eastern boundary upwelling system off central/southern Chile (2003-2012). ICES J. Mar. Sci **77**: 419–435. doi:10.1093/icesjms/fsz193

Morales, C. E., H. E. González, S. E. Hormazabal, G. Yuras, J. Letelier, and L. R. Castro. 2007. The distribution of chlorophyll-*a* and dominant planktonic components in the coastal transition zone off Concepción, central Chile, during different oceanographic conditions. Prog. Oceanogr. **75**: 452–469. doi:10.1016/j.pocean.2007.08.026

Paulmier, A., D. Ruiz-Pino, V. Garçon, and L. Farías. 2006. Maintaining of the eastern South Pacific oxygen minimum zone (OMZ) off Chile. Geophys. Res. Lett. **33**: L20601. doi:10.1029/2006GL026801

Peterson, W. 1998. Life cycle strategies of copepods in coastal upwelling zones. J. Mar. Syst. **15**: 313–326. doi:10.1016/S0924-7963(97)00082-1

Piiper, J., P. Dejaours, P. Haab, and H. Rahn. 1971. Concepts and basic quantities in gas exchange physiology. Respir. Physiol. **13**: 292–304. doi:10.1016/0034-5687(71)90034-x

Pino-Pinuer, P., R. Escribano, P. Hidalgo, R. Riquelme-Bugueño, and W. Schneider. 2014. Copepod community response to variable upwelling conditions off central-southern Chile during 2002-2004 and 2010-2012. Mar. Ecol. Prog. Ser. **515**: 83–95. doi:10.3354/meps11001

Riquelme Bugueño, R., I. Pérez Santos, N. Alegría, C. A. Vargas, M. A. Urbina, and R. Escribano. 2020. Diel vertical migration into anoxic and high pCO_2 waters: acoustic and net based krill observations in the Humboldt Current. Sci. Rep. **10**: 17181. doi:10.1038/s41598-020-73702-z

Rogers, N. J., M. A. Urbina, E. E. Reardon, D. J. McKenzie, and R. W. Wilson. 2016. A new analysis of hypoxia tolerance in fishes using a database of critical oxygen level (P_{crit}). Conserv. Physiol. **4**: cow012. doi:10.1093/conphys/cow012

Roman, M. R., S. B. Brandt, E. D. Houde, and J. J. Pierson. 2019. Interactive effects of hypoxia and temperature on coastal pelagic zooplankton and fish. Front. Mar. Sci. **6**: 139. doi:10.3389/fmars.2019.00139

Roman, M. R., and J. J. Pierson. 2022. Interactive effects of increasing temperature and decreasing oxygen on coastal copepods. Biol. Bull. **243**: 171–183. doi:10.1086/722111

Sampaio, E., C. Santos, I. C. Rosa, V. Ferreira, H. O. Portner, C. M. Duarte, L. A. Levin, and R. Rosa. 2021. Impacts of hypoxic events surpass those of future ocean warming and acidification. Nat. Ecol. Evol. **5**: 311–321. doi:10.1038/s41559-020-01370-3

Schmidtko, S., L. Stramma, and M. Visbeck. 2017. Decline in global oceanic oxygen content during the past five decades. Nature **542**: 335–339. doi:10.1038/nature21399

Schneider, W., D. Donoso, J. Garcés-Vargas, and R. Escribano. 2016. Water-column cooling and sea surface salinity increase in the upwelling region off central-south Chile driven by a poleward displacement of the South Pacific. Prog. Oceanogr. **141**: 38–58.

Seibel, B. A. 2011. Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. J. Exp. Biol. **214**: 326–336. doi:10.1242/jeb.049171

Seibel, B. A., and C. Deutsch. 2020. Oxygen supply capacity in animals evolves to meet maximum demand at the current oxygen partial pressure regardless of size or temperature. J. Exp. Biol. **223**: jeb210492. doi:10.1242/jeb.210492

Seibel, B. A., A. Andres, M. A. Birk, A. L. Burns, C. T. Shaw, A. W. Timpe, and C. J. Welsh. 2021. Oxygen supply capacity breathes new life into critical oxygen partial pressure (P_{crit}). J. Exp. Biol. **224**: jeb242210. doi:10.1242/jeb.242210

Sobarzo, M., and L. Djurfeldt. 2004. Coastal upwelling process on a continental shelf limited by submarine canyons, Concepción, central Chile. J. Geophys. Res.: Oceans **109**: C12012. doi:10.1029/2004JC002350

Sobarzo, M., L. Bravo, D. Donoso, J. Garces-Vargas, and W. Schneider. 2007. Coastal upwelling and seasonal cycles that influence the water column over the continental shelf off central Chile. Prog. Oceanogr. **75**: 363–382. doi:10.1016/j.pocean.2007.08.022

Stramma, L., G. C. Johnson, J. Sprintall, and V. Mohrholz. 2008. Expanding oxygen-minimum zones in the tropical oceans. Science **320**: 655–658. doi:10.1126/science.1153847

Stramma, L., S. Schmidtko, L. Levin, and G. C. Johnson. 2010. Ocean oxygen minima expansions and their biological impacts. Deep-Sea Res. I: Oceanogr. Res. Pap. **57**: 587–595. doi:10.1016/j.dsr.2010.01.005

Stramma, L., and others. 2012. Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes. Nat. Clim. Change **2**: 33–37. doi:10.1038/nclimate1304

Strub, P., J. Mesias, V. Montecino, J. Rutlland, and S. Salinas. 1998. Coastal ocean circulation off western South America, p. 273–313. *In* A. R. Robinson and K. H. Brink [eds.], The sea, v. **11**. John Wiley and Sons.

Tutasi, P., and R. Escribano. 2020. Zooplankton diel vertical migration and downward C flux into the oxygen minimum zone in the highly productive upwelling region off northern Chile. Biogeosciences **17**: 455–473. doi:10.5194/bg-17-455-2020

Vargas, C., R. Escribano, and S. Poulet. 2006. Phytoplankton food quality determines time windows for successful zooplankton reproductive pulses. Ecology **87**: 2992–2999. doi:10.1890/0012-9658(2006)87[2992:pfqdtw]2.0.co;2

Vargas, V., R. A. Martínez, R. Escribano, and N. A. Lagos. 2010. Seasonal relative influence of food quantity, quality, and feeding behavior on zooplankton growth regulation in coastal food webs. J. Mar. Biol. Assoc. **90**: 1189–1201. doi: 10.1017/S0025315409990804

Wilkinson, L. 1990. SYSTAT: the system for statistics. SYSTAT Inc.

Wishner, K. F., D. M. Outram, B. A. Seibel, K. L. Daly, and R. L. Williams. 2013. Zooplankton in the eastern tropical north Pacific: Boundary effects of oxygen minimum zone expansion. Deep-Sea Res. I: Oceanogr. Res. Pap. **79**: 122–140. doi:10.1016/j.dsr.2013.05.012

Wishner, K. F., and others. 2018. Ocean deoxygenation and zooplankton: Very small oxygen differences matter. Sci. Adv. **4**: eaau5180. doi:10.1126/sciadv.aau5180

Xiu, P., F. Chai, E. N. Curchitser, S. Frederic, and S. Castruccio. 2018. Future changes in coastal upwelling ecosystems with global warming: The case of the California Current System. Sci. Rep. **8**: 2866. doi:10.1038/s41598-018-21247-7

Zhou, Y., H. Gong, and F. Zhou. 2022. Responses of horizontally expanding oceanic oxygen minimum zones to climate change based on observations. Geophys. Res. Lett. **49**: e2022GL097724. doi:10.1029/2022GL097724

Acknowledgments

This work has been funded by Agencia Nacional para la Investigación y Desarrollo (ANID), Chile, grant FONDECYT 1210071 to MU and RE; the Instituto Milenio de Oceanografía (IMO), grant ICN12_019 to RE and MU, grant FONDECYT 1181682 to RE; the PhD Scholarship of Agencia Nacional de Investigacion y Desarrollo (ANID), grant 21181372 to LF and grant ANILLO ACT210073 to MU and RE. We are grateful to three anonymous reviewers whose comments and suggestions greatly improved our work.

Conflict of Interest

Authors declare no competing interests.

Submitted 23 March 2023
Revised 06 December 2023
Accepted 09 March 2024

Associate editor: Thomas Kiørboe

4.2 **Chapter 2**: “Reviews and synthesis on increasing hypoxia in eastern boundary upwelling systems: a major stressor for zooplankton”. Scientific manuscript submitted to “Biogeosciences” journal.

Summary:

Eastern boundary upwelling systems (EBUS) are ecologically and economically important marine regions of the world ocean. In these systems, zooplankton play a pivotal role in transferring primary production up through the food web. Recent studies show that global warming is causing a gradual deoxygenation of the world ocean, while in EBUS a vertical expansion of the subsurface oxygen minimum zone (OMZ) along with increased wind-driven upwelling is taking place, further exacerbating hypoxic conditions for zooplankton inhabiting the coastal upwelling zone. Hypoxia can affect zooplankton by disrupting their respiration, migration, reproduction, and development. These effects however depend on some specific adaptations of organisms that have evolved in habitats, permanently or episodically, subjected to low oxygen waters. Various metabolic, physiological, behavioral and morphological adaptations have been described in zooplankton interacting with the OMZ. Nevertheless, adaptive responses of zooplankton to withstand mild or severe hypoxia, and the eventual oxidative stress derived from highly fluctuating oxygen conditions have not been fully addressed in EBUS. The existence or lack of such adaptive responses can play a crucial role for zooplankton dynamics in EBUS with major consequences for their food web and biological productivity, and the aim of exploring this here.

Resumen

Los sistemas de surgencia de borde oriental (EBUS) son regiones marinas del océano mundial de importancia ecológica y económica. En estos sistemas, el zooplancton desempeña un papel fundamental en la transferencia de la producción primaria a través de la red alimentaria. Estudios recientes muestran que el calentamiento global está provocando una desoxigenación gradual de los océanos del mundo, mientras que en EBUS se está produciendo una expansión vertical de la zona mínima de oxígeno sub-superficial (OMZ) junto con un aumento de la surgencia impulsadas por el viento, lo que exacerba aún más las condiciones hipóxicas para el zooplancton que habita en la zona de surgencia costera. La hipoxia puede afectar al zooplancton al alterar su respiración, migración, reproducción y desarrollo. Sin embargo, estos efectos dependen de algunas adaptaciones específicas de los organismos que han evolucionado en hábitats, permanente o episódicamente, sujetos a aguas con poco oxígeno. Se han descrito diversas adaptaciones metabólicas, fisiológicas, conductuales y morfológicas en el zooplancton que interactúa con la OMZ. Sin embargo, las respuestas adaptativas del zooplancton para resistir una hipoxia leve o grave y el eventual estrés oxidativo derivado de condiciones de oxígeno altamente fluctuantes no se han abordado completamente en la EBUS. La existencia o falta de tales respuestas adaptativas puede desempeñar un papel crucial para la dinámica del zooplancton en EBUS con importantes consecuencias para su red alimentaria y productividad biológica, y el objetivo de explorar esto aquí.

Reviews and synthesis: increasing hypoxia in eastern boundary upwelling systems: a major stressor for zooplankton

Leissing Frederick[1], Mauricio A. Urbina[2, 3] and Ruben Escribano[3,4]

[1] Doctoral Program in Oceanography, University of Concepcion, Chile

[2] Department of Zoology, Faculty of Natural Sciences and Oceanography, University of Concepcion, Chile

[3] Millennium Institute of Oceanography, University of Concepcion, Chile

[4] Department of Oceanography, Faculty of Natural Sciences and Oceanography, University of Concepcion, Chile

Correspondence to: Ruben Escribano (rescribano@udec.cl)

Abstract. Eastern boundary upwelling systems (EBUS) are ecologically and economically important marine regions of the world ocean. In these systems, zooplankton play a pivotal role in transferring primary production up through the food web. Recent studies show that global warming is causing a gradual deoxygenation of the world ocean, while in EBUS a vertical expansion of the subsurface oxygen minimum zone (OMZ) along with increased wind-driven upwelling are taking place, further exacerbating hypoxic conditions for zooplankton inhabiting the upwelling zone. Hypoxia can affect zooplankton by disrupting their respiration, migration, reproduction, and development. These effects however depend on some specific adaptations of organisms that have evolved in habitats, permanently or episodically, subjected to low oxygen waters. Various metabolic, physiological, behavioural,

and morphological adaptations have been described in zooplankton interacting with the OMZ. Nevertheless, these adaptive responses of zooplankton to withstand mild or severe hypoxia, and the eventual oxidative stress derived from highly fluctuating oxygen conditions, may develop in association with trade-offs related to other metabolic/energy-demanding processes. New demands imply a reduction in energy otherwise available for growth, feeding and reproduction with further ecological consequences for the populations. This paper reviews and explores the existence or lack of such adaptive responses and their role for zooplankton dynamics in EBUS with major consequences for the pelagic food web and biological productivity.

1 INTRODUCTION

Currently it is widely recognized that the increase in atmospheric CO_2 and other greenhouse gasses is driving the warming of the Earth's surface and ocean (Oschlies et al., 2018). A warmer ocean drives several physical consequences, such as the increases in mean global sea surface temperature, increasing winds, more intense storms in some regions, and changes in ocean circulation (Schmidtko et al., 2017). The warming of the upper layers of the ocean also drives a greater stratification of the water column, reducing vertical mixing and thus affecting ocean ventilation. A warmer ocean also lowers oxygen solubility, and hence further challenging marine life. Under such a scenario, deoxygenation (a decline in oxygen) in the open ocean and coastal water has increased since the middle of the 20th century (Strama et al., 2008, Gregoire et al., 2021). Ocean warming also increases the metabolic rate of ectotherms, promoting a greater oxygen usage by marine communities, and further exacerbating the oxygen decline (Breitburg et al, 2018).

The decrease in oxygen concentration, evident in many areas of the ocean, becomes even more critical in regions with persistent and currently expanding oxygen minimum zones (OMZ) (Gregoire et al., 2021). These OMZ systems are defined by their extremely low oxygen concentrations (<20-45 µmol kg-1), covering large areas of the ocean, and associated with highly productive coastal and oceanic regions (Gilly et al., 2013). A vertical expansion of the OMZs has been evidenced and it is related to a shoaling of its upper boundary, and descent of the lower boundary, and thus increasing its total volume (Stramma et al., 2010). In some cases, the minimum oxygen concentrations in the OMZ cores have also been further reduced, intensifying the OMZ (Chan et al., 2008).

In the four major eastern boundary current systems (EBUS) (Chavez and Messié, 2009), the effect of climate change has been associated with an intensification of the physical forcings driving coastal upwelling (Bakun et al., 2010; Xiu et al. 2018), leading to several changes on the physical-chemical properties of the water column, including a gradual cooling in the last few decades (Santos et al., 2012; Schneider et al., 2016). The increase in upwelling favourable winds in EBUS brings colder water and more frequent occurrences of upwelling events (Breitburg et al., 2018). Stronger upwelling is ultimately thought to be a response to the strengthening of large-scale pressure gradients linked to global-scale climate change (Garcia-Reyes and Largier, 2009). With the intensification of the coastal upwelling, a shoaling of the oxygen minimum zones (OMZ) in coastal waters takes place and so compressing the upper highly oxygenated layer. The closely linked effects of increasing upwelling, cooling of the water column and shoaling of the OMZ in EBUS driven by global warming are illustrated in Fig. 1.

The ongoing combined processes, deoxygenation, increasing upwelling, and OMZ expansion will alter the oxygen conditions in upper layers (<50 m) in EBUS, where plankton becomes concentrated, with various ecological and biogeochemical consequences. Aerobic metazooplankton inhabiting the upwelling zone is thus expected to be exposed to variable levels of oxygenation from normoxia to mild or sever hypoxia, depending on their distribution and migrating behaviour. Their responses will also depend on the existence, absence, or development of new adaptations. In this paper, we review such adaptive responses of zooplankton and the ecological consequences driven by hypoxia, aiming at establishing the physiological/metabolic bases and directions when addressing issues related to the future of zooplankton dynamics in EBUS subjected to ongoing climate change.

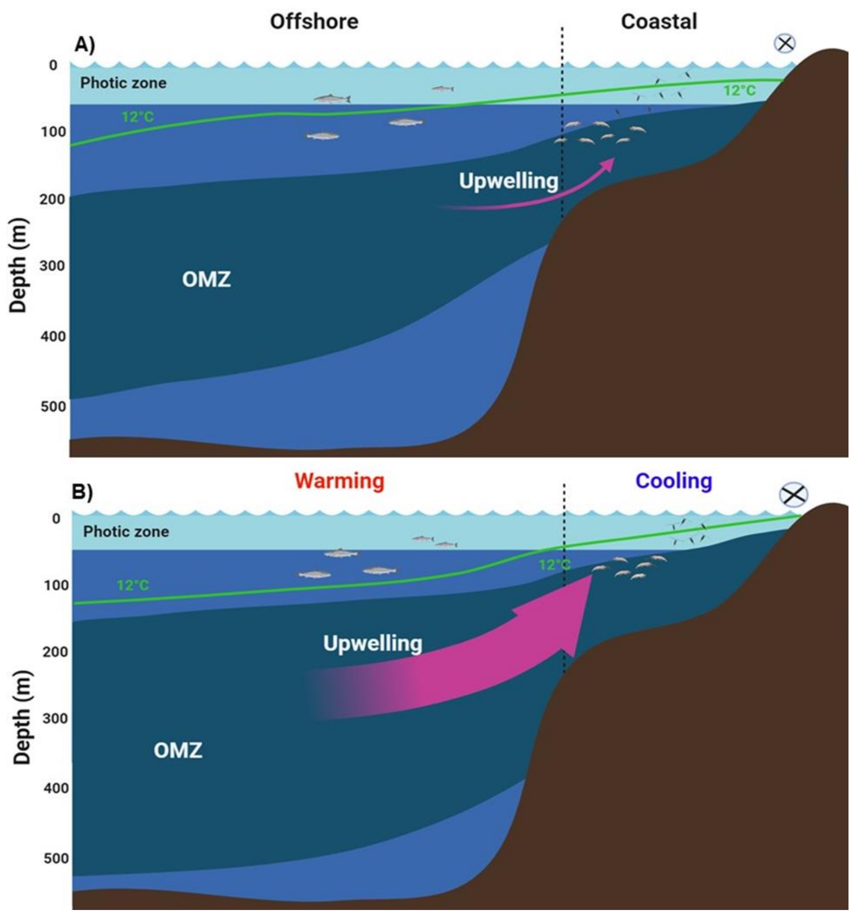

Figure 1: Projected effects of increasing upwelling in eastern boundary upwelling systems (EBUS). A) Under present (initial) conditions, wind-driven upwelling rises the OMZ system

and brings cold-water into shallow depths at the inshore as illustrated by the 12°C isotherm, and so fertilizes the photic zone and promotes plankton aggregation. B) Ocean warming effects manifest mainly at surface in the offshore region, while increased upwelling may instead cool down the coastal zone (B), also vertically expanding and further shoaling the OMZ at the inshore area, causing more hypoxia and reducing the oxygenated habitat.

2 ADAPTIVE RESPONSES OF ZOOPLANKTON TO HYPOXIA

Oxygen plays a key role in the structuring and functioning of marine ecosystems and so modulates the spatial-temporal distribution of many marine organisms. This is mainly because low oxygen levels challenge the maintenance of aerobic metabolism and can be harmful for most of the biota (Ekau et al., 2010; Wishner et al. 2018; Breitburg, 2018). The effects of depleted oxygen can affect organisms in many ways, including acute natatory and physiological impairment, diminished growth, and reproductive success, and altered behaviour of mobile forms when searching for more favourable oxygen regimes (Wishner et al., 2018).

At ecosystem level, the different tolerances to low oxygen across species will determine their survival, and so causing changes in community structure, the trophic webs, due to changes in predator-prey interactions because of changes in abundance, migration, and habitat compression (Tutasi and Escribano, 2020). While several species will be negatively affected (including commercially exploited species), others more hypoxia-tolerant may expand their range of distribution, exploit new niches (Stramma et al., 2010), and therefore have access to new resources. The adaptation of animals to low oxygen is triggered by a strong selective pressure to maintain aerobic metabolism by optimizing and enhancing oxygen uptake from hypoxic water (Childress and Seibel, 1998), or alternatively by suppressing their metabolic rate to reduce the oxygen demands (Seibel, 2011). At the upper extreme of the oxygen cascade, oxygen uptake is satisfied by two adaptive modes: as an oxygen-conformer organism by reducing aerobic metabolic rate as environmental oxygen decreases, or as an oxygen-regulator by maintaining the aerobic metabolism down to an oxygen level known as critical oxygen tension (P_{crit}) (Chisholm and Roff, 1990). The difference between these two daptive modes can be illustrated by the changes in the metabolic rate as a function of oxygen pressure (Fig. 2).

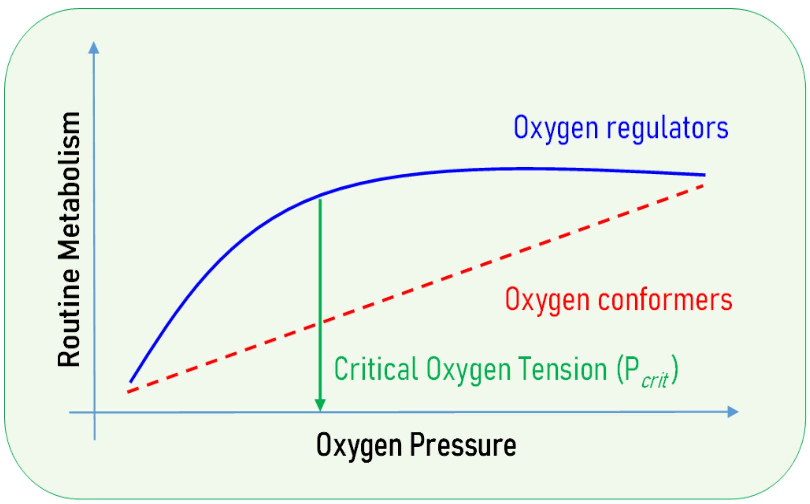

Figure 2: Two adaptive modes in marine zooplankton as a response to hypoxia (modified from Rogers et al., 2016).

The potential metabolic adaptation illustrated in Fig. 3, for instance by reducing P_{crit} to cope with severe hypoxia, or a lack of adjusting capacity, may vary with the species (Frederick et al. 2024), and this may therefore favour some species while others are negatively impacted. Fig. 3 represents the potential metabolic responses to a variable oxygen condition from normoxia to hypoxia. A non-adaptive response (Fig. 3A) is reflected in changing metabolic rate (MR) as a function of oxygen levels and this also implies a variable P_{crit}, whereas aadaptive response 1 (Fig. 3B) represents a constant MR at the cost of changing P_{crit}, and adaptive response 2 (Fig. 3C) will maintain a constant P_{crit} with a consequent change in MR. It has recently been suggested that P_{crit} is the point at which the physiological oxygen supply capacity reach its maximum, and as such is a species- and temperature-specific constant (Siebel et al., 2021). From past literature, in fact, P_{crit} is constant within a given species (Rogers et al., 2016) and thus supporting the validity of the maximum oxygen supply capacity within species. Yet, the potential plasticity of P_{crit} has not been explored on organisms inhabiting fluctuating oxygen regimes such as the ones living around OMZ zones. Furthermore, many of these pelagic organisms have several cohorts along the year, thus also allowing maternal effects on top of plasticity leading to shifts on either P_{crit} or MR on some species of planktonic copepods along the year (Frederick et al., 2024). Such differential responses will ultimately alter the species composition with consequences for the food web structure. However, even for those oxygen-regulator species, severe hypoxia may cause oxygen levels lower than P_{crit} with severe stress or deleterious effects on organisms.

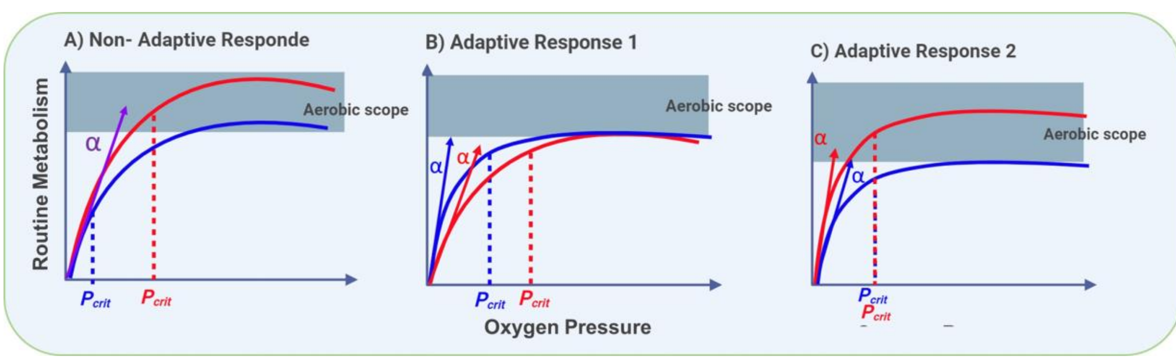

Figure 3: The expected metabolic rate (MR) as a function of environmental oxygen partial pressure (P_{crit}). The MR is theorized for two seasonal conditions: Autumn-winter (blue line)

and spring-summer (red line) in a coastal upwelling zone, where P_{crit} will move following changes in MR and the slope (α) of the oxyconforming segment remains constant (A), an adaptive response 1 in which P_{crit} increases upon a changing oxyconforming slope (α) to maintain a constant MR (B), and an adaptive response 2, where P_{crit} is maintained constant by increasing α during the upwelling season (C) (modified from Frederick et al, 2024). Here, the oxyconforming segment refers to the metabolic rate under oxygen levels below P_{crit}, while the aerobic scope represents the range of MR between the basal metabolism and its maximum for a given species.

Other adaptive responses include a shift to anaerobic metabolism when entering extremely low oxygen concentrations, such as those found in the core of the OMZ. This adaptation has been reported in actively migrating species, such as the krill *Euphausia* spp. (Riquelme-Bugueño et al., 2020). However, several adaptive responses have already been described for zooplankton, inhabiting, or entering the OMZ. For instance, crustaceans inside the OMZ can experience rapid physiological adjustments, and this involves various adaptations: enhanced ventilatory capability, enlarged gill surfaces, shortened diffusion distances, and increasing respiratory proteins with high oxygen affinity (Childress & Seibel 1998). For example, in vertically migrating zooplankton entering the OMZ, Antezana (2002) observed the presence of enlarged gill surfaces, along with active respiration and swimming under depleted oxygen in *Euphausia mucronata* indicating oxygen usage in the OMZ. In another krill species, *Meganyctiphanes norvergica* (Spicer et al, 1999), the anaerobic metabolism has been assessed showing that lactate concentration increased significantly when the oxygen concentration decreased down to hypoxic levels, and so being able to resist prolonged periods to such conditions. In *M. norvergica*, the production of lactate is rather low and so explaining a limited diel vertical migration of this species compared to *E. mucronata* in which the increase in lactate dehydrogenase is quite high allowing long periods of exposure to hypoxia (Gonzales & Quiñones, 2002).

Finally, changes in behaviour and distribution may also obey specific hypoxia adaptive responses, as found in many dominant zooplankton that avoid the OMZ by restricting their vertical migration (Escribano et al. 2009, Tutasi and Escribano 2019, Kiko et al. 2019). Also, in the copepod, *Acartia tonsa*, it has been possible to observe behavioural adaptations when copepods previously exposed to oxygen gradients avoided hypoxic bottom waters, while copepods not exposed to hypoxia did not avoid lethal oxygen concentrations (Decker et al 2003).

3 OXIDATIVE STRESS IN ZOOPLANKTON

An important biological response linked to variable oxygen levels, and rarely considered in the open ocean, is oxidative stress. The phenomenon can occur because variations in oxygen levels in the ocean range from normoxia to hypoxia at short spatial and temporal scales in some areas. Driven by such fluctuations, the oxidative stress appears related to a state of respiratory imbalance in terms of O_2 uptake, delivery, and usage, during which the animals cannot maintain a constant tissue oxygenation and, instead, undergo rapid changes between under-oxygenation and hyper-oxygenation (Tremblay et al., 2010). Therefore, as a product of aerobic respiration, the production of reactive oxygen species (ROS) can occur. ROS itself plays a crucial role as signalling molecule leaking out from the mitochondria, together with cytochrome c, AMP-activated protein kinase (AMPK), the release of mitochondrial DNA (mtDNA) and TCA (tricarboxylic acid) cycle metabolites (Martínes-Reyes & Chandel, 2022). Not surprisingly, all of them are highly dependent on the oxygen available for mitochondrial functioning, and thus their regulation is likely to be challenged under the unstable oxygen levels found in the vicinity of OMZ.

A higher production of ROS in the body may alter the DNA structure, result in modification of proteins and lipids, and trigger the activation of several stress-induced transcription factors (Birben et al., 2012). The available evidence suggests that oxidative stress can generate a significant physiological cost in life expectancy, reproduction, the immune response, in addition to the effect on metabolism and growth.

Molecular oxygen (O_2) is the primer biological electron acceptor, crucial in regulating cell functions. However, it is also the precursor of reactive oxygen species (ROS) formation because of normal cellular metabolism. The 3 major ROS of physiological significance are superoxide anion (O_2-.), hydroxyl radical (•OH), and hydrogen peroxide (H2O2) (Guérin et al., 2001). The generation of these reactive oxygen species (ROS) has been extensively studied (Welker et al., 2013, Moreira et al, 2016; Giraud-Billoud et al, 2019). When animals are re-oxygenated after hypoxic exposure, ROS formation occurs, and if not neutralized by the body's antioxidant defences, may cause oxidative damage and eventually cellular disorder and death. ROS production is species-dependent which can further vary as a function of the intensity of hypoxia and exposure time to an oxygen-deficient habitat. However, ROS are not only produced by re-oxygenation after hypoxic exposure, in fact the ROS production has also been reported to occur in hypoxic conditions for a variety of organisms, as reviewed by Hermes-Lima (2015). The exposure to hypoxia may lead to ROS and eventually to production of antioxidant compounds as an adaptive response, a phenomenon described as preparation for oxidative stress (POS) (Hermes- Lima et al 1998, 2015; Moreira et al, 2016). Tremblay et al. (2010) showed that krill species adapted to hypoxia have a sufficiently high antioxidant protection whereas less adapted species suffered a strong oxidative stress measurable as lipid peroxidation. Thus, antioxidants play an important role in neutralizing the oxidative action of free radicals. The mechanisms of cellular protection against ROS include several antioxidant enzymes such as Superoxide dismutase (SOD), Catalase (CAT), Peroxidase, Glutathion S-transferase (GST), and Glutathione Peroxidase (GPx), and non-enzymatic such as Ascorbic acid (Vitamin C), Glutathione (GSH, Tocopherol and Carotenoids, where their participation becomes relevant in ROS detoxification processes in organisms subjected to oxidative stress. An imbalance between the level of ROS and antioxidant protection, it can result in oxidative damage to tissues and a state of oxidative stress (Birben et al., 2012).

Linked to the presence of these radicals, a defence mechanism is the synthesis of antioxidant molecules capable of neutralizing the oxidative action of these free radicals. Several studies have described the presence of ROS and corresponding antioxidant responses in zooplankton, summarized in Table 1.

POS has been proposed as a mechanism to strengthen antioxidant defences (Hermes- Lima et al 1998, 2015; Moreira et al, 2016). However, how this mechanism operates in zooplankton inhabiting areas subjected to a shallow OMZ is an open question. The timing for developing antioxidant responses also becomes an important issue in both migrants and non-migrant zooplankton, because of the short-time (few hours) within which animals are exposed to hypoxia-normoxia conditions during migration or due to the irregular pulses of upwelling causing the ascent or descent of the OMZ. In the same context, and as mentioned above, most zooplankton avoid hypoxic waters by restricting their vertical distribution to the narrow and shallow normoxic layer in coastal waters, or by limiting the diel vertical migration (DVM) avoiding entering the OMZ or at least the extremely low oxygen layer found at the

OMZ core (Kiko & Haus, 2019, Tutasi & Escribano, 2019). The highly variable DVM behaviour may thus play a key role for the adaptive response to hypoxia in the context of ROS and POS processes. Zooplankton performing extensive DVM can indeed enter the core of the OMZ, as described in several euphausiid species (Escribano et al. 2009; Riquelme-Bugueño et al. 2020). The incursions in extremely low oxygen waters and rapid re-oxygenation when ascending to near surface at nighttime may trigger ROS and certainly antioxidant response.

Table 1: Zooplankton species exposed to different stress leading to reactive oxygen species (ROS). DVM (diel vertical migration) behavior can be performed and indicated by "Y" or not "N" (non-migration) and "NI" (no DVM information). Arrows in column 5 indicate the increase or decrease in the biomarker signal in relation at the stressor agent. * Indicates that the samples were obtained from areas under presence of an Oxygen Minimum Zone.

Group	Species	DVM	Stressor	Biomarker	Reference
Copepoda	*Acartia tonsa*	Y	Temp. (Heatwave)	↑GST	von Weissenberg et al, 2021
	Acartia sp.	Y	Temp/pH/DO	↑GST	Glippa et al, 2018
	Calanus pacificus	Y*	Temp/pH	↑GST	Engström-Öst et al, 2019
	Limnocalanus macrurus	Y	Contaminants	↑SOD/↓LPX	Vuori et al 2015
	Eurytemora affinis	Y	Temp/Sal	↑GST	Cailleaud et al, 2007
Euphausiacea	*Nictiphanes simplex*	Y*	Temp/Sal	↑SOD/CAT/GST	Tremblay et al, 2010
	Nematocelis difficilis	N*	Temp/Sal	↑SOD/CAT/GST	Tremblay et al, 2010
	Euphausia eximia	Y*	Temp/Sal	↑SOD/CAT/GST/↓LPX	Tremblay et al, 2010
Mysidae	*Neomysis awatschensis*	Y	DO	↓SOD/↑↓CAT-LDH	Wang et al, 2021
Pteropoda	*Limacina helicina*	Y*	Temp/pH	↑GR/CAT/↓LPX	Engström-Öst et al, 2019
Decapoda	*Scylla serrata*	N	Seasonal effect	↑SOD/CAT/GPX	Kong et al, 2008
Cephalopoda	*Sepiella maindroni*	NI	DO	↑↓SOD-CAT-POD-LDH	Wang et al, 2008

SOD: Superoxide dismutase; **CAT**: Catalase; **GST**: Glutathione S-transferase; **LPX**: Lipid peroxidation;

GPX: Glutathione peroxidase; **LDH**: Lactato deshidrogenasa; **POD**: Peroxidasa

A potential adaptive response to this periodical exposure to low-high oxygen is the possibility of doing POS during the hypoxic phase of DVM. The interplay between ROS and POS linked to DVM behaviour is illustrated in Fig. 4. In Euphausiids, which perform extensive DVM, the process of normoxic at oxygen levels >200 µM in the photic zone, and subsequent hypoxia ($O2 < 3$ µM), when diving down to 200 m at the sunrise, can take less than 3 hours (Riquelme-Bugueño et al., 2020), i,e. being exposed to >30% reduction/increasing in oxygenation per hour while swimming up or down. Such stressful oxygen conditions over a short-time scale can indeed trigger ROS (Tremblay et al. 2010), and potentially POS during the nighttime phase (Fig. 4).

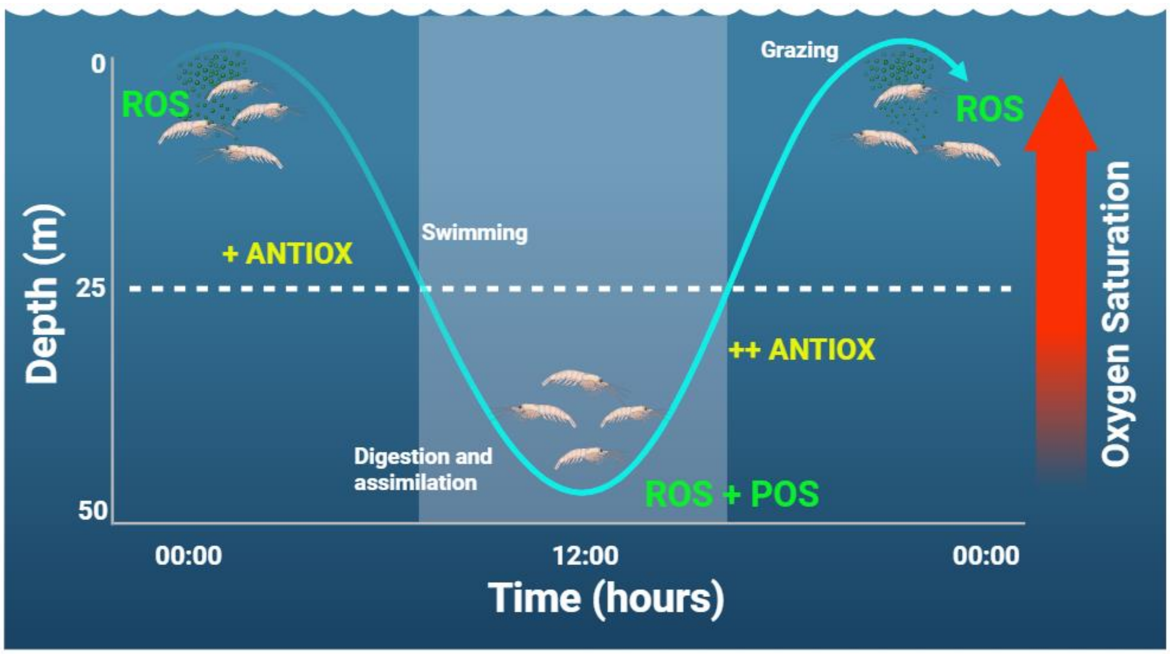

Figure 4: Changes in the daily vertical migration and short-term response to oxidative stress in zooplankton. Illuminated area shows the daytime.

The DVM behaviour, considered as an adaptive response evolved to avoid visual predators during daylight conditions (Giraud-Billoud et al, 2019), may at the same time require the evolution of POS and hence allow a mechanism to mitigate ROS effects. Diel cycles triggering antioxidant responses to ROS have been shown to occur in planktonic organisms exposed to light-dark conditions, such as *Daphnia pulex* (Cai et al., 2020), suggesting the existence of circadian rhythms of ROS and subsequent POS. However, not all zooplankton perform DVM, and indeed many species, mostly composed by copepods, which significantly contribute to the bulk of zooplankton biomass remain restricted to the upper 50 m layer (Escribano et al., 2009). These species however can still be exposed to hypoxia upon highly fluctuating upwelling intensity allowing the incursion of the upper limit of the OMZ (<3 kpa of O2) into near-surface water (Schneider et al., 2016). Upon strong upwelling such hypoxic conditions may even reach surface waters (Escribano et al., 2009).

In EBUS, depending on latitude, the active upwelling period can be strongly seasonal in temperate areas or having a weak seasonal signal being semi-permanent year-round in subtropical regions. For example, in the coast of Chile at mid-latitudes (30°-40° S) the Spring-Summer southern winds drives a very active upwelling period and consequently a shallow OMZ, whereas the Autumn-Winter exhibits a condition of depressed upwelling also known as downwelling period. This seasonal variation can temporarily expose non-migrating zooplankton populations to hypoxia during high frequency change (hours to days) in the upwelling season, and potentially demanding POS during the downwelling (spin-down) or transitional period before spin-up phase of upwelling. The combined effects of seasonal upwelling conditions, vertical distribution of the OMZ, and lack of DVM behaviour, promoting ROS and POS are illustrated in Fig. 5. Fig. 5A illustrates a condition of depressed upwelling (spin-down of upwelling or downwelling) with the upper limit of the OMZ below the photic zone where non-migrant zooplankton aggregates and normoxic conditions prevails. This conditions may trigger re-oxygenation and thus presence of ROS. Fig. 5B shows a condition of strong upwelling with shoaling of the OMZ which can intrude the photic zone, such that some of the non-migrant zooplankton becomes exposed to hypoxia and therefore POS may potentially occur as a response to a changing hypoxia-normoxia condition. Fig. 5C represents an exacerbated condition of hypoxia upon a much stronger and recurrent upwelling in a future condition.

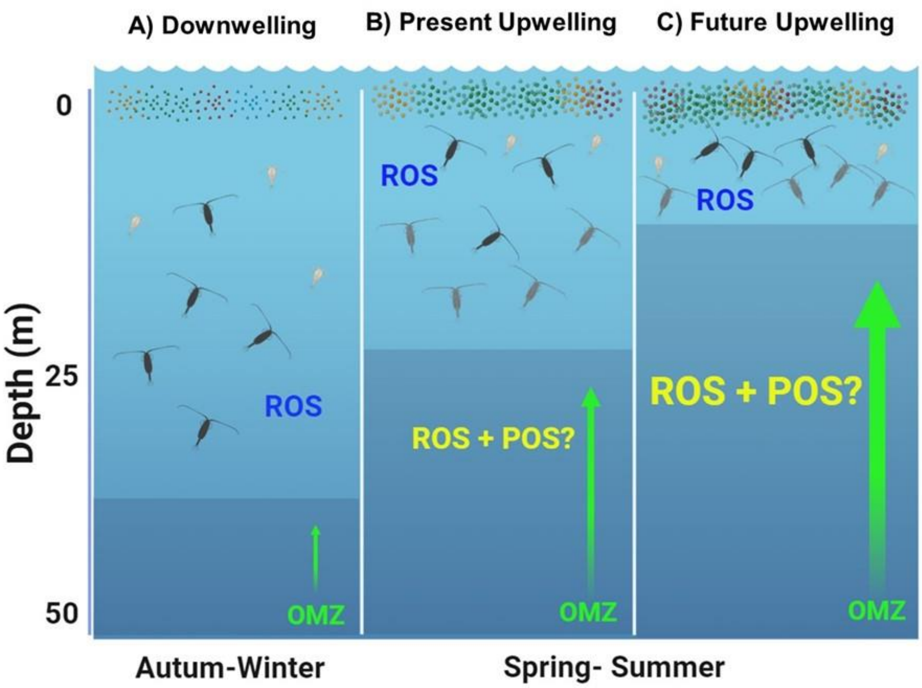

Figure 5: Oxidative stress (ROS) and the potential adaptive response (POS) of zooplankton subjected to seasonal upwelling-hypoxia conditions.

Both migrant and non-migrant zooplankton may have possibly evolved adaptive responses to ROS, although available information on this issue is scarce. Most studies on antioxidant response in marine organisms have been focused in coastal, intertidal, or benthic species.

It seems more difficult to carry out these studies in organisms inhabiting the water column, such as zooplankton, given the high variability in the physical-chemical parameters to which they are exposed. For example, planktonic copepods are very abundant, and they make up about 80% of the zooplankton biomass, so it is imperative to consider the effects of a changing environment and the response of these organisms to oxidative stress. Glippa et al. (2018) studied the copepod *Acartia* spp., which showed an increase in the enzymatic activity of GST over a two-weeks period when exposed to changes in temperature, pH and DO. An increase of enzymatic activity indicates the activation of an antioxidant defence mechanism front to environment changes. The same effect was reported by von Weissenberg et al. (2021) in *Acartia* spp. exposed to heatwaves, in experiments carried out in temperature range to 9-16°C. The authors observed a positive relationship between the increases in glutathione in response to increased environmental temperature and showed the deleterious effect over reproductive success during warming.

Regarding dominant zooplankton in EBUS, a relevant issue to consider is the short life cycle of copepods (<2 months) and euphausiids (<1 year). Under a seasonal upwelling regime, the exposure to hypoxia-normoxic may occur to different cohorts, and therefore the adaptive response (e.g. POS) might be seasonally adjusted. Although, if the OMZ continues its vertical expansion, there may not be sufficient time for developing adaptive responses with deleterious consequences for non-migrant populations, which comprise most upwelling inhabitant species.

3.1 NATURAL ANTIOXIDANT AGENTS FOR ZOOPLANKTON

Studies show that planktonic diatoms have a high antioxidant potential (Goiris et al, 2012). Diatoms are rich in carotenoids, such as fucoxanthin and astaxanthin that play a crucial role in protecting UV. Some diatoms as a *Skeletonema marinoi* and *Odontella aurita* can synthesize and accumulate ascorbic acid (Vitamin C) and phenolic compounds which also have antioxidant properties (Smerilli et al, 2019; Hemalatha et al, 2015).

The concentrations of antioxidants in diatoms are species-specific (Foo et al. 2017), where they quantified the total content of some antioxidants and their bioactive capacity in six diatom species, where *Chaetoceros calcitrans* and *Isochrysis galbana* showed the highest antioxidant activity, followed by *Odontella sinens* and *Skeletonema costatum* which exhibited moderate bioactivity. Meanwhile *Phaeodactylum tricornutum* and *Saccharina japonica* displayed the lowest antioxidant activity among the examined algae species.

In coastal upwelling environments, zooplankton must face a wide variability of the environmental parameters to which they are subject. For this reason, the presence of an exogenous source of antioxidants provided by diatoms would help mitigate the effect of oxidative stress due to environmental pressure.

4 CONCLUSION

Ocean deoxygenation and the loss of oxygen in EBUS is an ongoing phenomenon, and planktonic organisms must inevitably cope with this gradually increased hypoxia, and a changing dynamic of the ocean. The ecological consequences are far from understood and, will largely depend on the ability of zooplankton to strengthen their capacity to tolerate mild or severe hypoxia, exploit plasticity and maternal effects to their maximum, or to develop new adaptations. In any case these responses may likely come at some cost and likely with trade-offs on other metabolic/energy-demanding processes. The outcome from the new demands implies a reduction in energy otherwise available for growth, feeding and reproduction with further consequences in the population dynamics. Hypoxia conditions can also lead to changes in behaviour (upon stress) and spatial distribution, and so altering for example prey-predator interactions. Ultimately, the pelagic food-web and community structure will likely be affected with biogeochemical consequences in the context of the C and N recycling and ecosystem productivity. The assessment of plankton community structure through time series observations in the upwelling zone constitutes the most suitable proxy to examine the community responses to ongoing deoxygenation, and so long-term time series are extremely valuable for accurate predictions. The use of molecular methods to examine how individuals can modify their gene expression to cope with hypoxia, and eventually activate antioxidant responses, are also necessary approaches when aiming to the understanding and prediction of the ecological consequences upon expected severe conditions of an oxygen-deprived water column.

COMPETING INTEREST

The contact author has declared that none of the authors has any competing interests.

REFERENCES

Antezana, T.: Adaptive behaviour of *Euphausia mucronata* in relation to the oxygen minimum layer of the Humboldt Current, Oceanography of the Eastern Pacific, II, 29-40, 2002.

Bakun, A., Field, D. B., Redondo-Rodriguez, A., and Weeks, S. J.: Greenhouse gas, upwelling-favourable winds, and the future of coastal ocean upwelling ecosystems. Glob. Change Biol, 16, 1213–1228, https://doi.org/10.1111/j.1365-2486.2009.02094.x, 2010.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., and Kalayci, O.: Oxidative stress and antioxidant defence, World Allergy Organization Journal 5: 9-19, doi: 10.1097/WOX.0b013e3182439613, 2012.

Breitburg, D., Levin, L. A., Oschlies, A., Gregoire, M., Chavez, F.P., Conley, D.J., Garcon, V., Gilbert, D., Gutierrez, D., and Isensee, K.: Declining oxygen in the global ocean and coastal waters, Science 359, eaam7240, DOI: 10.1126/science.aam 7240, 2018.

Cai, M., Liu, Z., Yu, P., Jiao, Y., Chen, Q., Jiang, Q., and Zhao, Y.: Circadian rhythm regulation of the oxidation–antioxidant balance in *Daphnia pulex*, Comparative Biochemistry and Physiology, Part B 240, 110387, https://doi.org/10.1016/j.cbpb.2019.110387, 2020.

Cailleaud, K., Maillet, G., Budzinski, H., Souissi, S., and Forget-Leray, J.:Effects of salinity and temperature on the expression of enzymatic biomarkers in *Eurytemora affinis* (Calanoida, Copepoda), Comparative Biochemistry and Physiology, Part A 147, 841–849, DOI: 10.1016/j.cbpa.2006.09.012, 2007.

Chan, F., Barth, J.A., Lubchenco, J., Kirincich, A., Weeks, H., Peterson, W.T., and Menge, B.A.:Emergence of anoxia in the California Current large marine ecosystem, Science 319-920, DOI: 10.1126/science.1149016, 2008.

Chavez, F.P., and Messié, M.: A comparison of eastern boundary upwelling ecosystems, Prog. Oceanogr, 83, 80-96, https://doi.org/10.1016/j.pocean.2009.07.032, 2009.

Childress, J., and Seibel, B.: Life at stable low oxygen levels: Adaptations of animals to oceanic oxygen minimum layers, J. Exp.Biol., 201, 1223–1232, DOI: 10.1242/jeb.201.8.1223, 1998.

Decker, M. B., Breitburg, D.L., and Marcus, N. H.: Geographical differences in behavioural responses to hypoxia: local adaptation to anthropogenic stressor?, Ecol. Appl.13, 1104–9, https://www.jstor.org/stable/4134746, 2003.

Ekau, W., Auel, H., Portner, H. O., and Gilbert, D.: Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish), Biogeosci. 7, 1669–1699, https://doi.org/10.5194/bg-7-1669-2010, 2010.

Engström-Öst. J., Glippa, O., Feely, R. A., Kanerva, M., Keister, J. E., Alin, S. R.,Carter, B. R., McLaskey, A. K., Vuori, K. A., and Bednaršek, N.:Eco-physiological responses of copepods and pteropods to ocean warming and acidification, Scientific Report, 9, 4748, https://doi.org/10.1038/s41598-019-41213-1, 2019.

Escribano, R., Hidalgo, P., and Krautz, C.: Zooplankton associated with the oxygen minimum zone system in the northern upwelling region of Chile during March 2000, Deep-Sea Res. II., 56, 1083-1094, https://doi.org/10.1016/j.dsr2.2008.09.009, 2009.

Foo, S.Ch., Yusoff, F. Md., Ismail, M., Basri, M., Yau, S.K., Khong, N.M.H., Chan, K.W., and Ebrahimi, M.: Antioxidant capacities of fucoxanthin-producing algae as influenced by their carotenoid and phenolic contents, Journal of Biotechnology, 241, 175-183, https://doi.org/10.1016/j.jbiotec.2016.11.026, 2017.

Frederick, L., Urbina-Foneron, M., and Escribano, R.: Adjusting metabolic rates and critical oxygen tension in planktonic copepods under increasing hypoxia in highly productive coastal upwelling zones, Limnology and Oceanography, 9999, 1–14, doi: 10.1002/lno.12556, 2024.

Garcia-Reyes, M., and Largier, J.L.: Seasonality of coastal upwelling off central and northern California: New insights, including temporal and spatial variability, Journal of Geophysical Research, 117, C03028, https://doi.org/10.1029/2011JC007629, 2009.

Gilly, W.G., Beman, M., Litvin, S.Y., and Robison, B.H.: Oceanographic and biological effects of shoaling of the oxygen minimum zone, Annual Review Marine Science 5:393–420, DOI: 10.1146/annurev-marine-120710-100849, 2018.

Giraud-Billouda, M., Rivera-Ingrahamb, G., Moreira, D., Burmesterd,T., Castro-Vazqueza, A., Carvajalino-Fernándeze, J., Dafref, A., Cuijuan Niug, Tremblay N., Paital, B., Rosa, R., Storey, J., Vega,I., Zhangg, W., Yepiz-Plascencial, G., Zenteno-Savinm, T., Storey, K., and Hermes-Lima, M.: Twenty years of the 'Preparation for Oxidative Stress' (POS) theory: Ecophysiological advantages and molecular strategies, Comparative Biochemical and Physiology: Molecular and Integrative Physiology Part A, 234, 36-49, DOI: 10.1016/j.cbpa.2019.04.004, 2019.

Glippa, O., Engström-Öst, J., Kanerva, M., Rein, A., and Vuori, K.: Oxidative stress and antioxidant defense responses in *Acartia* copepods in relation to environmental factors, PLoS One, 13(4), e0195981, https://doi.org/10.1371/journal.pone.0195981, 2018.

Goiris, K., Muylaert, K., Fraeye, I., Foubert, I., De Brabanter, J., and De Cooman, L.: Antioxidant potential of microalgae in relation to their phenolic and carotenoid content, J. Appl. Phycol., 24, 1477–1486, https://doi.org/10.1007/s10811-012-9804-6, 2012.

Gonzalez, R., and Quiñones, R.:Ldh activity in *Euphausia mucronata* and *Calanus chilensis*: implications for vertical migration behavior, Journal of Plankton Research. 24(12), 1349-1356, https://doi.org/10.1093/plankt/24.12.1349, 2002.

Grégoire, M., Garçon, V., Garcia, H., Breitburg, D., Isensee, K., Oschlies, A., Telszewski, M., Barth, A., Bittig, H. C., Carstensen, J., Carval, T., Chai, F., Chavez, F., Conley, D., Coppola, L., Crowe, S., Currie, K., Dai, M., Deflandre, B., Dewitte, B., Diaz, R., Garcia-Robledo, E., Gilbert, D., Giorgetti, A., Glud, R., Gutierrez, D., Hosoda, S., Ishii, M., Jacinto, G., Langdon, Ch., Lauvset, S. K., Levin, L. A., Limburg, K. E., Mehrtens, H., Montes, I., Naqvi, W., Paulmier, A., Pfeil, B., Pitcher, G., Pouliquen, S., Rabalais, N., Rabouille, C., Recape,V., Roman, M., Rose, K., Rudnick, D., Rummer, J., Schmechtig, C., Schmidtko, S., Seibel, B., Slomp, C., Sumalia, U.R., Tanhua, T, Thierry, V., Uchida, H., Wanninkhof, R., Yasuhara, M.: A Global Ocean Oxygen Database and Atlas for Assessing and Predicting Deoxygenation and Ocean Health in the Open and Coastal Ocean, Frontiers in Marine Science. 8, 724913, https://doi.org/10.3389/fmars. 2021.72491, 2021.

Hemalatha, A., Parthiban, C., Saranya, C., Girija. K., and Anatharaman, P.: Evaluation of antioxidant activities and total phenolic contents of different solvent extracts of selected marine diatoms, Indian Journal of Geo-Marine Sciences, 44(10),1630-1636, https://doi.org/10.1186/s40538-017-0110-z, 2015.

Hermes-Lima, M., Storey, J.M., and Storey, K.B.: Antioxidant defences and metabolic depression. The hypothesis of preparation for oxidative stress in land snails, Comp. Biochem. Physiol. B., 120, 437-448, https://doi.org/10.1016/S0305-0491(98)10053-6, 1998.

Hermes-Lima, M., Moreira, D.C., Rivera-Ingraham, G.A., Giraud-Billoud, M., Genaro-Mattos, T.C., and Campos, E.G.: Preparation for oxidative stress under hypoxia and metabolic depression: Revisiting the proposal two decades later, Free Radic Biol Med., 89, 1122-43, DOI: 10.1016/j.freeradbiomed.2015.07.156, 2015.

Kiko, R., and Hauss, H.: On the Estimation of Zooplankton-Mediated Active Fluxes in Oxygen Minimum Zone Regions, Frontiers in Marine Science, 6, 741, https://doi.org/10.3389/fmars.2019.00741, 2019.

Kong, X., Wang, G., and Li, Sh.: Seasonal variation of ATPasa activity and antioxidant defences in gills of the mud crab Scylla serra (Crustacea, Decapoda), Marine Biology, 154, 269–276, DOI 10.1007/s00227-008-0920-4, 2008.

Martínez-Reyes, I., and Chandel, N.S.: Mitochondrial TCA cycle metabolites control physiology and disease, 11, 102, https://doi.org/10.1038/s41467-019-13668-3, 2022.

Moreira, D., Venancio, L., Sabino, M., and Hermes-Lima, M.: How widespread is preparation for oxidative stress in the animal kingdom, Comparative Biochemical and Physiology: Molecular Integrative and Physiology, 200, 64-78, https://doi.org/10.1016/j.cbpa.2016.01.023, 2016.

Oschlies, A., Brandt,P., Stramma, L., and Schmidtko, S.: Drivers and mechanisms of ocean deoxygenation, Nature Geoscience, 11, 467-473, https://doi.org/10.1038/s41561-018-0152-2, 2018.

Riquelme Bugueño, R., Pérez Santos, I., Alegría, N., Vargas, C. A., Urbina, M. A., and Escribano, R.: Diel vertical migration into anoxic and high pCO2 waters: acoustic and net based krill observations in the Humboldt Current Ramiro, Nature, Sci. Rep., 10, 17181, https://doi.org/10.1038/s41598-020-73702-z , 2020.

Rogers, N. J., Urbina,M. A., Reardon, E. E., McKenzie, D. J. and Wilson, R. W.: A new analysis of hypoxia tolerance in fishes using a database of critical oxygen level (Pcrit), Conserv. Physiol., 4 (1), cow012, DOI: 10.1093/conphys/cow012, 2016.

Santosa, F., Gomez-Gesteira, M., de Castro, M., and Alvarez, I.: Differences in coastal and oceanic SST trends due the strengthening of coastal upwelling along the Benguela Current System, Cont. Shelf Res. 34, 79–86, https://doi.org/10.1016/j.csr.2011.12.004, 2012.

Schmidtko, S., Stramma, L., and Visbeck, M.: Decline in global oceanic oxygen content during the past five decades, Nature, 542, 335–339, https://doi.org/10.1016/j.csr.2011.12.004, 2017.

Schneider, W., Donoso, D., Garcés-Vargas, J., and Escribano, R.: Water-column cooling and sea surface salinity increase in the upwelling region off central-south Chile driven by a poleward displacement of the South Pacific, Prog. Oceanogr., 141, 38-58, https://doi.org/10.1016/j.pocean.2016.11.004, 2016.

Seibel, B. A.: Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones, J. Exp. Biol., 214, 326-336, DOI: 10.1242/jeb.049171, 2011.

Smerilli, A., Balzano, S., Maselli, M., Blasio, M., Orefice, I., Galasso, Ch., Sansone, C., and Brunet, Ch.: Antioxidant and photoprotection networking in the coastal diatom *Skeletonema marinoi*, Antioxidant, 8, 154, DOI: 10.3390/antiox8060154, 2019.

Spicer, J.I., Thomasson, M.A., and Strömberg, J.: Possessing a poor anaerobic capacity does not prevent the diel vertical migration of Nordic krill *Meganyctiphanes norvegica* into hypoxic waters, Marine Ecology Progress Series, 185, 181-187, https://www.jstor.org/stable/24853208, 1999.

Stramma, L., Johnson, G.C., Sprintall, J., and Mohrholz. V.: Expanding oxygen minimum zones in the tropical oceans, Science, 320, 655–658, DOI: 10.1126/science.1153847, 2008.

Stramma, L., Schmidtko, S., Levin, L., and Johnson, G.: Ocean oxygen minima expansions and their biological impacts, Deep-Sea Res., 57, 587-595, https://doi.org/10.1016/j.dsr.2010.01.005, 2010.

Tremblay, N., Gómez-Gutiérrez, J., Zenteno-Savín, T., Robinson, C.J., and Sánchez-Velascoa, L.: Role of oxidative stress in seasonal and daily vertical migration of three krill species in the Gulf of California, Limnol. Oceanogr. 55, 2570–2584, https://doi.org/10.4319/lo.2010.55.6.2570, 2010.

Tutasi, P., and Escribano, R.: Zooplankton diel vertical migration and downward C flux into the oxygen minimum zone in the highly productive upwelling region off northern Chile, Biogeosci., 17, 455–47, https://doi.org/10.4319/lo.2010.55.6.2570, 2020.

Vuori, K.A., Lehtonen, K.K., Kanerva, M., Peltonen, H., Nikinmaa, M., Berezina, N.A., and Boikova, E.: Oxidative stress biomarkers in the copepod *Limnocalanus macrurus* from the northern Baltic Sea: effects of hydrographic factors and chemical contamination, Marine Ecology Progress Series, 538, 131–144, doi:10.3354/meps11471, 2015.

Wang, C.L., Wu, D. H., Dong, T. Y., and Jiang, X. M.: Oxygen consumption rate and effects of hypoxia stress on enzyme activities of *Sepiella maindron*, Chin J Appl Ecol. 19, 2420–2427, 2008.

Wang, Q., Li, X, Yan, T., Song, J., Yu, R., and Zhou, M.: Detrimental impact of hypoxia on the mortality, growth, reproduction, and enzyme activities of planktonic mysid *Neomysis awatschensis*, Aquat Ecol. 55, 849–859, https://doi.org/10.1007/s10452-021-09864-3, 2021.

von Weissenberg. E., Jansson, A., Vuori, K.A., and Engström-Öst, J.: Copepod reproductive effort and oxidative status as responses to warming in the marine environment, Ecol and Evol, 12, e8 594, https://doi.org/10.1002/ece3.8594, 2021.

Welker, A.F., Moreira, D.C., Campos, E.G., and Hermes-Lima, M.: Role of redox metabolism for adaptation of aquatic animals to drastic changes in oxygen availability, Comparative Biochemistry and Physiology: Molecular and Integrative Physiology 165, 384–404, https://doi.org/10.1016/j.cbpa.2013.04.003, 2013.

Wishner, K.F., Seibel, B.A., Roman, C., Deutsch, C., Outram, D., Shaw, C.T., Birk, M.A., Mislan, K.S., Adams, T. J., Moore, D., and S. Riley.:Ocean deoxygenation and zooplankton: Very small oxygen differences matter, Sci. Adv. 4(12), eaau5180 doi:10.1126/sciadv.aau5180, DOI: 10.1126/sciadv.aau5180, 2018.

Xiu, P., Chai, F., Curchitser, E. N., Frederic, S., and Castruccio, S.: Future changes in coastal upwelling ecosystems with global warming: The case of the California Current System, Sci. Rep. 8, 2866., https://doi.org/10.1038/s41598-018-21247-7, 2018.

5. DISCUSSION

Ocean deoxygenation and the loss of oxygen in EBUS is an ongoing phenomenon, and it can be stated that planktonic organisms must inevitably cope with gradually increased hypoxia. Understanding the impact of the expansion of zones of minimum oxygen in EBUS systems is of vital importance to understand the changes in the community structure associated with these systems and their possible effects on carbon transfer in the food webs. The ecological consequences however largely depend on the ability of zooplankton to develop new adaptations. The degree of adaptation of zooplankton to hypoxic conditions will be measured by the duration of hypoxic events. Under conditions of persistent hypoxia, it is likely that behavior, for example avoidance of areas with low oxygen, will be persistent, but not under sporadic conditions of hypoxia. Thus, hypoxic zones can be a selective force for zooplankton.

Copepods inhabiting in the coastal upwelling zone exhibit a high ecological flexibility to withstand this physical and chemically unstable environment (Peterson. 1998). They can also couple their annual life cycle to seasonal upwelling variation to optimize their temperature-dependent growth rates (Escribano et al. 2014), and their feeding behavior upon seasonal changes in the quality and quantity of food resources (Vargas et al. 2006). Also in copepods, the reproduction may be coupled to seasonal upwelling (Hidalgo & Escribano 2007), such that even though reproduction can occur year-round, it increases during the spring-summer active upwelling, coinciding with a major phytoplankton bloom. Under these conditions, a greater feeding rate may induce increased metabolic rates of copepods, as suggested by previous studies (Lamper. 1984; Ikeda et al. 2000, Donoso & Escribano 2014), allowing them to maximize the utilization of food resources, thus fueling the intensified reproductive activity.

In our respirometry experiments, the results to *C. patagoniensis* which can almost double their metabolic rate during the spring-summer. Contrastingly, *P.* cf. *indicus* and *A. tonsa* can maintain their metabolic rate throughout the year allowing them to exploit alternate seasonal sources of food for year-round growth and reproduction (Hidalgo & Escribano 2007). Our results show that metabolic rate is regulated over several hours up to the critical partial pressure of oxygen, without showing any signs of changes in metabolic substrate utilization or shortage.

Some studies mention the allometric effect on respiration rates. Respiration has been found to decrease with copepod size (allometric effect) (Hirst & Sheader 1997, Roman and Pierson 2022). A small size in copepods can lead to a greater oxygen absorption capacity, because it is necessary to transport oxygen through the cell membrane to the mitochondria; and for smaller cells there is a greater surface area and shorter diffusion distances (Verberk et al. 2021). However, some species-dependent adaptations can cause allometric deviations in animals inhabiting minimal oxygen zones (Childress & Seibel 1998), and it has been suggested that in several animals the oxygen supply capacity is adjusted to meet the metabolic demands of independently of its size (Seibel & Deutsch 2020). This is the case in our results, where it is observed that *C. patagoniensis* had a much higher metabolic rate (10 times higher) than the other two species despite its greater body mass.

Higher temperature can increase metabolic rate (Roman et al. 2019), as expected for any ectotherm. Recent data synthesis about the interaction of temperature and oxygen on metabolic processes, suggested that the respiration rate of copepods increases by approximately 7% per degree Celsius (Heine et al. 2019). This suggests that in warm waters copepod respiration will increase faster. However, species-specific responses can be affected by the temperature range they experience and their thermal tolerance.

In our result, a higher metabolic rate in *C. patagoniensis* during spring-summer was associated with warmer water in these seasons. The temperature at St.18 was in the range of 11.8-13.6 °C at 10 m depth (mean=12.5 °C), with an average of 12.0 °C for the entire 50 m layer. m. During an annual cycle, copepods are exposed to a full range of 10.0–15.0 C° in this layer (0–50 m). All of our experiments were performed at a constant temperature of 13°C, so the effect of temperature on metabolic rate was controlled in the laboratory. However, in the field, temperature varied by 5 °C between winter and summer in the upper 50 m layer where most copepods reside, and therefore, when extrapolating our results to the field, both metabolic rate and The critical partial pressure of oxygen had to be adjusted to in situ temperature. This adjustment altered estimates of the critical partial pressure of oxygen by increasing/decreasing its value by approximately 5% per degree Celsius, suggesting that warmer conditions may reduce copepods' tolerance to hypoxia by increasing their critical partial pressure of oxygen and therefore finding critical levels of oxygen in shallower waters.

When interpreting and understanding the effects of seasonal upwelling on critical oxygen partial pressure it must be considered that copepods are multigenerational organisms with a life span in the range of 10-40 days for most species. The small *P.* cf. *indicus* may have >30 cohorts a year (Escribano et al. 2014), and although we lack information on the number of cohorts per year on *A. tonsa*, based on its size, it may have a similar number of generations per year to that of *P.* cf. *indicus*. This means that different cohorts are exposed to different oxygen regimes, suggesting the possibility of seasonal adaptation to the prevailing oxygen levels. However, during the spring-summer, when hypoxic conditions prevail in the water column, we observed greater critical oxygen partial pressure values which will be encountered at shallower depths in the water column. In contrast during winter, with a more oxygenated water column, lower values of critical oxygen partial pressure were found, allowing individuals to distribute deeper under oxygen levels higher than their P_{crit}. This response is not accompanied by seasonal changes in metabolic rate in *P*. cf. *indicus* and *A. tonsa*. Earlier Fry and Hart's. 1948, realized that an increase in metabolic rate will mathematically result in a greater critical oxygen partial pressure if the slope of the conforming segment remains constant. This slope, so called alpha (α) and recently subject to debate, is the line of respiratory dependence. In fact, it has been proposed that critical oxygen partial pressure is the result of reaching the maximum oxygen supply capacity (Seibel et al. 2021) on an organism, this is "the maximum amount of oxygen that can be supplied per unit time and oxygen pressure" and is a species- and temperature-specific constant.

According to our initial hypothesis, we would expect a change in adaptations driven by hypoxia but our findings suggest the existence of differential responses to hypoxia in the species under study. *P*. cf. *indicus* and *A. tonsa* are able to maintain their metabolic rate throughout the season (Fig. 5B), possibly to sustain year-round reproduction and growth, but at the cost of increasing the critical partial pressure of oxygen (P_{crit}) and therefore become less tolerant to upwelling-driven hypoxia. On the other hand, *C. patagoniensis* seems to optimize its metabolic rate during the upwelling season (Fig. 5C) and take better advantage of the spring-summer phytoplankton bloom, it is capable of maintaining a critical partial pressure of oxygen and therefore with a greater capacity to cope with seasonal hypoxia. Both types of adaptive responses involve a change in oxygen delivery capacity (or "conductance," Piiper et al. 1971), which should remain constant in the face of a non-adaptive response. To our knowledge, the present study is the first to show seasonal changes in oxygen supply capacity (α), probably possible by the existence of different cohorts between contrasting seasons and therefore selecting for elevated metabolic capacity when intensified hypoxia. This emergence of more "athletic" individuals has been hypothesized to occur under persistent hypoxia (Seibel et al. 2021), as an upwelling system, and mediated by enhanced conductance along the oxygen cascade (Farrel et al. 2021). Therefore, modification of oxygen delivery capacity should be considered as a species-dependent physiological adaptation in seasonal cohorts of copepods, to optimize metabolic outcomes under seasonally varying food sources, temperature, and hypoxia.

The adaptive response to hypoxia (Fig. 5B) can have major ecological consequences for copepods under a scenario of increasing upwelling in eastern boundary upwelling systems (Schneider et al. 2016; Xiu et al. 2018). The shoaling of the OMZ upon increased upwelling will lead to an increased occurrence of conditions with oxygen levels equal or lower than critical oxygen partial pressure, implying an effective vertical reduction of the normoxic environment. Our monthly time series of dissolved oxygen in the water column shows that out of 163 monthly observations, there were 111 occasions (i.e. ca 70%) having oxygen levels lower than the critical oxygen partial pressure of all species tested within the upper 50 m layer. This implies strong limitations on the aerobic metabolism of copepods, which given the frequency of occurrence may have drastic implications for the whole planktonic community and food webs. Studies show negative effects of hypoxia on the occurrence and abundance of zooplankton (Roman et al. 2019; Auel & Verheye 2007; Ekau et al. 2010), with unpredictable consequences for the whole pelagic food web due to alterations at basal levels.

The vertical distribution and potential migration of copepods becomes crucial to maximize aerobic metabolism in the water column. Our zooplankton sampling in 2008 shows that copepods can be found at any depth within the upper 50 m during a winter condition in the coastal area. For spring-summer conditions we have a previous study carried out in a nearby area during January 1985 (Castro et al. 2007). The study showed that *Acartia tonsa* is concentrated in the upper 20 m, *Paracalanus* cf. *indicus* (previously named as *P. parvus*) in the upper 30 m, and *C. patagoniensis* appear to be more widely distributed in the upper 50 m. Interestingly, the summer vertical distribution matches the measured critical partial pressure of oxygen specific to each species, so its physiological limit to sustain aerobic metabolism coincides with its ability to exploit different habitat volumes. We do not have information on the daily vertical migration (DVM) of these species in this area, but for these small and medium-sized copepods the vertical amplitude of the DVM cannot be greater than 30 m (Tutasi & Escribano 2020), so even carrying out DVM, they can hardly avoid being exposed to hypoxic water within the upper 50 m. Differential distribution can influence the impact of hypoxia on the population of the species but also on the entire community. For example, *A. tonsa* may be less affected if it resides in shallow, nearshore waters, which are typically highly oxygenated, in contrast to *C. patagoniensis* and *P.* cf. *indicus*, both live at greater depths under the influence of the waters of the OMZ.

Despite the potentially differential impact of hypoxia, depending on vertical distribution, and on the adaptive responses shown (Fig. 5), the three species are indeed frequently exposed to hypoxic events, which become greatly increased during the spring-summer conditions. In our experiments, copepod mortality was greater than 70% after reaching critical oxygen partial pressure, suggesting that copepods in the field are experiencing severe episodes of mortality. Previous studies in this region have already shown that increased upwelling negatively impact copepod abundances (Pino-Pinuer et al. 2014; Escribano et al. 2012), and this impact may relate to increased hypoxia upon upwelling intensification.

Ocean deoxygenation appears as a gradual global process triggered by global warming (Breitburg et al. 2018). However, observations and modeling regarding the responses of highly productive coastal eastern boundary upwelling systems to global warming, also indicate and predict the intensification of the wind-driven upwelling (Schneider et al. 2016; Xiu et al. 2018, Bakun et al. 2010). More upwelling implies a shoaling of the OMZ at the upwelling zone, and a consequent shoaling of the "depth of critical oxygen partial pressure", so further reducing the normoxic habitat of copepods. Hypoxia conditions can lead to changes in behavior upon stress and distribution, and so altering for example prey-predator interactions. Our results highlight the vulnerability of planktonic copepods to global warming, even in highly variable environments.

The assessment of plankton community structure through time series observations in the upwelling zone constitutes the most suitable proxy to examine the community responses to ongoing deoxygenation. However, the use of molecular methods to examine how individuals can modify their gene expression to cope with hypoxia, and eventually activate antioxidant responses, are also necessary approaches when aiming to the understanding and prediction of the ecological consequences upon expected severe conditions of an oxygen-deprived water column.

6. CONCLUSION

- It is observed that the copepods that inhabit the OMZ off central-southern Chile are exposed to hypoxic conditions close to their P_{crit} approximately 70% of the time. Despite this high exposure, adaptive mechanisms are not observed in all species, only in *C. patagoniensis*, which has a slightly deeper vertical distribution. In general, copepods that inhabit the surface layer of the water column do not present adaptive mechanisms to cope with a hypoxic condition. This response supports hypothesis 1, indicating that copepod species exposed to hypoxia would have a species-dependent adaptive response.
- Pelagic copepods that are associated with OMZ are multigenerational organisms (>30 cohorts per year). This indicates that different cohorts are exposed to different oxygen regimes, suggesting the possibility of seasonal adaptation to the prevailing oxygen levels. However, in our results we did not observe a coupling between the seasonal cycle and adaptation to hypoxia. During spring-summer the P_{crit} values were higher, while during winter, when the water column is more oxygenated, the P_{crit} values were lower. Furthermore, this response is not accompanied by seasonal changes in the metabolic rate in *P.* cf. *indicus* and *A. tonsa*. These results reject hypothesis 2 since although there is a species-specific response, the seasonal cycle of hypoxia is not coupled with greater tolerance, in response to low oxygen conditions in all species.

7. REFERENCES

Anabalón, V., Morales, C.E, Escribano, R. & Varas, M.A. 2007. The response of nano- and microplanktonic assemblages in the upper layer (0-30 m) to varying oceanographic conditions in the upwelling area off Concepción, central Chile. Progr. Oceanogr. 75: 396-414.

Auel, H & Verheye, H. 2007. Hypoxia tolerance in the copepod *Calanoides carinatus* and the effect of an intermediate oxygen minimum layer on copepod vertical distribution in the northern Benguela Current upwelling system and the Angola–Benguela Front. J. Exp. Mar. Biol. Ecol. 352: 234-243.

Auel, H., Hagen, W., Ekau, W. & Verheye, H. M. 2005. Metabolic adaptations and reduced respiration of the copepod *Calanoides carinatus* during diapause at depth in the Angola-Benguela Front and northern Benguela upwelling regions. Afr. J. Mar. Sci. 27: 653-657.

Bakun, A., Field, D. B., Redondo-Rodriguez, A. & Weeks, S. J. 2010. Greenhouse gas, upwelling-favorable winds, and the future of coastal ocean upwelling ecosystems. Glob. Change Biol. 16: 1213–1228.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S. & Kalayci, O. 2012. Oxidative stress and antioxidant defense. WAOjournal. 5: 9-19.

Birk, M. A. 2021. Respirometry: Tools for Conducting & Analyzing Respirometry Experiments. R package version 1.3.0. https://CRAN.R-project.org/package=respirometry.

Blanco, J. L., Thomas, A. C., Carr, M. E. & Strub. P.T. 2001. Seasonal climatology of hydrographic conditions in the upwelling region off northern Chile. J. Geophys. Res. 106: 11451–11467.

Bograd, S. J., Castro, C. G., Di Lorenzo, E., Palacios, D. M., Bailey, H., Gilly, W. & Chavez, F. P. 2008. Oxygen declines and the shoaling of the hypoxic boundary in the California Current. Geophys. Res. Lett. 35: L12607.

Bopp, L., Resplandy, L., Orr, J. C., Doney, S. C., Dunne, J. P., Gehlen, M., et al. 2013. Multiple stressors of ocean ecosystems in the 21st century: projections with CMIP5 models. Biogeosci. 10: 6225–6245.

Breitburg, D., L. A. Levin, A. Oschlies, M. Gregoire, F.P. Chavez, D.J. Conley, V. Garcon, D. Gilbert, D. Gutierrez, & Isensee, K. 2018. Declining oxygen in the global ocean and coastal waters. Science 359: eaam7240.

Castro, L. R., Troncoso, V. A., & Figueroa, D. R. 2007. Fine-scale vertical distribution of coastal and offshore copepods in the Golfo de Arauco, central Chile, during the upwelling season. Prog. Oceanogr. 75: 486–500.

Chan, F., Barth, J.A., Lubchenco, J., Kirincich, A., Weeks, H., Peterson, W.T. & Menge, B.A. 2008. Emergence of anoxia in the California Current large marine ecosystem. Science. 319: 920.

Childress, J., & Seibel, B. 1998. Life at stable low oxygen levels: Adaptations of animals to oceanic oxygen minimum layers. J. Exp. Biol. 201: 1223–1232.

Connett, R. J., Honig, C. R., Gayeski, T. E. & Brooks, G. A. 1990. Defining hypoxia: a systems view of VO2, glycolysis, energetics, and intracellular pO_2. J. Appl. Physiol. 68, 833.

Dam, H.G. 2012. Evolutionary Adaptation of Marine Zooplankton to Global Change. Annu. Rev. Mar. Sci. 5:349-370.

Daneri, G., Dellarossa, V., Quiñones, R., Jacob, B., Montero, P. & Ulloa, O. 2000. Primary production and community respiration in the Humboldt Current System off Chile and associated oceanic areas. Mar. Ecol. Prog. Ser. 197: 41-49.

Donoso, K., & Escribano, R. 2014. Mass-specific respiration of mesozooplankton and its role in the maintenance of an oxygen-deficient ecological barrier (BEDOX) in the upwelling zone off Chile upon presence of a shallow oxygen minimum zone. J. Mar. Syst. 129: 166-171.

Deutsch, C., Brix, H., Ito, T., Frenzel, H. & Thompson, L. 2011. Climate-forced variability of ocean hypoxia. Science 333: 336–339.

Ekau, W., Auel, H., Portner, H. O., & Gilbert, D. 2010. Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish). Biogeosci. 7: 1669–1699.

Elder, LE & Seibel, B.A. 2015. Ecophysiological implications of vertical migration into oxygen minimum zones for the hyperiid amphipod, *Phronima sedentaria*. J. Plankton Res 37:897–911.

Escribano, R. 2006. Zooplankton interactions with the oxygen minimum zone in the eastern south pacific, Gayana (Concepc.), 70: 19–21.

Escribano, R., Hidalgo, P., & Krautz, C. 2009. Zooplankton associated with the oxygen minimum zone system in the northern upwelling region of Chile during March 2000. Deep-Sea Res. II. 56: 1083-1094.

Escribano, R., Hidalgo, P., Fuentes, M., & Donoso, K. 2012. Zooplankton time series in the coastal zone off Chile: variation in upwelling and responses of the copepod community. Prog. Oceanogr. 97-100: 174-186.

Escribano, R., Bustos-Ríos, E., Hidalgo, P. & Morales, C.E. 2016. Non-limiting food conditions for growth and production of the copepod community in a highly productive upwelling zone. Cont. Shelf Res. 126: 1-14.

Farrell, A. P., C. A. Mueller., & Seymour, R. S. 2021. Coming up for air. J. Exp. Biol. 224(17): jeb243101.

Froelicher, T. L., Joos, F., Plattner, G. K., Steinacher, M., & Doney, S. C. 2009. Natural variability and anthropogenic trends in oceanic oxygen in a coupled carbon cycle-climate model ensemble. Glob. Biogeoch. Cycles. 23.

Garcia-Reyes, M., & Largier, J. L. 2009.Seasonality of coastal upwelling off central and northern California: New insights, including temporal and spatial variability. J. Geophys. Res. 117: C03028.

García-Reyes, M., Sydeman, W.J., Schoeman, D.S., Rykaczewski, R.R., Black, B.A., Smit, A.J., & Bograd, S.J. 2015. Under pressure: climate change, upwelling, and eastern boundary upwelling ecosystems. Front. Mar. Sci. 2:109

Gilly, W.G., Beman, M., Litvin, S.Y. & Robison B.H. 2018. Oceanographic and biological effects of shoaling of the oxygen minimum zone. Annu. Rev. Mar. Sci. 5:393–420.

Giraud-Billouda, M., Rivera-Ingrahamb, G., Moreira, D., Burmesterd,T., Castro-Vazqueza, A., Carvajalino-Fernándeze, J., Dafref, A., Cuijuan Niug, Tremblay N., Paital, B., Rosa, R., Storey, J., Vega,I., Zhangg, W., Yepiz-Plascencial, G., Zenteno-Savinm, T., Storey, K. &

Gong, H., Li, Ch., & Zhou, Y. 2021. Emerging Global Ocean Deoxygenation Across the 21st Century. Geophys. Res. Lett. 1-9

Gonzalez, R., & Quiñones, R. 2002. Ldh activity in *Euphausia mucronata* and *Calanus chilensis*: implications for vertical migration behavior. J. Plankton Res. 24(12): 1349-1356.

Grantham, B.A., Chan, F., Nielsen, K.J., Fox, D.S., Barth, J.A., Huyer, A., Lubchenco, J. & Menge, B.A. 2004. Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes in the northeast Pacific. Nature 429: 749-754.

Guérin, P., El Mouatassim, S., & Ménézo, Y. 2001. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Human Reproduction Update. 7(2):175-189.

Heine, K.B., A. Abebe, A.E. Wilson., & Hood, W.R. 2019. Copepod respiration increases by 7% per °C increase in temperature: A meta-analysis. Limnol. Oceanogr. Letters. 4:53–61.

Helm, K. P., Bindoff, N. L. & Church, J. A. 2011. Observed decreases in oxygen content of the global ocean. Geophys. Res. Lett. 38: L23602.

Hermes-Lima, M. 2019. Twenty years of the 'Preparation for Oxidative Stress' (POS) theory: Ecophysiological advantages and molecular strategies. Comp. Biochem. Physiol., Part A. 234: 36-49**.**

Herring, P. J., Fasham, M. J. R., Weeks, A. R., Hemmings, J. C. P., Roe, H. S. J., Pugh, P. R., Holley, S., Crisp, N. A., & Angel, M. V. 1998. Across-slope relations between the biological populations, the euphotic zone and the oxygen minimum layer off the coast of Oman during the southwest monsoon (August, 1994). Prog. Oceanogr. 41: 69–109.

Hidalgo, P., & Escribano, R. 2007. The coupling of life cycles of the copepods Calanus chilensis and Centropages brachiatus to upwelling variability in the Central-South upwelling region off Chile. Progr. Oceanogr. 75: 501-517.

Hirst, A. G., & Sheader, M. 1997. Are in situ weight specific growth rates body-size independent in marine planktonic copepods? A re-analysis of the global syntheses and a new empirical model. Mar. Ecol. Prog. Ser. 154: 155–165.

Huyer, A. 1983. Coastal upwelling in the California Current System. Prog. Oceanogr. 12:259–284.

Ikeda, T., Torres, J.J., Hernández-León, S. & Geiger, S.P. 2000. Metabolism. In Harris, R., Wiebe, P. H., Lenz, J., Skjoldal, H. R. and Huntley, M. (eds), ICES Zooplankton Methodology Manual. Academic Press, London. 455–532.

Invidia, M., Sei, S., & Gorbi, G. 2004. Survival of the copepod *Acartia tonsa* following egg exposure to near anoxia and to sulfide at different pH values, Mar. Ecol. Prog. Ser. 276: 187–196.

Katajisto, T. 2004. Effects of anoxia and hypoxia on the dormancy and survival of subitaneous eggs of *Acartia bifilosa* (Copepoda: Calanoida), Mar. Biol., 145(4), 751–757, 2004.

Keeling, R., Körtzinger, A., & Gruber, N. 2010. Ocean deoxygenation in a warming world. Annual Review Mar. Sci. 2: 199–229.

Kiko, R., Hauss, H., Dengler, M., Sommer, S., & Melzner, F. 2015. The squat lobster, *Pleuroncodes monodon* tolerates anoxic dead zone conditions off Peru. Mar. Biol., 162: 1913-1921.

Kowaltowski, A. J., de Souza-Pinto, N.C., Castilho, R. F. & Vercesi, A. E. 2009. Mitochondria and reactive oxygen species. Free Rad. Biol. Med. 47(4):333-343**.**

Lampert, W. 1984. The measurement of respiration. In: Downing JA, Rigler FH (eds). A manual on methods for the assessment of secondary productivity in fresh waters, 2nd ed, Blackwell, Oxford. 413–468.

Landaeta, M.F., Veas, R., Letelier, L. & Castro, L.R. 2008. Larval fish assemblages off central Chile upwelling ecosystem. Revta. Biol. Mar. Ocean. 43: 569-584.

Levin, L. A., Ekau, W., Gooday, A. J., Jorissen, F., Middelburg, J. J., Naqvi, S. W. A., et al. 2009. Effects of natural and human-induced hypoxia on coastal benthos. Biogeosciences 6: 2063–2098.

Long, M. C., Deutsch, C., & Ito, T. 2016. Finding forced trends in oceanic oxygen. Glob. Biogeoch. Cycles 30(2): 381–397.

Longhurst, A. R. 1967. Vertical distribution of zooplankton in relation to the eastern Pacific oxygen minimum. Deep-Sea Res. 14: 51–63.

Lutz, R. V., Marcus, N. H., & Chanton, J. 1994. Hatching and viability of copepod eggs at two stages of embryological development: anoxic/hypoxic effect. Mar. Biol. 119: 199–204.

Maas, A. E., Wishner, K. F., & Seibel, B.A. 2012. Metabolic suppression in thecosomatous pteropods as an effect of low temperature and hypoxia in the eastern tropical north Pacific. Mar. Biol. 159:1955–67.

McQuoid, M. R., Godhe, A., & Nordberg, K. 2002. Viability of phytoplankton resting stages in the sediments of a coastal Swedish fjord. Eur. J. Phycol., 37(2): 191–201.

Merino-Campos, V., De Pol-Holz, R., Southon, J., Latorre, C., & Collado-Fabbri, S. 2019. Marine radiocarbon reservoir age along the Chilean continental. Radiocarbon. 61(1):195–210.

Mitrovic, D., Dymowska, A., Nilsson, G. E. & Perry, S. F. 2009. Physiological consequences of gill remodeling in goldfish (*Carassius auratus*) during exposure to long-term hypoxia. Am. J. Physiol. 297: R224-R234.

Montero, P., Daneri, G., Cuevas, L.A., Humberto, G., Jacob, B., Lizárraga, L., & Menschel, E. 2007. Productivity cycles in the coastal upwelling area off Concepción: The importance of diatoms and bacterioplankton in the organic carbon flux. Prog. Oceanogr. 75 (3) 518-530.

Morales, C.E., González, H. E., Hormazabal, S.E., Yuras, G., Letelier, J. & Castro, L.R., 2007. The distribution of chlorophyll-a and dominant planktonic components in the coastal transition zone off Concepción, central Chile, during different oceanographic conditions. Progr. Oceanogr. 75(3):452-469.

Moreira, D., Venancio, L., Sabino, M., & Hermes-Lima, M. 2016. How widespread is preparation for oxidative stress in the animal kingdom. Comp. Biochem. and Physiol. 200:64-78.

Oschlies, A. 2019. Ocean deoxygenation from climate change. In ‘Laffoley, D. & Baxter, J.M. (eds.). Ocean deoxygenation: Everyone’s problem - Causes, impacts, consequences, and solutions. Gland, Switzerland: IUCN. xxii+562pp.

Oschlies, A., Schulz, K. G., Riebesell, U. & Schmittner, A. 2008. Simulated 21st century’s increase in oceanic suboxia by CO2-enhanced biotic carbon export. Glob. Biogeochem. Cycles 22: GB4008.

Oschlies, A., Brandt, P., Stramma, L., & Schmidtko, S. 2018. Drivers and mechanisms of ocean deoxygenation. Nature Geosciences, 11: 467–473.

Paulmier, A. 2017. “Oxygen and the ocean,” in The Ocean revealed, eds Euzen, A., Gaill, F., Lacroix, D., & Cury, P. Paris: CNRS. 48–49.

Pauly, D., & Christensen, V. 1995. Primary production required to sustain global fisheries. Nature 374: 255–257.

Peterson, W. 1998. Life Cycle Strategies of Copepods in Coastal Upwelling Zones. J. Mar. Syst.15: 313-326.

Piiper, J., Dejaours, P., Haab, P., & Rahn, H. 1971. Concepts and basic quantities in gas exchange physiology. Respir. Physiol. 13: 292-304.

Pino-Pinuer, P., Escribano, R., Hidalgo, P., Riquelme-Bugueño, R., & Schneider, W. 2014. Copepod community response to variable upwelling conditions off central-southern Chile during 2002-2004 and 2010-2012. Mar. Ecol. Progr. Ser. 515: 83-95.

Pitcher, G. C., Aguirre, A., Breitburg, D., Cardich, J., Carstensen, J., Conley, D. J., et al. 2021. System controls of coastal and open ocean oxygen depletion. Prog. Oceanogr. 197:102613.

Robinson, C., Steinberg, D. K., Anderson, T. R., Aristegui, J., Carlson, C. A., Frost, J. R., Ghiglione, J.-F., Hernández-León, S., Jackson, G. A. & Koppelmann, R. 2010. Mesopelagic zone ecology and biogeochemistry – a synthesis. Deep-Sea Res. Part II, 57: 1504-1518.

Rogers, N. J., Urbina, M. A., Reardon, E. E., McKenzie, D. J., & Wilson, R. W. 2016. A new analysis of hypoxia tolerance in fishes using a database of critical oxygen level (P_{crit}). Conserv. Physiol. 4 (1): cow012.

Roman, M. R., Gauzens, A. L., Rhinehart, W. K. & White, J. R. 1993. Effects of low oxygen waters on Chesapeake Bay zooplankton. Multi. Digital Publi. Inst. 38(8): 1603–1614.

Roman, M. R., Brandt, S. B., Houde, E. D., & Pierson, J. J. 2019. Interactive Effects of Hypoxia and Temperature on Coastal Pelagic Zooplankton and Fish. Front. Mar. Sci. 6.

Roman, M., & Pierson, J.J. 2022. Interactive Effects of Increasing Temperature and Decreasing Oxygen on Coastal Copepods. Biol. Bull. 243: 171–183.

Saltzman, J. & Wishner, K. F.1997. Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 2. Vertical distribution of copepods. Deep-Sea Res. Part I 44: 931–954.

Sameoto, D., Guglielmo, L., & Lewis, M. 1987. Day/night vertical distribution of euphausiids in the eastern tropical Pacific. Mar. Biol. 96:235–45.

Schmidtko, S., Stramma, L., & Visbeck, M. 2017. Decline in global oceanic oxygen content during the past five decades. Nature 542: 335–339.

Schneider, W., Donoso, D., Garcés-Vargas, J., & Escribano, R. 2016. Water-column cooling and sea surface salinity increase in the upwelling region off central-south Chile driven by a poleward displacement of the South Pacific. Prog. Oceanogr. 141: 38-58.

Sedlacek, C., & Marcus, N.H. 2005. Egg production of the copepod Acartia tonsa: the influence of hypoxia and food concentration. J. Exp. Mar. Biol. Ecol. 318:183–90

Seibel, B. A. 2011. Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. J. Exp. Biol. 214: 326-336.

Seibel, B.A., Hafker, S., Trubenbach, K., Zhang, J., Portner, H.O., Rosa, R., & Storey, K.B. 2014. Energy metabolism during hypoxic exposure in an oxygen minimum zone squid, *Dosidicus gigas*. J. Exp. Biol. 217:2555–68.

Seibel, B. A., Jillian, L., Schneider, S. K., Wishner K.F., & Daly, K. L. 2016. Hypoxia Tolerance and Metabolic Suppression in Oxygen Minimum Zone Euphausiids: Implications for Ocean Deoxygenation and Biogeochemical Cycles. Integr. Comp. Biol. 56(4): 510-523.

Seibel, B. A., & Deutsch, C. 2020. Oxygen supply capacity in animals evolves to meetmaximum demand at the current oxygen partial pressure regardless of size or temperature. J. Exp. Biol. 223: jeb210492.

Seibel, B. A., Andres, A., Birk, A. M. A., Burns, L., Shaw, C. T., Timpe, A. W., & Welsh, C. J. 2021. Oxygen supply capacity breathes new life into critical oxygen partial pressure (P_{crit}). J. Exp. Biol. 224(8): jeb242210.

Sobarzo, M., & Djurfeldt, L. 2004. Coastal upwelling process on a continental shelf limited by submarine canyons, Concepción, central Chile. J. Geophys. Res. 109: C12012.

Sobarzo, M., Bravo, L., Donoso, D., Garces-Vargas, J. & Schneider, W., 2007. Coastal upwelling and seasonal cycles that influence the water column over the continental shelf off central Chile. Prog. Oceanogr. 75: 363–382.

Steinberg, D. K., & Landry, M. R. 2017. Zooplankton and the ocean carbon cycle, Annual Rev. Mar. Sci., 9, 413–444.

Stendardo, I., Rhein, M., & Hollmann, R. 2016. A high resolution salinity time series 1993–2012 in the North Atlantic from Argo and Altimeter data. Limnol. Oceanogr. 121(4): 2523-2551.

Stocker, T.F., Qin, D. & Plattner, G. K. 2013.Technical summary. In: Climate change 2013: the physical science basis. Contribution of working group Ito the fifth assessment report of the intergovernmental panel on climate change. Cambridge: Cambridge University Press. 2013.

Stramma, L., Johnson, G.C., Sprintall, J. & Mohrholz, V. 2008. Expanding oxygen-minimum zones in the tropical oceans. Science 320: 655–658.

Stramma, L., Schmidtko, S., Levin, L., & Johnson, G. 2010. Ocean oxygen minima expansions and their biological impacts. Deep-Sea Res. 57: 587-595.

Stramma, L., Prince, E., Schmidtko, S., Luo, J., Hoolihan, J. P., Visbeck, M., Wallace, D. W. R., Brandt, P., & Körtzinger, A. 2012. Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes. Nature Climate Change. 2: 33–37.

Strub, P., Mesias, J., Montecino, V., Rutlland, J. & Salinas, S.1998. Coastal ocean circulation off western south America, in The Sea Vol 11, The Global Coastal Ocean, Regional Studies and Synthesis, edited by A. R. Robinson and K. H. Brink. 10: 273 – 313.

Svetlichny, L. S., Hubareva, E. S., Erkan, F. & Gucu, A. C. 2000. Physiological and behavioral aspects of *Calanus euxinus* females (Copepoda: Calanoida) during vertical migration across temperature and oxygen gradients. Mar. Biol. 137: 963- 971.

Tremblay, N., Colley, R.C., Saunders, T.J., Healy, G.N. & Owen, N. 2010. Physiological and health implications of a sedentary lifestyle. Applied Physiology, Nutrition and Metabolism 35(6):725-40.

Tutasi, P., & Escribano, R. 2020. Zooplankton diel vertical migration and downward C flux into the oxygen minimum zone in the highly productive upwelling region off northern Chile. Biogeosciences 17: 455–473.

Vargas, C., Escribano, R., & Poulet, S. 2006. Phytoplankton food quality determines time windows for successful zooplankton reproductive pulses. Ecology 87: 2992-2999.

Vargas. V. 2010. Seasonal relative influence of food quantity, quality, and feeding behavior on zooplankton growth regulation in coastal food webs. J. Mar. Biol. Assoc. UK 90(06): 1189-1201.

Verberk, W.C.E.P., Atkinson, D., Hoefnagel, K.N., Hirst, A.G., Horne, C.R., & Siepel, H. 2021. Shrinking body sizes in response to warming: explanations for the temperature–size rule with special emphasis on the role of oxygen. Biol. Review. 96(1): 247-268.

Ward, B.A., Dutkiewicz, S., Jahn, O., & Follows, M. J. 2012. A size-structured food-web model for the global ocean. Limnol. Oceanogr. 57(6): 1877-1891

Welker, A.F., Moreira, D.C., Campos, E.G. & Hermes-Lima, M. 2013. Role of redox metabolism for adaptation of aquatic animals to drastic changes in oxygen availability. Comp. Biol. Physiol. 165:384–404.

Wilkinson, L. 1990. SYSTAT: the system for statistics. SYSTAT Inc., Evanston, IL.

Wishner, K. F., Gowing, M. M., & Gelfman, C. 1995. Living in suboxia: Ecology of an Arabian Sea oxygen minimum zone copepod. Limnol. Oceanogr. 45: 1576–1593.

Wishner, K. F., Gelman, C., Gowing, M. M., Outram, D. M., Rapien, M. & Williams, R. L. 2008. Vertical zonation and distributions of calanoid copepods through the lower oxycline of the Arabian Sea oxygen minimum zone. Prog. Oceanogr. 78:163-191.

Wishner, K. F., Outram, D. M., Seibel, B. A., Daly, K. L., & Williams, D R. L. 2013. Zooplankton in the eastern tropical north Pacific: Boundary effects of oxygen minimum zone expansion. Deep-Sea Res. Part I 79:122–140.

Wishner, K. F., Seibel, B. A., Roman, C., Deutsch, C., Outram, D., Shaw, C. T., Birk, M.A., Mislan, K.A.S., Adams, T. J., Moore, D., & Riley, S. 2018. Ocean deoxygenation and zooplankton: Very small oxygen differences matter. Sciences Adv. 4(12): eaau5180.

Xiu, P., Chai, F., Curchitser, E. N., Frederic, S., & Castruccio, S. 2018. Future changes in coastal upwelling ecosystems with global warming: The case of the California Current System. Sci. Rep. 8:2866.

8. ANNEXES

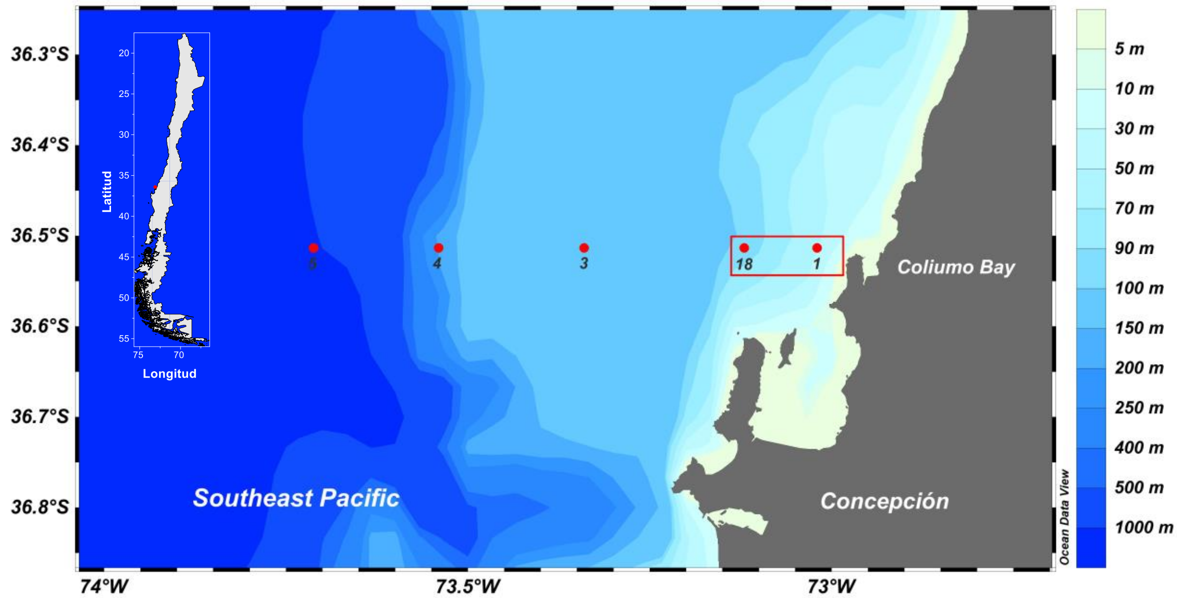

Figure. S1: The coastal upwelling zone of central-southern Chile in the Southeast Pacific, illustrating the sampling stations where oceanographic variables were measured and zooplankton samples were obtained. The square shows the area where copepods were captured for the experiments. The time series for monthly observations (2002-2020) on temperature, oxygen and chlorophyll-a was carried out at Station 18.

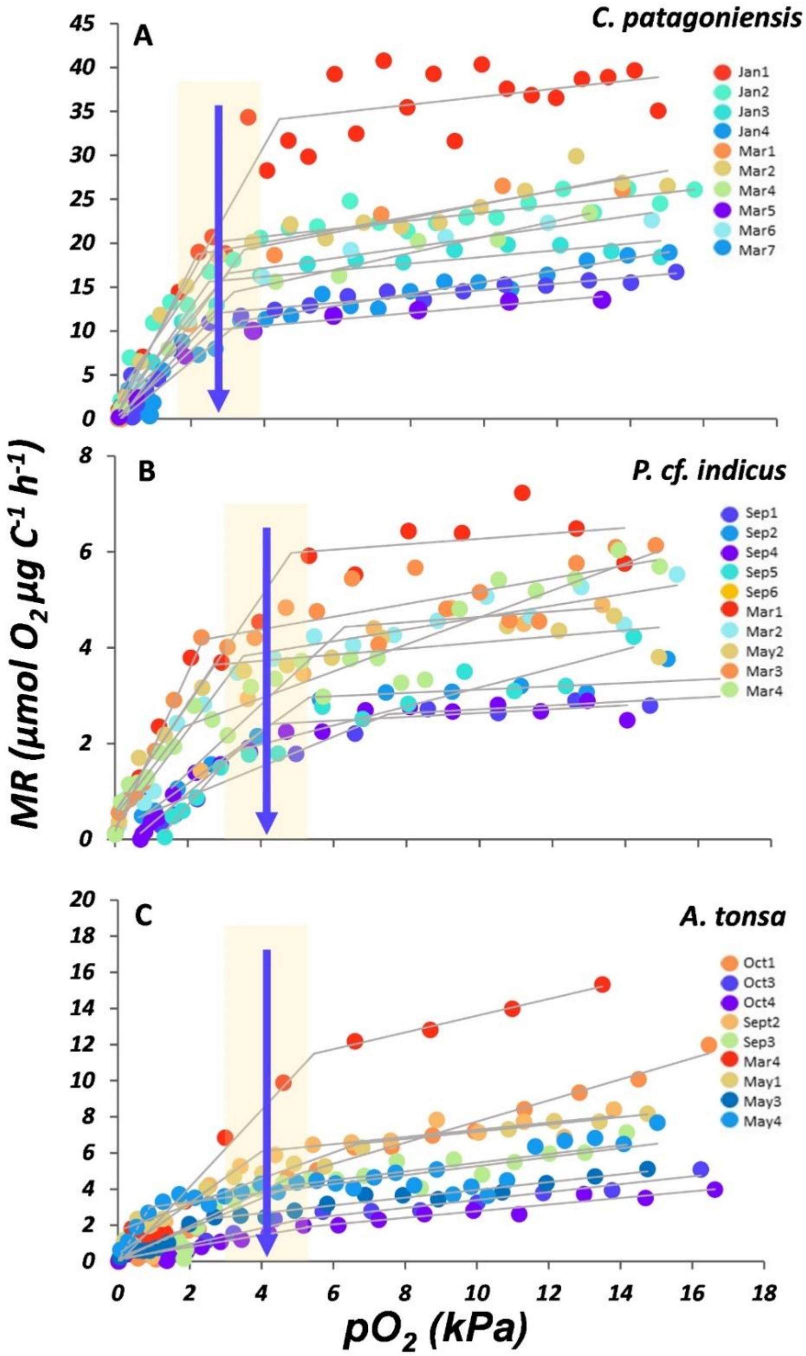

Figure S2: Annual P_{crit} for three species of planktonic copepods inhabiting the coastal upwelling of central-southern Chile. A: *C. patagoniensis*, B: *P.* cf. *indicus* and C: *A. tonsa*. Colors represent experiments conducted at different seasons. Vertical arrows signal average P_{crit} values, and its standard deviation is signaled by the light-yellow shading.

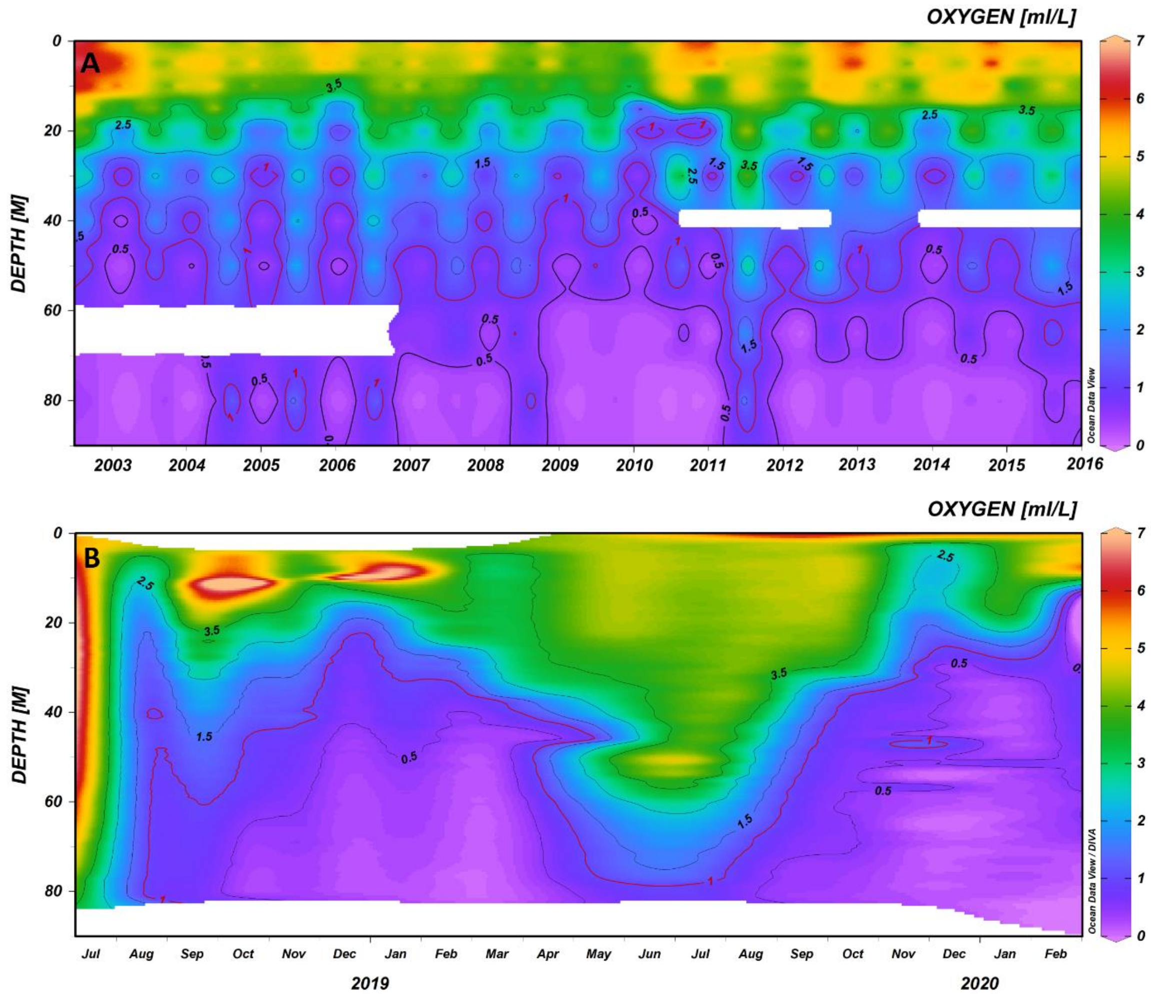

Figure. S3: Variation of the oxygen content in the water column obtained from the time series at Station 18 (A) and Zeus monitoring (B) for the study area. Oxygen profiles were obtained with a SeaBird SBE Conductivity Temperature Depth (CTD) device, equipped with a SeaBird SBE-43 oxygen sensor.

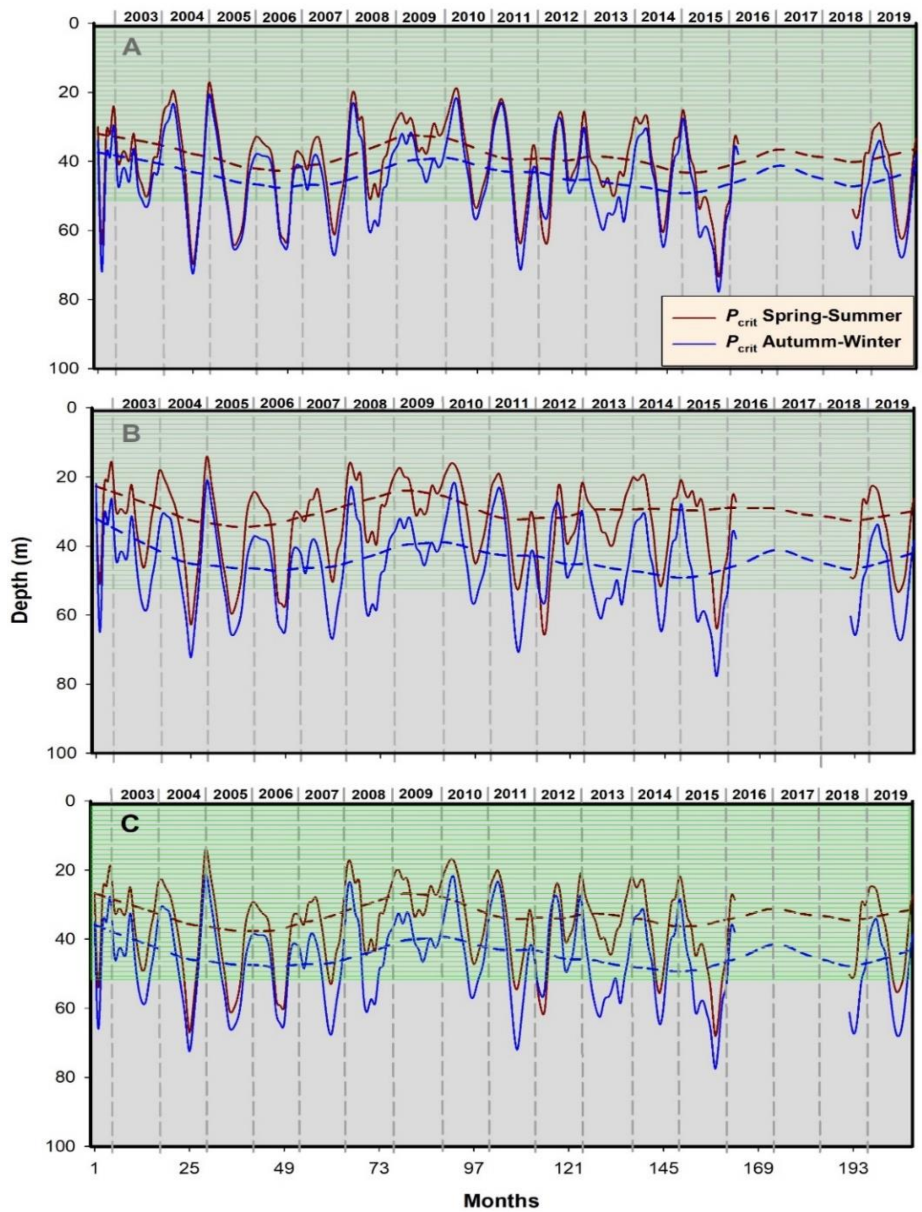

Figure S4: Monthly variability in calculated vertical position of P_{crit}. Historical monthly variation in the depth at which P_{crit} values of three copepods species are encountered in the water column during the time series study (2002-2019) at Station 18 of central/southern Chile. A: *Calanoides patagoniensis*, B: *Paracalanus* cf. *indicus*, C: *Acartia tonsa*. P_{crit} were empirically estimated in two seasons: spring-summer and autumn-winter. Dotted-lines are smoothed series (3-months moving average).

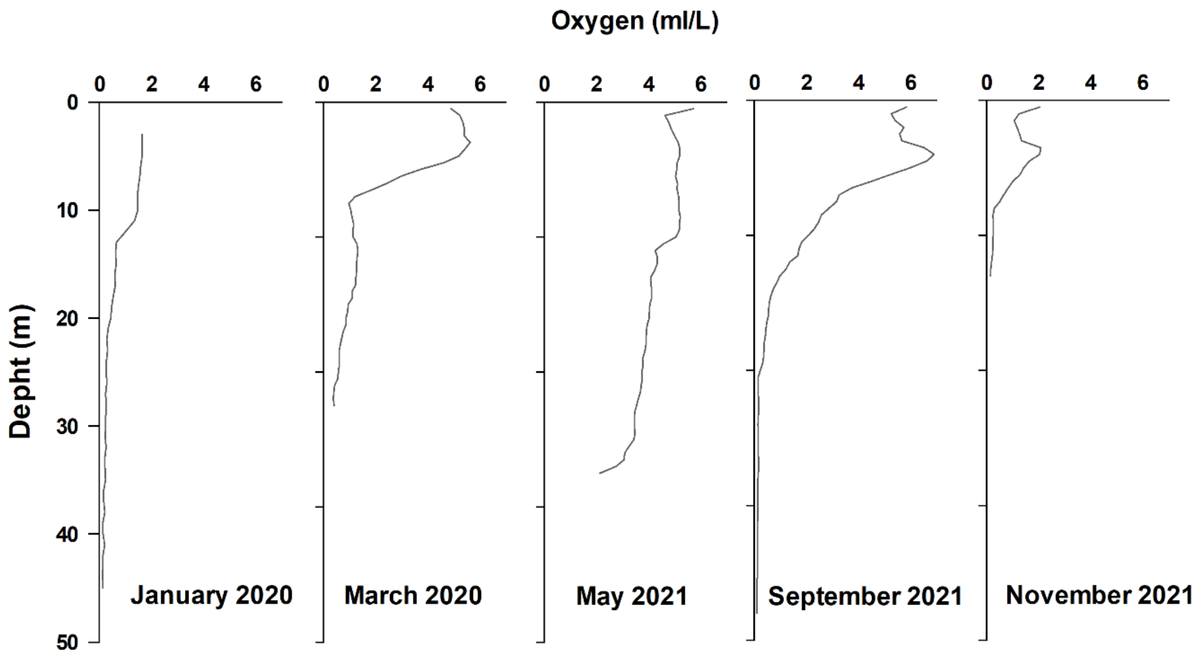

Figure S5: Vertical oxygen profiles obtained during plankton sampling to carry out experiments on respiration rate of copepods. Profiles were obtained with a SeaBird SBE Conductivity Temperature Depth (CTD) device, equipped with a SeaBird SBE-43 oxygen sensor.

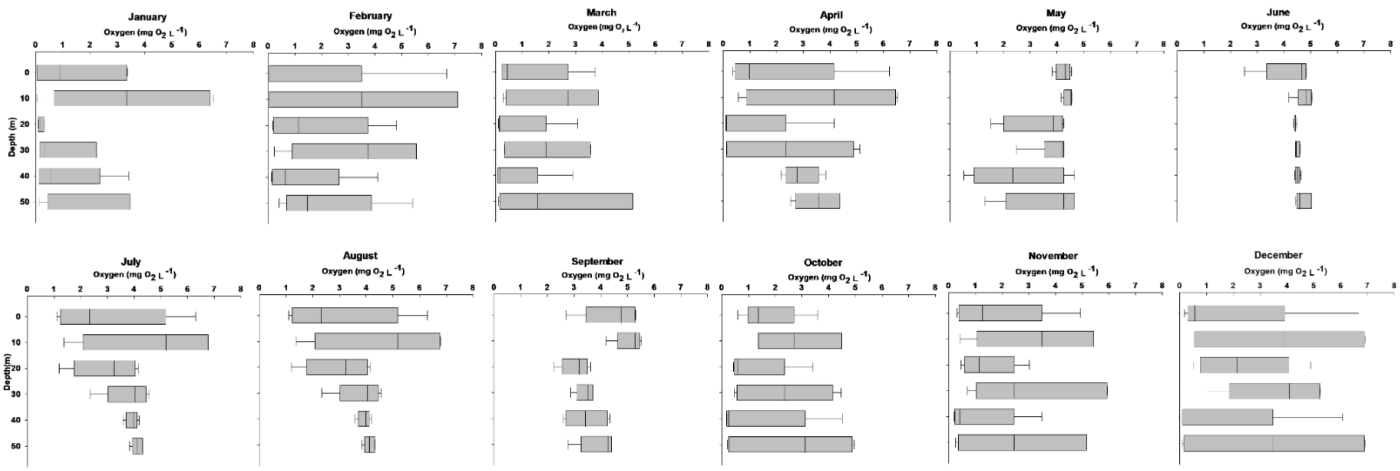

Figure S6: Monthly variation in the oxygen concentration in the first 50 m of depth, obtained from the time series at Station 18 (shown in Fig. S1). Oxygen profiles were obtained with a SeaBird SBE Conductivity Temperature Depth (CTD) device, equipped with a SeaBird SBE-43 oxygen sensor.

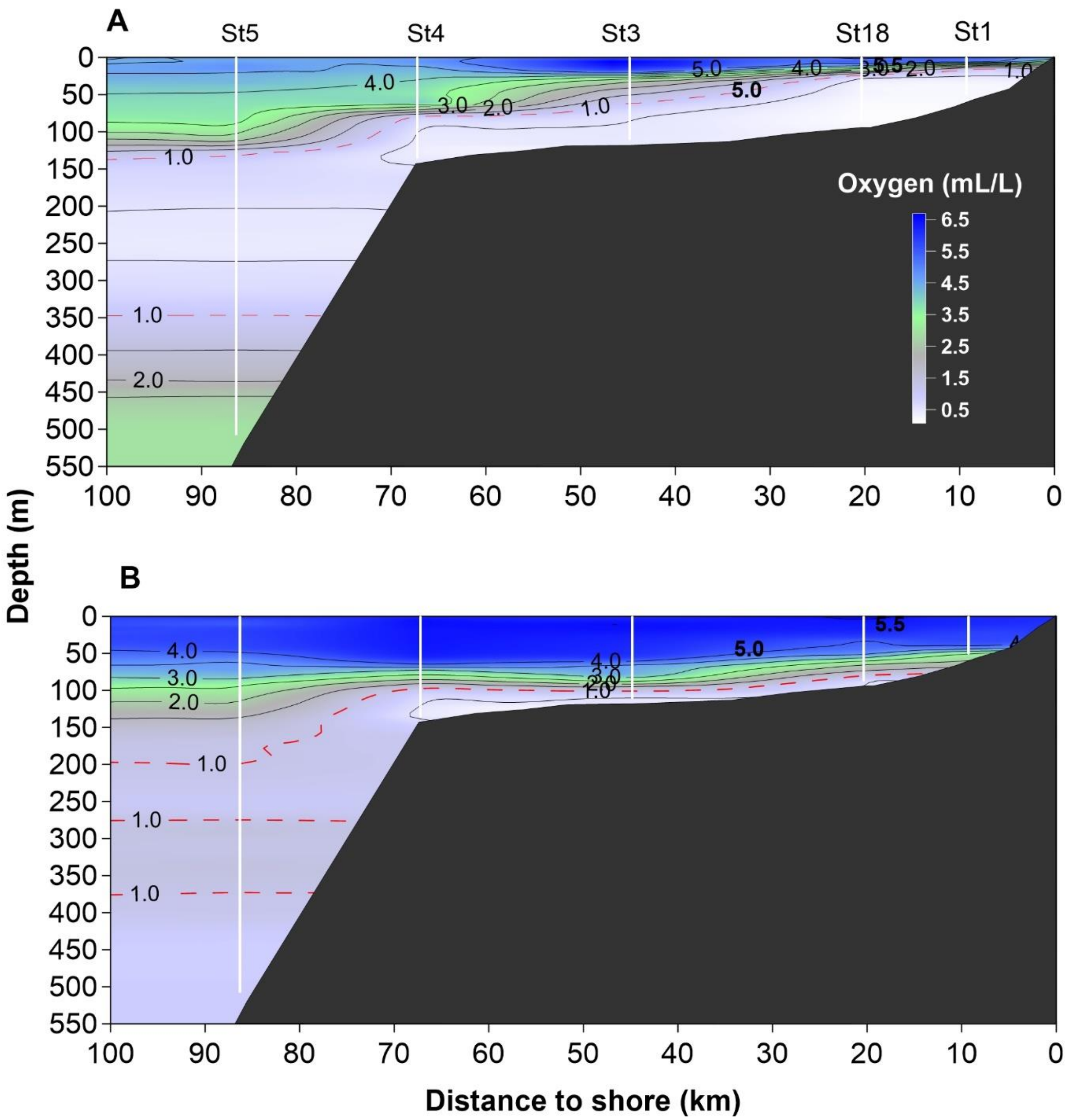

Figure S7: Cross-shelf section of oxygen concentration in the coastal upwelling zone of central-southern Chile during the spring 2008 and the winter 2009 at the latitude of 36°30 S, illustrating the presence of the oxygen minimum zone (OMZ) in the subsurface layer. The dotted red line shows the upper limit of the OMZ (1 mL O_2 L^{-1}).

Table S1: Summary of respiration rate experiments for three planktonic copepods carried out between January 2021 and October 2021 in central-southern Chile. n=number of individuals per vial and Inc. time is the incubation time. Temperature was kept constant at 13°C for all experiments in a coldroom and dark condition.

Season	Date	Species	Replicates	N° of vials	n	Inc. time (hours)
Spring-Summer	Jan 2021	*C. patagoniensis*	2	8	10-15	8
	Sept 2021	*P.* cf. *indicus*	2	12	40-50	8
	Sept 2021	*A. tonsa*	1	5	30-40	10
	Oct 2021	*A. tonsa*	1	5	30-40	11
Autumn-Winter	March 2021	*C. patagoniensis*	2	8	10-15	9
	March 2021	*P.* cf. *indicus*	2	4	50	9
	May 2021	*C. patagoniensis*	1	4	10-15	8
	May 2021	*P.* cf. *indicus*	1	4	40-50	9
	May 2021	*A. tonsa*	1	3	35-40	10

Table S2: Two-way ANOVA to test the effects of season and species on the respiratory rate (MR) and P_{crit} of three copepod species, *Calanoides patagoniensis*, *Paracalanus* cf. *indicus* and *Acartia tonsa.*

Dependent variable	Effect	d.f	F-ratio /P-value
MR (μmol O_2 μg $C^{-1}h^{-1}$)	Season 1	5.448	0.028*
	Species 2	70.87	0.000**
	Season x species 2	2.488	0.103
P_{crit} (kPa)	Season 1	24.51	0.000**
	Species 2	0.214	0.809
	Season x species 2	3.202	0.058

Table S3: One-way ANOVA to test the effect of season on the respiratory rate (MR) and P_{crit} of three copepods from the upwelling zone of central-southern Chile. *=Significant effect (P<0.05), **=Highly significant effects (P<0.01), ns=non-significant.

Species	Variable	F-ratio	P-value
C. patagoniensis	MR	6.525	0.03*
	P_{crit}	1.012	0.34 ns
P. cf. *indicus*	MR	0.283	0.61 ns
	P_{crit}	17.013	0.00**
A. tonsa	MR	1.627	0.24 ns
	P_{crit}	22.425	0.00**

Table S4: Mean ± standard deviation of copepod sizes from variable experimental periods. (n) is the number of organisms measured. t-test was applied to *C. patagoniensis* and *A. tonsa*, while for *P.* cf. *indicus* we applied a one-way ANOVA. ns=non-significant differences (P>0.05).

Species	Month	Size µm (n)	t-test/ANOVA
C. patagoniensis	January	2019 ± 168.9 (13)	0.177 ns
	August	1925 ± 153.2 (10)	
*P.*cf. *indicus*	April	746 ±21.61 (14)	0.472 ns
	July	753 ± 28.68 (16)	
	August	766 ± 20.41 (6)	
A. tonsa	May	965 ±83.27 (19)	0.618 ns
	October	971 ±75.23 (13)	

Table S5: Mean and standard deviation of P_{crit} (kPa) to 10, 13 and 15°C to copepods estimated from slope of curve (P_{crit} vs MR).

Species	**Season**	***P_{crit}* (kPa)**		
		10°C	**13°C**	**15°C**
C. patagoniensis	S-S	3.29±3.192	3.83±10.220	5.09±4.571
	A-W	2.30±0.710	3.18±2.803	4.02±1.570
P.cf. *indicus*	S-S	3.59±0.803	4.64±0.571	5.22±1.885
	A-W	1.99±0.593	2.77±0.638	2.962±1.170
A. tonsa	S-S	3.85±0.551	4.90±2.025	3.21±0.853
	A-W	1.72±0.785	2.33±0.775	2.81±1.359

Table S6: The slopes ± Standard deviation of the oxyconforming regression after fitting the two-step regression model of metabolic rate as a function of oxygen pressure (kPa) to estimate P_{crit} in three copepods species. Seasonal effects on the slopes were tested by two-tailed t-test. *Significant differences ($P<0.05$), ns=non-significant differences ($P>0.05$).

Species	**Slope 1**	**Slope 2**	**d.f**	**t-test**
C. patagoniensis	0.604±0.132	0.430±0.162	11	2.76*
P. cf. *indicus*	0.071±0.020	0.102±0.028	11	2.99*
A. tonsa	0.104±0.051	0.132±0.080	9	0.90ns

Slope 1= spring-summer

Slope 2= autumn-winter

d.f= degrees of freedom

t-test= two-tailed Student test

Table S7: Time to reaching P_{crit} (T) and time to survival (TS) after reaching P_{crit}, and percentage of survivors (NS) after reaching P_{crit}.

Species	P_{crit} (kPa)	T (hours)	TS	NS
C. patagoniensis	3.42	4	3	28%
P. cf. *indicus*	3.79	5	7	24%
A. tonsa	4.05	8	5	26%

Table S8: Location and depth strata used for zooplankton sampling performed in August 2008 in the coastal upwelling zone of central-southern Chile. Sampling was carried out with a midi-type Hydrobios Multinet horizontally towed. Station locations are also illustrated in Fig. S1.

Station	latitude	Longitude	Depth strata
St1	36.5	73.02	40-30 30-20 20-10 10-0
St18	36.5	73.12	80-60 60-40 40-20 20-0
St3	36.5	73.34	100-70 70-50 50-20 20-0
St4	36.5	73.54	200-90 90-60 60-30 30-0
St5	36.5	73.712	500-300 300-200 200-100 100-0