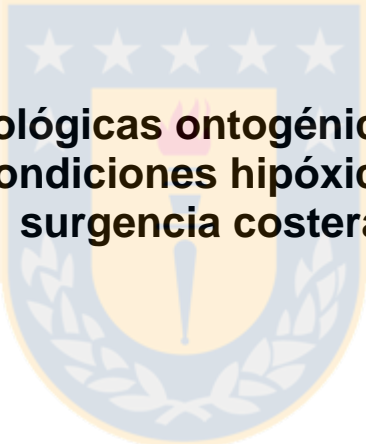




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Dirección de Postgrado
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Oceanografía



**Respuestas fisiológicas ontogénicas de copéodos
pelágicos bajo condiciones hipóxicas en sistemas de
surgencia costera**

Tesis para optar al grado de Doctor en Oceanografía

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A mis padres.

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Resumen

Respuestas fisiológicas ontogénicas de copépodos pelágicos bajo condiciones hipóxicas en sistemas de surgencia costera

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Las zonas de mínimo oxígeno (ZMOs) son áreas de bajas concentraciones de oxígeno o de hipoxia natural presentes en el océano, asociadas a los sistemas de surgencia costera, como el Sistema de Corrientes de Humboldt (SCH). Las ZMOs han sido consideradas verdaderos laboratorios naturales para el estudio de los cambios que podría enfrentar el ecosistema pelágico ante la desoxigenación del océano asociada al cambio climático, la cual produce la expansión, intensificación y somerización de estas aguas. Esto se ha convertido en un tema relevante debido a las perturbaciones que podría generar en la ecología de los organismos, como la reducción de la extensión de la capa superficial donde habita la mayoría del zooplancton. Los cambios en el ecosistema pelágico pueden ser estudiados mediante respuestas fisiológicas del plancton, descritos como bioindicadores de cambios en las condiciones ambientales. La presente investigación abordó, desde una perspectiva experimental, las potenciales respuestas ontogénicas (de distintos estadios de desarrollo) en las tasas vitales y sobrevivencia de los copépodos *Calanus chilensis* y *Acartia tonsa* en condiciones de hipoxia y, desde la perspectiva *in situ*, el rol de la hipoxia asociada a la ZMO en la abundancia y distribución de huevos de los copépodos *A. tonsa*, *Paracalanus* cf. *indicus* y *C. chilensis* en la zona norte de Chile, durante el 2013 y 2014. Área que fue utilizada como modelo para el estudio de los potenciales efectos que puede generar la expansión y somerización de las ZMOs sobre los copépodos pelágicos en sistemas de surgencia costera.

Los copépodos pelágicos presentaron respuestas ontogénicas a la hipoxia. Las hembras de *C. chilensis* fueron tolerantes al bajo oxígeno a diferencia de los estadios tempranos. Mientras las hembras y estadios tempranos de *A. tonsa* fueron susceptibles a la hipoxia. La

ecofisiología de los copépodos en estudio, también presentó respuestas especie-específicas. Las hembras de *C. chilensis* (especie endémica del SCH) fueron más tolerantes que las de *A. tonsa* (especie cosmopolita), las cuales sufrieron efectos letales a concentraciones de hipoxia severa. Los estadios tempranos de ambas especies fueron afectados negativamente por la hipoxia, sin embargo, concentraciones de bajo oxígeno disuelto sólo disminuyeron la tasa de crecimiento naupliar de *C. chilensis*. Esta disminución en la tasa de crecimiento podría resultar en una consecuente reducción del tamaño final de los organismos, potencialmente afectando las tramas tróficas del ambiente pelágico.

Los resultados obtenidos a partir de la data *in situ* permitieron observar que las mayores abundancias de huevos de *A. tonsa* y *P. cf. indicus* se encontraron en aguas superficiales sobre la ZMO y que la distribución de ambas especies fue correlacionada positivamente con la clorofila-*a* y la estratificación. Los huevos de *C. chilensis* presentaron una distribución más profunda que no fue correlacionada con ninguna variable abiótica, mientras la distribución de las hembras de esta especie se asoció con eventos de surgencia. La distribución vertical de los huevos de copépodos en el SCH puede ser explicada por la variabilidad abiótica, la profundidad de desove, las velocidades de hundimiento de los huevos y el efecto de resuspensión producto de las velocidades verticales de la surgencia, que retienen los huevos de copépodos pequeños en aguas someras sobre la ZMO mientras, los huevos de *C. chilensis* se hunden llegando a aguas hipóxicas en menos de dos días. Adicionalmente, el estudio de la morfología externa de estos huevos permitió identificar diferencias interespecíficas entre las tres especies y diferencias intraespecíficas en *A. tonsa*, con la presencia de tres tipos de huevos según el largo de sus espinas, lo que podría influir en los tiempos de eclosión y desarrollo de esta especie.

Las condiciones de hipoxia asociadas a la ZMO del norte de Chile pueden influir sustancialmente en la dinámica poblacional de copépodos dominantes, al disminuir la sobrevivencia y tasas vitales de estadios tempranos de especies como *C. chilensis* y por la disminución de la sobrevivencia y potencial reproductivo en copépodos como *A. tonsa*, cambios que podrán influir en sus abundancias y tiempos generacionales. La variabilidad en las condiciones ambientales, como la intensificación de la surgencia, somerización y expansión de las ZMOs podrían afectar la productividad del sistema pelágico en ecosistemas de surgencia costera.

Abstract

Ontogenetic physiological responses of pelagic copepods under hypoxic conditions in coastal upwelling systems

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The Oxygen Minimum Zones (OMZs) are low oxygen or natural hypoxia areas that occur in the ocean, usually associated with coastal upwelling systems, such as the Humboldt Current System (HCS). The OMZs have been considered as natural laboratories for the study of changes that could face the pelagic ecosystem due to the ocean deoxygenation linked to climate change. Global warming may cause the expansion, intensification and shoaling of the OMZs. This has become a relevant issue due to perturbations that could generate on the ecology of organisms, such as the reduction of the extent of the surface layer where most zooplankton inhabit. Changes in the pelagic ecosystem can be studied through physiological responses of plankton, which have been described as bioindicators of changes in environmental conditions. The present research, from an experimental perspective, dealt with ontogenetic responses in vital rates and survival of the copepods *Calanus chilensis* and *Acartia tonsa* under hypoxia conditions. From the *in situ* perspective, the role of the hypoxia associated with the OMZ in the abundance and distribution of copepod eggs *A. tonsa*, *Paracalanus cf. indicus* and *C. chilensis* were studied in northern Chile during 2013 and 2014. This area was used as a model to study the potential effects of the OMZs expansion and shoaling on pelagic copepods in coastal upwelling systems.

The pelagic copepods showed ontogenetic responses to hypoxia. *C. chilensis* females were tolerant to low oxygen as opposed to early stages. Whereas, both the females and early stages of *A. tonsa* were susceptible to the hypoxia. The ecophysiology of copepods under study also exhibited species-specific responses. Females of *C. chilensis* (an endemic species of HCS) were more tolerant than those of *A. tonsa* (a cosmopolitan species), which suffered

lethal effects at concentrations of severe hypoxia. The survival of early stages of both species were negatively affected by hypoxia, but low oxygen reduced the naupliar growth rate only for *C. chilensis*. This decrease in the growth rate could result in a consequent reduction in the final size of the organisms, potentially affecting the pelagic trophic web.

The results obtained from the *in situ* data allowed us to find out that the highest abundances of *A. tonsa* and *P. cf. indicus* eggs were found in surface waters above the OMZ. The distribution of both species was positively correlated with chlorophyll-*a* and stratification. In contrast, the eggs of *C. chilensis* had a deeper distribution and were not correlated with any abiotic variable. However, the distribution of the females of this species was associated with upwelling events. The vertical distribution of copepod eggs in HCS can be explained by abiotic variability, spawning depth, egg sinking rates and the resuspension effect resulting from vertical velocities of upwelling, which retain eggs from small copepods in shallow waters above the OMZ, whereas the eggs of *C. chilensis* sink to hypoxic waters in less than two days. In addition, the study of the external morphology of these eggs allowed us to identify interspecific differences between the three species and intraspecific differences between *A. tonsa* eggs. The presence of three types of eggs in according to the length of their spines, could influence the hatching and development of *A. tonsa*.

The hypoxia associated with the OMZ of northern Chile may have a significant influence on the population dynamics of dominated copepods by decreasing the survival and vital rates of early stages of species such as *C. chilensis*, and by decreasing survival and reproductive potential copepods such as *A. tonsa*, changes that may influence on their abundances and generational times. The variability in environmental conditions such as intensification of upwelling, somerization and expansion of OMZs could affect the productivity of the pelagic system in coastal upwelling ecosystems.

1. INTRODUCCIÓN

1.1. Sistemas de surgencia y zonas de mínimo oxígeno

Los sistemas de surgencia costera se encuentran ubicados en los bordes orientales de los océanos Atlántico y Pacífico, y en el borde occidental del Índico. Los sistemas de surgencia más relevantes se encuentran asociados a las corrientes de California, Humboldt, Canarias y Benguela (Fig. 1). En estas zonas, el estrés inducido por el viento paralelo a la costa y con dirección hacia el Ecuador (a excepción del Océano Índico) desplaza las aguas superficiales costa afuera, dando lugar al ascenso de aguas subsuperficiales más frías, ricas en nutrientes y pobres en oxígeno disuelto hacia la zona eufótica, sosteniendo elevados niveles de producción biológica en la capa superficial (Mann & Lazier 1991).

La alta producción biológica favorece la presencia de aguas pobres en oxígeno disuelto a profundidades intermedias, producto de la escasa ventilación de las aguas subsuperficiales y las elevadas tasas de remineralización (respiración aeróbica) de materia orgánica proveniente de la capa superficial (Wyrтки 1962; Keeling et al. 2010; Wang et al. 2015). Estas zonas de hipoxia natural, han sido denominadas zonas de mínimo oxígeno (ZMOs) debido a que presentan concentraciones $< 0.5 \text{ mL L}^{-1}$ ($\sim 22.3 \text{ }\mu\text{M}$) (Paulmier & Ruiz-Pino 2009; Ulloa & Pantoja 2009; Keeling et al. 2010; Stramma et al. 2010), llegando incluso a presentar condiciones de anoxia total (Ulloa et al. 2012).

Las ZMOs más intensas ocupan cerca del 8% (30.4 ± 3 millones de km^2) del área total de océano mundial (Paulmier & Ruiz-Pino 2009) (Fig. 1). Se caracterizan por presentar una estructura vertical típica, comenzando con: 1) un fuerte gradiente superior ($\sim 0.05 \text{ mL L}^{-1} \text{ m}^{-1} \approx 0.07 \text{ mg L}^{-1} \text{ m}^{-1} \approx 1.6 \mu\text{M m}^{-1}$) cuatro veces más oxigenado que el núcleo ($\sim 2.08 \text{ mL L}^{-1} \approx 2.97 \text{ mg L}^{-1} \text{ m}^{-1} \approx 65 \mu\text{M}$), 2) un núcleo con valores de oxígeno altamente deficientes ($\sim 0.48 \text{ mL L}^{-1} \approx 0.69 \text{ mg L}^{-1} \approx 15 \mu\text{M}$) y, 3) un gradiente inferior más oxigenado ($\sim 3.2 \text{ mL L}^{-1} \approx 4.57 \text{ mg L}^{-1} \approx 100 \mu\text{M}$) pero menos intenso ($\sim 0.0012 \text{ mL L}^{-1} \text{ m}^{-1} \approx 0.0017 \text{ mg L}^{-1} \text{ m}^{-1} \approx 0.04 \mu\text{M m}^{-1}$) que el gradiente superior (Paulmier & Ruiz-Pino 2009).

El ambiente reductor de las ZMOs favorece el reciclamiento de nutrientes como fosfatos y silicatos, la biodisponibilidad de metales traza (*e.g.* Fe^{+2}) y la acumulación de carbono inorgánico disuelto ($\text{CID} > 2225 \text{ }\mu\text{mol kg}^{-1}$), constituyendo grandes reservorios de CO_2 (Paulmier et al. 2011). Además, estas aguas se encuentran enriquecidas en nitrito (NO_2^-)

y empobrecidas en nitrato (NO_3^-) debido a que sostienen una gran diversidad microbiana que contribuye a la pérdida de nitrógeno (como N_2 y N_2O) hacia la atmósfera mediante procesos de desnitrificación y anammox, cumpliendo un rol fundamental en los ciclos biogeoquímicos y en el reciclamiento de la materia orgánica (Paulmier & Ruiz-Pino 2009; Ulloa et al. 2012; Gilly et al. 2013).

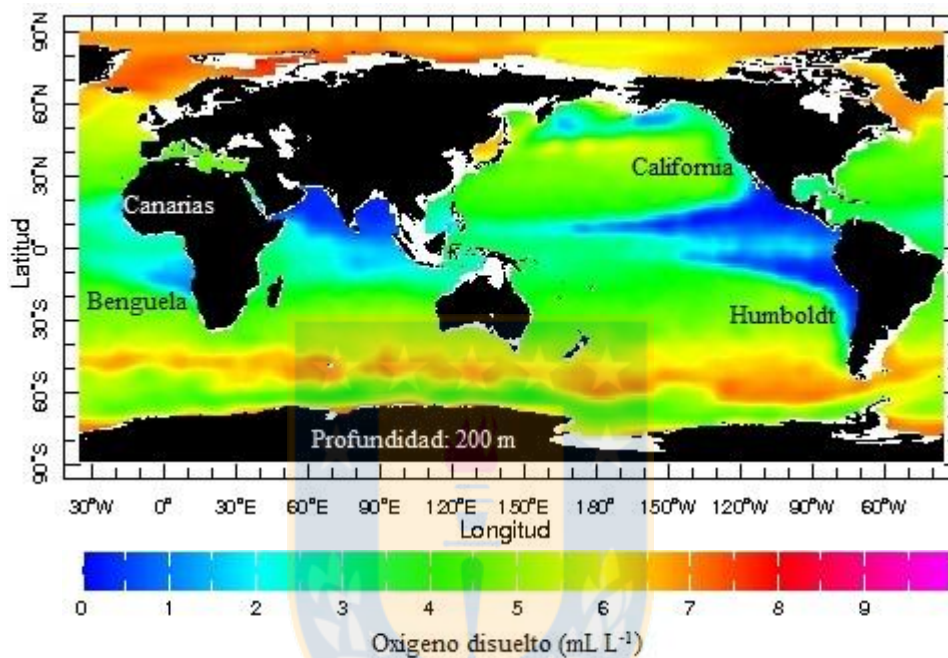


Figura 1.- Distribución del oxígeno disuelto (mL L^{-1}) a 200 m de profundidad. zonas de mínimo oxígeno (color azul) asociadas a los sistemas de surgencia de las corrientes de Canarias, Benguela, California y Humboldt (Fuente: <http://iridl.ldeo.columbia.edu/>, último acceso, 25 de febrero de 2017).

Actualmente, es de gran interés comprender cómo el cambio climático podría afectar la estructura, abundancia, distribución y fenología del plancton en sistemas de surgencia costera, atributos que podrían ser utilizados para comprender y cuantificar los efectos ecológicos y socioeconómicos generados por el aumento de las concentraciones de CO_2 atmosférico (Hays et al. 2005) y el aumento de la temperatura global, lo cual ha llevado a una mayor estratificación de la columna de agua producto del calentamiento superficial del océano (Bograd et al. 2008; Richardson 2008). El cambio climático ha demostrado favorecer la desoxigenación del océano y, por lo tanto, la expansión de las ZMOs producto de procesos

biológicos y cambios en la ventilación natural (Ekau et al. 2010; Keeling et al. 2010; Pelejero et al. 2010; Stramma et al. 2010).

Se ha observado que en los sistemas de surgencia de borde oriental, los vientos favorables a la surgencia se han intensificado, generando enfriamiento costero y somerización de las ZMOs (Bakun 1990; Rykaczewski & Checkley 2008; Falvey & Garreaud 2010). En el Océano Pacífico Suroriental (PSO), el SCH se distribuye latitudinalmente desde 42°S hasta latitudes cercanas al Ecuador (Montecino et al. 2005). Este sistema, la ZMO se encuentra asociada a Aguas Ecuatoriales Subsuperficiales y comprende una extensión volumétrica cercana a los $2.7 \times 10^6 \text{ km}^3$, lo cual corresponde al ~11% del total global (Morales et al. 1999; Schneider et al. 2006). Al menos tres cuartos de la costa chilena forman parte del SCH (~ 42°S - 18°S) (Thiel et al. 2007). Debido a su gran extensión y al patrón de vientos predominante en las zonas norte y centro-sur de Chile, distintos regímenes de surgencia semipermanente y estacional predominan en cada área, respectivamente (Piñones et al. 2007; Escribano et al. 2012). Patrones de surgencia que se pueden ver afectados por la intensificación de los vientos favorables (Wang et al. 2015) y que, por lo tanto, podrían generar cambios el ecosistema pelágico el SCH.

Los cambios físicos y químicos del ambiente marino inducidos por el cambio climático podrían generar nuevas condiciones ambientales e influir sobre la biología del océano. Los cambios en la estructura de poblaciones dependen principalmente de las limitaciones fisiológicas de los organismos que las componen, lo que podría alterar patrones de dispersión, relaciones interespecíficas, riqueza y diversidad de especies (e.g. en ecosistemas polares, tropicales y sistemas de surgencia), guiar cambios en la estructura comunitaria, flujos de energía, material particulado, ciclos biogeoquímicos y la estructura de los ecosistemas (Doney et al. 2012).

1.2. Ecología del zooplancton: copépodos como modelo de estudio

Las ZMOs constituyen verdaderos laboratorios naturales para el estudio de las variaciones del ecosistema pelágico ante problemáticas ambientales asociadas al cambio climático global, como la acidificación y desoxigenación del océano (Paulmier et al. 2011). La variabilidad del sistema pelágico suele ser estudiada mediante respuestas fisiológicas del plancton, debido a que son considerados buenos bioindicadores de cambios en las condiciones

oceanográficas, principalmente debido a sus cortos ciclos de vida y rápidas respuestas a cambios ambientales (Hays et al. 2005).

En el ambiente pelágico, el plancton cumple un rol importante en la “Bomba biológica” del carbono. El fitoplancton mediante el proceso de fotosíntesis convierte el CO₂ atmosférico en carbono orgánico y lo deja disponible para entrar a las tramas tróficas (Chisholm 2000). Mientras que el zooplancton es capaz de incorporar, almacenar y transferir el carbono orgánico hacia niveles tróficos superiores (Longhurst 1991) y hacia aguas profundas, mediante transporte pasivo y/o activo (Honjo 2004; Hidalgo et al. 2005b; Yáñez et al. 2012) (Fig. 2).

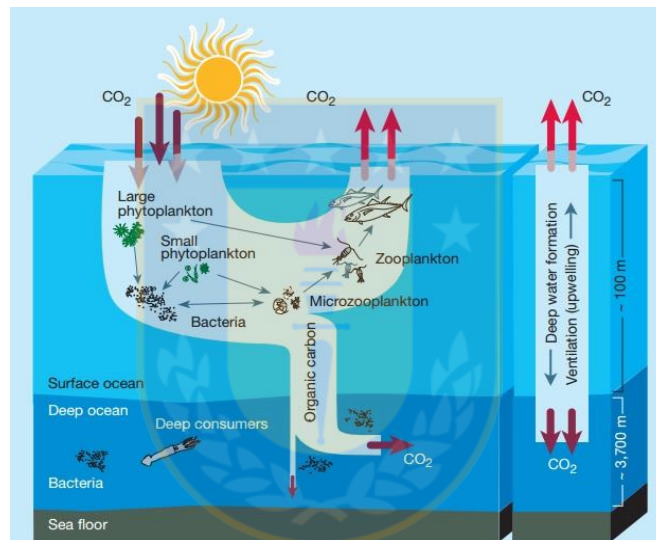


Figura 2. “Bomba biológica” del carbono (izquierda) guiada por la complejidad de las tramas tróficas pelágicas, donde es posible observar el rol del zooplancton en niveles intermedios, y como complemento, la “Bomba de solubilidad” (derecha) que se encuentra regulada por procesos físico-químicos que ocurren en la columna de agua (Fuente: Chisholm 2000).

El componente más abundante del zooplancton son los copépodos (Kiørboe 1998) y en el SCH, al igual que en otras regiones oceanográficas, constituyen aproximadamente cerca del 80% de la composición y abundancia (Escribano & Hidalgo 2000; Hidalgo & Escribano 2001; Escribano et al. 2007; Hidalgo et al. 2010). Los copépodos han sido ampliamente utilizados como grupo modelo para el estudio de estimaciones de producción zooplanctónica, principalmente a través de tasas vitales (*e.g.* tasas reproductivas y de crecimiento), para una mejor comprensión de la ecología de poblaciones y los factores que controlan la producción secundaria en el océano (Huntley & López 1992; Hirst & Lampitt 1998; Runge & Roff 2000).

La producción de una especie dada, considera la biomasa y la tasa de crecimiento instantánea (g) de la población estudiada. Sin embargo, la dificultad de determinar g ha llevado al desarrollo de distintos métodos de estimación de producción zooplanctónica y, por lo tanto, distintos resultados que tienden a subestimar o sobreestimar los valores reales de producción (Rey-Rassat et al. 2004). Entre los métodos utilizados, se encuentran el método de incremento en peso, tasas de muda, producción de huevos, actividad enzimática, razón de ácidos nucleicos, métodos de cohorte natural y artificial, además de algunos modelos empíricos. Sin embargo, hasta la fecha aún no existe un método unificado para estimar producción.

La mayoría de los métodos antes mencionados han sido desarrollados en copépodos, lo cual se dificulta debido a la corta duración y la complejidad de sus ciclos de vida. El ciclo de vida u ontogenia de los copépodos comprende 13 estadios de desarrollo: el huevo (estadio embrionario), seis estadios naupliares (N1 – N6) y seis estadios copepoditos (CI – CVI), donde el estadio CVI corresponde al estado adulto con dimorfismo sexual (Trujillo-Ortiz 1986; Peterson 1998; Hidalgo et al. 2012) (Fig. 3). Cada etapa puede presentar una propia tasa de crecimiento instantánea, la cual generalmente es obviada al utilizar un valor poblacional específico. No obstante, incluir distintos estadios de desarrollo y complementar el uso de más de un método de producción, podría ser una buena estrategia para reducir el error en este tipo de estimaciones. Por ejemplo, tasas de mudas en estadios tempranos y/o tardíos en conjunto con la producción de huevos en adultos (e.g. Richardson & Verheye 1999).

Los copépodos pueden ser clasificados en dos grupos de acuerdo a su comportamiento posterior al desove, lo cual influye en la sobrevivencia de los huevos en el ambiente. Existe un grupo filogenéticamente más evolucionado que se caracteriza por permitir el desarrollo embrionario bajo cuidado paternal mediante la formación de sacos ovígeros. Mientras que los representantes del segundo grupo desovan sus huevos libremente en el ambiente pelágico. Entre los huevos de libre desarrollo existen huevos esféricos de superficie lisa (e.g. *Calanus*) y huevos con implementos de flotación, los cuales son evolutivamente más desarrollados (e.g. *Eucalanus*, *Centropages* y *Tortanus*) que aquellos sin ornamentaciones (Koga 1968).

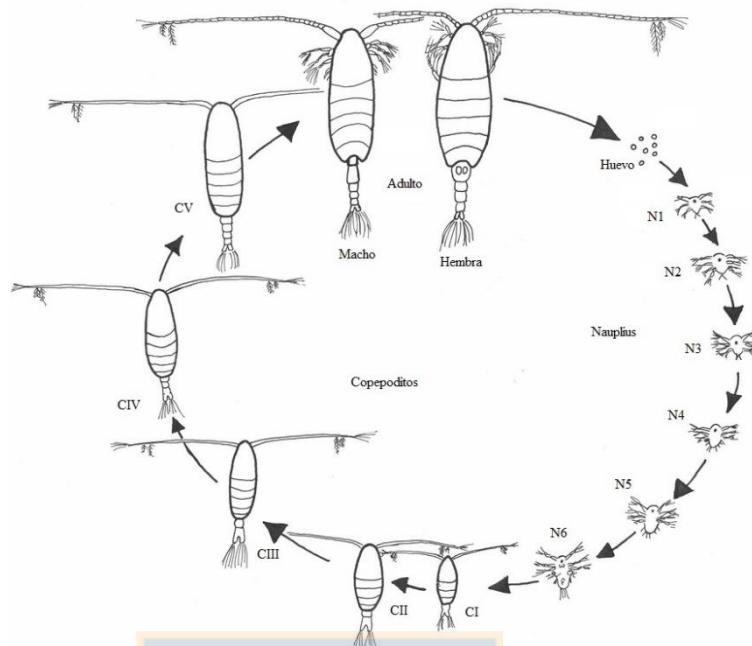


Figura 3. Ciclo de vida de un copépodo (Fuente: <http://www.st.nmfs.noaa.gov/copepod>, último acceso 6 de marzo de 2017).

Respecto a la variabilidad intraespecífica se han descrito tres tipos de huevos en copépodos: 1) huevos subitáneos, que eclosionan después del desove (24 a 72 horas) bajo condiciones óptimas de desarrollo y que podrían retrasar su desarrollo ante condiciones desfavorables; 2) huevos en diapausa, que corresponde a un estado refractario hormonalmente inducido, cuya eclosión ocurre al terminar dicho periodo; y 3) los huevos de eclosión tardía, que presentan una tasa de eclosión más lenta que la de huevos subitáneos, pero sin entrar en un estado refractario (Chen & Marcus 1997; Drillet et al. 2011). El tipo de huevo en copépodos ha sido relacionado a diferencias morfológicas y al éxito de eclosión en condiciones (no) favorables para su desarrollo. La morfología de huevos subitáneos y en reposo puede diferenciarse a nivel ultramicroscópico, luciendo similar en un microscopio convencional o pueden ser morfológicamente distinguibles con la presencia de largas espinas en el corion (estructura externa) de huevos en diapausa y cortas espinas o la ausencia de ellas en huevos subitáneos (Castellani & Lucas 2003; Belmonte & Pati 2007). Condiciones adversas pueden inducir el reposo en huevos subitáneos o inducir la producción de huevos en estado de reposo como estrategia de sobrevivencia a escala estacional (Chen & Marcus 1997; Katajisto 2004; Wu et al. 2009; Drillet et al. 2011). Parte de las diferencias morfológicas y el éxito de eclosión asociado podrían estar dadas por la semipermeabilidad de la membrana que permite el ingreso

de pequeñas moléculas de H₂O, O₂, CO₂ y NH₄⁺ al interior del huevo, dejando a los embriones expuestos a las condiciones del ambiente circundante (Zirbel et al. 2007), diferencias que podrían ser importantes en la sobrevivencia y abundancia de copépodos dominantes en sistemas de surgencia costera, en cuanto a la capacidad de mantenerse en aguas aptas para el desarrollo embrionario, por ejemplo, al disminuir sus velocidades de hundimiento y evitar las condiciones de hipoxia asociadas a las ZMOs en aguas subsuperficiales.

La alta variabilidad espacio-temporal del ambiente pelágico regula los patrones de distribución y las tasas de producción del zooplancton y, por lo tanto, de los copépodos. El crecimiento de estos organismos ha sido descrito como dependiente de la temperatura (Bèlehràdek 1957; Fernández-Álamo & Färber-Lorda 2006) y de la calidad y cantidad de alimento disponible (Hirst & Lampitt 1998). Se ha demostrado que floraciones algales con baja calidad nutritiva u organismos tóxicos son capaces de reducir el crecimiento y la fecundidad en copépodos (Turner & Tester 1997; Ianora et al. 1999; Poulet et al. 2007). Asimismo, procesos advectivos (Escribano 1998), la intensidad de la surgencia (Escribano et al. 2012) y bajas concentraciones de oxígeno disuelto en la columna de agua pueden actuar como reguladores de la distribución, abundancia y producción zooplanctónica (Roman et al. 1993; Stalder & Marcus 1997; Manríquez et al. 2009; Hidalgo et al. 2010).

1.3. El rol del oxígeno disuelto en la ecofisiología y distribución de copépodos en zonas de mínimo oxígeno

El oxígeno disuelto cumple un rol fundamental en la estructura de los ecosistemas y en la regulación de la distribución espacio-temporal de la mayoría de los organismos marinos, desde microbios a peces (Gilly et al. 2013). Las condiciones de bajo oxígeno disuelto pueden ser letales para una gran proporción de especies, dependiendo del tiempo de exposición, intensidad de las condiciones de hipoxia y por supuesto, de la especie propiamente tal. Muchos grupos taxonómicos presentan metabolismo aeróbico, manteniéndolo en condiciones de hipoxia o presentando adaptaciones para sobrevivir en ambientes bajos en oxígeno (Spicer 2016). En este contexto, las condiciones de hipoxia asociadas a las ZMOs constituyen verdaderas barreras para la distribución vertical de la mayoría del zooplancton, entre ellos los copépodos. Estos organismos forman agregaciones sobre el límite superior de las ZMOs, definida por la profundidad que presenta una concentración de 1 mL O₂ L⁻¹ ($\approx 1.4 \text{ mg L}^{-1} \approx$

44.6 μM) y que, por lo general, suele ser bastante somera (< 50 m) (Morales et al. 1999; González et al. 2000; Hidalgo & Escribano 2001; Hidalgo et al. 2005a; Auel & Verheye 2007; Escribano et al. 2009; Manríquez et al. 2009; Wishner et al. 2013).

En el SCH, sólo algunas especies han desarrollado estrategias que les permiten sobrellevar la hipoxia asociada a las ZMO, entre ellas los euphausíidos como *Euphausia mucronata* y *E. eximia* y el copépodo *Eucalanus inermis*. Especies que realizan migraciones verticales diurnas-nocturnas hacia el interior de la ZMO, utilizando estas aguas como áreas de desove o refugio contra la predación (González et al. 2000; Escribano et al. 2000; Hidalgo et al. 2005b). Recientemente, se observó que adultos de *Calanus chilensis* son capaces de habitar el interior de la ZMO de sur del Perú (Hirche et al. 2014), mientras que poblaciones de copépodos como *Acartia tonsa*, *Paracalanus* cf. *indicus* pueden sufrir efectos negativos ante la exposición a la hipoxia a corto plazo en sistemas de surgencia costera (Yáñez et al. 2012; Ruz et al. 2015). El efecto del oxígeno disuelto sobre la dinámica poblacional de copépodos pelágicos ha demostrado que las especies pueden tener respuestas especie-específicas a la hipoxia (Auel & Verheye 2007; Ruz et al. 2015).

En el SCH, se ha observado que *C. chilensis* y *Centropages brachiatus* presentan ciclos de vida fuertemente influenciados por la surgencia, con pulsos reproductivos ligados a la profundidad de la termoclina y la oxiclina en condiciones de alimento no limitante (Hidalgo & Escribano 2007, 2008). Se ha observado que los estadios de desarrollo más avanzados de *E. inermis*, suelen permanecer cerca de la base de la oxiclina (30 - 60 m) y en la zona superior de la ZMO (60–200 m), mientras que el desove parece ser realizado en el interior de la ZMO y los estadios naupliares ascienden a alimentarse hacia la capa oxigenada (Hidalgo 2005; Hidalgo et al. 2005b). En la OMZ del Mar Arábigo, los distintos estadios de desarrollo de *Lucicutia grandis* también presenta diferencias en su distribución vertical, donde los adultos se distribuyen a mayores profundidades que los estadios jóvenes posiblemente debido las limitantes fisiológicas e interacción ecológicas propias de cada estadio (Wishner et al. 2000). Actualmente, existe escasa información sobre el efecto de la hipoxia en respuestas ontogénicas de copépodos dominantes asociados al SCH. Considerar las potenciales variaciones de las respuestas fisiológicas de distintos estadios de copépodos ante cambios en las condiciones ambientales, podría ser de gran relevancia para realizar mejores aproximaciones para el estudio de la ecología de copépodos dominantes.

En la zona norte de Chile la comunidad de copépodos pelágicos habita principalmente la capa superficial oxigenada (< 50 m) sobre la ZMO. En este sistema, dominan en abundancia especies de copépodos como *P. indicus*, *A. tonsa*, *Oithona similis*, *C. brachiatus*, *Oncaea* spp. *C. chilensis* y *Corycaeus typicus* cuya distribución vertical se ve restringida por la ZMO, además de las especies asociadas a aguas hipóxicas como *E. inermis* y *Rhincalanus nasutus* (Hidalgo et al. 2005b; Escribano et al. 2009; Escribano et al. 2012). Debido a lo anterior, es de gran importancia conocer los potenciales efectos de la expansión y la somerización de la ZMO sobre el crecimiento, biomasa y abundancia de copépodos dominantes en la zona norte de Chile, además de conocer cuáles son las capacidades o limitantes fisiológicas que presentan estos organismos ante los continuos cambios en el contenido de oxígeno disuelto que puedan estar expuestos, y al potencial efecto del incremento de las condiciones de hipoxia en la columna de agua que conlleva a la restricción de hábitat del zooplancton en la capa superficial oxigenada. En el contexto de cambio climático, la expansión, somerización e intensificación de las ZMOs podrían afectar la productividad biológica de los sistemas de surgencia costera, principalmente al influir en el flujo de carbono hacia niveles tróficos superiores.

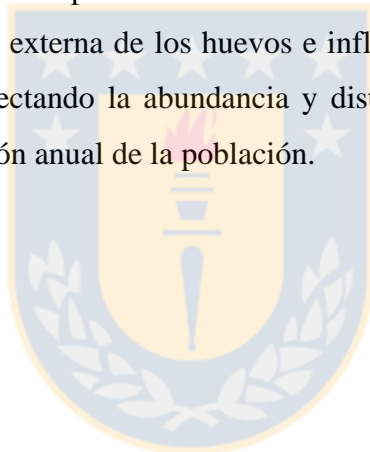
La presente investigación abordó, desde una perspectiva experimental, las potenciales respuestas ontogénicas en las tasas vitales y sobrevivencia de los copépodos *C. chilensis* y *A. tonsa* en condiciones de hipoxia y, desde la perspectiva *in situ*, el rol de la hipoxia asociada a la ZMO en la abundancia y distribución de huevos y hembras de los copépodos *A. tonsa*, *P. cf. indicus* y *C. chilensis* en la zona norte de Chile en el SCH, como un área modelo para el estudio de los potenciales efectos que puede generar la expansión y somerización de la ZMO sobre las respuestas de copépodos pelágicos en sistemas de surgencia costera.

1.4. Hipótesis

La distribución vertical de la mayoría de los copépodos pelágicos se encuentra restringida por las zonas de mínimo oxígeno (ZMOs), por lo cual las hipótesis planteadas en la presente investigación son las siguientes:

Hipótesis 1: La hipoxia asociada a la zona de mínimo oxígeno afecta significativamente las tasas respiratorias, tasas de crecimiento y respuestas adaptativas ontogénicas de copépodos dominantes, reflejadas en cambios poblacionales en la producción, abundancia y biomasa de estos organismos en el sistema de surgencia del norte de Chile, en el Sistema de Corrientes de Humboldt.

Hipótesis 2: Las condiciones de hipoxia de la zona de mínimo oxígeno, inducen cambios significativos en la morfología externa de los huevos e influyen en la duración del desarrollo embrionario de copépodos, afectando la abundancia y distribución vertical de los huevos y consecuentemente, la producción anual de la población.



1.5. Objetivo General

Comprender el efecto de las condiciones de hipoxia sobre las tasas de respiración, tasas de crecimiento y potenciales respuestas adaptativas ontogénicas de copépodos dominantes y cómo estos cambios se podrían ver reflejados en la dinámica poblacional de estos organismos en el Sistema de Corrientes de Humboldt, como sensor de potenciales cambios poblacionales asociados a la expansión y somerización de la zona de mínimo oxígeno.

1.6. Objetivos específicos

1. Determinar el efecto de la hipoxia sobre las tasas de respiración y crecimiento ontogénicas de copépodos dominantes en la zona norte del SCH, 23° S.
2. Identificar cambios morfológicos en la estructura externa de huevos inducidos por condiciones de hipoxia en la zona norte del SCH, 23° S.
3. Determinar variaciones en la morfología, abundancia y distribución vertical de los huevos de copépodos dominantes asociados a las condiciones oceanográficas en la zona norte (23°S) del SCH.
4. Desarrollar un modelo conceptual de la interacción de copépodos dominantes y la ZMO, en la zona norte (23°S) del SCH.

2. MATERIALES Y MÉTODOS

2.1. Área de estudio

La presente investigación se llevó a cabo en la zona de surgencia costera en la Bahía de Mejillones (23°S) durante dos años consecutivos (2013-2014), en periodo de primavera/verano y otoño/invierno, integrando actividades experimentales y de terreno. El muestreo se realizó en el área cercana a las coordenadas oceanográficas $23^{\circ} 0,2'S - 70^{\circ} 28,2'W$ correspondiente a la Estación 3 (St-3; profundidad máxima = 120 m) de la Serie de Tiempo de Antofagasta localizada en la Bahía de Mejillones (Escribano et al. 2012) (Fig. 4)



Figura 4. Área de estudio en la Bahía de Mejillones en el norte de Chile. El lugar de muestreo se encuentra indicado por un asterisco (*).

Objetivo 1: Determinar el efecto de la hipoxia sobre las tasas de respiración y crecimiento ontogénicas de copépodos dominantes en la zona norte del SCH, 23°S .

2.2. Trabajo de campo

En la estación de muestreo previo a los muestreos de zooplancton se realizó un lance simultáneo de dos perfiladores oceanográficos, un CTD-O SeaBird 19 para (conductividad, temperatura (T), densidad, oxígeno disuelto (OD) y fluorescencia) y una sonda multiparámetro YSI EXO2 (T, densidad y OD) hasta 90 m de profundidad para determinar el borde superior de la ZMO (profundidad con $1,0 \text{ mL O}_2 \text{ L}^{-1}$). Los perfiles de la sonda fueron inmediatamente visualizados a bordo para determinar el perfil de OD. Con una botella Niskin de 10-L se

obtuvo agua desde la capa oxigenada y de la ZMO. Se recolectó zooplancton desde la capa oxigenada mediante lances oblicuos con red tipo WP-2 de 200 μm de poro. En un periodo no superior a dos horas, las muestras fueron cuidadosamente transportadas al laboratorio en una nevera para mantener las condiciones de temperatura del medio en el cual fueron recolectadas.

2.3. Trabajo de laboratorio

Debido a la alta abundancia de *Calanus chilensis* y *Acartia tonsa* durante el periodo de muestreo, hembras ovígeras de ambas especies fueron seleccionadas desde las muestras de zooplancton mediante el uso de lupas estereoscópicas (2x y 4x). Mientras que estadios tempranos, como huevos y nauplios, fueron obtenidos desde los experimentos de producción de huevos, seguidos por experimentos de éxito de eclosión y crecimiento naupliar.

Las tasas vitales y la sobrevivencia de *C. chilensis* fueron estimadas en normoxia ($\sim 5 \text{ mL O}_2 \text{ L}^{-1}$) e hipoxia ($\sim 0,5 \text{ mL O}_2 \text{ L}^{-1}$). En el caso de *A. tonsa* los mismos parámetros fueron estudiados además una condición de hipoxia intermedia ($\sim 0,9 \text{ mL O}_2 \text{ L}^{-1}$) entre las concentraciones de oxígeno antes mencionadas. En laboratorio, el agua de mar obtenida en terreno fue filtrada por $0,7 \mu\text{m}$ para reducir la respiración microbiana durante las incubaciones. El OD fue medido con un sensor óptico OXY - 4 Optode PreSens (nivel de detección $0,1 \mu\text{L L}^{-1}$) y controlado mediante la inyección de nitrógeno gaseoso (N_2). El sensor de oxígeno fue previamente calibrado usando dos puntos de calibración, 100% y 0% de saturación, siguiendo las instrucciones del fabricante. Se obtuvieron mediciones de OD al inicio y al final de las incubaciones. La temperatura fue controlada con un termostato y un baño termorregulado a 14°C . La temperatura seleccionada corresponde a la temperatura *in situ* promedio de la columna de agua durante el periodo de estudio. La sobrevivencia de hembras fue estimada bajo lupa al término de las incubaciones. Mientras que la sobrevivencia de huevos y nauplios sólo fue estimada en *C. chilensis*, utilizando el protocolo de tinción de Rojo Neutro, método que permite identificar organismos vivos (teñidos) y muertos (no teñidos) (Tang et al. 2006; Elliott y Tang 2009; Yáñez et al. 2012). Finalmente, todos los organismos (hembras, nuevos y nauplios) recolectados al final de los experimentos fueron preservados en formalina al 4%.

2.3.1. Tasa de producción de huevos

Se estimó la tasa de producción de huevos de dos especies dominantes, *Calanus chilensis* y *Acartia tonsa*. Un mínimo de 20 hembras ovígeras por especie fueron incubadas individualmente en contenedores herméticos de 100 mL para obtener la variabilidad intraespecífica en la tasa de producción de huevos (huevos hembra⁻¹ día⁻¹) de acuerdo al método básico descrito por Runge & Roff (2000).

2.3.2. Aproximación bioquímica para el estudio del metabolismo en copépodos

Se realizaron tres experimentos para estudiar el metabolismo de hembras de *C. chilensis* en condiciones de hipoxia y normoxia mediante una aproximación bioquímica. Las hembras fueron incubadas en el interior botellas de vidrio a densidades de 10 hembras L⁻¹. Luego de 24 horas, las hembras fueron recolectadas con un tamiz de 200- μ m y sólo aquellas que se encontraron vivas fueron seleccionadas y congeladas inmediatamente en nitrógeno líquido (-196 °C). En el laboratorio se analizó 1) el crecimiento somático mediante la actividad de la enzima aminoacil-ARNt sintetasa (AARS) (Yebra y Hernández-León 2004; Yebra et al. 2005, Yebra et al. 2011). 2) La respiración potencial mediante la actividad del Sistema de Transporte de Electrones (ETS) (Owens y King 1975) fue medida de forma continua (Maldonado et al. 2012) y corregida a la temperatura de incubación (Packard et al. (1975) y, 3) la biomasa mediante ensayo estandarizado (Smith et al. 1985). La actividad de AARS y ETS fueron estandarizadas para obtener tasas proteína-específicas. El consumo de oxígeno potencial obtenido desde ETS fue comparado con el consumo de oxígeno obtenido desde las mediciones iniciales y finales obtenidas desde las botellas de incubación. Un modelo lineal generalizado (GLM) ($\alpha= 0.05$) y el test a posteriori Tukey fueron utilizados para determinar diferencias de los parámetros metabólicos entre experimentos (Quinn & Keough 2002). Previa comprobación de supuestos paramétricos, correlaciones de Pearson fueron realizadas para testear relaciones lineales entre los parámetros metabólicos.

2.3.3. Éxito de eclosión y tasa de crecimiento naupliar

Los huevos utilizados para estimar el éxito de eclosión fueron obtenidos desde 1) experimentos de producción de huevos realizados en incubaciones individuales de hembras, donde el número inicial de huevos se obtuvo desde el conteo de carcasas de huevos

eclosionados y los huevos presentes al final de la incubación; y 2) desde grupos de 50 o 100 hembras incubadas en baldes de 3-L en condiciones de normoxia durante 24 horas. Se procuró evitar el canibalismo, separando hembras de huevos con un tamiz de (200µm) en el interior. Los huevos fueron recolectados desde el fondo de cada balde con pipetas plásticas, separados en grupos (20, 50 o 100) bajo el estereoscopio (4x) e inmediatamente incubados en condiciones de hipoxia y normoxia en viales de 100-mL. El éxito de eclosión fue evaluado por el porcentaje de huevos que eclosionaron a nauplios en todas las incubaciones y estandarizado a 24 horas.

El volumen específico de los huevos y nauplios, fue estimado desde mediciones de diámetro de los huevos, y largo y ancho de los nauplios realizadas en un estereoscopio (8X) y microscopio (40X), siguiendo la metodología utilizada en Torres & Escribano (2003). El crecimiento naupliar se analizó mediante el modelo exponencial linealizado: $g = \ln(V_{i+1} / V_i) / t$ donde, g es la tasa de crecimiento instantáneo volumen-específico (día^{-1}), V_{i+1} y V_i son volúmenes naupliares (μm^3) entre dos observaciones subsecuentes y t corresponde al intervalo de tiempo (día) entre observaciones. El tiempo de desarrollo naupliar corresponde al inverso de g . Los estadios naupliares fueron identificados siguiendo literatura especializada.

Los análisis Chi-cuadrado y Test-G fueron utilizados para determinar si la sobrevivencia y las tasas vitales eran dependientes del OD. El efecto del OD sobre las tasas vitales, diámetro de huevos y talla de copépodos fue analizado mediante T-Student ($\alpha=0.05$) una vez comprobados los supuestos paramétricos, su homólogo no paramétrico Mann-Whitney fue aplicado en caso de ser necesario (Zar, 1999).

Objetivo Específico 2 y 3: Identificar cambios morfológicos en la estructura externa de huevos inducidos por condiciones de hipoxia en la zona norte del SCH, 23° S. Determinar variaciones en la morfología, abundancia y distribución vertical de los huevos de copépodos dominantes asociados a las condiciones oceanográficas en la zona norte (23°S) del SCH.

2.4. Abundancia y distribución vertical de hembras y huevos de copépodos.

Se realizaron lances de CTD-O y sonda YSI EXO2 para obtener la variabilidad oceanográfica de datos de temperatura, salinidad, densidad, OD y fluorescencia de 0 a 90 m. La abundancia y distribución vertical de huevos fue obtenida mediante muestreos

estratificados a 5, 10, 20, 30, 40, 50, 70 y 80 m de profundidad. Se obtuvieron 20 L de agua por profundidad con una botella Niskin (10-L). La abundancia y distribución de hembras fueron analizadas a partir de lances estratificados con una red WP-2 (200 μ m) en los estratos correspondientes a la capa oxigenada, la oxiclina y la ZMO. Se estimó la producción de huevos *in situ* según el método de Edmonson (Edmonson 1968).

El agua de mar obtenida en cada estrato fue filtrada por un tamiz de 20 μ m para retener los huevos de copépodos, los cuales fueron preservados en formalina al 2%. Las muestras de zooplankton fueron preservadas en formalina al 4%. Análisis multivariados fueron utilizados para determinar cambios espaciales y temporales en las abundancias y las variables ambientales usando E-PRIMER (v. 7.0.11) (Clarke & Gorley 2015). Diferencias en la distribución vertical fue analizada con un Análisis de similaridad (ANOSIM) basado en permutaciones no paramétricas. Las variables predictoras de las abundancias se determinaron mediante la rutina BEST (Clarke & Gorely, 2006).

2.5. Estudio de la morfología de huevos de copépodos

Los huevos obtenidos en los experimentos de la sección 2.2.1 y 2.2.3 previamente fijados en formalina al 4%, fueron medidos y categorizados por tipo de superficie y color (huevos sin Rojo Neutro). Los huevos fueron fotografiados con una lupa estereoscópica (8X), microscopio convencional (40x-100x) y/o microscopía electrónica de barrido (SEM, Scanning Electron Microscope). Se realizó un análisis descriptivo de los tipos de huevos encontrados.

Objetivo 4: Desarrollar un modelo conceptual de la interacción de copépodos dominantes y la ZMO, en la zona norte (23°S) del SCH.

2.6. Elaboración de modelo conceptual

Se esquematizó un modelo conceptual de la dinámica poblacional de copépodos dominantes de la zona norte del SCH asociados a cambios en la distribución vertical del oxígeno disuelto. Se integró los resultados obtenidos de los objetivos 1, 2 y 3 y se complementó con datos de las especies en estudio a partir de referencias bibliográficas de la zona norte de Chile (23°S).

3. RESULTADOS

3.1. Capítulo 1

Efectos de la hipoxia sobre hembras y estadios tempranos de *Calanus chilensis* en el Sistema de Corrientes de Humboldt (23°S)

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Resumen

La desoxigenación del océano se ha convertido en un problema crítico en los sistemas de afloramiento costero expuestos a zonas mínimo oxígeno (ZMOs). La somerización de la ZMO podría alcanzar la zona fótica causando condiciones hipóxicas en aguas someras, afectando a los organismos pelágicos que habitan en ella. Durante los inviernos 2013 y 2014, el copépodo *Calanus chilensis* fue recolectado en el norte de Chile para evaluar cómo las condiciones de oxígeno disuelto pueden afectar sus tasas vitales y las respuestas fisiológicas de sus estadios de desarrollo. Hembras adultas, huevos y nauplios tempranos (N1 a N3) fueron expuestos en el laboratorio a condiciones de hipoxia ($\sim 1 \text{ mg L}^{-1}$) y normoxia ($\sim 8 \text{ mg L}^{-1}$). La hipoxia no afectó la sobrevivencia de las hembras ni su producción de huevos. No hubo diferencias significativas en el crecimiento somático ni en la respiración potencial de las hembras entre ambas condiciones de oxígeno. Sin embargo, los estadios temprano fueron fuertemente afectados por bajas concentraciones de oxígeno disuelto. El éxito de la eclosión disminuyó al menos un 30% desde normoxia a hipoxia. Mientras, el crecimiento naupliar fue significativamente más bajo en hipoxia ($0,15 \pm 0,02 \text{ d}^{-1}$) que en normoxia ($0,24 \pm 0,01 \text{ d}^{-1}$). Se sugiere que la expansión de las ZMOs en sistemas de surgencia podría influir sustancialmente en la dinámica copépodos, como se ha observado en el Sistema de Corrientes de Humboldt, donde la hipoxia es capaz de suprimir el crecimiento y disminuir la sobrevivencia de los estadios tempranos de *C. chilensis*.

Hypoxia effects on females and early stages of *Calanus chilensis* in the Humboldt Current ecosystem (23°S)

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Highlights:

- Ontogenetic responses to hypoxia were observed in the copepod *Calanus chilensis*.
- In hypoxia, longer development times of early stages may affect population dynamics.
- Females of *C. chilensis* coped hypoxia better than eggs and early nauplii.

Keywords: Hypoxia, Humboldt Current System, Oxygen Minimum Zone, Copepods, *Calanus chilensis*, Ecophysiology.

Abstract

Deoxygenation of the ocean has become a critical issue in coastal upwelling systems, which are subjected to shallow Oxygen Minimum Zones (OMZs). The OMZ can expand into the photic zone causing hypoxic conditions and affecting pelagic organisms. During the winters 2013 and 2014, the copepod *Calanus chilensis* was collected off northern Chile to evaluate how oxygen conditions may affect its vital rates and to assess the physiological responses of developmental stages to oxygen. Adult females, eggs, and early nauplii (N1 to N3) were exposed to hypoxia ($\sim 1 \text{ mg L}^{-1}$) and normoxia ($\sim 8 \text{ mg L}^{-1}$) in the laboratory. Survival of females and their egg production were not affected by hypoxia. No significant differences in female somatic growth and potential respiration between hypoxic and normoxic conditions were found. Early stages, however, were strongly affected by hypoxia. Hatching success diminished by at least 30% from normoxia to hypoxia. Naupliar growth was significantly lower under hypoxia ($0.15 \pm 0.02 \text{ d}^{-1}$) than normoxia ($0.24 \pm 0.01 \text{ d}^{-1}$). These findings suggest that expansion of OMZs in upwelling systems may substantially influence copepod dynamics by suppressing growth and survival of early life stages.

Introduction

Ocean deoxygenation has become a crucial issue in coastal upwelling systems. Global warming and eutrophication processes, guided by anthropogenic activity, are causing the spread of hypoxic and anoxic conditions in coastal and open oceans (Ekau et al., 2010; Keeling et al., 2010; Stramma et al., 2010). Oxygen availability is crucial for the successful development of aerobic marine biota (Marcus et al., 2004) and plays a direct role in the biogeochemical cycling of nutrients (Feely et al., 2004); therefore, the capacity of organisms to avoid or to adapt to stressful oxygen conditions may be vital to promote their continued success in a changing ocean.

The Oxygen Minimum Zones (OMZs) present in Eastern Boundary Upwelling Systems (EBUS) may be considered as natural laboratories to understand how organisms can deal with and inhabit oxygen-poor environments. The OMZs are mid-depth regions of the water column which contain low oxygen concentrations due to the natural decomposition of sinking organic material and aerobic respiration; they mostly occur in the eastern boundaries of the Pacific and Atlantic oceans, (Wyrтки, 1962). Due to climate-related deoxygenation, the hypoxic conditions of OMZs are intensifying and, consequently, expanding their distribution area at faster rates

than the natural OMZ formation (Keeling et al., 2010; Wang et al., 2015). Moreover, the intensification of upwelling-favorable winds due to global warming may even strengthen the shoaling of these low-oxygen layers, and thus vertically compress the normally oxygenated surface layer and affect zooplankton and fishes inhabiting these waters (Gilly et al., 2013). This in turn may change ecosystem structure, vertical fluxes, the biological pump, distribution, behavior, and metabolic rates of marine organisms, benthic-pelagic coupling, and fisheries (Levin, 2003; Paulmier et al., 2008; Ekau et al., 2010; Stramma et al., 2010; Seibel, 2011; Wishner et al., 2013).

OMZs have been described as effective barriers that restrict the vertical distribution of most zooplankton and benthic macrofauna (Wishner et al., 1995; Levin, 2003; Escribano et al., 2009), where only some species that perform diel or ontogenetic vertical migration can move into them (Hidalgo and Escribano, 2005a; Hidalgo and Escribano, 2005b; Escribano et al., 2009; Wishner et al., 2013; Hirche et al., 2014). In epipelagic environments associated with an OMZ, the aggregation of zooplankton in waters just above the OMZ may also contribute to oxygen loss through enhanced respiration at the base of the oxycline (Donoso and Escribano, 2014). Hidalgo et al. (2010) described that species diversity and richness are higher in oxygenated shallow waters than in deeper hypoxic waters. Therefore, the vertical intrusion of the OMZ into the surface layer may lead to natural mortality of species that inhabit shallow waters, like the copepods *Paracalanus* cf. *indicus* (Yáñez et al., 2012) and *Acartia tonsa* who decrease hatching success and female survival at concentrations $<0.9 \text{ mL O}_2 \text{ L}^{-1}$ ($\sim 1.3 \text{ mg O}_2 \text{ L}^{-1}$) (Ruz et al., 2015). Similar effects also have been reported for *A. tonsa* in environments dominated by seasonal bottom hypoxia (Richmond et al., 2006; Marcus et al., 2004).

The Mejillones Bay is one of the most important upwelling centers off northern Chile (Marín et al., 2001; Thiel et al., 2007) that is characterized by a shallow OMZ ($\leq 26 \text{ m}$) as result of a semi-permanent upwelling regime (Pizarro et al., 1994; Morales et al., 1999; Sobarzo et al., 2007; Piñones et al., 2007; Thiel et al., 2007; Hidalgo and Escribano, 2008; Ruz et al. 2015). In this zone, most of the copepod abundance and biomass are retained in well-oxygenated surface waters (Hidalgo et al. 2005b; Escribano et al., 2012, Ruz et al. 2015). The upper boundary of the OMZ is defined by the depth where the dissolved oxygen concentration is $1 \text{ mL O}_2 \text{ L}^{-1}$ ($\sim 1.39 \text{ mg O}_2 \text{ L}^{-1}$) (Morales et al., 1999; Hidalgo and Escribano,

2008). Whereas the OMZ reaches lower concentrations ($< 0.5 \text{ mL O}_2 \text{ L}^{-1} \sim 0.71 \text{ mg O}_2 \text{ L}^{-1}$) or even total anoxia (Ulloa et al., 2012).

Copepods correspond to *ca.* 80% of zooplankton abundance in Mejillones Bay and have been considered a model group to evaluate changes in bio-oceanography, especially for their rapid response to changing conditions such as temperature, food quantity and quality, oxygen, changes in upwelling intensity and El Niño Southern Oscillation (Escribano and Hidalgo, 2000, 2001; Vargas et al., 2006; Hidalgo et al., 2010; Aguilera et al., 2011; Escribano et al., 2012, 2014). However, in the Humboldt Current System (HCS), early copepodite stages have rarely been studied to evaluate population changes in abundance, growth and distributions (Torres and Escribano, 2003; Hidalgo et al., 2005a; Vargas et al., 2006; Hidalgo and Escribano, 2007; 2008; Ruz et al., 2015). Early stages are a key component for zooplankton dynamics and production, especially in areas where hypoxia can be a selective force for the zooplankton of coastal waters (Dam, 2013). Understanding how copepods and their early stages may cope with oxygen-deficient water-levels seems highly relevant to comprehend how their populations respond to a changing ocean.

The ontogenetic development of copepods includes shifts in swimming speed and nutritional demands; therefore, metabolism and growth rate are stage-specific and plays a relevant role in the depths inhabited by copepods (Mauchline, 1998; Hidalgo et al., 2005b; Hidalgo and Escribano, 2008). Among the copepod community in the HCS, *Calanus chilensis* Brodsky 1959 is one of the dominant species (Hidalgo et al., 2010; Escribano et al., 2012). *C. chilensis* exhibits continuous reproduction year-round (Hidalgo and Escribano, 2008; Ruz et al., 2015), and seem to be vertically restricted by the shallow OMZ to the well-oxygenated surface layer (50 m depth), with few individuals entering hypoxic waters (Escribano et al., 2009). However, recent evidence shows that later life stages of *C. chilensis* may be able to inhabit the OMZ off south Peru (Hirche et al., 2014), and that adult females off northern Chile (23°S) are able to tolerate short-term exposure to hypoxia (Ruz et al., 2015). This suggests that *C. chilensis* may be a suitable species to assess the effect of hypoxia associated with the OMZ, and the potential effects of low-oxygen conditions through the species' ontogeny.

The main goal of this research was to determine whether survival and stage-specific vital rates of the copepod *C. chilensis* are affected by low oxygen concentrations associated with the OMZ. We hypothesized that low oxygen conditions would limit the metabolism of *C.*

chilensis and that responses to hypoxia would be stage-specific, reflected in a potential reduction of metabolic and survival rates; and thus may affect population dynamics of *C. chilensis* within a scenario of expansion and intensification of the OMZ in northern Chile.

Methods

Sampling

Experiments on female metabolism, egg production rate (EPR), egg hatching success, naupliar growth, and naupliar development rate of the copepod *Calanus chilensis* were conducted from zooplankton collected at Station 3 (23° 00.2' S, 70° 28.2' W; maximum depth = 120 m) in Mejillones Bay during austral winters 2013 and 2014. Station 3 corresponds to the outermost station along a coast to ocean transect of the Antofagasta Zooplankton Time Series Program conducted by the University of Concepción (Fig. 1 in Escribano et al., 2012).

Prior to each zooplankton sampling, a CTD-O (Conductivity, Temperature, Density and Dissolved Oxygen) Seabird 19 plus and a YSI EXO2 multiparameter sonde (C, T and DO) were deployed to 90 m depth, to assess the depth of the upper boundary of the OMZ; profiles were immediately visualized onboard from the YSI EXO2 multiparameter data. Once the oxygen profile was analyzed, seawater for experiments was collected from the oxygenated layer (for normoxic treatments) at ~10 m depth and from inside the OMZ (for hypoxic treatments) at ~60 m depth using a 10 L Niskin Bottle to maintain chemical characteristics, temperature, and salinity of *in situ* seawater. Zooplankton were collected by gentle oblique trawls with a WP-2 net (200 µm mesh size) within the oxygenated upper layer. Once onboard, live zooplankton was kept in an insulated cooler diluted with surface seawater and transported to the laboratory within 2 h.

Within the first two hours after returning to the laboratory, healthy ovigerous females of *C. chilensis* were carefully picked from the live samples under a stereoscopic microscope. Groups of five females were placed into several 500 mL glass jars filled with 0.7 µm filtered seawater and stored for two hours at 14 °C. Meanwhile, incubation media for the experiments was prepared: seawater collected from 10 m for normoxic treatments, and from 60 m depth for hypoxic treatments, was filtered through 0.7-µm glass fiber filters. Copepods and media were stored in a cold room at 14 °C, which is the typical mean *in situ* temperature of the ~30 m layer during non-El Niño years (Ruz et al., 2015).

Controlling dissolved oxygen concentration of seawater

Experiments were carried out at two oxygen levels: hypoxic ($\sim 1 \text{ mg O}_2 \text{ L}^{-1}$) and normoxic ($\sim 8 \text{ mg O}_2 \text{ L}^{-1}$). The filtered seawater (one carboy at a time) was bubbled with highly purified N_2 to achieve the experimental oxygen concentrations, especially to decrease oxygenation and oversaturation caused by filtering. The DO concentrations were verified with an optical sensor OXY-4 Micro-Optode PreSens, by collecting 20-mL seawater subsamples by overflow using optical sensors that had been calibrated using a two-point calibration (100% and 0% oxygen saturation) following the manufacturer's instructions.

To carry out EPR, hatching success, and naupliar growth experiments, 100-mL gas-tight vials were filled by overflow with treatment water. The dissolved oxygen of three randomly-selected bottles per treatment was measured before transferring the organisms into them. Immediately after carefully placing the organisms inside the vials using a plastic pipette, the vials were sealed without bubbles with a rubber stopper and crimp seal. Control vials were only filled with treated seawater. For each experiment, two replicate controls were run per treatment.

To study female metabolism (detailed below), 1-L glass bottles were filled and oxygen measurements were done following the procedure described above. After transferring the females, the bottles were sealed with rubber septa and closed with a screw cap for 24 hours. Both the vials and bottles were placed in a water bath at 14°C to minimize temperature variation during the incubation period. At the incubation end-points, final oxygen was recorded inside the same bottles initially measured.

Female survival and egg production rate

After the acclimation period at 14°C , six experiments were carried out in 100-mL vials with a total of 194 ovigerous females of *C. chilensis* to estimate EPR. Four experiments were conducted in hypoxia and normoxia, whereas two additional experiments were carried out only in normoxia (Table 1). Females were individually incubated for 24 hours. After the incubation time, the vials were opened and one of two different procedures was followed: 1) the females were removed with a pipette and the vials refilled (generally with 1 or 2 mL) of either hypoxic or normoxic filtered seawater before recapping to follow hatching success and naupliar growth, or 2) the female and eggs were gently collected onto a $23\text{-}\mu\text{m}$ sieve, counted

under a stereomicroscope, and preserved in 4% formalin solution. EPR ($\text{eggs} \cdot \text{females}^{-1} \cdot \text{day}^{-1}$) of those females was then calculated as:

$$\text{EPR} = N_{\text{eggs}} * (24/ t) \quad (1)$$

where EPR is the experimental egg production rate; N_{eggs} is the number of eggs laid per female during the incubation time t (in hours) (Runge and Roff, 2000).

The survival of eggs and females was evaluated using the Neutral Red technique adjusted for copepods (Yáñez et al., 2012) after the incubation time in both treatments. This technique allows discrimination of live (red stained) from dead (non-stained) organisms to estimate mortality, and has often been applied in research associated with estimates of natural or non-predatory mortality of copepods in the field (Tang et al., 2006; Elliott and Tang, 2009; Yáñez et al., 2012). The eggs and females were stained by adding 0.1 mL of Neutral Red solution [0.5% p/v, 1:1000] in 2 mL of seawater. After 10 minutes the samples were preserved in 4% formalin solution buffered with sodium acetate for later analysis. The samples were analyzed within two months of the experiments to avoid degradation of the stain. In the laboratory, we sieved (200 μm) and rinsed the formalin solution from the samples prior to putting them in counting chambers. Then the samples were acidified to $\text{pH} < 7$ by the addition of five drops (~ 0.5 mL) of acetic acid ($\text{C}_2\text{H}_4\text{O}_2$), to obtain the red stain's color in the organisms that were alive at the end of the experiments.

Female metabolism

Between July 23 and August 2 2014 (austral winter), three experiments were carried out with a total of 280 ovigerous females to study female metabolism in hypoxic (170 females) and normoxic (110 females) conditions (Table 1). The females were incubated in 1-L glass bottles in batches at a density of 10 females $\cdot \text{L}^{-1}$. After 24h incubation, the content of each bottle was filtered with a 23- μm sieve to collect the females. Only live females were selected and immediately frozen in liquid nitrogen (-196°C) for later biochemical analysis.

In the laboratory, frozen samples were homogenized and the following parameters were assessed on each female batch. 1) The activity of the enzymes aminoacyl-tRNA synthetases (AARS) was measured as index of copepod somatic growth as in Yebra and Hernández-León

(2004) modified by Yebra et al. (2011). AARS activity was monitored for 10 min at 25 °C. AARS activities were calculated as in Herrera et al. (2014) and corrected for the incubation temperature by applying an activation energy of 8.57 kcal·mol⁻¹ (Yebra et al. 2005) to the Arrhenius equation. 2) The activity of the Electron Transport System (ETS) was calculated as a measure of potential respiration following the method of Owens and King (1975). ETS activity was read continuously for 8 min at 20°C, calculated as in Maldonado et al. (2012) and corrected for the incubation temperature with the Arrhenius equation with an activation energy of 15 kcal·mol⁻¹ (Packard et al., 1975). 3) Biomass was measured as protein content following the bicinchoninic acid (BCA) assay by Smith et al. (1985), using a Pierce BCA protein assay kit and bovin serum albumin (BSA) as standard. AARS and ETS activities were standardized by protein to obtain protein-specific rates, hereafter called spAARS (nmol PPI·mg protein⁻¹·h⁻¹) and spETS (μL O₂·mg protein⁻¹·h⁻¹), respectively. Potential oxygen consumption obtained from ETS was also standardized by the number of individuals per sample (μL O₂·ind⁻¹·h⁻¹) to allow comparison with the oxygen consumption (μL O₂·ind⁻¹·h⁻¹) obtained from the initial and final DO measurements in the EPR incubation bottles.

Hatching success

Hatching success was calculated as the percentage of eggs that hatched into nauplii, standardized to 24 h. The eggs used to estimate hatching success came from two sources: 1) the EPR experiments described above, which produced eggs from individual females. Here, the initial number of eggs was obtained by counting intact eggs and empty membranes that remained from hatched eggs at the end of the incubation. 2) Eggs produced from batches of females to obtain a larger number of eggs than those collected from individual incubations. Groups of 50 to 100 females were incubated in 3-L buckets filled with normoxic water to lay eggs over 24 hours; females were suspended in a 200-μm mesh sieve to prevent cannibalism of the eggs. After 24-hrs, the sieve containing the females was removed and the eggs were gently pipetted from the bottom of the bucket. The eggs were then separated into groups (20, 50 or 100) under a stereomicroscope and immediately incubated in vials at hypoxic or normoxic conditions. The groups of eggs varied in number depending on the total number of eggs collected in the batches (Table 1).

Female production, naupliar growth rates and development time

Naupliar development and growth rates of *C. chilensis* were estimated from the same vials in which hatching success was estimated. Every 24 h for 5 days, at least two vials were randomly selected to collect unhatched eggs and nauplii with a 23- μm sieve. Eggs and nauplii were counted, stained with Neutral Red, preserved, measured (details below), and analyzed to determine live from dead organisms. The naupliar stages N1 and N2 were measured from the top of the head to the bottom of the cephalosome; due to the curved body of N3 an additional measurement was made from the bottom of the cephalosome to the bottom of the naupliar body. The sum of these two measurements corresponds to the total length of the nauplii (Rey et al., 2001); width was measured across the middle of the naupliar cephalosome. We staged the first three naupliar stages (N1, N2 and N3) under a microscope (10x – 40x), following the similarities in the external morphology of *C. chilensis* with other *Calanus* spp., like *C. australis* (Bradford et al., 2010), *C. finmarchicus* (Ogilvie, 1953) and *C. pacificus* (Marshall and Orr, 1955), because *C. chilensis* naupliar stages descriptions have not yet been published.

All measurements (length, width and diameter) were registered using a Nikon SMZ 1000 stereoscopic microscope equipped with a digital camera and the software Micrometric SE Premium 4. The females and eggs from EPR experiments were measured ($\pm 0.01\mu\text{m}$) to register prosome length (PL) and cephalosome width of females, and the egg diameter ($\mu\text{m} \pm \text{SD}$). The egg diameter, length and width of nauplii from the hatching success, naupliar growth and development experiments were also measured under the stereomicroscope.

We calculated the biovolume of females and nauplii following the equation used by Torres and Escribano (2003):

$$V = 4/3 \cdot A \cdot (w/2) \quad (2)$$

where V corresponded to the volume (μm^3), A is the copepod area (μm^2) in a dorsal view and, w the copepod width (μm). The biovolume of eggs was estimated assuming the area and volume of a sphere.

The biovolume of females, eggs, and nauplii were converted to carbon biomass using the factor of $0.045 \text{ mg C} \cdot \mu\text{L}^{-1}$ proposed by Omori and Ikeda (1984). The weight-specific

growth rates (g) from females and eggs were indirectly estimated from the relationship used in Kiørboe and Sabatini (1995):

$$g = \ln(W_{fem}/W_{egg})/D \quad (3)$$

where W_{fem} and W_{egg} are the carbon content of females and eggs, respectively. D was the development time (d^{-1}) from egg to adult (CVI) obtained from the generational time of *C. chilensis* of northern Chile, that correspond to 35 d (Hidalgo & Escribano 2008)

The naupliar growth was estimated by:

$$W = W_i e^{gt} \quad (4)$$

where W_i correspond to the initial naupliar weight ($\mu\text{g C}$), g is the specific instantaneous growth rate (day^{-1}) and t is time (days). From Equation (4), g between subsequent stages and from egg to N3 was estimated as:

$$g = \ln(W_{i+1}/W_i)/t \quad (5)$$

where W_{i+1} and W_i correspond to naupliar weight ($\mu\text{g C}$) between two subsequent stages and, t to the incubation time. Naupliar growth rate was calculated according the equations 4 and 5, whereas naupliar development time corresponded to the inverse of equation 5.

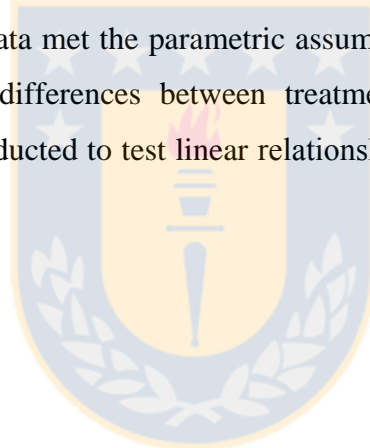
Data analysis

Chi-square tests, used with a level of significance (α) of 0.05, were run to test if *Calanus chilensis* survival (%) (females, eggs, and nauplii) was independent of the oxygen levels under study. G Tests were used to evaluate if hatching success and the abundance of eggs and nauplii were independent of oxygen concentrations.

The effects of oxygen on EPR, hatching success, biomass and size of eggs and nauplii, naupliar growth, and development time were statistically analyzed with Student-t Tests ($\alpha=0.05$), after testing that samples from both treatments came from normal populations with

equal variances (Zar, 1999). Female size differences between treatments were also evaluated with a Student-t Test to discard any effect of body size on results. When the data did not meet the parametric assumptions, the non-parametric test Mann-Whitney was carried out. All experimental data were reported as mean \pm 1 SE (Standard error), whereas morphological parameters of *C. chilensis*, such as size, volume, and weight were expressed as mean \pm 1 SD (Standard deviation). The eggs and nauplii (N1 to N3) collected at the end of the experiments were expressed as the total number of individuals and its percentage (%) in relation with the total number of eggs initially incubated per treatment. Additionally, the abundance of eggs and nauplii collected were expressed as the mean percentage (\pm 1 SE) per stage (eggs, N1, N2 and N3) found each day, from day 1 to day 5.

To test for statistical differences in metabolic parameters among experiments and oxygen conditions, a two-factor nested General Linear Model (GLM) was applied with $\alpha=0.05$, after checking that the data met the parametric assumptions. A post-hoc Tukey test was done to identify significant differences between treatments (Quinn and Keough, 2002). Pearson correlations were conducted to test linear relationships between these parameters ($\alpha=0.05$).



Results

Female survival and egg production rate

Mean oxygen concentrations from the experiments are given in Table 2. The 24-h survival of *C. chilensis* females was not related to the oxygen concentration (Chi-square = 4.32, df = 1, p = 0.12): 94.3% of females survived under normoxic conditions and 82.9% under hypoxic conditions from a total of 118 and 76 females, respectively.

The mean size of *C. chilensis* females was $2373.8 \pm 165.1 \mu\text{m}$ (range = 2066.7 – 2666.7 μm , n = 47) and $2400.0 \pm 159.1 \mu\text{m}$ (range = 2000.0 – 2733.3 μm , n = 47) in hypoxia and normoxia, respectively. Female sizes incubated in both treatments were not statistically different (t= 0.79, df = 94, p= 0.43). EPR showed high variability under both oxygen conditions, and no significant differences were found between them (t= -0.46, df = 192, p = 0.64). EPR ranged from 0 to 85 eggs·female⁻¹·d⁻¹, with mean values of 10.9 ± 2.09 eggs·female⁻¹·d⁻¹ in hypoxia, and 9.7 ± 1.5 eggs·female⁻¹·d⁻¹ in normoxia.

Metabolic parameters of females

The metabolic parameters of *C. chilensis* showed that females remained active over the 24-h they were held under hypoxic and normoxic conditions (Fig. 1). The biomass of individual *C. chilensis* females showed high variability, but no significant differences were observed between females set up in hypoxic and normoxic treatments for experiments during winter 2014, although a slight decreasing trend was observed over the 10-day window of collections (Fig. 1a). SpAARS, spETS, and individual oxygen consumption showed no significant differences among experiments and between DO levels (Figs. 1b – f; Table 3). The biomass of females was negatively correlated with somatic growth (spAARS) (Table 3). Mean values of metabolic parameter estimates are given in Table 4.

Hatching success and naupliar abundance

The hatching success of *C. chilensis* eggs obtained from experiments using single females (G = 128.7, df = 1, p < 0.001) and from batches (of females) (G = 197.6, df = 1, p < 0.001) was dependent on oxygen. Hatching success from single female experiments showed significant differences (t = -4.15, df = 48, p < 0.001) between hypoxia and normoxia, with values of $22.9 \pm 9.7\%$ (n = 15) and $72.3 \pm 6.6\%$ (n = 35), respectively. Similarly, the

experiments from female batches showed significant differences ($t = 4.10$, $df = 36$, $p < 0.001$), with values of $26.8 \pm 6.8\%$ ($n = 17$) and $55.5 \pm 3.7\%$ ($n = 24$) of hatching in hypoxia and normoxia, respectively.

The Neutral Red technique was used to evaluate survival, the method did not allow us to evaluate whether mortality and disintegration by bacterial activity prior to counting and staining the eggs and nauplii occurred. Retrieval of eggs and nauplii in normoxic treatment was very high indicating that experimental protocols did not result in the loss of organisms; thus missing individuals were assumed to have died and disintegrated. In this context, eggs and nauplii coming from randomly selected vials that were stained with the stain showed that 75.6% of 1,324 and 93.9% of 1,910 individuals survived in hypoxia and normoxia, respectively. Dead eggs were much more common than dead nauplii in the hypoxic treatments (24.4% versus 6.1% of the total, respectively). The same pattern occurred in normoxia, with 4.3% and 1.8% dead eggs and nauplii, respectively.

Between oxygen conditions, naupliar and female lengths were not significantly different, whereas egg diameter and naupliar width of N2 and N3 stages were. Consequently, the biovolume calculations that include these parameters also were significantly different (Table 5). Smaller eggs and thinner nauplii (N2 – N3) were present in hypoxia compared to normoxia, whereas changes in size were not evident in the first naupliar stage (Table 5).

Over a period of five days, a lower proportion of the initial numbers of eggs were recovered as nauplii in hypoxia than normoxia (Fig. 2). In hypoxia, the abundance of unhatched eggs recovered was 869 (72.4% of the total recovered), N1 was 252 (20.1%), N2 was 44 (3.7%) and N3 was 36 (3.0%); whereas in normoxia, 519 eggs (28.7%) did not hatch, followed by a higher presence of nauplii than in hypoxia; 861 (74.5%) N1, 159 (8.78%) N2 and 272 (15.02%) N3. The relative abundance of unhatched eggs and early naupliar stages showed that early stage survival was dependent on oxygen ($G = 582.8$, $df = 3$, $p < 0.001$). The relative abundance of stage N1 showed a decreasing trend due to naupliar development to subsequent stages. Only at the third day, N2 and N3 stages were found in hypoxia (Fig. 2a). In normoxia, from day 1, N1 showed a decreasing trend whereas N2 had high abundance at day 3 and N3 at day 5 (Fig. 2b).

Female production, naupliar growth and development rates

The female weight specific-growth rates estimated from females and eggs in both conditions were not statistically different as also observed by the AARS activities, with values of $0.2 \pm 0.01 \text{ d}^{-1}$ and $0.3 \pm 0.01 \text{ d}^{-1}$ under hypoxia and normoxia, respectively. However, changes in naupliar body length showed that nauplii reached larger size in normoxic ($\text{Ln}(y) = 5.07 \cdot x + 0.0904$; $R^2 = 0.37$, $\text{df} = 391$, $p < 0.001$) than in hypoxic ($\text{Ln}(y) = 5.27 \cdot x + 0.0063$; $R^2 = 0.03$, $\text{df} = 152$, $p = 0.49$) conditions (Fig. 3). The growth rates from egg to N3 showed significant differences (Fig. 4a); the averaged naupliar growth was $0.15 \pm 0.015 \text{ d}^{-1}$ in hypoxia and $0.24 \pm 0.014 \text{ d}^{-1}$ in normoxia ($t = -7.45$, $\text{df} = 5$, $p < 0.001$). Therefore, naupliar development took on average $6.5 \pm 0.35 \text{ d}$ and $4.2 \pm 0.15 \text{ d}$ in hypoxia and normoxia, respectively ($t = 6.77$, $\text{df} = 5$, $p < 0.001$) (Fig. 4b).

Discussion

Expansion, intensification, and shoaling of OMZs is a main concern related to global warming due to the negative impacts that may have over the survival of some pelagic species, affecting the ecosystem structure, vertical fluxes, and productivity of EBUS (Ekau et al., 2010; Keeling et al., 2010; Seibel, 2011). One of the major effects of the increasing hypoxia in OMZs is the compression of the oxygenated surface layer, where organisms tend to concentrate to avoid low oxygen conditions (Escribano et al., 2009; Hidalgo et al., 2010; Ekau et al., 2010; Teuber et al., 2013). The shoaling of OMZs diminishes the extension of the usable habitat of zooplankton (Manríquez et al., 2009), small-pelagic (Bertrand, et al., 2010) and large size fishes such as billfishes and tunas who have shown to decrease their diving depths, increasing their exposure to overfishing due to their shallower distribution (Stramma et al., 2011).

Non-vertically migrating zooplankton may be more limited by the avoidance of hypoxia, increasing the chance to be negatively affected by the vertical movements of the OMZ, which are mainly modulated by coastal upwelling, since EBUS have shown intensification under upwelling-favorable winds (Bakun, 1990; Rykaczewski and Checkley, 2008; Wang et al., 2015). On the other hand, some species have developed metabolic adaptations (*e.g.* low aerobic or anabolic metabolism) to successfully inhabit or refuge from predation inside the OMZ. Some copepods of the families Eucalanidae and Metridinidae (Hidalgo et al., 2005b;

Auel and Verheye, 2007; Teuber et al., 2008) may be less affected by ocean deoxygenation if these adaptations occur in the early stages as well as adults.

As in previous experiments with copepods (see Runge and Roff, 2000; Yáñez et al., 2012; Escribano et al., 2014; Ruz et al., 2015), our experiments did not expose females to a prolonged acclimation period prior to incubation, so these results may be considered as indications of short-term, or episodic, effects of hypoxia on *Calanus chilensis*. The reproductive potential and metabolism of *C. chilensis* females were not affected by hypoxia, which is consistent with previous EPR estimates under low oxygen (Ruz et al., 2015), and also with the capacity of stage CV and adults of *C. chilensis* to inhabit the OMZ off northern Peru (Hirche et al., 2014). Short-term exposure can occur in the field as strong spatial and temporal variability in upwelling can cause shoaling and deepening of the OMZ within hours, thus limiting acclimation time for individuals in the field.

The increased mortality of copepods due to hypoxia associated with OMZs has been reported for copepods such as *Paracalanus cf. indicus* (Yáñez et al., 2012), *Acartia tonsa* (Ruz et al., 2015), *Calanoides carinatus* (Auel and Verheye, 2007) and nauplii of *C. chilensis* (this study); species which mostly inhabit the oxygenated surface layer. Ontogenetic vertical migration and diapause of CV stages below the OMZ play a key role in enabling *C. carinatus* to cope with hypoxia (Auel and Verheye, 2007), whereas, in bottom-hypoxia environments, resting eggs help *A. tonsa* to deal with stressful conditions and maintain populations over time (Marcus, 1984; Marcus and Taulbee, 1992; Chen and Marcus, 1997). Although, neither resting stages nor ontogenetic vertical migrations have been reported for *C. chilensis* in the HCS, it has been described as the only species of the *Calanidae* family of which older stages can tolerate hypoxia (Hirche et al., 2014). This is supported by the high 24-h survival (> 82%) of *C. chilensis* females in hypoxia observed in this study.

Although adult females of *C. chilensis* seemed unaffected by low oxygen and maintained protein synthesis rates (spAARS activity) under low oxygen DO conditions, our results clearly revealed strong effects of hypoxia on early life stages. Development and growth of eggs and nauplii were very sensitive to short-term hypoxia, a response that could potentially affect the timing of cohort development and affect copepod recruitment and population size. As development and survival of early stages are critical for the success of zooplankton

populations (Vargas et al., 2006), the depth at which the females spawn their eggs in OMZ regions is highly relevant to ensure hatching and naupliar growth.

The center (DWA) of *C. chilensis* female and eggs population typically occurs between 10 and 27 m and 13 and 46 m depth, respectively. This suggests that females may lay their eggs at shallow depths often above the OMZ, whereas eggs tend to sink at $\sim 27 \text{ m}\cdot\text{d}^{-1}$, reaching hypoxic waters in less than two days if upwelling vertical velocities are not strong enough to refloat eggs into the photic layer (Ruz et al., 2017). The lack of an effect of hypoxia on EPR observed in this work and by Ruz et al. (2015), suggests that short-term exposure to hypoxia, such as that experienced during diel vertical migration or strong wind-driven ascent of the OMZ to shallow waters, may not influence the reproductive potential of *C. chilensis*, whereas a more extended hypoxia in very shallow waters may increase the mortality of early stages.

Due to metabolic rates increase with decreasing size in metazoans (Kleiber, 1961), the weight-specific respiration rate in pelagic organism tends to decrease with increasing body weight (Ikeda, 1970). The early stages of *C. chilensis* may be more sensitive to hypoxia than CV and adults due their higher weight-specific metabolism and therefore, the need for more oxygen to supply their energetic demands. Furthermore, Calanoida have a heart (Mauchline, 1998) that in early stages may not be fully developed, so they may not circulate oxygen as well as adults. Body mass and habitat temperature together contribute more than 95% to the variance in the oxygen consumption of marine copepods (Ikeda et al., 2001). This would favor the development of early stages in the warm, oxygenated, and food-rich surface layer. The physiology of zooplankton larvae in response to hypoxia is poorly known in the HCS. Ruz et al. (2015) also studied EPR and hatching success of *C. chilensis* and *A. tonsa* under different oxygen concentrations, showing that *C. chilensis* coped better with low oxygen than *A. tonsa*. However, they did not follow naupliar development into later stages than N1.

A selective pressure can be induced by hypoxia intensity. Adults and later stages of the genus *Calanus* seem adapted to the typical local oxygen concentration in their habitat. For example, *C. chilensis* from the HCS and *C. euxinus* from the Black Sea withstand more intense hypoxia (Svetlichny et al., 1998; Besiktepe et al., 2005; Hirche et al., 2014; Ruz et al., 2015; this study) than *C. pacificus* from Puget Sound, Washington (USA) (Keister and Tuttle, 2013; Grodzins et al., 2016) and *C. sinicus* (Wang et al., 2013) from Qingdao (China) coastal waters. The severe hypoxia that occurs in the HCS is due the presence of the OMZ (Escribano

et al., 2009). However, the severe hypoxia of the Black Sea and Puget Sound are mostly driven by poor ventilation of subsurface waters leading to decreased oxygen by respiration processes (Svetlichny et al., 1998; Besiktepe et al., 2005; Warner et al., 2001). In environments where the OMZ is permanent, copepods such as *Calanus* spp. seem to be more tolerant and better adapted than species that inhabit environments with seasonal and weaker ($\sim 2 \text{ mg}\cdot\text{L}^{-1}$) bottom hypoxia. To have a better understanding of the adaptations to local hypoxia, further research is needed in the HCS to know if *C. chilensis* and other zooplankton species have adaptive characteristics to cope with low-oxygen waters, like low metabolic rates, anaerobic activity, or metabolic suppression as has been observed in other species associated with the OMZ (Siebel et al., 2011; Teuber et al., 2013). In this context, it is important to know the critical oxygen levels that zooplanktonic organism can tolerate, and to investigate the coupled effects of temperature and oxygen. Climate change is pushing DO levels in OMZs below the capacity of oxygen extraction by some organisms, potentially changing the diversity of these ecosystems towards fauna capable of coping with hypoxia (Siebel, 2011).

Other species, such as *A. tonsa*, decrease their reproduction, survival, hatching success and development under hypoxia, resulting in smaller adult body size (Richmond et al. 2006) and delaying their embryonic development (Lutz et al., 1994; Marcus and Lutz, 1994). In other ecosystems, hypoxia and anoxia can induce resting or dormancy stages in copepods (Lutz et al., 1994; Katajisto, 2004). Also, the exposure to stressful factors, such as food limitation, may promote the production of resting eggs in marine copepods (Drillet et al., 2011). Off northern Chile, *C. chilensis* populations prevail all year round, with early stages and eggs continuously present, thanks to the consistent food availability due to the semipermanent upwelling that injects nutrients into the photic zone (Hidalgo and Escribano, 2008). Additionally, we found that growth rates of *C. chilensis* nauplii decreased in hypoxia, resulting in longer development times to stage N3. We did not follow the development to the adult stage, but we did observe that in low oxygen, nauplii were thinner than in normoxia. This effect of oxygen on size may ultimately decrease the final biomass of copepod populations. In upwelling areas, a potential decrease in growth rate and hence biomass of *C. chilensis* populations may be driven by a combination of intensification of hypoxia and coastal cooling that may occur due to upwelling intensification (Wang et al., 2015).

The hatching success estimates carried out in this study may be influenced by the exposure to normoxia prior incubations. The eggs collected from the buckets that were moved to hypoxia were older and had been in normoxia for a longer portion of the time before hatching than others, so the significant effect on hatching probably would have been even stronger if all were placed into hypoxia immediately after spawning. However, the significant negative impacts on hatching in the hypoxic treatment, even though in the batch cultures eggs were not exposed to hypoxia for their full development is highly relevant for the population dynamics of *C. chilensis*. In the field, if the eggs are spawned in shallow waters, they may encounter the transition from normoxia to hypoxia before hatching (Ruz et al. 2017) or carry out their whole embryonic development inside the OMZ if the females laid their eggs deeper. The hatching success of *C. chilensis* from northern Chile may have a seasonal response. In this study, conducted in winter, we found that hatching success and survival was affected by low oxygen, in contrast to similar experiments run in summer (Ruz et al., 2015). Although, oxygen can act as a limiting factor for early development, other factors (*e.g.* temperature and low food concentrations) associated with life traits of the females collected for experiments may have influenced hatching success and naupliar growth of *C. chilensis* populations; potentially arresting the development and reducing the growth rate of *C. chilensis* nauplii and other copepod species (Torres and Escribano, 2003; Vargas et al., 2006; Poulet et al., 2007).

Since all the responses to hypoxia evaluated in this study were experimental, the extrapolation of the data can only be done to potential population-level responses associated with the reduction in size and early stage's vital rates (survival and growth). In this context, the productivity of *C. chilensis* in the HCS may decrease due to a reduction in biomass by a combined effect of low oxygen and temperature. Further research is needed to evaluate synergistic or antagonistic effects of environmental variables on copepod production to better understand how these organisms may respond to different combinations of DO and temperature in the HCS. Given that we observed significant effects of hypoxia on eggs and nauplii, it is very important to understand how this factor influences copepod early stage development and growth, mainly in oxygen deficient environments as OMZs, which are subject to and highly impacted by climate change.

Conclusions

The copepod *Calanus chilensis*, a dominant species in the HCS, responded ontogenetically to hypoxia. Early stages (eggs and nauplii) were more vulnerable than adult females. No significant effects were observed on 24-h survival, metabolism and egg production rates of females between hypoxia and normoxia. In contrast, hatching success diminished more than 30% under low oxygen conditions. Nauplii were able to develop independently of the oxygen level. However, a significantly slower growth occurred in hypoxia than in normoxia, leading to long development times when the early stages of *C. chilensis* grew under low oxygen conditions. Exposure of early stages to critical oxygen levels may be a key issue for maintenance of copepod populations over time and have long-term implications in pelagic ecosystems associated with coastal upwelling processes.

Our findings suggest that the expansion and shoaling of OMZs in upwelling systems can substantially influence copepod dynamics by suppressing growth and survival of early stages, due the shrinking of the oxygenated surface layer where most zooplanktonic organism tend to inhabit. These results provided a better understanding of how short-term hypoxia may affect stage-specific vital rates of this species which constantly deals with hypoxic events associated with shoaling of the OMZ.

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Tables

Table 1. Summary of the experiments indicating the number of vials/bottles as replicates, the stage, the number or range of individuals incubated (n) and the range of incubation time (days). Hatching success, naupliar growth and development were estimated from two approaches: from EPR experiments on individual females (1) and groups of females (2).

Experiment	Date	N° vials/bottles		Stage	n	Duration (d)
		Hypoxia	Normoxia			
<i>Egg Production Rate</i>						
	14/07/2013	33	33	Female	1	1
	16/07/2013	15	11		1	1
	23/07/2014	15	12		1	1
	23/07/2014	13	14		1	1
	24/07/2014	-	24		1	1
	01/08/2014	-	24		1	1
<i>Biochemical and metabolic parameters</i>						1
	23/07/2014	6	5	Female	10	1
	26/07/2014	3	3		10	1
	02/08/2014	8	3		10	1
<i>Hatching success, naupliar growth and development (1)</i>						1
	15/07/2013	4	2	Egg	10 – 64	1 – 5
	17/07/2013	11	13		3 – 64	1 – 5
	24/07/2014	-	5		21 – 58	1 – 5
	25/07/2014	-	15		2 – 72	1 – 5
<i>Hatching success, naupliar growth and development (2)</i>						1 – 5
	15/07/2013	4	3	Egg	20 – 100	1 – 5
	20/07/2013	2	-		100	1 – 5
	19/07/2014	3	3		20	1 – 5
	25/07/2015	8	10		20 – 50	1 – 5

Table 2. Summary of dissolved oxygen concentrations ($\text{mg O}_2\cdot\text{L}^{-1}$) measured during the experiments. Mean ($\pm\text{SE}$) and range (min. - max. value) among different bottles are given. Maximum and minimum values of the range correspond to final and initial oxygen condition, respectively.

	Hypoxia ($\text{mg O}_2\cdot\text{L}^{-1}$)		Normoxia ($\text{mg O}_2\cdot\text{L}^{-1}$)	
	Mean \pm SE	Range	Mean \pm SE	Range
Experiment				
<i>Egg production rate</i>	1.01 ± 0.05	0.62 – 1.94	8.65 ± 0.28	6.10 – 10.69
<i>Female metabolism</i>	1.29 ± 0.12	0.95 – 1.73	9.02 ± 0.21	8.67 – 9.39
<i>Egg hatching, naupliar growth and development</i>	0.84 ± 0.05	0.23 – 1.73	8.36 ± 0.10	7.30 – 9.48



Table 3. Two factors nested General Linear Model to test dissolved oxygen (DO) (hypoxia vs. normoxia) and the three experiments (nested factor) carried out between July 23 and August 2 of 2014. Also, Pearson correlations were run among these parameters. $\alpha = 0.05$; significant p are marked in bold.

General Linear Model			
Source	<i>df</i>	<i>F</i>	<i>p</i>
Biomass			
Treatments	1	1.20	0.29
Experiments	4	1.25	0.34
spAARS			
Treatments	1	0.01	0.93
Experiments	4	0.94	0.47
spETS			
Treatments	1	0.42	0.53
Experiments	4	0.75	0.57
Pearson Correlations			
		Biomass	spAARS
spAARS	<i>r</i>	-0.612	
	<i>p</i>	0.005	
	<i>n</i>	16	
spETS	<i>r</i>	-0.209	0.312
	<i>p</i>	0.391	0.193
	<i>n</i>	16	16

Table 4. Mean (\pm SE) of biochemical and metabolic parameters of *C. chilensis* females during winter 2014.

Experiments	spAARS (nmol PPi·mg protein ⁻¹ ·h ⁻¹)		spETS (μ L O ₂ ·mg protein·h ⁻¹)		Potential oxygen consumption (μ LO ₂ ·ind ⁻¹ ·h ⁻¹)		DO consumption (μ LO ₂ ·ind ⁻¹ ·h ⁻¹)							
	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia						
23-07-2014	14.64	\pm 3.51	18.63	\pm 4.20	5.63	\pm 1.46	3.51	\pm 1.65	0.63	\pm 0.32	1.35	\pm 0.21	0.72	\pm 0.06
	17.20	\pm 2.20	24.28	\pm 6.98	4.84	\pm 3.57	7.57	\pm 4.94	0.30	\pm 0.47	0.47	\pm 0.24	0.41	\pm 0.16
27-07-2014	31.52	\pm 8.50	19.36	\pm 0.35	7.91	\pm 2.68	12.65	\pm 9.84	0.55	\pm 0.43	0.75	\pm 0.57	0.57	\pm 0.65
	8.50	\pm 0.35	19.36	\pm 0.35	7.91	\pm 2.68	12.65	\pm 9.84	0.55	\pm 0.43	0.75	\pm 0.57	0.57	\pm 0.65



Table 5. Estimates of egg, nauplii, and female volume and weight in carbon units (mean \pm SD) of *C. chilensis* under experimental oxygen conditions (hypoxia and normoxia) reared at constant temperature (14°C). Significant differences between DO treatments are showed in bold. The statistical parameters of T-student ($\alpha = 0.05$) shown are: T-value, degrees of freedom (df) and p-value (p).

	Eggs	N1	N2	N3	Female
<u>Hypoxia</u>					
Length/diameter					2373.81 \pm
(μm)	158.3 \pm 9.54	186.89 \pm 13.80	216.23 \pm 21.75	285.63 \pm 43.44	165.06
Width (μm)	-	111.56 \pm 11.10	112.39 \pm 17.70	115.57 \pm 14.34	-
					1066.47 \pm
Volume ($10^6 \mu\text{m}^3$)	2.09 \pm 0.39	1.23 \pm 0.27	1.47 \pm 0.54	2.06 \pm 0.75	249.90
Weight ($\mu\text{g C}$)	0.09 \pm 0.018	0.06 \pm 0.01	0.07 \pm 0.02	0.09 \pm 0.03	37.93 \pm 8.95
N	438	38	48	5	49
<u>Normoxia</u>					
Length/diameter					2400 \pm 159.14
(μm)	161.4 \pm 10.13	188.01 \pm 16.97	216.76 \pm 13.03	273.69 \pm 20.69	
Width (μm)	-	113.45 \pm 10.67	123.13 \pm 8.71	131.57 \pm 7.97	-
					1091.16 \pm
Volume ($10^6 \mu\text{m}^3$)	2.25 \pm 0.48	1.28 \pm 0.28	1.73 \pm 0.29	2.54 \pm 0.45	252.91
Weight ($\mu\text{g C}$)	0.10 \pm 0.02	0.06 \pm 0.01	0.08 \pm 0.01	0.11 \pm 0.02	38.88 \pm 9.23
N	116	80	120	241	47
<u>T-Student (df, p)</u>					
		-0.35 (116,			
Length/diameter	-3.07 (552, 0.002)	0.72)	-0.19 (166, 0.85)	1.24 (244, 0.22)	-0.79 (94, 0.43)
		-0.89	-5.26 (116,	-4.36 (244,	
Width	-	(116,0.38)	<0.001)	<0.001)	-
	-3.73 (552,	-0.92	-4.03 (166,		
Volume	<0.001)	(116,0.36)	<0.001)	-2.33 (244, 0.02)	-0.48 (94, 0.62)
	-5.19 (552,	0.00 (116,	-4.31 (166,		
Weight	<0.001)	1.00)0	<0.001)	-2.19 (244, 0.03)	-0.51 (94, 0.61)

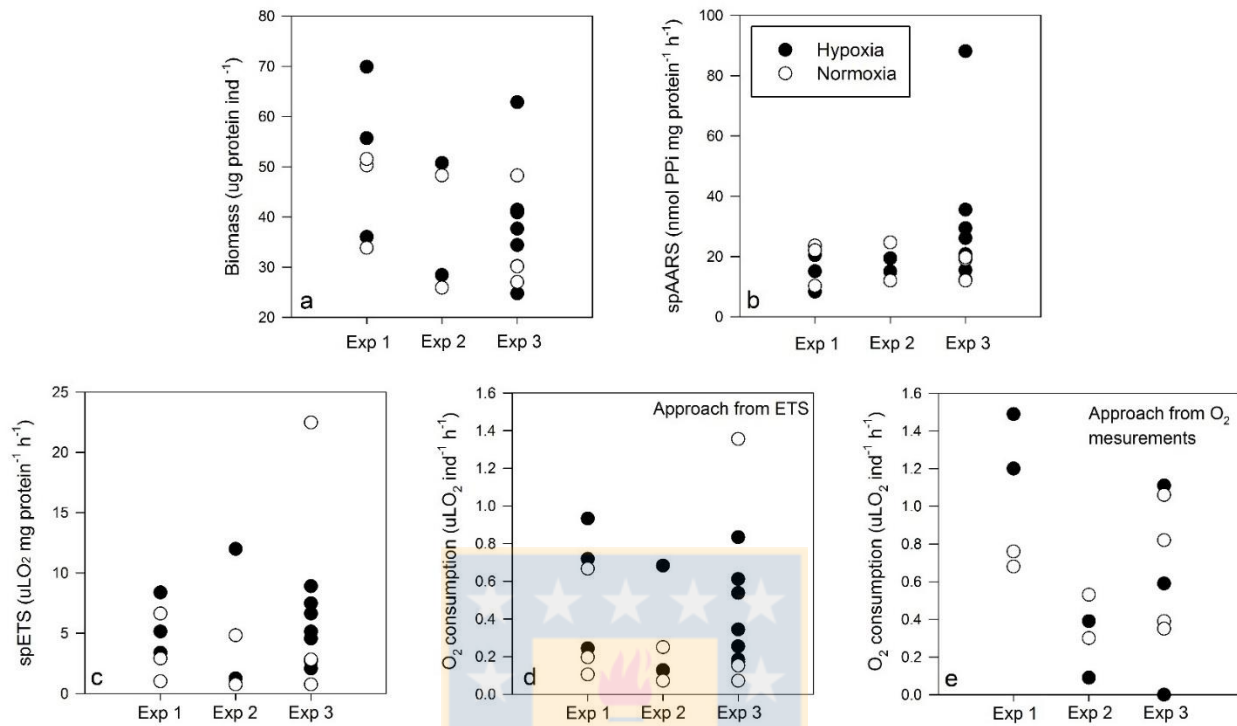


Figure 1.- Summary of biochemical and metabolic parameters of *C. chilensis* females under two oxygen levels during winter 2014. Three experiments with replicates were carried out on females collected from the field between July 23 and August 2 to obtain a) biomass ($\mu\text{g protein}\cdot\text{ind}^{-1}$); b) specific aminoacyl-tRNA synthetases activity (spAARS, $\text{nm PPi}\cdot\text{mg protein}^{-1}\cdot\text{h}^{-1}$); c) specific electron transport system activity (spETS, $\mu\text{L O}_2\cdot\text{mg protein}^{-1}\cdot\text{h}^{-1}$); d) potential oxygen consumption from spETS ($\mu\text{L O}_2\cdot\text{ind}^{-1}\cdot\text{h}^{-1}$) and; e) oxygen consumption ($\mu\text{L O}_2\cdot\text{ind}^{-1}\cdot\text{h}^{-1}$) from direct measurements.

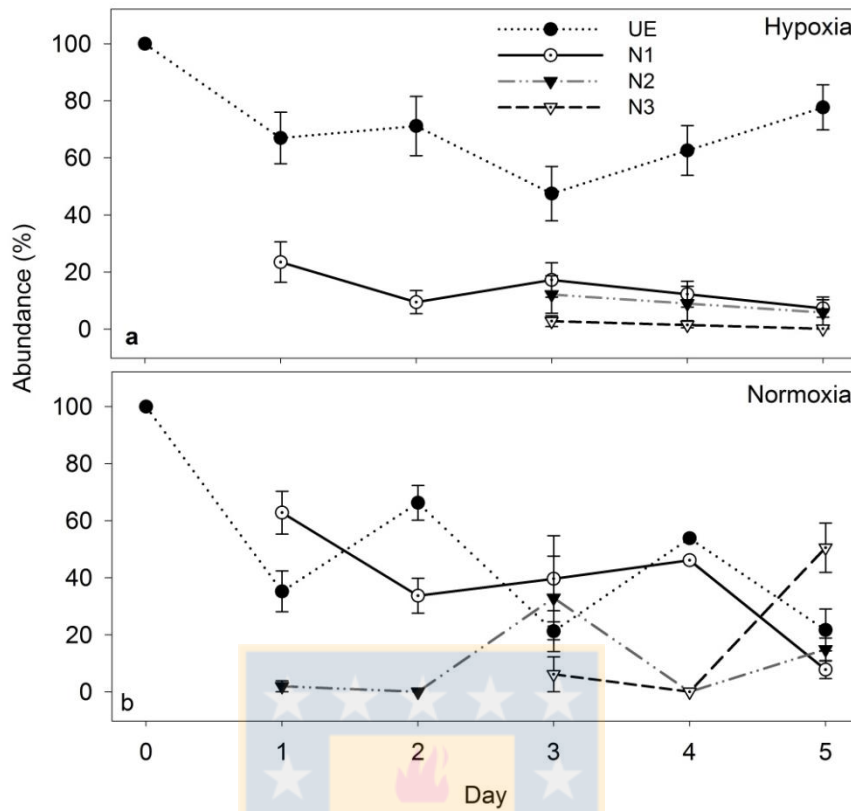


Figure 2.- Abundance (%) of unhatched eggs (UE) and naupliar stages 1 to 3, of *C. chilensis* reared under: a) hypoxia and b) normoxia during 5 days.

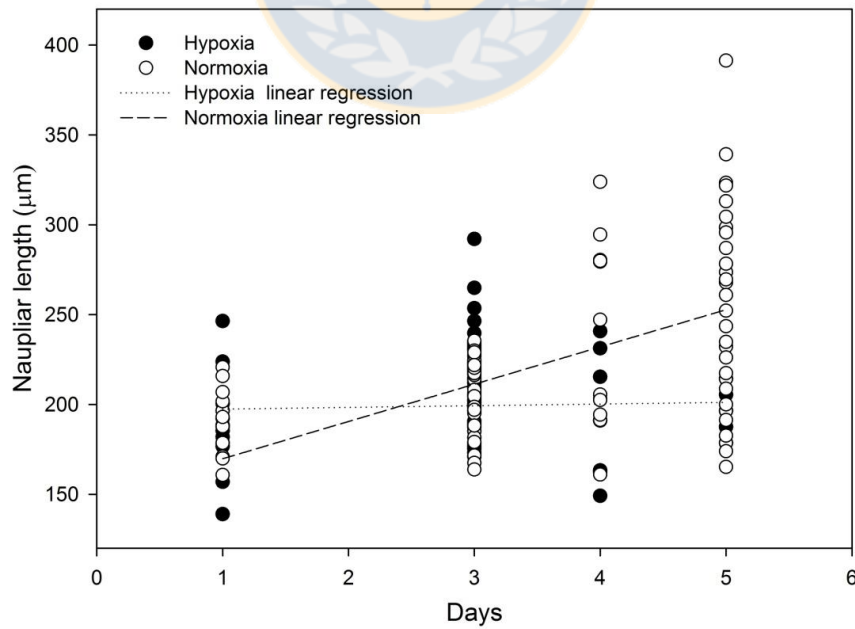


Figure 3.- Body length increases of *C. chilensis* nauplii reared in the laboratory under hypoxia and normoxia.

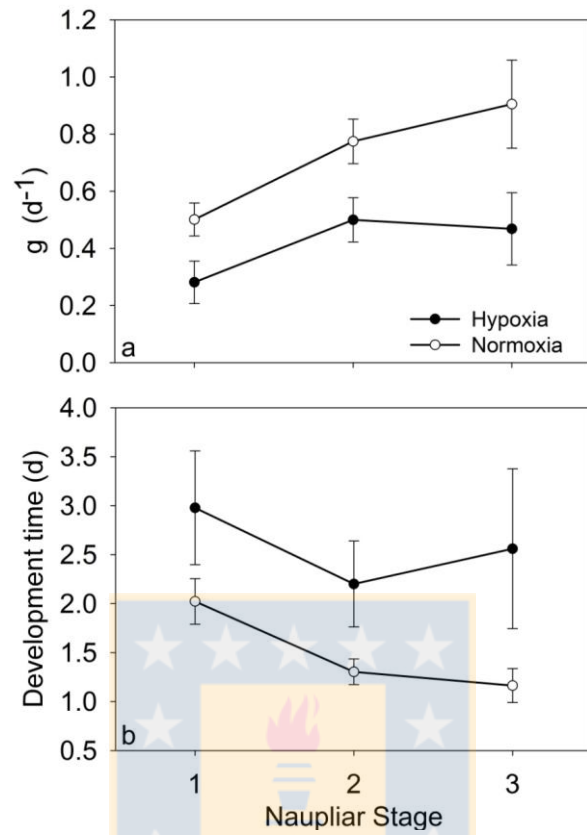


Figure 4.- Growth rate (d^{-1}) and b) naupliar development time (d) of *C. chilensis* under hypoxia and normoxia, respectively.

3.2. Capítulo 2

Efectos de la hipoxia en la producción de huevos y estadios tempranos de *Acartia* (*Acanthacartia*) *tonsa* en el Sistema de Corrientes de Humboldt (23°S)

Artículo en preparación

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Resumen

La desoxigenación de océano debido al cambio climático se ha convertido en un tema clave en los sistemas de surgencia costera, debido a la expansión, somerización e intensificación de las zonas de mínimo oxígeno (ZMOs). Los movimientos verticales de las ZMOs restringen la distribución vertical del zooplankton, lo que puede afectar desde su fisiología hasta la estructura del ecosistema. Durante 2013 y 2014, el copépodo *Acartia tonsa* fue recolectado en el norte de Chile para evaluar cómo las condiciones de oxígeno limitantes tienen un efecto sobre su sobrevivencia y tasas vitales. Hembras adultas, huevos y nauplios tempranos (N1 a N2) fueron expuestos a hipoxia severa ($<0,4 \text{ mL L}^{-1}$), hipoxia leve ($\sim 0,8 \text{ mL L}^{-1}$) y normoxia ($> 5 \text{ mL L}^{-1}$). La hipoxia severa causó efectos letales (100%) en las hembras y, por lo tanto, una producción de huevos (PH) extremadamente baja. La hipoxia leve indujo efectos subletales (48%) en hembras, sin evidenciar diferencias significativas en la PH al comparar con condiciones normóxicas. El éxito de la eclosión y el crecimiento naupliar se observó sólo en hipoxia leve y normoxia. El éxito de eclosión disminuyó en hipoxia, mientras el crecimiento naupliar ($\sim 0.27 \text{ d}^{-1}$) fue similar en ambas condiciones de oxígeno. Se sugiere que las hembras y huevos de *A. tonsa* son fuertemente afectados por la hipoxia, por lo tanto, variaciones en intensificación de las condiciones de hipoxia en las ZMOs podrían afectar la dinámica poblacional de los copépodos, debido a una disminución en el potencial reproductivo y por lo tanto, la productividad del ecosistema pelágico.

Palabras claves: Hipoxia, zona de mínimo oxígeno, Copépodos, *Acartia tonsa*, norte de Chile.

Efecto de la hipoxia sobre la producción de huevos y estadios tempranos de *Acartia* (*Acanthacartia*) *tonsa* en el Sistema de Corrientes de Humboldt (23°S)

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Introducción

En la actualidad, la desoxigenación del océano producto del cambio climático global, ha generado la intensificación de las bajas concentraciones de oxígeno disuelto y la expansión de las áreas de hipoxia natural presentes en los sistemas de surgencia costera (Keeling et al. 2010, Wang et al. 2015). Este incremento en la pérdida de oxígeno podría producir cambios en la estructura de estos ecosistemas, viéndose reflejado principalmente en cambios en los ciclos biogeoquímicos, comportamiento y tasas metabólicas de organismos marinos, acoplamiento bento-pelágico, y en las altas tasas de producción pesquera característica de los sistemas de surgencia (Levin, 2003, Paulmier et al. 2008, Ekau et al. 2010, Keeling et al. 2010, Stramma et al. 2010, Seibel 2011, Wishner et al. 2013).

Los sistemas de surgencia costera se encuentran en los bordes orientales de las corrientes de California, Benguela, Canarias y Humboldt y se caracterizan por presentar aguas pobres en oxígeno disuelto a profundidades intermedias, debido a la escasa ventilación de las aguas subsuperficiales y a las elevadas tasas de remineralización de materia orgánica que sedimenta desde la capa superficial hacia aguas profundas (Wyrski 1962, Keeling et al. 2010, Wang et al. 2015). Estas zonas de hipoxia natural, denominadas zonas de mínimo oxígeno (ZMOs), suelen presentar concentraciones de oxígeno disuelto $< 0.5 \text{ mL L}^{-1}$ ($\sim 22.3 \text{ } \mu\text{M}$) (Keeling et al. 2010, Stramma et al. 2010), llegando incluso a alcanzar condiciones de anoxia total (Ulloa et al. 2012). Debido a estas características, las ZMOs han sido reconocidas como verdaderas barreras naturales para la distribución vertical de los organismos con metabolismo aeróbico, como la mayoría del zooplancton, peces pelágicos y macrofauna bentónica (Wishner et al. 1995, Levin 2003, Escribano et al. 2009, Stramma et al. 2010, Seibel 2011, Wishner et al. 2013, Donoso & Escribano 2013).

Las ZMOs han sido consideradas laboratorios naturales para el estudio de los cambios que puede enfrentar el ecosistema pelágico ante problemáticas ambientales asociadas al cambio climático global, como la acidificación y desoxigenación del océano (Paulmier et al. 2011). Los cambios en el sistema pelágico pueden ser estudiado mediante las respuestas fisiológicas del fitoplancton y zooplancton, los cuales han sido descritos como bioindicadores de cambios en las condiciones oceanográficas, principalmente gracias a sus cortos ciclos de vida y rápidas respuestas a cambios ambientales (Hays et al. 2005).

La alta producción biológica del Sistema de Corriente de Humboldt (SCH) sostiene una de las pesquerías más grandes del mundo (Thiel et al. 2007). En la zona norte de Chile ($18 - 28^{\circ}\text{S}$) predominan vientos favorables para la surgencia durante todo el año, permitiendo el ascenso de aguas subsuperficiales de forma semipermanente (Sobarzo et al. 2007, Piñones et al. 2007). Específicamente en la Bahía de Mejillones (23°S), la distribución vertical del oxígeno disuelto se caracteriza por presentar una estrecha capa superficial oxigenada donde habita la mayoría del zooplancton, seguida de una severa oxiclina (Ruz et al. 2017) y de una ZMO somera que llega hasta el fondo de la bahía y cuyo límite superior ($1 \text{ mL O}_2 \text{ L}^{-1}$) suele encontrarse a una profundidad promedio de $\sim 25 \text{ m}$ (Escribano et al. 2012, Hidalgo & Escribano, 2008, Escribano et al. 2009, Ruz et al. 2015). Debido a las altas abundancias de copépodos (*c.a.* $\geq 80\%$), este grupo ha sido utilizado como modelo para comprender los

potenciales efectos que puedan ocasionar cambios en las condiciones oceanográficas como variaciones en temperatura, calidad y cantidad de alimento, oxígeno disuelto (Escribano et al. 1996, Escribano et al. 1998, Escribano and Hidalgo 2000, Vargas et al. 2006, Escribano et al. 2007, Hidalgo et al. 2010, Aguilera et al. 2011, Escribano et al. 2014), intensidad de la surgencia (Escribano et al. 2012) y la presencia de El Niño en el ambiente pelágico del norte de Chile (Hidalgo & Escribano 2001).

Estadios tempranos de copépodos en el Sistema de Corrientes de Humboldt (SCH) han sido estudiados para estimar tasas de crecimiento y variabilidad espacio-temporal de abundancia y distribución respecto a cambios en las condiciones ambientales (Torres & Escribano 2003, Hidalgo et al. 2005, Vargas et al. 2006, Hidalgo & Escribano 2007, Hidalgo & Escribano 2008, Ruz et al. 2015, 2017). Sin embargo, escasos estudios han considerado el efecto de la hipoxia asociada a la ZMO sobre adultos y estadios tempranos de copépodos y sus potenciales respuestas a nivel ontogénico (Ruz et al. 2015, *enviado*), donde variaciones en la talla, capacidad natatoria y preferencias alimentarias, podrían influir en la actividad metabólica de estos organismos (Mauchline 1998). El desarrollo exitoso de estadios temprano es esencial para en la dinámica poblacional de los copépodos (Tang et al. 1998, Turner 2004), por lo cual, conocer cómo podrían responder las hembras, huevos y nauplios temprano a cambios oceanográficos, podría ser de gran relevancia en ambientes donde la hipoxia puede constituir una presión de selección sobre poblaciones zooplanctónicas (Dam 2013).

En el norte de Chile (23°S), la comunidad de copépodos habita principalmente la capa superficial oxigenada sobre los 50 m de profundidad y dominan en abundancia especies como *Acartia (Acanthacartia) tonsa* Dana 1849, *Centropages brachiatus* (Dana 1849), *Paracalanus* cf. *indicus* Wolfenden 1905 y *Calanus chilensis* Brodsky 1959, entre otras (Hidalgo et al. 2010, Escribano et al. 2009, Escribano et al. 2012, Razouls et al. 2005-2017). *A. tonsa* es una especie nerítica que forma parte importante de una gran cantidad de tramas tróficas costeras alrededor del mundo (Roman et al. 1993, Marcus et al. 2004, Richmond et al. 2006, Razouls et al. 2005 – 2017). En el SCH, se ha observado que la reproducción de *A. tonsa* se relaciona negativamente con aguas bajas en pH (Aguilera et al. 2013), disponibilidad de alimento (Aguilera et al. 2011) y condiciones hipóxicas, las que aparentemente reducen la sobrevivencia de hembras y el éxito de eclosión de sus huevos (Ruz et al. 2015). Por otro lado, los huevos de copépodos como *A. tonsa* y *P. cf. indicus* tienden a permanecer en aguas

someras bien oxigenadas sobre la ZMO, debido a que son resuspendidos en la columna de agua por acción de la surgencia, lo que ayuda a mantener a estas poblaciones en aguas someras (Ruz et al. 2017).

Estudios previos señalan que los movimientos verticales de la ZMO hacia aguas superficiales podrían aumentar la mortalidad de copépodos pelágicos en el SCH (Yáñez et al. 2012). Sin embargo, se desconoce si la sobrevivencia y tasas vitales del copépodo *A. tonsa* se ven afectadas por cambios en las concentraciones de oxígeno disuelto en la zona norte de Chile. Para esto se plantea como hipótesis, que las condiciones de hipoxia asociadas a la ZMO tienden a disminuir la sobrevivencia y las tasas vitales de hembras y estadios tempranos de *A. tonsa*, potencialmente afectando su reclutamiento y dinámica poblacional en el SCH.

Materiales y Métodos

Trabajo de campo

Experimentos de producción de huevos, éxito de eclosión, tasa de crecimiento y desarrollo naupliar del copépodo *Acartia tonsa*, fueron realizados a partir de muestras de zooplancton recolectadas en la Estación 3 (23° 00.2' S, 70° 28.2' W; profundidad máxima = 120 m) de la Bahía de Mejillones durante los años 2013 y 2014. La Estación 3 corresponde a la estación más externa de la transecta costa-océano de la Serie de Tiempo de Antofagasta, realizada por la Universidad de Concepción (Fig. 1 en Escribano et al. 2012).

Previo a la recolección de zooplancton, perfiles de CTD (Conductividad, Temperatura y Densidad) SeaBird 19 equipado con sensor de oxígeno disuelto (OD) y una sonda multiparámetro YSI EXO2 (C, T y OD) fueron realizados hasta 90 m de profundidad para determinar el borde superior de la ZMO (profundidad con 1,0 mL O₂ L⁻¹); los perfiles de la sonda fueron inmediatamente visualizados a bordo. Una vez analizado los perfiles de oxígeno, se obtuvo agua para los experimentos desde la capa oxigenada (~ 10 m) y de la ZMO (~60 m) usando una botella Niskin de 10-L, con el fin de mantener las características químicas *in situ*. El zooplancton fue recolectado en la capa oxigenada mediante lances oblicuos con una red WP-2 de 200 µm de tamaño de poro. Una vez a bordo, el zooplancton fue almacenado en una nevera con agua de mar y transportados al laboratorio dentro de dos horas.

Trabajo de laboratorio

En el laboratorio, inmediatamente y durante un plazo máximo de dos horas se realizó la selección de hembras ovígeras de *A. tonsa* bajo una lupa estereoscópica. Las hembras fueron seleccionadas en grupos de 10 individuos y dejadas en pocillos de vidrio de 500 mL con agua de mar filtrada (0,7 μ m) en el interior de una cámara fría a 14°C, para su aclimatación por un período máximo de dos horas extras. Luego se procedió a preparar el agua de mar recolectada para las incubaciones. El agua de la capa superficial y de la ZMO fue filtrada en filtros de fibra de vidrio de 0,7 μ m y almacenada por separado a la temperatura promedio (14°C) de la columna de agua durante un año normal sin condiciones de El Niño (Ruz et al. 2015).

Los experimentos de producción de huevos fueron llevados a cabo, utilizando el agua previamente filtrada y aclimatada a 14°C, en tres concentraciones de oxígeno: hipoxia severa (< 0,4 mL O₂ L⁻¹), hipoxia leve (~0,8 mL O₂ L⁻¹) y normoxia (>5,0 mL O₂ L⁻¹). El agua de la ZMO y el agua de la capa oxigenada fue almacenada en bidones de 20 L por separado, burbujeada con nitrógeno gaseoso (N₂) altamente purificado hasta alcanzar las concentraciones experimentales de oxígeno, especialmente para disminuir el exceso de oxígeno disuelto del agua obtenido durante el proceso de filtración. Con una delgada manguera de silicona se recolectaron submuestras de 20 mL de agua de mar mediante llenado por rebalse, para medir el oxígeno disuelto con un sensor óptico OXY-4 Micro-Optode PreSens y así obtener la concentración deseada para cada tratamiento. El sensor de oxígeno fue previamente calibrado usando dos puntos de calibración, 100% y 0% de saturación, siguiendo las instrucciones del fabricante.

Al menos tres viales fueron elegidos al azar para medir la concentración de oxígeno inicial antes de introducir los organismos y un mínimo de dos viales, fueron solamente llenados con agua de mar filtrada para llevar el control del oxígeno en cada experimento. Inmediatamente después, los individuos de *A. tonsa* fueron introducidos en los viales cuidadosamente utilizando pipetas plásticas para las hembras y micropipetas para los huevos. Los viales fueron sellados herméticamente con tapones de goma y sellos de aluminio, cuidando no dejar burbujas en su interior, y luego almacenados en un contenedor con agua aclimatada a 14°C, para minimizar las variaciones de temperatura al interior de la cámara fría.

Experimentos de producción de huevos

Ocho experimentos fueron llevados a cabo para estimar la tasa de producción de huevos de hembras ovígeras de *A. tonsa* durante 24 horas. Seis experimentos fueron realizados en condiciones de hipoxia y normoxia, mientras dos experimentos fueron llevados a cabo sólo en hipoxia (Tabla 1). Luego del período de incubación, los viales fueron abiertos y uno de los siguientes procedimientos fueron utilizados: 1) las hembras fueron removidas con una pipeta y los viales rellenos (1 o 2 mL) con agua de mar en las mismas condiciones de oxígeno, antes de sellarlo nuevamente para continuar con el éxito de eclosión y crecimiento naupliar. 2) Las hembras y los huevos fueron cuidadosamente recolectados con un tamiz (23µm), contados bajo lupa estereoscópica y preservados en formalina al 4%.

La sobrevivencia de las hembras fue evaluada en 206 individuos, bajo lupa al final de las incubaciones en todos los tratamientos y posteriormente, la tasa de producción de huevos (huevos hembra⁻¹ día⁻¹) fue calculada utilizando la siguiente fórmula:

$$PH = N_h \times (24/t) \quad (1)$$

donde *PH* es la tasa de producción de huevos experimental, *N_h* es el número de huevos desovados por hembra durante el tiempo de incubación *t* (en horas) (Runge & Roff 2000).

Éxito de eclosión

El éxito de eclosión fue evaluado por el porcentaje de huevos que eclosionaron a nauplios en todas las incubaciones y estandarizado a 24 horas. Los huevos utilizados para estimar el éxito de eclosión, fueron obtenidos de dos formas: 1) desde los experimentos de producción de huevos descritos anteriormente desde incubaciones individuales de hembras. Aquí el número inicial de huevos fue obtenido desde el conteo de carcasas de los huevos eclosionados y los huevos presentes al final de la incubación; y 2) desde grupos de 50 o 100 hembras que fueron incubadas en condiciones de normoxia durante 24 horas en baldes de 3-L modificados con un tamiz de (200µm) en el interior, para separar las hembras de los huevos y así, evitar el canibalismo. Los huevos fueron recolectados desde el fondo de los baldes con pipetas plásticas, separados en grupos bajo el estereoscopio (2x – 4x) e inmediatamente incubados en condiciones hipóxicas y normóxicas en viales de 100 mL. Los huevos fueron agrupados en

grupos de 20 o 50 huevos por vial (Tabla 1), dependiendo del número de huevos recolectados. El éxito de eclosión y el crecimiento naupliar (siguiente sección) fueron realizados en normoxia e hipoxia leve, debido a la baja sobrevivencia de las hembras y, por lo tanto, a la escasa producción de huevos en hipoxia severa.

Tabla 1.- Resumen de los experimentos (Exp.) de tasa de producción de huevos (A) y de éxito de eclosión en conjunto con crecimiento y desarrollo naupliar (B). Se indica el número de réplicas (viales), el estadio de incubación inicial, el número o rango de individuos (n) incubados y la duración de las incubaciones en días.

Exp.	Fecha	N° viales			Estadio	n	Días
		Hipoxia severa	Hipoxia leve	Normoxia			
A	01/03/2013	34	-	23	Hembra	1	1
A	13/03/2013	-	8	8	Hembra	1	1
A	14/03/2013	-	8	-	Hembra	1	1
A	15/03/2013	-	8	8	Hembra	1	1
A	20/07/2013	-	14	15	Hembra	1	1
A	11/07/2014	-	22	23	Hembra	1	1
A	14/07/2014	-	9	-	Hembra	1	1
A	25/07/2014	-	17	9	Hembra	1	1
B	21/07/2013	-	4	2	Huevos	10 – 64	1 – 3
B	11/01/2014	-	11	13	Huevos	3 – 64	1 – 3
B	25/01/2014	-	-	5	Huevos	21 – 58	1 – 3
B	15/07/2013	-	4	3	Huevos	20 – 100	1 – 3
B	20/07/2013	-	2	-	Huevos	100	1 – 3
B	19/07/2014	-	3	3	Huevos	20	1 – 3
B	25/07/2015	-	8	10	Huevos	20 – 50	1 – 3

Crecimiento y tiempo de desarrollo naupliar.

Las mediciones de talla huevos y nauplios se realizaron en una lupa esterescópica (4X – 8X), modelo Nikon SMZ 1000, equipada con una cámara digital y el software Micrometric SE Premium 4 ($\pm 0.01\mu\text{m}$). Los diámetros de huevos de los experimentos de producción de huevos

fueron medidos ($\mu\text{m} \pm 1 \text{ DE}$), asimismo, se registró el largo y ancho de los nauplios recolectados en los experimentos de éxito de eclosión y crecimiento naupliar.

La tasa de crecimiento y el tiempo de desarrollo fueron estimados con los nauplios eclosionados desde las incubaciones experimentales del éxito de eclosión (Tabla 1). Cada 24 horas durante 3 días, al menos tres viales fueron elegidos al azar para recolectar los huevos no eclosionados y los nauplios en un tamiz de $23 \mu\text{m}$. Los huevos fueron contados y su diámetro registrado. Mientras el cefalosoma de los estadios naupliares (N1 y N2) fue medido bajo lupa con ocular graduado y calibrado. Los nauplios fueron fotografiados e identificados bajo lupa (8x) y microscopio (10x – 40x) siguiendo las similitudes de la morfología externa de *A. tonsa* con sus congéneres *A. clausi* Giesbrecht, 1889 (Ogilvie 1953), *A. bifilosa* (Giesbrecht, 1881) (Yoon 1998) y *A. californiensis* Trinast, 1976 (Trujillo-Ortíz 1986).

Se calculó el biovolumen de las hembras y los nauplios de acuerdo a Torres & Escribano (2003) y Ruz et al. (2015):

$$V = 4/3 \times A \times (w/2) \quad (2)$$

donde V es el volumen en (μm^3), A corresponde al área (μm^2) en una vista dorsal y w al ancho del copépodo. EL biovolumen de los huevos se estimó asumiendo el área y el volumen de una esfera.

El biovolumen de las hembras, nauplios y huevos de *A. tonsa*, fue convertido a biomasa de carbono por un factor de $0.045 \text{ mgC } \mu\text{L}^{-1}$ (Omori e Ikeda 1984). El crecimiento de peso específico de las hembras y huevos fue indirectamente estimado a partir de la aproximación de Kiørboe & Sabatini (1995):

$$g = \ln(W_{he}/W_{hu})/D \quad (3)$$

donde W_{he} y W_{hu} corresponde al contenido de carbono de hembras y huevos, respectivamente. D corresponde al tiempo de desarrollo total (d^{-1}) de *A. tonsa*.

El crecimiento naupliar fue estimado a partir de la ecuación:

$$W = W_i e^{gt} \quad (4)$$

donde W_i corresponde al peso naupliar inicial (μgC), g es la tasa instantánea de crecimiento específico (día^{-1}) y t es el tiempo en (días). A partir de la Ecuación (4), las tasas de crecimiento de estadios subsecuentes de huevo a N2 fue estimada desde la siguiente ecuación:

$$g = \ln(W_{i+1}/W_i)/t \quad (5)$$

donde W_{i+1} y W_i corresponden al peso naupliar (μgC) entre dos estadios subsecuente y t el intervalo de tiempo (días) entre observaciones. El crecimiento naupliar fue calculado desde las Ecuaciones 4 y 5, mientras que el tiempo de desarrollo corresponde al inverso de la Ecuación 5.

Análisis de datos

Para testear si la sobrevivencia de las hembras, el éxito de eclosión y las abundancias de huevos y nauplios de *A. tonsa*, es independiente de la concentración de oxígeno disuelto, se utilizó el Test G de independencia ($\alpha = 0.05$). Posteriormente, la producción de huevos, el éxito de eclosión, el crecimiento y desarrollo naupliar, además del tamaño de huevos y nauplios fueron estadísticamente analizados mediante el Test de Student ($\alpha = 0.05$), previo a la comprobación de los supuestos de normalidad, homocedasticidad e independencia de datos (Zar 1999). El tamaño de las hembras también fue analizado mediante el Test de Student para discriminar cualquier efecto de la talla en los resultados. Todos los datos experimentales fueron reportados como el promedio \pm EE (error estándar), mientras que los parámetros morfológicos de *A. tonsa*, como talla, peso y volumen fueron expresados en promedio \pm DE (desviación estándar). Finalmente, el número de huevos y nauplios (N1 y N2) fue expresado en abundancia relativa (%) al número total de huevos usados por tratamiento. Asimismo, la abundancia promedio \pm EE por cada estadio, fue expresada en porcentaje (%) de abundancia relativa a los estadios encontrados.

Resultados

Sobrevivencia y producción de huevos

La sobrevivencia de hembras de *Acartia tonsa* se estimó en 156 individuos, indicando que es dependiente de la concentración de oxígeno disuelto (Test-G = 145,9; g.l. = 2; $P < 0,001$). En normoxia hubo una sobrevivencia del 100% (n = 86), en hipoxia leve ($0,91 \pm 0,04$) sobrevivió el 33.3% de 88 hembras incubadas y en hipoxia severa ($0,21 \pm 0,01$), ninguna hembra (n = 34) fue capaz de sobrevivir. Los valores de las condiciones de oxígeno experimentales se encuentran registradas en la Tabla 2.

Tabla 2. Concentraciones de oxígeno disuelto ($\text{mL O}_2\text{L}^{-1}$) obtenidas en los experimentos de *A. tonsa*. Se entrega el promedio, error estándar (EE) y el rango (valor min. - max.) para condiciones de normoxia, hipoxia leve y severa.

Condición	Oxígeno disuelto (mL L^{-1})	
	Prom. \pm EE	mín. - max.
Normoxia	$5,77 \pm 0,11$	3,59 - 6,92
Hipoxia leve	$0,64 \pm 0,03$	0,37 - 1,32
Hipoxia severa	$0,22 \pm 0,01$	0,09 - 0,33

Debido a la escasa sobrevivencia de las hembras de *A. tonsa* en condiciones de hipoxia leve y severa, la producción de huevos demostró ser dependiente de la concentración de oxígeno disuelto (Test-G = 27,7; g.l. = 1; $P < 0,001$). La producción de huevos no presentó diferencias significativas entre normoxia e hipoxia leve, pero si entre normoxia e hipoxia severa (T-test; $T = -6,60$; g.l. = 103; $P < 0,001$), y entre hipoxia leve y severa (T-test; $T = -10,4$; g.l. = 112; $P < 0,001$) (Fig. 1). Los valores de producción de huevos fueron en normoxia, hipoxia leve y severa fueron $3,65 \pm 0,47$ (rango = 0 – 19, n = 86), $4,32 \pm 0,56$ (rango = 0 – 24, n = 86) y $0,39 \pm 0,10$ (rango = 0 – 5, n = 34), respectivamente.

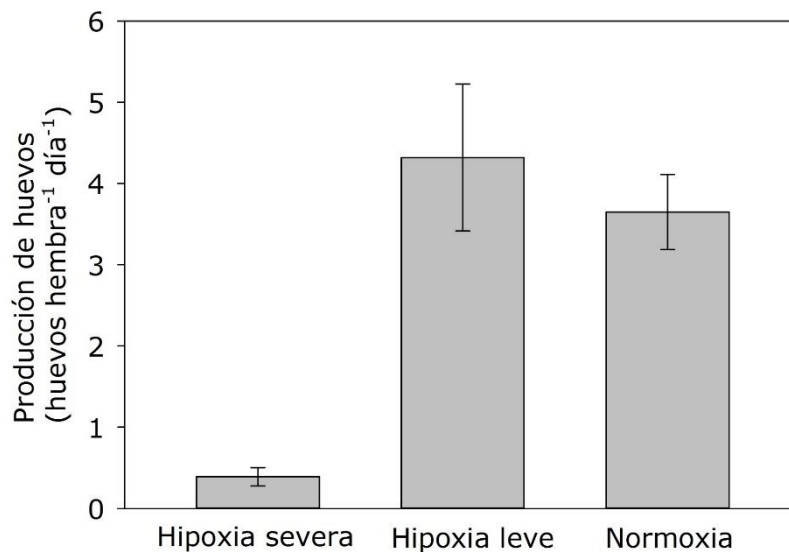


Figura 1.- Producción de huevos de hembras de *A. tonsa* expuestas a diferentes condiciones de oxígeno disuelto: hipoxia severa, hipoxia leve y normoxia.

Éxito de eclosión y crecimiento naupliar

Para evaluar el éxito de eclosión, crecimiento y tiempo de desarrollo naupliar de *A. tonsa* se incubó un total de 578 huevos en hipoxia leve y 368 huevos en normoxia. Al término del período de incubación, en hipoxia sólo se recuperó el 32,2% correspondiente a 95 huevos sin eclosionar, 80 nauplios en estadio N1 y 11 en estadio N2. En normoxia el 83,4% de los huevos incubados fue recuperado al final de las incubaciones, de los cuales, 148 huevos no habían eclosionado, 122 eran nauplios N1 y 37 N2. Los organismos no encontrados se consideraron muertos y degradados por acción bacteriana, valores que alcanzaron el 67,8% y 16,6% hipoxia y normoxia, respectivamente.

En condiciones de hipoxia, se observó una disminución en la abundancia de huevos, con un leve estancamiento entre los días 1 y 2. Los estadios N1 y N2 presentaron un día de desfase en aparecer, una disminución del estadio N1 en el día 2 coincidente con el aumento en abundancia del estadio N2 (Fig. 2a). En cambio, en normoxia se observa una continua disminución de la abundancia de huevos, la cual es coincidente con el aumento en la abundancia de N1 y posterior incremento en abundancia de N2 (Fig.2b). Los individuos no encontrados, fueron considerados muertos y desintegrados, indicando que la sobrevivencia de los estadios temprano de *A. tonsa* es dependiente de la concentración de oxígeno disuelto (G-

Test; $G = 218,4$; g.l. = 1; $P < 0,001$). El éxito de eclosión de *A. tonsa* también fue dependiente del oxígeno disuelto (G-Test; $G = 44,5$; g.l. = 1; $P < 0,001$) y demostró ser significativamente menor (T-test; $T = -29,3$; g.l. = 77; $P < 0,001$) en hipoxia ($10,4 \pm 2,4$ %) que en normoxia (37.6 ± 5.36 %).

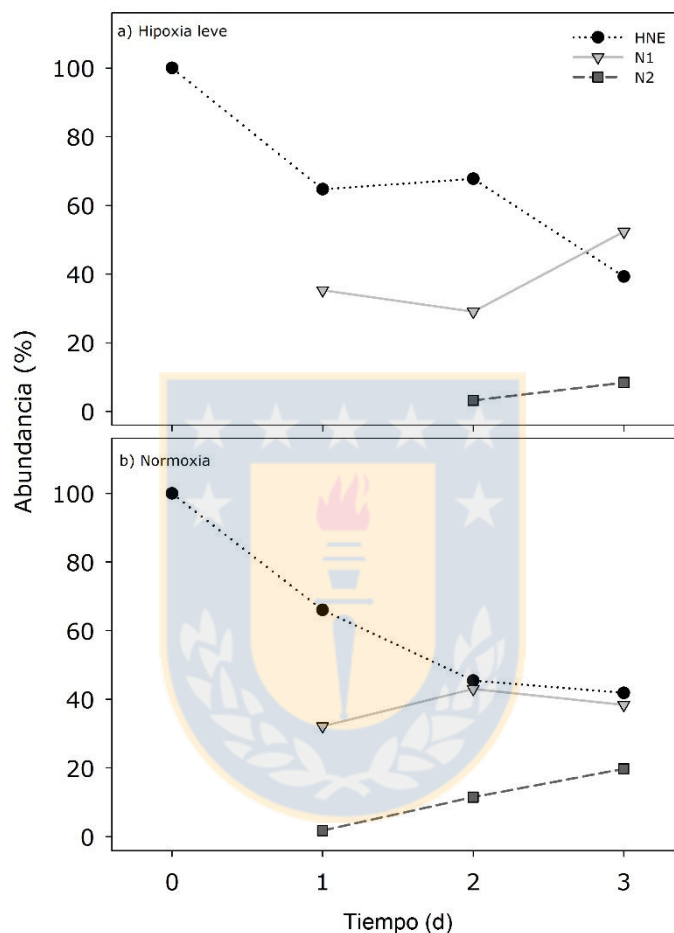


Figura 2.- Abundancia (%) de huevos no eclosionados (HNE), estadios naupliars N1 y N2 de *A. tonsa* durante el período de incubación: a) hipoxia leve y b) normoxia.

El diámetro de los huevos (Test-T; $T = 2,13$; g.l. = 133; $P = 0,035$) y el largo de los estadios N1 (Test-T; $T = 4,22$; g.l. = 140; $P < 0,001$) y N2 (Test-T; $T = 3,29$; g.l. = 41; $P = 0,002$) fueron significativamente diferentes en condiciones de normoxia e hipoxia, mientras que el ancho de los nauplios no fue afectado por la condición de oxígeno. El volumen específico de los huevos se estimó desde las mediciones del diámetro, mientras el volumen de

los nauplios fue calculado considerando las mediciones de largo y ancho. Las estimaciones de volumen de huevos y de los estadios N1 y N2, tampoco se vieron afectadas por el oxígeno disuelto (Tabla 3). El contenido de carbono en los estadios tempranos de *A. tonsa* expuesto a hipoxia y normoxia, sólo mostró diferencias significativas en el estadio N2 (Test-T; T = 2,39; g.l. = 41; P = 0,021) (Tabla 3).

Tabla 3. Estimaciones del volumen y peso en carbono (promedio \pm desviación estándar) de huevos y nauplios de *A. tonsa* en condiciones experimentales de oxígeno (normoxia e hipoxia leve) realizadas a temperatura constante (14°C). Diferencias significativas entre condiciones de oxígeno se encuentran destacadas en negritas.

	Huevos	N1	N2
Normoxia			
Largo/Diámetro (μm)	88,00 \pm 3,61	106,64 \pm 8,35	134,22 \pm 6,06
Ancho (μm)	-	53,61 \pm 5,71	62,99 \pm 5,46
Volumen ($10^5 \mu\text{m}^3$)	3,62 \pm 0,43	1,64 \pm 0,44	2,81 \pm 0,54
Peso ($\mu\text{g C}$)	0,016 \pm 0,002	0,007 \pm 0,002	0,013 \pm 0,002
n	65	63	34
Hipoxia leve			
Largo/Diámetro (μm)	86,00 \pm 6,73	100,38 \pm 11,24	127,02 \pm 4,79
Ancho (μm)	-	52,63 \pm 8,16	60,34 \pm 7,80
Volumen ($10^5 \mu\text{m}^3$)	3,43 \pm 0,78	1,49 \pm 0,56	2,47 \pm 0,76
Peso ($\mu\text{g C}$)	0,015 \pm 0,004	0,007 \pm 0,003	0,011 \pm 0,003
n	70	79	9

La tasa de crecimiento de huevo a N2 no mostró diferencias significativas; el crecimiento naupliar fue $0,27 \pm 0,03 \text{ d}^{-1}$ en hipoxia y $0,26 \pm 0,01 \text{ d}^{-1}$ en normoxia, consecuentemente el tiempo fue similar en ambas condiciones, demorando $3,8 \pm 0,5 \text{ d}$ en hipoxia y $3,9 \pm 0,2 \text{ d}$ en normoxia.

Discusión

El oxígeno cumple un rol fundamental en la estructura de los ecosistemas y en la regulación de la distribución espacio-temporal de la mayoría de los organismos marinos, desde microorganismos a peces (Gilly et al. 2013). En áreas de surgencia costera, los organismos tienden a agregarse en aguas someras ricas en oxígeno por sobre la ZMO (Escribano et al. 2009, Manríquez et al. 2009). Producto del cambio climático global, la somerización y expansión de las ZMOs ha sido inminente, lo que restringe aún más el hábitat de la mayoría de los organismos pelágicos hacia aguas más someras (Bertrand et al. 2010, Stramma et al. 2011).

Algunas especies han desarrollado adaptaciones metabólicas (*e.g* supresión metabólica) y/o realizan migraciones verticales para habitar o refugiarse dentro las ZMOs, como ha sido observado en copépodos de las familias Calanidae, Metridinidae y Eucalanidae (Hidalgo et al. 2005, Auel & Verheye 2007, Teuber et al. 2008). Sin embargo, lugares donde la intensificación de los vientos favorable para la surgencia está ocurriendo (Bakun 1990, Rykaczewski & Checkley 2008, Wang et al. 2015), evitar las condiciones de hipoxia asociadas a los movimientos verticales de las ZMO podría ser una desventaja en especies que no realizan migraciones verticales.

La producción de huevos de *Acartia tonsa* en el presente estudio, fue similar ($24 - 0$ huevos hembra⁻¹ día⁻¹) a valores previamente estimados en el SCH (50 y 3 huevos hembra⁻¹ día⁻¹) (Vargas et al. 2006, Aguilera et al. 2011, 2013, Ruz et al. 2015), excepto en condiciones de hipoxia severa. La existencia de mortalidad inducida por hipoxia en copépodos se ha reportado en *Calanus chilensis* (Ruz et al. 2017), *Paracalanus. cf. indicus* (Yáñez et al. 2012) y *A. tonsa* (Ruz et al. 2015) en el SCH. Lo cual coincide con experimentos realizados en ecosistemas de hipoxia de fondo estacional, donde la hipoxia reduce el crecimiento y aumenta la mortalidad de *A. tonsa* y otros copépodos (Stalder & Marcus 1997, Marcus et al. 2004, Richmond et al. 2006). Nuestros resultados indican que la sobrevivencia y la producción de huevos de *A. tonsa* se encuentra afectada por condiciones de hipoxia $\leq 0,2$ mL O₂ L⁻¹ y por concentraciones entre $1,2$ y $0,5$ mL O₂ L⁻¹, las cuales serían consideradas condiciones de hipoxia letales y subletales, respectivamente (Fig. 3).

Las concentraciones de oxígeno subletales y letales estimadas para *A. tonsa* son menores que valores previamente obtenidos en sistemas de hipoxia de fondo estacional (Roman et al. 1993, Richmond et al. 2006, Elliot et. al., 2013), probablemente debido a que la hipoxia de la ZMO es más intensa que la hipoxia de fondo estacional, generando una presión

de selección sobre la población de *A. tonsa* en el norte de Chile. En esta zona, el hábitat óptimo para las hembras de *A. tonsa* serían aguas con concentraciones sobre 1.5 mL O₂ L, las cuales se ubican sobre la ZMO. Mientras que las hembras que tienen la capacidad de sobrevivir en condiciones de hipoxia subletal podrían sobrellevar los movimientos verticales de la ZMO asociados a la surgencia costera, logrando el éxito de las futuras generaciones de *A. tonsa* en el SCH. Sin embargo, es necesario diseñar experimentos que evalúen con mayor precisión los rangos de oxígeno críticos para las poblaciones de *A. tonsa* y otros copépodos, y así, comprender con mayor precisión como la intensificación de las ZMOs podría afectar al zooplancton y consecuentemente a la productividad en los sistemas de surgencia.

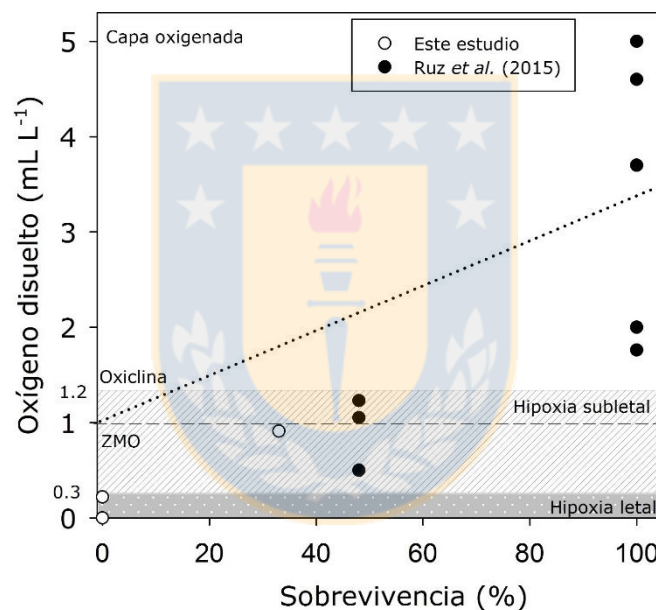


Figura 3.- Esquema de la supervivencia (%) de *A. tonsa* en relación al oxígeno disuelto (mL L⁻¹). Se indican las capas típicas de un perfil de oxígeno en zonas de surgencia costera: capa oxigenada, oxiclina (línea punteada) y zona de mínimo oxígeno (ZMO, línea discontinua). Se muestran las concentraciones de hipoxia subletales y letales para esta especie en el norte de Chile.

Acartia tonsa parece presentar una mayor sensibilidad que *C. chilensis* a la hipoxia asociada a la ZMO en el norte de Chile, indicando la presencia de respuestas especie-específicas (Ruz et al. 2015). Recientemente, respuestas estadio-específicas a la hipoxia han sido observadas en *C. chilensis*, donde las hembras son capaces de sobrevivir en hipoxia e

ingresar al núcleo de la ZMO (Hirche et al. 2014). Mientras que el éxito de eclosión de los huevos de *C. chilensis* pareciera tener una respuesta estacional, su tasa de crecimiento naupliar (N1 – N3) tiende a disminuir en condiciones de hipoxia (Ruz et al. *en revisión*). Nuestros resultados indicaron una alta mortalidad de estadios temprano y una disminución del éxito de eclosión de *A. tonsa* en hipoxia, similar a lo estimado previamente para *A. tonsa* en la Bahía de Mejillones (Ruz et al. 2015). Asimismo, la tasa de crecimiento naupliar de *A. tonsa* no mostró diferencias significativas al contrario de lo estimado en *C. chilensis*, lo cual indicó que el mayor efecto negativo de la hipoxia sobre la población de *A. tonsa* está dado en la sobrevivencia de estadios adultos y huevos, mientras que los nauplios tempranos podrían subsistir condiciones de hipoxia por un corto período, por ejemplo, durante procesos de mezcla de capa oxigenada con aguas provenientes de la ZMO.

En el norte de Chile, la dinámica del zooplancton se encuentra afectada por eventos de surgencia persistentes durante el año, por lo cual los organismos tienen que lidiar con un ambiente altamente heterogéneo, sujetos a cambios en temperatura, estratificación, intensidad de la surgencia y movimientos verticales de la ZMO (Escribano et al. 1998, 2012, 2014, Hidalgo & Escribano, 2008, Ruz et al. 2017). El oxígeno disuelto actúa como un factor modulador de la estructura de los ecosistemas y en sistemas de surgencia costera, como el norte de Chile y determina el hábitat disponible para los organismos que habitan las aguas oxigenadas. La intrusión de la ZMO en aguas someras puede inducir a una mayor mortalidad de las especies que habitan preferentemente en estas aguas, como *Paracalanus cf. indicus* (Yáñez et al. 2012), *A. tonsa* (Ruz et al. 2015, este estudio) y estadios temprano de *C. chilensis* (Ruz et al. 2015). Es importante realizar investigaciones futuras para comprender como podrían responder a la hipoxia otras especies o grupos taxonómicos dominantes del zooplancton, para poder comprender cómo potencialmente podría cambiar la estructura comunitaria de los sistemas de surgencia, ante un escenario de expansión, intensificación y somerización de las ZMOs asociados al calentamiento global.

Las aproximaciones experimentales realizadas en el presente estudio permiten comprender la ecofisiología de hembras adultas y estadios tempranos de *A. tonsa* en el SCH. Además, han permitido establecer que *A. tonsa* es una especie altamente vulnerable en su estado adulto y primeros estadios de desarrollo a las condiciones de hipoxia asociadas a la ZMO, las cuales son capaces de generar efectos subletales y letales, perjudicando la

producción de huevos y, por ende, la permanencia de la población en el ecosistema. Debido a esto, es de gran relevancia comprender como la hipoxia por si sola o en sinergia con otras variables ambientales podrían influenciar la dinámica poblacional de copépodos en sistemas de surgencia costera.



3.3. Capítulo 3

3.3.1. Distribución vertical de huevos de copépodos en la zona de mínimo oxígeno de la Bahía de Mejillones (23°S) en el Sistema de Corrientes de Humboldt

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Resumen

En el Sistema de Corriente de Humboldt (SCH) en el norte de Chile (23°S), se realizaron seis muestreos entre 2013 y 2014, cada uno a ocho profundidades de 5 a 80 m para estudiar el rol de la variabilidad ambiental en la abundancia y distribución vertical de huevos de copépodos dominantes, con énfasis en la hipoxia asociada a la ZMO. Las más altas abundancia de huevos de *Acartia tonsa* y *Paracalanus* cf. *indicus* se encontraron en aguas superficiales sobre la OMZ y la distribución de ambas especies se correlacionó positivamente con Chl-a y la estratificación (Frecuencia de Brunt-Väisälä). Los huevos de *Calanus chilensis* presentaron una distribución más profunda, pero su distribución no se correlacionó con ninguna variable abiótica, en cambio, la distribución de las hembras de esta especie se asoció con eventos de surgencia debido a una correlación positiva con el transporte de Ekman y la salinidad. La distribución vertical de los huevos de copépodos en el SCH puede ser explicada por la variabilidad abiótica, las velocidades de hundimiento de los huevos y el efecto de resuspensión producto de las velocidades verticales de la surgencia, que retienen los huevos de copépodos más pequeños en aguas someras sobre la ZMO mientras, los huevos de *C. chilensis* se hunden llegando a la ZMO en menos de dos días. La supervivencia de los estadios tempranos de copépodos es vital para el éxito de las poblaciones. Cambios en las condiciones ambientales, como intensificación de la surgencia, somerización y expansión de ZMOs podrían afectar la productividad del sistema pelágico en ecosistemas de surgencia costera.

Vertical distribution of copepod eggs in the oxygen minimum zone off Mejillones Bay (23° S) in the Humboldt Current System

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ABSTRACT: In the Humboldt Current System (HCS) off northern Chile (23° S), 6 samplings were conducted in 2013 and 2014, each at 8 depths ranging from 5 to 80 m, to study the role of environmental variability on the abundance and vertical distribution of the eggs of dominant copepods, with special emphasis on hypoxia associated with the oxygen minimum zone (OMZ). The highest levels of abundance of *Acartia tonsa* and *Paracalanus cf. indicus* eggs were in surface waters above the OMZ. The distribution of eggs of both species correlated positively with chlorophyll *a* and stratification (Brunt-Väisälä frequency). *Calanus chilensis* eggs were found at greater depths, and their distribution did not correlate with any abiotic variables, whereas the distribution of *C. chilensis* females was associated with subsurface water upwelling due to a positive correlation with Ekman transport and salinity. The vertical distribution of copepod eggs in the HCS can be explained by abiotic variability, the sinking velocities of the eggs and the resuspension effect of upwelling vertical velocities, all of which may retain the eggs of smaller copepods in waters above the OMZ while *C. chilensis* eggs sink more rapidly, reaching the OMZ in less than 2 d. The survival of early-stage copepods is vital for the success of populations; therefore, any changes in conditions, like upwelling intensification, shoaling, and expansion of OMZs due to climate change, can affect the productivity of the pelagic realm in coastal upwelling ecosystems.

KEY WORDS: *Acartia tonsa* · *Paracalanus cf. indicus* · *Calanus chilensis* · Northern Chile · Sinking velocity

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INTRODUCTION

The Humboldt Current System (HCS) is one of the major Eastern Boundary Upwelling Ecosystems (EBUEs) (Chavez & Messié 2009). It extends from ~42° S to approximately the equator in the southeastern Pacific Ocean (Montecino et al. 2005, Thiel et al.

2007). It is considered one of the most productive EBUEs because it supports high levels of abundance and biomass of planktonic organisms and pelagic fish (Chavez et al. 2003, Alheit & Niquen 2004, Hidalgo et al. 2010).

Biological production in the HCS is driven by frequent upwelling events that cause shoaling of oxy-

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gen-poor Equatorial Subsurface Water, which forms the oxygen minimum zone (OMZ) in the southeastern Pacific and results in deficient levels of dissolved oxygen (DO; $<21.7 \mu\text{mol kg}^{-1}$, $\sim 22.3 \mu\text{M}$) near the surface ($<50 \text{ m}$) (Mann & Lazier 1991, Escribano 1998, Morales et al. 1999). DO in the upper boundary of the OMZ reaches levels of $43.6 \mu\text{mol kg}^{-1}$ ($44.6 \mu\text{M}$, $\sim 1 \text{ ml O}_2 \text{ l}^{-1}$), which limits the aerobic metabolism of zooplankton (Morales et al. 1999, Hidalgo et al. 2005a, Fuenzalida et al. 2009, Ruz et al. 2015) and acts as an oxygen-deficient ecological barrier ('barrera ecológica deficiente en oxígeno', BEDOX) to plankton biomass, abundance, and diversity, causing plankton to aggregate in oxygenated surface waters in the photic zone (Donoso & Escribano 2014). Below the BEDOX layer, DO concentrations decrease dramatically to as low as $4.36 \mu\text{mol kg}^{-1}$ ($4.46 \mu\text{M}$, $\sim 0.1 \text{ ml O}_2 \text{ l}^{-1}$) and even reach anoxic levels (Ulloa et al. 2012).

Approximately 80% of zooplankton in this ecosystem are copepods (Escribano & Hidalgo 2000, Hidalgo & Escribano 2001, Escribano et al. 2007), with 119 species identified (Hidalgo & Escribano 2001, Hidalgo et al. 2010, Fierro et al. 2016). However, the copepod community in the HCS off the Chilean coast is mainly dominated by calanoid copepods such as *Acartia tonsa*, *Calanus chilensis*, *Centropages brachiatus*, and *Paracalanus cf. indicus*, among others (Hidalgo et al. 2010, 2012, Escribano et al. 2012, Pino-Pinuer et al. 2014).

The temporal distribution of copepods in the HCS (23° S) has been studied for *C. chilensis* (Escribano 1998, Escribano & McLaren 1999, Hidalgo & Escribano 2007, 2008, Ruz et al. 2015), *A. tonsa* (Ruz et al. 2015), *C. brachiatus* (Hidalgo & Escribano 2007, 2008), and *Eucalanus inermis* (Hidalgo et al. 2005b). Eggs, nauplii, copepodites, and adults are abundant throughout the year, suggesting continuous reproduction or several reproductive events in the northern coastal zone of Chile (Hidalgo & Escribano 2008, Ruz et al. 2015). These studies have primarily included analyses of samples from integrated vertical trawls (Escribano 1998) or from specific strata, especially for eggs, which are often collected from water samples above 15 m because the chlorophyll *a* (chl *a*) maximum is typically located around this depth (Iriarte et al. 2000). Although a high abundance of eggs and early stages (size $<200 \mu\text{m}$) has been reported close to this depth (15 m) off south-central and northern Chile (Hidalgo & Escribano 2007, 2008), the depths at which females spawn and the eggs continue developing in the HCS have not yet been studied.

Upwelling prevails throughout the year off the northern Chilean coast ($18\text{--}27^\circ \text{ S}$) (Thiel et al. 2007,

Díaz-Ochoa et al. 2011, Escribano et al. 2012), continuously exposing copepods to abrupt changes in temperature, food availability, and low oxygen conditions, which are key factors that influence copepod physiology and may produce stress on their populations (Escribano et al. 2012, Ruz et al. 2015). Most copepods, like *P. cf. indicus* and *A. tonsa*, tend to inhabit well-oxygenated surface waters, where conditions are favorable for them to complete their life cycles, while others such as *E. inermis* engage in diel vertical migration to avoid predators, releasing their eggs in the OMZ (Hidalgo et al. 2005b, Escribano et al. 2009). Research on the vertical distribution of copepods associated with the OMZ in the HCS has only included copepodite and adult stages (Escribano et al. 2009, Hirche et al. 2014), leaving a gap in our knowledge about the vertical distribution of eggs.

Mejillones Bay is among the most important upwelling centers off northern Chile (Marín & Olivares 1999, Thiel et al. 2007) and is characterized by cold hypoxic subsurface waters due to upwelling throughout the year (Marín & Olivares 1999) and a shallow upper boundary of the OMZ ($\leq 26 \text{ m}$), which supports year-round copepod reproduction in well-oxygenated surface waters (Hidalgo et al. 2005b, Ruz et al. 2015). In this area, copepod reproduction is apparently not limited by food availability, and the main factors that affect the population dynamics are changes in temperature and DO associated with upwelling intensity (Escribano et al. 1998, 2012, Hidalgo & Escribano 2008, Hidalgo et al. 2010). While information is available about the vertical distribution of later and adult copepod stages (Escribano et al. 2009), we know little about the vertical distributions of copepod eggs and their relationship with abiotic variability, mainly temperature, density, and the depth of the OMZ.

Recent experimental evidence has indicated inter- and intra-specific variability among copepods in terms of tolerance to hypoxia. Female survival and hatching success of *A. tonsa* are apparently more vulnerable to lower oxygen concentrations (ca. $43.6 \mu\text{mol kg}^{-1}$) than those of *C. chilensis* (Ruz et al. 2015), which is not significantly affected. However, the responses of *C. chilensis* to hypoxia may have a seasonal signal. P. M. Ruz et al. (unpubl. data) observed a 30% reduction in the hatching success of *C. chilensis* and a notable decrease in the growth rates of the early stages of nauplii under hypoxia ($<34.8 \mu\text{mol O}_2 \text{ kg}^{-1}$), whereas the metabolism of females was not significantly affected by short-term exposure to hypoxia. If the low oxygen concentrations of the OMZ are a limiting factor for the growth and development of copepods at early stages, it is expected

that reproduction and egg laying take place in a suitable habitat for egg development and hatching before eggs reach the OMZ by natural processes of particle sedimentation. In this context, laying eggs in surface, well-oxygenated, and food-rich waters could be a survival strategy to maintain copepod populations over time.

In this study, we determined whether the vertical distribution of the eggs of dominant copepods in the HCS in northern Chile is associated with abiotic variability as a result of upwelling. The variability of the upwelling intensity results in changes of temperature, water density, and vertical movements of the OMZ that could reduce or expand the oxygenated surface layer that most copepods inhabit, especially in areas where upwelling intensity and the OMZ's extension are increasing due to climate change. We hypothesized that the hypoxia associated with the OMZ restricts the vertical distribution and abundance of eggs of dominant copepods to well-oxygenated shallow waters in Mejillones Bay, which in turn may play a key role in annual population production. To evaluate this hypothesis, discrete seasonal vertical copepod egg profiles were obtained in the austral winters and summers of 2013 and 2014. These data were correlated with abiotic and biotic parameters and stratified abundances of females of the 3 dominant copepods in the Humboldt Current ecosystem.

MATERIALS AND METHODS

Field studies

To study the vertical distribution of eggs of dominant epipelagic copepods in the coastal upwelling zone off Mejillones Bay (23° S), we conducted hydrographic surveys, net tows, and water sampling in 2013 and 2014. The study site was Stn 3 (23° 00.2' S, 70° 28.2' W) of the Antofagasta Time-Series at Mejillones Bay (Fig. 1) (Escribano et al. 2012). Two autonomous oceanographic profilers calibrated by their respective suppliers, the CTD-O Seabird 19 plus (Sea-Bird Electronics) and the EXO2 Multiparameter Sonde (YSI), were deployed at Stn 3 to obtain measurements of salinity (S), temperature (T), density (σ_t), DO, and fluorescence down to ~100 m depth. On 15 March and 16 July 2013, and 18 January and 29 July 2014, we used the 2 devices to obtain 2 independent profiles: one as backup and the other for semi-instantaneous visualization. The data were extracted using the EXO2 profiler to determine the



Fig. 1. Study area in northern Chile (23° S). The black star denotes the location of Stn 3 in Mejillones Bay

depth of the OMZ. Once the upper limit of the OMZ was identified ($\sim 43.6 \mu\text{mol kg}^{-1}$, Morales et al. 1999, Hidalgo et al. 2005b, Fuenzalida et al. 2009), 3 strata were categorized: the oxygenated surface layer, the oxycline at depths with a gradient of $> 2.05 \mu\text{mol kg}^{-1} \text{ m}^{-1}$ ($\sim 2.1 \mu\text{M m}^{-1}$; Paulmier & Ruiz-Pino 2009), and the OMZ ($< 43.6 \mu\text{mol kg}^{-1}$).

Six profiles were collected with Niskin bottles (10 l) to obtain the abundance of eggs of the dominant copepods at depths of 5, 10, 20, 30, 40, 50, 70, and 80 m, which comprised the 3 DO layers previously described. Four profiles were collected in 2013 and 2 in 2014. Additionally, zooplankton samples were obtained using a WP-2 net (200 μm mesh size) equipped with a flowmeter and a closed net system, to collect females of the dominant species under study. Vertical stratified trawls were conducted at varying depths, according to the DO profile and the 3 previously categorized layers.

Oceanographic data

Coastal upwelling favorable conditions were calculated using the cumulative Ekman transport (CET), which is the cumulative transport effect over time. The CET is the wind-generated mass transport per unit of width over depth of the Ekman layer and is expressed as $\text{kg m}^{-1} \text{ s}^{-1}$ (a typical wind stress of 0.1 N m^{-2} at 45° gives a mass transport of $1000 \text{ kg m}^{-1} \text{ s}^{-1}$; Tomczak & Godfrey 2003). CET is useful for visualizing the beginning and end dates of upwelling/down-

welling periods at a seasonal scale (Bograd et al. 2009). The CET was integrated monthly from 1 January 2013 to 31 December 2014 and integrated from 10 d (synoptic scale) to the sampling date to know the upwelling conditions at that time. The CET was computed from the daily-averaged sea level pressure fields at 1° resolution centered at 22° 30' S and 70° 30' W. The data were obtained from the US Navy Fleet Numerical Meteorology and Oceanography Center (FNMOC; data retrieved from <http://coastwatch.pfeg.noaa.gov/erddap/index.html>). Ekman transport is defined as negative for northerly winds (downwelling) and positive for southerly winds (upwelling).

To characterize water column conditions, the mean and standard deviations of abiotic variables T, S, DO, chl *a*, σ_t , and stratification were estimated per stratum (oxygenated, oxycline, and OMZ). The stability or stratification of the water column was estimated as the Brunt-Väisälä (BV) or buoyancy frequency from CTD density profiles. The BV was expressed in s^{-1} and calculated as:

$$BV = [(g/\rho) \times (\partial\rho/\partial z)]^{1/2} \quad (1)$$

where g is gravitational acceleration (9.8 m s^{-2}), ρ is the density of seawater (1.025 g cm^{-3}), and $\partial\rho/\partial z$ is the density distribution as a function of depth z . BV frequencies >0 show that the water column is stratified (stable), while low BV values suggest that the water column is well mixed (Stull 1995, Landaeta et al. 2013).

Chl *a* profiles were derived from fluorescence (Flu) using a linear regression model between these 2 variables ($\log \text{ chl } a = -0.2925 + 1.4105 \times \log \text{ Flu}$; $n = 286$, $R^2_{\text{adj}} = 0.879$, $p < 0.0001$, authors' unpubl. data)

Sample analysis

Females of *Acartia tonsa*, *Paracalanus cf. indicus*, and *Calanus chilensis* were identified in the laboratory and counted from half of each sample, using a Nikon SMZ1000 stereoscopic microscope at 2× and

4× magnification. As well, 19 l of seawater were obtained with Niskin bottles and sieved at 20 μm to collect eggs, which were then preserved in a 2% buffered formalin solution to estimate their abundance. The eggs of these species were identified and counted at 8×, 10×, and 40× magnification. Identification was based on egg size and morphological characteristics (e.g. spiny or smooth surface) according to earlier egg production experiments with these species (Hidalgo 2005, Ruz et al. 2015, P. M. Ruz unpublished data), as well as morphological characteristics from published data on *A. tonsa* (Hansen et al. 2010a,b) and *Paracalanus parvus* (Checkley 1980) (Table 1). Using these features, the *P. cf. indicus*, *A. tonsa*, and *C. chilensis* eggs were distinguished from those of other calanoid copepods in the region. Female and egg abundances were reported as the numbers of individuals (or eggs) m^{-3} . A single parameter was calculated for females and eggs of each species, to represent the population's center of gravity over the vertical axis. To this end, depth-weighted averages (DWA) were calculated as:

$$\text{DWA} = \frac{\sum_{n=1}^n (x_i \cdot z_i)}{\sum_{n=1}^n x_i} \quad (2)$$

where x_i is the abundance of each species in stratum i (ind. m^{-3}), z_i is the corresponding mid depth (m) of stratum i , and n is the total number of sampled strata. These values were related to the concentrations of DO and environmental variables.

The abundances of females and eggs were used to estimate the integrated *in situ* egg production rate (EPR) using the approach of Edmonson (1968) for broadcast spawning species, estimated for the copepods *Clausocalanus forcatus* (Bi & Benfield 2006), *Calanus chilensis*, *Centropages brachiatus*, and *A. tonsa* (Hidalgo & Escribano 2007, 2008, Ruz et al. 2015), which is defined as:

$$\text{EPR} = (N_{\text{eggs}}/N_{\text{females}})/\text{DT} \quad (3)$$

where EPR is the *in situ* egg production rate (eggs $\text{female}^{-1} \text{ d}^{-1}$), N_{eggs} is egg abundance (number m^{-3}), N_{females} is female abundance (number m^{-3}), and DT

Table 1. Summary of size and morphological characteristics of the eggs of dominant copepods in northern Chile (23° S)

Species	Diameter (μm , mean \pm SD)	Surface	Color
<i>Acartia tonsa</i>	75 \pm 1.2, range = 71–83 (14°C) ^a ; 85 \pm 4.0 (6°C) to 80 \pm 3.0 (24°C) ^b	No spines or short spines ^c	Brown, yellowish, or orange to brown ^d ; opaque light pink ^e
<i>Paracalanus cf. indicus</i>	~73 (<i>P. parvus</i>) (18°C) ^f ; ~70 ^g	Smooth ^g	Opaque light yellow ^g
<i>Calanus chilensis</i>	160 \pm 0.3, range = 143–176 (14°C) ^a	Smooth ^g	Opaque light white ^g

^aRuz et al. (2015); ^bHansen et al. (2010a); ^cMarcus & Wilcox (2007); ^dHansen et al. (2010b); ^eP. M. Ruz unpubl. data;

^fCheckley (1980); ^gHidalgo (2005)

represents the embryonic development time (d) as a function of temperature as described by Bělehrádek (1935) and determined using:

$$DT = a \times (T - t_0)^{-b} \quad (4)$$

where T is the temperature ($^{\circ}\text{C}$), and a , t_0 , and b are constants. The parameters a and t_0 are species specific, whereas b is a fixed value for copepods ($= 2.05$; McLaren et al. 1969). The species-specific equations of Bělehrádek embryonic development time were obtained from published data for *A. tonsa* (McLaren et al. 1969, Mauchline 1998), *P. cf. indicus* (Escribano et al. 2014), and *C. chilensis* (Escribano et al. 1998, Hidalgo & Escribano 2007, Hidalgo & Escribano 2008). To solve these equations, DT values were estimated using the *in situ* temperatures at each depth where eggs were collected, according to the following equations:

$$DT = 489 \times (T - 1.8)^{-2.05} \text{ (for } A. \textit{ tonsa)} \quad (5)$$

$$\ln(DT + 1) = -0.112 + 0.078 \times T \text{ (for } P. \textit{ cf. indicus)} \quad (6)$$

$$DT = 947.7 \times (T + 11.0)^{-2.05} \text{ (for } C. \textit{ chilensis)} \quad (7)$$

Data analysis

Multivariate statistics were used to assess spatial and temporal changes in egg and female abundances and environmental variables following the methods described by Clarke (1993), using E-PRIMER (v. 7.0.11) (Clarke & Gorley 2015) set at 999 permutations and a significance level of 0.1% ($\alpha = 0.01$). Differences in the vertical distribution of *A. tonsa*, *P. cf. indicus*, and *C. chilensis* among layers were assessed by 1-way analysis of similarity (ANOSIM) for the abundance of eggs and females of the 3 species. ANOSIM is a permutation-based non-parametric test (analogue of 1-way analysis of variance), which uses the difference between ranked values of similarity measures (e.g. Bray-Curtis) of the abundance of organisms among sample groups. The abundances were transformed by applying the fourth root and the Bray-Curtis similarity index was used to obtain resemblance matrices (Quinn & Keough 2002). Environmental variables (T , S , DO , density, BV , CET and $\text{chl } a$) were used as predictors and normalized in order to obtain comparable scales of magnitude (Clarke et al. 2014).

The BEST routine was used to identify environmental variables that best predict egg and female abundance. The BEST routine works by linking multivariate biotic patterns to sets of environmental variables to search which environmental variables are

most likely driving the observed pattern in the abundances (Clarke & Gorley 2015). BEST was used with the BIO-ENV method, which examines all possible combinations of variables, from each environmental variable separately, through to all at the same time (analogue of stepwise multiple correlation). This method computes a rank correlation between the elements of similarity matrices for environmental and biological data giving the 'best fit' (higher Spearman rank correlation) of environmental variables explaining changes in biotic data. The best 10 results were included in the analysis. The abiotic and biotic data are reported as the mean \pm SD.

RESULTS

Environmental conditions

Mejillones Bay was strongly influenced by coastal upwelling as indicated by positive CET for most of the study period, except June 2013 and August 2014. The index ranged from $-41\,550$ to $898\,185 \text{ kg s}^{-1} \text{ km}^{-1}$ for August (winter) and January (summer) 2014, respectively. Coastal upwelling prevails year-round, with lower CET values from March/April to August in 2013 and 2014 (Fig. 2, Table 2).

The vertical structure of the water column showed some variability on a seasonal scale. The vertical temperature profiles suggest the presence of 2 periods denoted by changes of the sea surface temperature (SST), with warmer waters at the surface layer in austral summer (sea surface temperature 17°C) than in winter (14.5°C), whereas a gradual decrease with depth was observed until subsurface waters reached temperatures close to 13°C during the sampling period. Variability in salinity profiles also indicates 2 periods, with less saline waters in March and January (summer) than in July (winter) (Fig. 3a,c,e,g, Table 3)

Vertical DO distribution was sharply defined, with the permanent presence of oxygen-poor waters in the subsurface layer (Table 3). An oxygenated surface layer was present only in July, whereas in March 2013 and January 2014 the oxycline reached surface waters. The thickness of the oxycline ranged between 12 and 33 m, and its DO gradient varied from 3.3 to $24.6 \mu\text{mol kg}^{-1} \text{ m}^{-1}$. The depth of upper boundary of the OMZ was very heterogeneous, ranging from 11 to 44 m in the water column, whereas below 45 m to the bottom, low-oxygen waters associated with the OMZ were consistently present throughout the study period, regardless of the intensity of CET (Fig. 3a,c,e,g, Table 2).

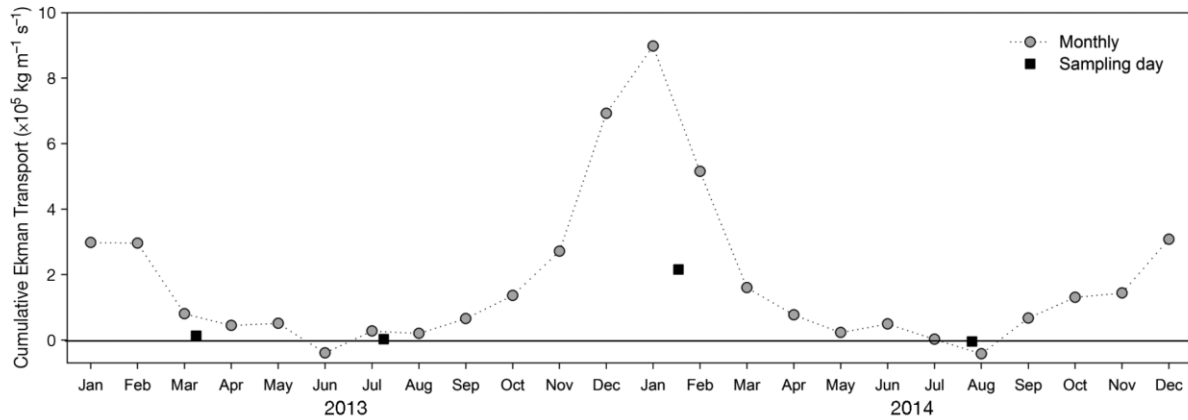


Fig. 2. Temporal variability of cumulative Ekman transport ($\text{kg m}^{-1} \text{s}^{-1}$) off Mejillones Bay, Chile (23°S). Black squares are cumulative transports for each sampling day, and grey circles are cumulative transports for each month during the study period

Table 2. Vertical structure of the dissolved oxygen (DO) profile and synoptic cumulative Ekman transport (CET) during the study period. OSL: oxygenated surface layer, OXY: oxycline, OMZ: oxygen minimum zone (OMZ). Dates are given as mm/dd/yyyy. -: no data, as no OSL was present in summer

Date	Season	OSL Depth (m)	OXY			OMZ		CET ($\text{kg s}^{-1} \text{km}^{-1}$)
			Beginning (m)	Bottom (m)	Thickness (m)	DO gradient ($\mu\text{mol kg}^{-1} \text{m}^{-1}$)	Upper boundary (m)	
03/13/2013	Summer	-	5	27	22	6.4	26	13827.5
07/16/2013	Winter	19	19	47	28	3.3	44	3028.9
01/18/2014	Summer	-	1	12	12	24.6	11	215883.6
07/29/2014	Winter	15	15	30	15	6.0	29	-3968.3

The density profiles showed similar patterns throughout the study period, but with slightly lower values in winter of 2013, led by changes in temperature and salinity (Fig. 3b,d,f,h). BV frequencies varied from $(0.15 \pm 0.21) \times 10^{-4}$ to $(6.98 \pm 5.21) \times 10^{-4} \text{ s}^{-1}$, which demonstrated a high degree of stability in the first 20 m of the water column, except in winter 2014, when a well-mixed water column was present (Fig. 3d,h, Table 3).

Chl *a* profiles ranged from 7.06 to 0.03 mg m^{-3} from the surface to the sea floor. Values $>2.0 \text{ mg m}^{-3}$ were found above 12 m depth, whereas a second chl *a* maximum was observed in subsurface waters close to 40 m in summer 2014, with values ranging from 2.01 to 2.15 mg chl a m^{-3} (Fig. 3a,c,e,g).

Vertical distribution of copepod eggs and females

The abundance and distribution of eggs of the dominant copepods *A. tonsa*, *P. cf. indicus*, and *C. chilensis* were associated with changes in abiotic conditions. The vertical distribution of these species in Mejillones Bay were heterogeneous and were

associated with changes in oceanographic conditions as the result of different intensities of upwelling events during the study period (Figs. 2 & 3), reflected in egg and female densities (Table 4).

Acartia tonsa

The eggs of *A. tonsa* had a mean diameter of $79.5 \pm 3.6 \mu\text{m}$ (range = 72.1–85.9 μm , $n = 45$). The vertical distribution of egg abundance differed significantly (1-way ANOSIM, $R = 0.09$, $p = 0.04$). The highest levels of abundance were found when the oxycline was in shallow waters and tended to decrease with depth into the OMZ (pairwise test, $R = 0.18$, $p = 0.005$), except in the samples collected in July of both years, when no eggs were found (Fig. 3b,d,f,h). The vertical distribution in the abundance of *A. tonsa* females was similar to that of eggs, with the greatest abundance in shallow water (1-way ANOSIM, $R = 0.09$, $p = 0.02$). There were significant differences in the abundance of females in the oxycline and the OMZ (pairwise test, $R = 0.14$, $p = 0.007$; Table 4)

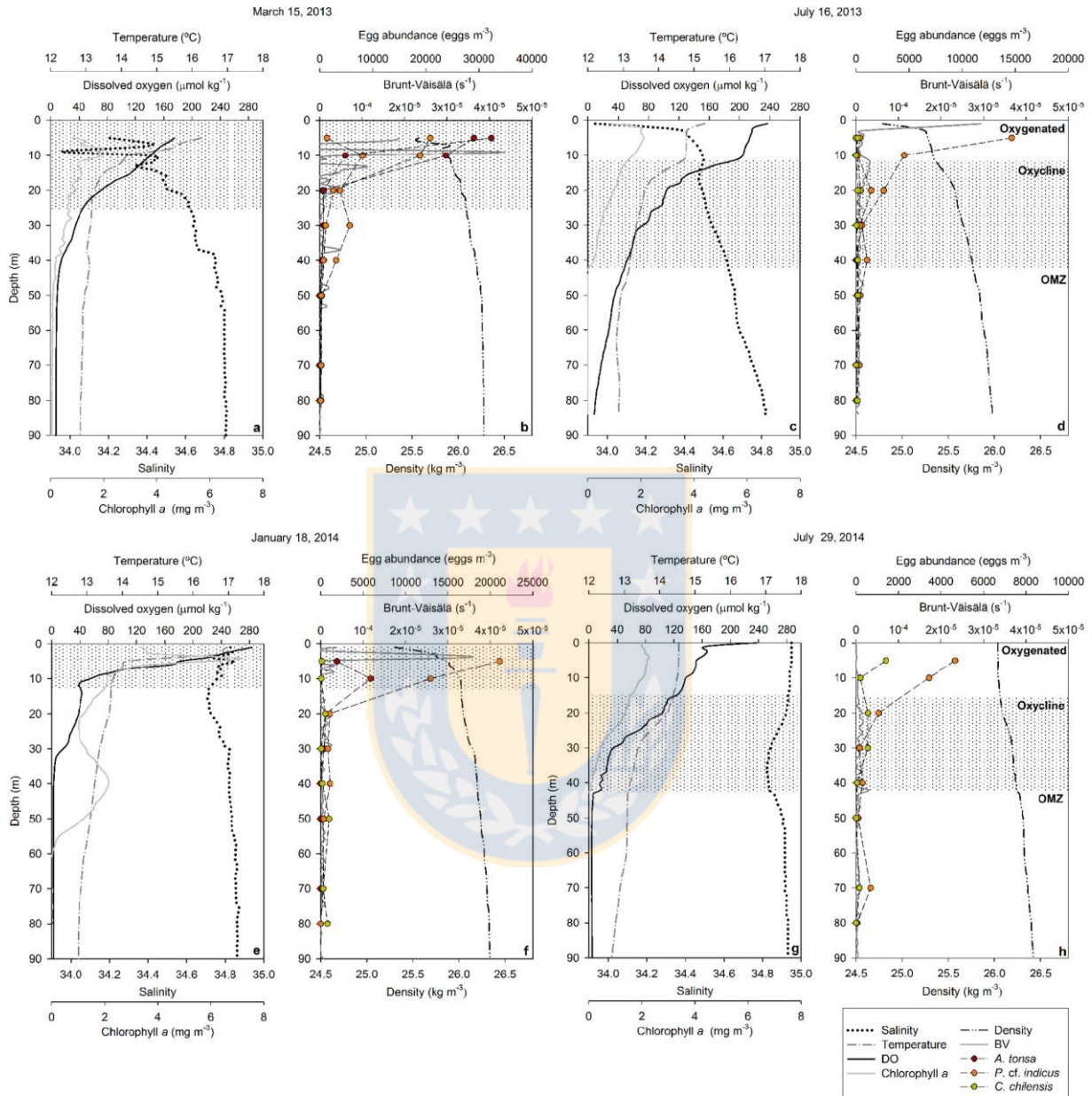


Fig. 3. Vertical profiles of temperature ($^{\circ}\text{C}$), salinity, dissolved oxygen (DO) ($\mu\text{mol kg}^{-1}$), chlorophyll *a* (mg m^{-3}), Brunt-Väisälä frequency (s^{-1}), and density (σ_t , kg m^{-3}), and the vertical distributions of egg abundance (eggs m^{-3}) of *Acartia tonsa*, *Paracalanus cf. indicus*, and *Calanus chilensis* at Stn 3 in Mejillones Bay, Chile (23°S ; see Fig. 1) for (a,b) 15 March 2013, (c,d) 16 July 2013, (e,f) 18 January 2014, and (g,h) 29 July 2014. The layers were categorized according to the vertical distribution of DO: oxygenated surface layer, oxycline (grey background), and oxygen minimum zone (OMZ). Panels (a–d) intentionally present 2 egg abundance data sets to better show their variability in the field

A multivariate analysis of the variations in *A. tonsa* eggs compared to that of environmental conditions (BEST routine) showed that the strongest Spearman's rank correlation was obtained with chl *a* ($\rho = 0.41$, $p =$

0.001), followed by a combination of BV and chl *a* ($\rho = 0.32$, $p = 0.001$). The strongest correlation for the same analysis for *A. tonsa* females was a combination of salinity and chl *a* ($\rho = 0.528$, $p = 0.001$; Table 5).

Table 3. Summary (mean \pm SD) of abiotic conditions and chlorophyll *a* in Mejillones Bay, Chile. The data are grouped by year, sampling date, and layers according to the dissolved oxygen profile, oxygenated layer, oxycline, and oxygen minimum zone (OMZ)

Year	Season	Layer	Depth (m)	Temperature (°C)	Salinity	Dissolved oxygen ($\mu\text{mol kg}^{-1}$)	Density σ_t (kg m^{-3})	Brunt-Väisälä frequency ($\times 10^{-4} \text{ s}^{-1}$)	Chl <i>a</i> (mg m^{-3})
2013	March 15 (Summer)	Oxycline	5–25	13.98 \pm 1.00	34.44 \pm 0.16	102.36 \pm 43.92	25.94 \pm 0.14	4.52 \pm 11.3	2.11 \pm 0.88
		OMZ	26–99	12.94 \pm 0.10	34.77 \pm 0.06	11.81 \pm 8.25	26.24 \pm 0.06	0.29 \pm 0.74	0.48 \pm 0.24
	July 16 (Winter)	Oxygenated	0–10	14.59 \pm 0.03	33.70 \pm 0.22	192.61 \pm 15.88	24.29 \pm 0.17	6.98 \pm 5.21	1.23 \pm 0.37
Oxycline		10–43	14.25 \pm 0.23	35.00 \pm 0.33	126.73 \pm 27.94	25.7 \pm 0.29	3.09 \pm 3.25	0.74 \pm 0.49	
2014	January 18 (Summer)	Oxycline	1–13	14.64 \pm 1.32	34.77 \pm 0.04	167.44 \pm 82.60	25.85 \pm 0.21	4.71 \pm 11.03	3.34 \pm 1.57
		OMZ	11–90	13.12 \pm 0.32	34.82 \pm 0.05	10.81 \pm 13.99	26.21 \pm 0.10	0.35 \pm 3.13	1.33 \pm 0.61
	July 29 (Winter)	Oxygenated	0–15	14.49 \pm 0.05	35.94 \pm 0.01	149.58 \pm 27.34	26.04 \pm 0.01	0.15 \pm 0.21	2.01 \pm 0.20
		Oxycline	15–37	13.74 \pm 0.42	35.86 \pm 0.05	83.34 \pm 21.97	26.15 \pm 0.05	0.68 \pm 0.517	0.81 \pm 0.47
		OMZ	32–103	12.90 \pm 0.23	35.89 \pm 0.04	8.62 \pm 8.82	26.34 \pm 0.07	0.32 \pm 0.46	0.23 \pm 0.11

Table 4. Summary (mean \pm SD) of the abundances of eggs (m^{-3}) and females (m^{-3}) of *Acartia tonsa*, *Paracalanus cf. indicus*, and *Calanus chilensis* in Mejillones Bay (Stn 3), Chile, during the sampling period. The data are grouped by year, season, and layers according to the dissolved oxygen profile: oxygenated layer, oxycline, and oxygen minimum zone (OMZ). Different superscript letters denote significant differences ($\alpha = 0.01$) among layers within species and season

Year	Season	Layer	Eggs m^{-3}			Females m^{-3}		
			<i>A. tonsa</i>	<i>P. cf. indicus</i>	<i>C. chilensis</i>	<i>A. tonsa</i>	<i>P. cf. indicus</i>	<i>C. chilensis</i>
2013	Summer	Oxycline	15219.6 \pm 16201.2 ^a	9237.4 \pm 6713.4 ^a	0	276.61 \pm 272.48 ^a	808.21 \pm 579.33 ^a	1.53 \pm 1.95 ^a
		OMZ	243.3 \pm 298.7 ^b	1203.6 \pm 1420.8 ^b	0	10.55 \pm 7.25 ^b	23.02 \pm 14.37 ^b	0.04 \pm 0.08 ^b
	Winter	Oxygenated	0	4938.2 \pm 3650.9 ^{a,c}	73.1 \pm 103.3	7.74 \pm 5.42 ^{a,b}	233.81 \pm 100.57 ^{a,c}	6.55 \pm 4.34 ^{a,c}
		Oxycline	0	891.1 \pm 781.3 ^c	154.6 \pm 135.7	0.72 \pm 0.45 ^a	141.46 \pm 219.73 ^c	6.93 \pm 4.85 ^c
2014	Summer	Oxycline	3911.8 \pm 2786.8 ^a	17029.4 \pm 5781.6 ^a	88.2 \pm 41.6	147.48 \pm 177.92 ^a	693.21 \pm 557.37 ^a	29.39 \pm 5.25 ^a
		OMZ	162.9 \pm 260.0 ^b	600.9 \pm 455.0 ^b	467.2 \pm 359.4	5.62 ^b	19.74 ^b	0.74 ^b
	Winter	Oxygenated	0	4047.2 \pm 871.2 ^{a,c}	798.9 \pm 848.6	0	2.61 \pm 1.09 ^{a,c}	0.17 \pm 0.18 ^{a,c}
		Oxycline	0	605.9 \pm 627.6 ^c	546.5 \pm 8.4	0	0.06 \pm 0.02 ^c	0.04 \pm 0.05 ^c
		OMZ	0	281.5 \pm 285.3 ^b	51.4 \pm 74.1	0	0.003 \pm 0.003 ^b	0.001 \pm 0.002 ^b

The DWA for *A. tonsa* showed that eggs and females were concentrated above 15 m depth, while the integrated *in situ* EPR ranged between 4.4 and 13.2 eggs female⁻¹ d⁻¹. The EPR per stratum showed lower EPR in the OMZ than the oxycline, while no eggs were found in July of either year (Table 6).

Paracalanus cf. indicus

P. cf. indicus eggs had a mean diameter of 76.3 \pm 4.2 μm (range = 66.9–84.8 μm , $n = 61$). There was significant variation in the vertical distribution of egg abundance (1-way ANOSIM, $R = 0.23$, $p = 0.001$). Similar to *A. tonsa*, the highest levels of abundance were

found in the shallow surface layer in oxygenated waters or in the oxycline. There were significant differences in egg abundance between the oxycline and OMZ (pairwise test, $R = 0.26$, $p = 0.01$), and between

Table 5. Spearman rank correlations ($p = 0.001$) from the BEST routine for eggs and females of *Acartia tonsa*, *Paracalanus cf. indicus*, and *Calanus chilensis*. The table shows the 3 best results. The variables are chlorophyll *a* (chl *a*), Brunt-Väisälä frequency (BV), salinity (S), dissolved oxygen (DO), cumulative Ekman transport (CET), and temperature (T)

BEST results for:	<i>Acartia tonsa</i>		<i>Paracalanus cf. indicus</i>		<i>Calanus chilensis</i>	
	ρ	Variables	ρ	Variables	ρ	Variables
Eggs	0.414	Chl <i>a</i>	0.348	Chl <i>a</i>	0.367	CET
	0.320	BV, chl <i>a</i>	0.315	BV, chl <i>a</i>	0.287	S, CET
	0.278	S, BV, chl <i>a</i>	0.300	DO, BV, chl <i>a</i>	0.275	T, S, CET
Females	0.528	S, chl <i>a</i>	0.584	S, BV, chl <i>a</i>	0.361	T
	0.518	S	0.574	S, BV	0.358	T, BV
	0.447	S, BV, chl <i>a</i>	0.564	S, chl <i>a</i>	0.352	T, DO, BV

Table 6. Depth-weighted averages (DWA) (m) of females and eggs of *Acartia tonsa*, *Paracalanus cf. indicus*, and *Calanus chilensis* in Mejillones Bay, Chile, during the study period. Index of *in situ* egg production rate (EPR) (eggs female⁻¹ d⁻¹) of the 3 species is given by strata (oxygenated, oxycline, and oxygen minimum zone [OMZ]) and integrated from 0 to 80 m depth. -: no data

Species	Parameter	2013		2014	
		March	July	January	July
DWA					
<i>A. tonsa</i>	Females	8.1	14.8	13.7	-
	Eggs	5.0	-	7.7	-
<i>P. cf. indicus</i>	Females	10.3	23.3	14.4	15.7
	Eggs	11.0	9.3	6.8	11.2
<i>C. chilensis</i>	Females	10.2	26.6	16.2	19.8
	Eggs	-	29.2	45.6	13.1
EPR					
<i>A. tonsa</i>	Oxygenated	-	-	-	-
	Oxycline	15.7	-	4.9	-
	OMZ	6.7	-	0	-
	Integrated	13.2	-	4.4	-
<i>P. cf. indicus</i>	Oxygenated	-	7.5	-	12.3 ^a
	Oxycline	3.2	2.8	5.0	2.7 ^a
	OMZ	21.2	2.9	8.8	11.7 ^a
	Integrated	4.0	5.4	5.2	5.2 ^a
<i>C. chilensis</i>	Oxygenated	-	9.2	-	191.9 ^a
	Oxycline	-	17.3	4.9	99.8 ^a
	OMZ	-	60.8	532.3	80.9 ^a
	Integrated	-	18.2	18.1	115.3 ^a

^aBecause almost no females were found, the EPR was estimated from the mean female abundances recorded in July 2013 and 2014

the oxygenated layer and the OMZ (pairwise test, $R = 0.29$, $p = 0.04$; Fig. 3b,d,f,h, Table 4). The vertical distribution of female abundance was similar to that of eggs, with the highest abundances in shallow waters (1-way ANOSIM, $R = 0.33$, $p = 0.001$), with significant differences between the oxycline and the OMZ (pairwise test, $R = 0.38$, $p = 0.001$), and between the oxygenated layer and the OMZ (pairwise test, $R = 0.37$, $p = 0.01$; Table 4).

The strongest Spearman rank correlation of the BEST routine for variations in *P. cf. indicus* eggs was with chl *a* ($\rho = 0.35$, $p = 0.001$) followed by a combination of BV and chl *a* ($\rho = 0.31$, $p = 0.001$), while the strongest correlation for females was with a combination of salinity, BV, and chl *a* ($\rho = 0.584$, $p = 0.001$; Table 5).

The DWA showed that *P. cf. indicus* eggs prevailed above 12 m depth, while females were more concentrated above 24 m depth. The integrated *in situ* EPR of *P. cf. indicus* ranged from 4.0 to 5.4 eggs female⁻¹ d⁻¹, whereas higher values were found in estimates per stratum (Table 6).

Calanus chilensis

C. chilensis eggs were larger than those of *A. tonsa* and *P. cf. indicus*, with a mean diameter of 155.9 ± 8.0 μm , (range = 140.0–169.2 μm , $n = 85$). No significant differences were found in the vertical distribution of *C. chilensis* among the 3 layers (Fig. 3b,d,f,h), while in relation to the distribution of females there were differences (1-way ANOSIM, $R = 0.20$, $p = 0.001$) between the oxycline and OMZ (pairwise test, $R = 0.26$, $p = 0.001$), and between the oxygenated surface layer and the OMZ (pairwise test, $R = 0.20$, $p = 0.04$).

The BEST routine for *C. chilensis* eggs indicated the strongest Spearman's rank correlation was with Ekman transport ($\rho = 0.37$, $p = 0.001$), followed by a combination of salinity and Ekman transport ($\rho = 0.29$, $p = 0.001$), whereas the best correlation for females was with temperature ($\rho = 0.36$, $p = 0.001$; Table 5).

The DWA for *C. chilensis* was deeper than that for *A. tonsa* and *P. cf. indicus* (Table 5). *C. chilensis* females were more prevalent in shallow waters in austral summer than in winter, while eggs were found at depths between 13.1 and 45.6 m.

The integrated *in situ* EPR of *C. chilensis* were 18.2 and 18.1 eggs female⁻¹ d⁻¹ in winter 2013 and summer 2014, respectively. No eggs were found in summer 2013, while the extremely low number of *C. chilensis* females in winter 2014 showed high values in all estimates of EPR (Tables 4 & 6).

DISCUSSION

We assessed the effects of abiotic factors (T, S, DO, density, BV, and CET) and chl *a* variability on the vertical distribution of eggs and females of the dominant copepods *Acartia tonsa*, *Paracalanus cf. indicus*, and *Calanus chilensis* in Mejillones Bay (23° S). The responses of the 3 species to variability in abiotic factors and chl *a* differed. *A. tonsa* and *P. cf. indicus* eggs and females were primarily distributed in shallow waters, whereas the eggs of *C. chilensis* were distributed in deeper waters than were the females, which were found from the oxycline to shallow waters.

Our results showed the vertical distribution of copepod eggs in the water column and how they correlate

with abiotic conditions on short time scales. Longer-scale processes cannot be assessed from our data, although 2 well-defined periods were identified. Ruz et al. (2015) described a warm (summer/autumn) and cold (winter/spring) pattern in Mejillones Bay, which we observed in addition to slight differences in temperature between austral winters and summers (2013–2014). These changes may be primarily a response of increased solar radiation on the ocean surface in the summer, which affects thermal stratification, whereas haline stratification prevails in winter as indicated by the BV frequency.

Upwelling-favorable winds occur year-round in Mejillones Bay, which is evidenced by the mostly positive Ekman transport estimates and that concurred with previous records for the region (Piñones et al. 2007, Hidalgo & Escribano 2008, Escribano et al. 2012, Ruz et al. 2015). Upwelling events maintained a shallow OMZ throughout the year (mean ~28.3 m), near the range previously described (21–26 m; Hidalgo et al. 2005b, Ruz et al. 2015). Although the OMZ was deeper during winter months, the upwelling favorable conditions that occurred in this season may favor mixing of the water column, as indicated by the BV frequencies in winter.

The highest abundances of eggs, nauplii, and copepodites of *C. chilensis*, *Centropages brachiatus*, and *A. tonsa* in Mejillones Bay have been reported in shallow oxygenated waters above 10–15 m, considering these depths as an index of variability in egg stock in the entire water column (Hidalgo & Escribano 2007, 2008, Ruz et al. 2015), given the similarity to egg abundance in integrated water samples (Escribano 1998, Escribano & McLaren 1999). Our study involved high-resolution vertical sampling, with 8 discrete depths to study copepod egg distribution in Mejillones Bay. Between 70 and 95% of *A. tonsa* and *P. cf. indicus* eggs were found above 12 m depth, supporting the view that depths close to 10 m represent a good index to estimate egg stocks given the presence of the chl *a* maximum (Escribano 1998, Iriarte et al. 2000, Hidalgo & Escribano 2007, 2008). However, while this layer appears as a good approximation for small copepods like *A. tonsa* and *P. cf. indicus*, the DWA for eggs of the mid-sized copepod *C. chilensis* was between 13.1 and 45.6 m. The vertical egg distribution was supported by a positive correlation of egg copepod abundance with chl *a* and stratification for *A. tonsa* and *P. cf. indicus*, whereas the egg abundance of *C. chilensis* correlated positively with Ekman transport and with the combined effect between Ekman and salinity, suggesting that the appearance of *C. chilensis* (females and eggs) is related to strong

upwelling events that bring subsurface waters to shallower waters.

In other study areas, *A. tonsa* nauplii, copepodites, and eggs tend to occur above the pycnocline (McLaren 1966, Hansen et al. 2010a, 2012, Landaeta et al. 2013), whereas copepod eggs and *P. cf. indicus* copepodites are usually found below the pycnocline (Landaeta et al. 2013). These tendencies are consistent with our estimates for the 2 species in Mejillones Bay, where the DWA for *A. tonsa* eggs and females was slightly shallower than that of *P. cf. indicus*, suggesting that eggs were spawned in shallow waters under adequate abiotic conditions for the growth and development of nauplii and copepodites.

Peterson & Bellantoni (1987) suggested that the variability of phytoplankton abundance is partially controlled by changes in water column stratification. Therefore, stratification and phytoplankton abundance could be partly responsible for changes in the growth and fecundity of coastal copepods. Upwelling is also a key process for copepod population dynamics, given that abundance and biomass are linked to an optimal window of upwelling intensity (Escribano et al. 2012). For example, strong upwelling induces an acutely shallow OMZ, which may restrict zooplankton in a thin oxygenated surface layer (Manríquez et al. 2009). Donoso & Escribano (2014) described the OMZ as an oxygen-deficient ecological barrier (BEDOX layer) that restricts zooplankton and, therefore, copepods to the oxygenated surface layer where oxygen is not deficient for growth and development (Hidalgo & Escribano 2008, Ruz et al. 2015).

The OMZ acts as a barrier to active organism, like nauplii, copepodites, and especially adult copepods, which avoid oxygen-poor waters, while most copepod eggs tend to sink in the water column until they hatch (Salzen 1956, Marcus & Fuller 1986, Miller & Marcus 1994, Tang et al. 1998, Knutsen et al. 2001, Wang et al. 2005, Jiang et al. 2006, Svetlichny et al. 2010). As occurs in other taxa and ecosystems, if the eggs of copepods reach critical oxygen levels below their survival threshold, they may suppress their metabolism, as has been observed among organisms associated with oceanic OMZs (Seibel 2011). They may also suffer non-predatory mortality, as had been observed for nauplii of *A. tonsa* in Chesapeake Bay, USA (Elliott et al. 2013), or they can rest in sediments until appropriate conditions exist for successful development, as has been reported for copepods in other marine coastal areas as well as in freshwater environments (Dahms 1995, Marcus 1996, Williams-Howze 1997, Katajisto 2003).

Although oxygen limitation in sediments and the OMZ restricts egg development, other factors, like the density structure of the water column, may play a more important role in retaining eggs in favorable environment conditions (Peterson & Bellantoni 1987, Hansen et al. 2012, Landaeta et al. 2013). As well, eggs with floating devices, like the spines of *A. tonsa* eggs, may help retain them in shallow waters (Marcus & Wilcox 2007, Hansen et al. 2010b). On the other hand, females may play a key role in the selection of the spawning depth according to the optimal environment associated with the physiological requirements of eggs for successful growth and development. Temperature (Escribano et al. 2014), food (Vargas et al. 2006, Aguilera et al. 2011), and oxygen (Donoso & Escribano 2014, Ruz et al. 2015) are key factors for the success of zooplankton populations. Tolerance to environmental factors is a species-specific response that depends on the physiology of organisms and their adaptive plasticity; therefore, the deeper vertical distribution of *C. chilensis* eggs may be related to better adaptation to hypoxic conditions than evidenced by *A. tonsa* (Ruz et al. 2015) and *P. cf. indicus*. In this context, oxygenated surface waters are a suitable environment for the development of early and successive stages to ensure population success.

We suggest that females optimize the use of their habitat. The depth (strata) where most eggs were found may represent the optimal habitat for the species in highly heterogeneous environments, such as Mejillones Bay. For example, *Eucalanus inermis* spawns in the OMZ to avoid predation, and in the naupliar stages, individuals leave the OMZ to feed, while adult stages mostly inhabit the OMZ (Hidalgo 2005, Hidalgo et al. 2005a). Adult *A. tonsa* females are less tolerant to low DO than *C. chilensis* (Ruz et al. 2015), which is reflected in the *in situ* data of the vertical distribution of the eggs and females of the 2 species. Given that the vertical distribution of *P. cf. indicus* is similar to that of *A. tonsa*, it is possible that the 2 species have similar tolerance to hypoxia and co-exist in the same habitats, as occurs with *C. chilensis* and *Centropages brachiatus* in the HCS (Hidalgo & Escribano 2007, 2008).

Although females may lay their eggs at a specific depth (e.g. 10 m), the eggs of each species have distinct sinking rates that depend on factors like water density and viscosity, gravitational acceleration (9.81 m s^{-2}), and egg parameters like size and density (see Eq. A4 in Ju et al. 2006). The sinking velocities of the eggs were calculated based on *in situ* water density (1.0259 g cm^{-3}) and viscosity ($0.01243 \text{ g cm}^{-1} \text{ s}^{-1}$), the

radius of *A. tonsa* (0.040 cm), *P. cf. indicus* (0.038 cm), and *C. chilensis* (0.078 cm) eggs, and the densities of the eggs of the same species or congeners, obtained from the literature. The density values used were 1.0836 g cm^{-3} for *A. tonsa* (Miller & Marcus 1994, Svetlichny et al. 2010, Kozak et al. 2017), 1.0836 g cm^{-3} (*P. parvus*) for *P. cf. indicus* (Checkley 1980), and 1.0552 g cm^{-3} for *C. chilensis*, which is the average of 3 congeners, *C. finmarchicus*, *C. gracialis*, and *C. exinus* (Salzen 1956, Knutsen et al. 2001, Svetlichny et al. 2010). Therefore, the sinking velocities of the dominant copepod eggs in Mejillones Bay were 13.8, 16.3, and 26.9 m d^{-1} for *A. tonsa*, *P. cf. indicus*, and *C. chilensis*, respectively. From these theoretical data, the sinking speed of *C. chilensis* partially explains why its eggs are distributed at greater depths than those of *A. tonsa* and *P. cf. indicus* and why egg and female abundances in the OMZ are not always consistent. However, *C. chilensis* females may enter the OMZ and lay eggs there, especially in summer when the restricted oxygenated surface layer is more evident due to increased upwelling (Ruz et al. 2015).

In a hypothetical homogeneous water column, the eggs of the 3 species reach the OMZ in less than 2 d if they are spawned at 10 m depth. However, water stratification slows the sinking speed of eggs, which can be reinforced by the velocity of vertical upwelling. If the upwelling velocity is 14 m d^{-1} (Renault et al. 2012) and the sinking speeds of *A. tonsa*, *P. cf. indicus*, and *C. chilensis* eggs are 13.8, 16.3, and 26.9 m d^{-1} , respectively, then by Day 3, *A. tonsa* eggs spawned at 10 m depth will be at 9.5 m; *P. cf. indicus* eggs will be at 16.8 m; and *C. chilensis* eggs will be at 49.0 m. Consequently, the eggs of the first 2 species have time to develop in the oxygenated layer or the oxycline when mild to moderate upwelling events occur in Mejillones Bay, while *C. chilensis* eggs may reach the OMZ by the first day, which could explain why *C. chilensis* cope with hypoxia better than the smaller copepods *A. tonsa* and *P. cf. indicus* (Ruz et al. 2015, P. M. Ruz unpubl. data).

The *in situ* estimates of EPR from egg and female abundance showed that *C. chilensis* in northern Chile has reproductive pulses that take place at any time of the year, with a mean value of 4.22 (Hidalgo & Escribano 2008) and 30 eggs $\text{female}^{-1} \text{ d}^{-1}$ (Ruz et al. 2015), values that are consistent with the estimates in this study, except for a possible overestimation for July 2014 due to the low number of females that were found. In the case of *P. cf. indicus*, the values reported in this study are the first estimates of *in situ* EPR for this species. *P. cf. indicus* presented high

in situ EPR values, which may explain the high and permanent abundance of this copepod year round (Escribano et al. 2012). On the other hand, the *in situ* EPR for *A. tonsa* (0 to 12.5 eggs female⁻¹ d⁻¹) was lower than a previous estimation (120 eggs female⁻¹ d⁻¹; Ruz et al. 2015). The difference may be because females were sampled at greater depths (0–80 m) in our study than in the study by Ruz et al. 2015 (0–30 m depth). However, *in situ* EPR estimations could have been influenced by the retention of eggs in shallow waters due to stratification and vertical upwelling velocities, and consequently overestimations could have occurred. Greater effort was made with samplings (8 discrete depths for eggs and 3 strata for female abundance) than with previous estimates, where eggs gathered at only 1 depth (10 m) and females in 1 stratum (0–30 m) were used for *in situ* EPR estimations (Ruz et al. 2015). Our sampling provided more accurate data to estimate *in situ* EPR, which was used as an index of female production in a highly heterogeneous habitat due the presence of year-round upwelling and shallow OMZ.

Further research is needed at higher temporal resolution to better understand *in situ* egg production, and the abundance of females and early stages of dominant copepods in highly heterogeneous environments like the upwelling zone off northern Chile (23°S). Species at early and adult stages have different ontogenetic responses to changes in abiotic conditions, and consequently, may inhabit different depths according to their physiological requirements. In addition, the density of each developmental stage is highly determining for their sinking speeds, which in EBUSs may be subjected to a resuspension effect given by the upwelling vertical velocities. The survival of early stages is vital for the success of populations; therefore, any changes in abiotic conditions, such as upwelling intensification, shoaling, and expansion of OMZs, can affect the productivity of the pelagic realm in coastal upwelling ecosystems. Understanding how egg abundance and distribution is influenced by abiotic variability (e.g. from upwelling events), stratification, and vertical movements of the OMZ can be the basis to predict changes in copepod population dynamics in EBUES as a result of climate change.

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3.3.2. Morfología de huevos de copépodos dominantes en el norte de Chile (23°S)

Artículo en preparación (resultados preliminares)

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Resumen

Se estudió la morfología de la capa externa o corión de los huevos de tres especies de copépodos dominantes en el Sistema de Corrientes de Humboldt (SCH). Los huevos de *Calanus chilensis*, *Acartia tonsa* y *Paracalanus* cf. *indicus* fueron obtenidos desde experimentos de producción de huevos y desde perfiles verticales realizados en la Bahía de Mejillones (23°S) durante los años 2013 y 2014. Los huevos fueron fotografiados mediante microscopía convencional (8X) y microscopía electrónica de barrido (600X-3000X). Se observó (8X) que la morfología externa de los huevos de *C. chilensis* y de algunos huevos de *A. tonsa* presentaron un corión liso. Mientras un segundo tipo de huevo, con espinas largas fue encontrado en *A. tonsa*. Las fotografías obtenidas mediante microscopía SEM mostraron la presencia de ultraestructuras en el corión. *C. chilensis* presentó un corión estriado con aspecto de pequeñas ramificaciones que cubren el huevo por completo. *A. tonsa* presentó tres tipos de huevos, con espinas largas (Tipo 1), espinas muy cortas similares a protuberancias (Tipo 2) y espinas cortas (Tipo 3). Mientras que los huevos de *P. cf. indicus* no presentaron ornamentaciones ni implementos de flotación. Los resultados preliminares obtenidos en la presente investigación sugieren que las diferencias en la morfología del corión y la presencia de implementos de flotación o estriaciones en él, podrían influir en la sobrevivencia de los huevos en condiciones de hipoxia asociada a la zona de mínimo oxígeno (ZMO) en el SCH, debido a un incremento de la superficie semipermeable mediante prolongaciones del corión (*e.g.* estrías y espinas) para aumentar la superficie de contacto y mejorar la captación de oxígeno en condiciones limitantes y/o incrementar la superficie de contacto para disminuir las velocidades de hundimientos, lo que les podría permitir mantenerse por más tiempo en aguas oxigenadas por sobre la ZMO.

Metodología

Área de estudio y trabajo de campo

El estudio de la morfología externa (corión) de huevos de los copépodos *C. chilensis*, *A. tonsa* y *P. cf. indicus* fue realizada a partir de muestras de zooplancton y de agua recolectadas en la Estación 3 (23° 00.2' S, 70° 28.2' W; profundidad máxima = 120 m) de la Bahía de Mejillones durante los años 2013 y 2014, estación que corresponde al punto más alejado de la costa en la Serie de Tiempo de Antofagasta, realizada por la Universidad de Concepción (Fig. 1 en Escribano et al. 2012)

Lances oblicuos de 30 a 0 m fueron realizados con una red zooplanctónica tipo WP-2 de 200 µm de abertura de malla para recolectar hembras de las especies mencionadas, para experimentos de producción de huevos en condiciones de laboratorio. Una vez recolectado el zooplancton, las muestras fueron almacenadas en una nevera y transportadas al laboratorio en un lapso no mayor a dos horas (ver Materiales y Métodos de Capítulo 1 y 2). Adicionalmente, huevos provenientes de lances realizados con una botella Niskin (10-L) a diferentes profundidades (5, 10, 20, 30, 40, 50, 70 y 80 m) (ver Materiales y Métodos de Capítulo 3) fueron utilizados para analizar la morfología externa de huevos de copépodos de las tres especies.

Trabajo de laboratorio

Los huevos provenientes de los experimentos de producción de huevos descritos en el Capítulo 1 y 2, fueron identificados y fotografiados en una lupa esteresocópica Nikon SMZ 1000 con una magnificación de 8X. Los huevos fueron recolectados desde los perfiles verticales analizados en el Capítulo 3 y fueron identificados utilizando estereomicroscopios (8X) y microscopía electrónica de barrido (SEM, por su acrónimo en inglés). Para esto, se seleccionó aleatoriamente 30 huevos, o en su defecto la cantidad existente de las tres especies en estudio *C. chilensis*, *A. tonsa* y *P. cf. indicus*. Muestras que fueron almacenadas en tubos Eppendorf de 2 ml y fijados con glutaraldehído 2,5 %.

La preparación de muestras para la microscopía SEM consistió en deshidratar con etanol a distintas concentraciones en escala creciente. En primera instancia se utilizó etanol al 50% durante 10 minutos y posteriormente, se repitió el mismo procedimiento en etanol al 70%, 90% y 100%. Luego se realizó una segunda deshidratación en CO₂ líquido, se efectuó

secado de punto crítico y finalmente, las muestras fueron metalizadas con oro. El equipo utilizado fue un microscopio electrónico de barrido modelo JEOL JSM-6380 LV con una magnificación entre 600X y 3000X.

Resultados

Los huevos de *Calanus chilensis* fotografiados en el estereomicroscopio presentaron una superficie lisa (Fig. 1a, b). Mientras que en los huevos de *Acartia tonsa* fue posible observar dos tipos de morfología externa, la primera con una superficie lisa (Fig. 2a, b) y la segunda con la presencia de espinas (Fig. 2c). Con esta técnica no se obtuvo fotografías de *P. cf. indicus*.

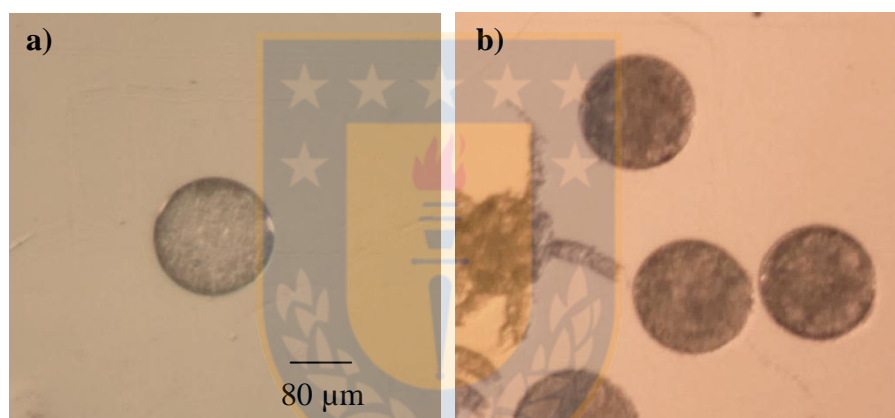


Figura 1.- Fotografías *C. chilensis* obtenidas en un estereomicroscopio (8X): a) huevos de 40 m de profundidad (julio de 2013) y b) huevos obtenidos desde experimentos de hipoxia (julio 2013).

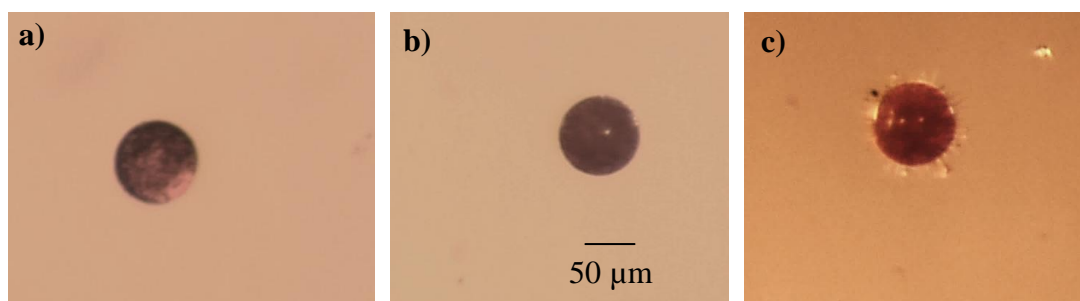


Figura 2.- Fotografías de *A. tonsa* obtenidas en estereomicroscopio (8X) con tinción de Rojo Neutro: a) huevo sin espinas visibles obtenido desde experimento de normoxia (julio de 2013); b) huevo sin espinas visibles recolectado desde experimento de hipoxia (enero de 2014) y c) huevo con espinas obtenido desde experimento de hipoxia (julio 2014).

Mediante el uso de microscopía SEM se logró realizar una identificación más detallada de la morfología externa de los huevos de *C. chilensis*, *A. tonsa* y *P. cf. indicus*, no observada en la microscopía convencional. Los huevos del copépodo *C. chilensis* presentaron un corión estriado alrededor de todo el huevo, presentando una apariencia de pequeñas ramificaciones (Fig. 3a, b).

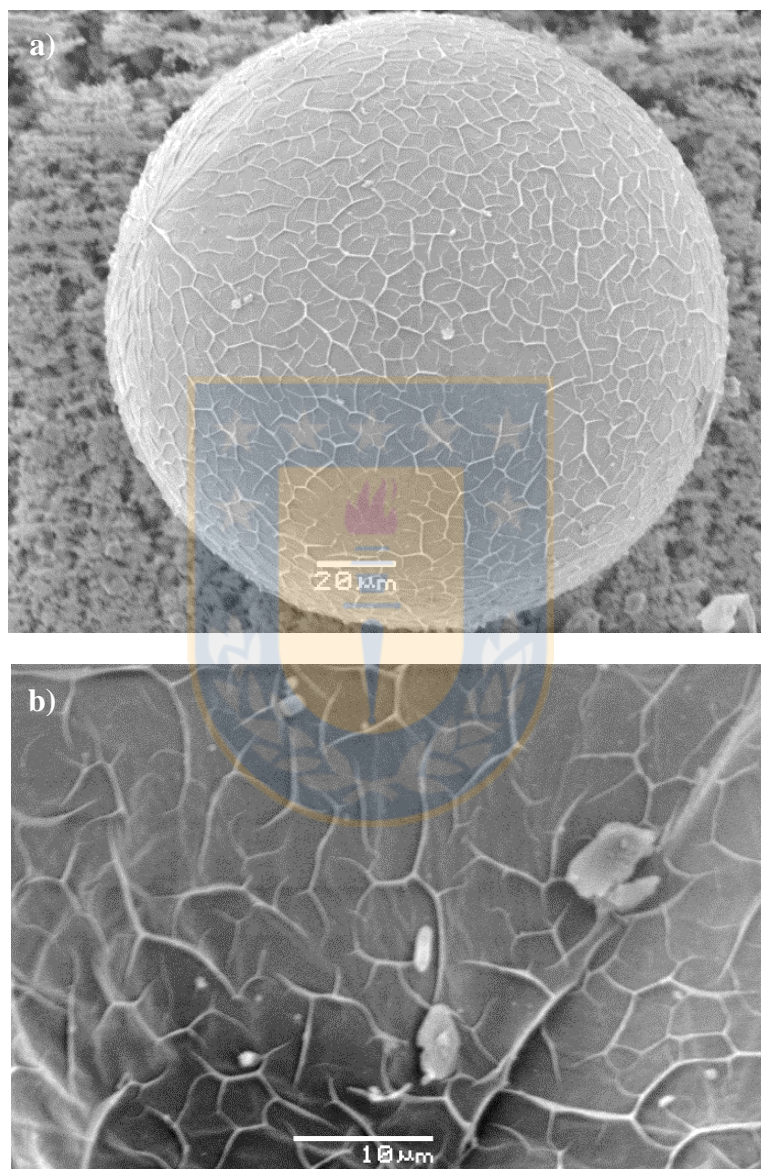


Figura 3.- Fotografías SEM de la morfología externa de huevos de *C. chilensis*: a) ultraestructura estriada del corión y b) magnificación de las estrías del corión.

En el caso de *A. tonsa*, tres tipos de huevos fueron identificados mediante microscopía SEM. El primer tipo (Tipo 1) de huevo presentó un corión liso y con espinas de longitud *c.a.* a 20 μm , que aparentemente constituyen prolongaciones del corión (Fig. 4a). La base de las espinas presentó un mayor grosor que en la región distal. Las espinas presentaron una consistencia blanda con algunos extremos curvos, cuya disposición no siguió un patrón uniforme con separaciones aproximadas entre 5 y 10 μm (Fig. 4b).

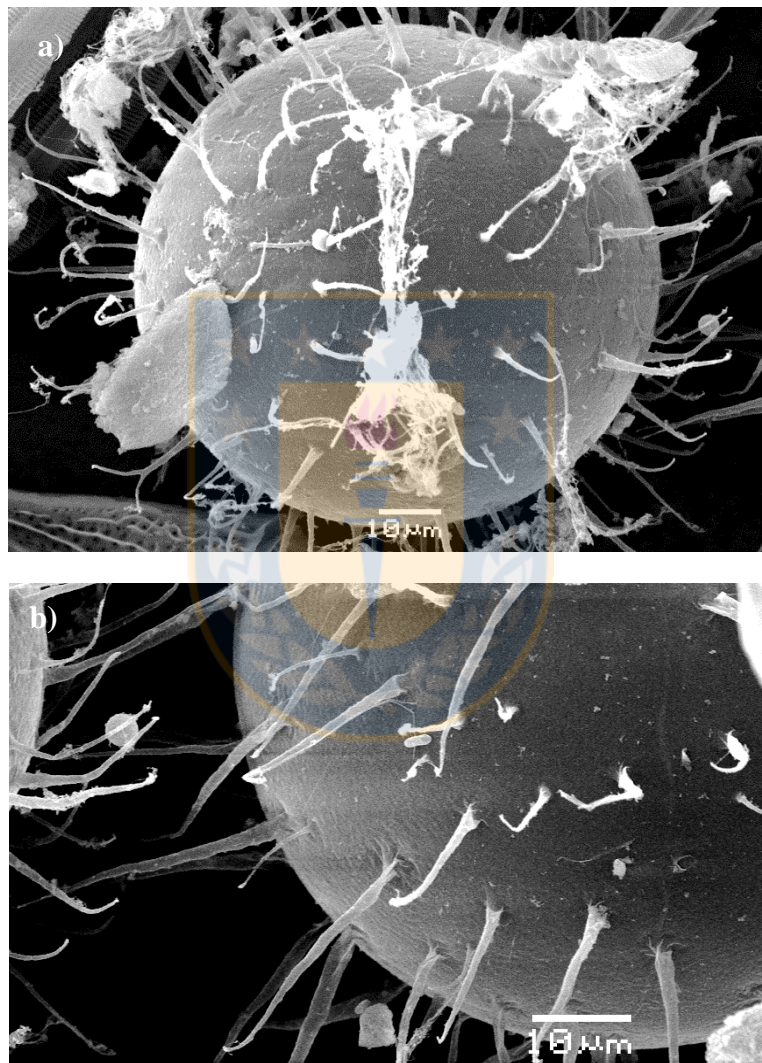


Figura 4.- Fotografías SEM de la morfología externa de huevos de *A. tonsa*: a) ultraestructura lisa del corión con espinas y b) acercamiento de las espinas del corión.

El segundo tipo (Tipo 2) de huevo de *A. tonsa* presentó un corión con pequeñas protuberancias similares a espinas (espinas truncadas) muy cortas de longitud no superiores a 0.2 μm de largo, que cubren completamente la superficie externa del huevo entregando un aspecto rugoso (Fig. 5a). El tercer tipo (Tipo 3) de huevo de *A. tonsa*, es muy similar al huevo Tipo 1 presentando un corión liso y con espinas que se prolongan desde él. La longitud de las espinas fue alrededor de cuatro veces menor que las del huevo Tipo 1, con un largo cercano a 5 μm . La base de las espinas fue más gruesa que la región distal, de consistencia blanda y una disposición que no sigue un patrón uniforme pero que cubre la mayoría de la superficie del huevo (Fig. 5b).

A diferencia de los huevos de *C. chilensis* y *A. tonsa* fotografiados en microscopía SEM, los huevos de *P. cf. indicus* presentaron un corión liso libre de protuberancias, espinas o estructuras estriadas como ornamentación (Fig. 6a, b).

Conclusiones

La morfología externa de los huevos de copépodos puede presentar estructuras ornamentales que no es posible identificar mediante microscopía convencional como ocurre en el caso de *C. chilensis* y *A. tonsa*, especies que al observarlas en microscopía de mayor resolución (SEM) presentaron estructuras estriadas y espinas en el corión, respectivamente. Mientras que los huevos de *P. cf. indicus* mostraron un corión libre de ornamentaciones.

Los huevos de *A. tonsa* presentaron mayor variabilidad intraespecífica que *C. chilensis* y *P. cf. indicus*, presentando tres tipos de huevos, los cuales lograron ser identificados principalmente debido a la longitud de las espinas del corión. Espinas que podrían ser consideradas como implementos de flotación.

Las diferencias en la morfología de los huevos de copépodos dominantes del SCH podrían influir en la sobrevivencia de los huevos en condiciones de hipoxia asociada a la ZMO, debido al aumento de la superficie semipermeable para un mayor intercambio gaseoso (e.g. estrías y espinas) y/o a la disminución en las velocidades de hundimientos al incrementar la superficie de contacto con los implementos de flotación presentes en ellos.

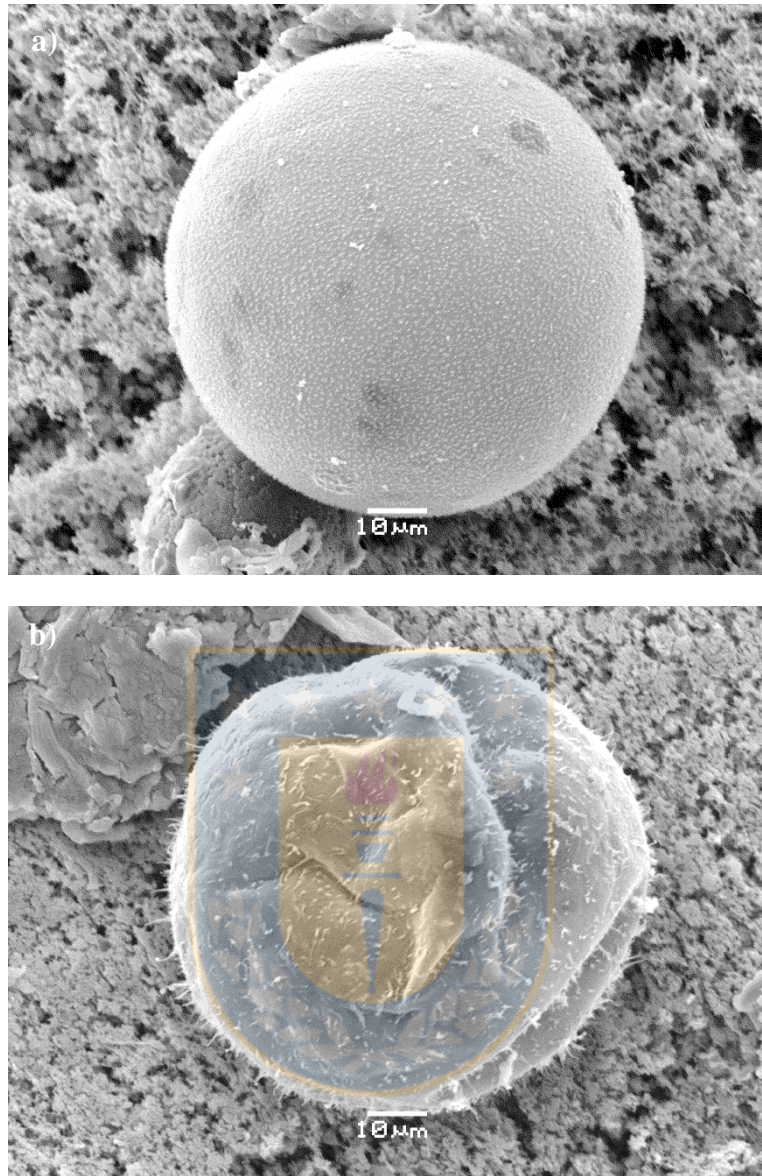


Figura 5.- Fotografías SEM de la morfología externa de huevos de *A. tonsa*. Se muestra la ultra estructura lisa del corión con a) espinas muy cortas y b) con espinas.

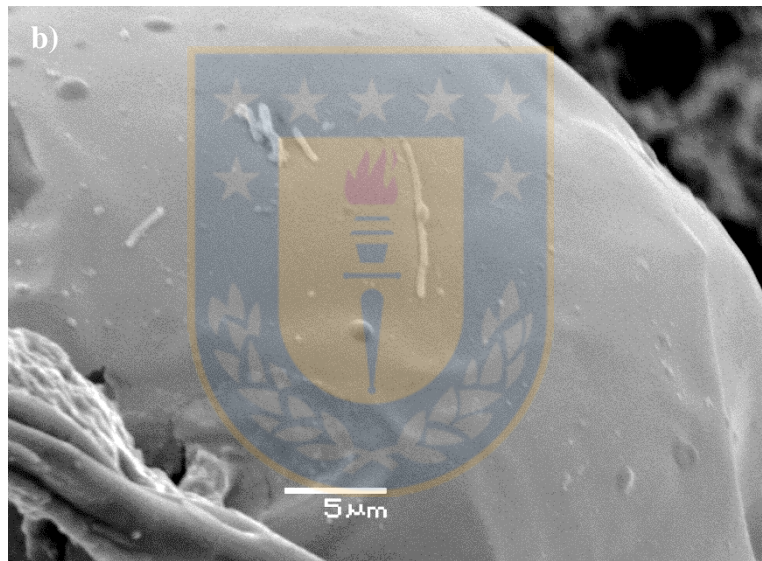
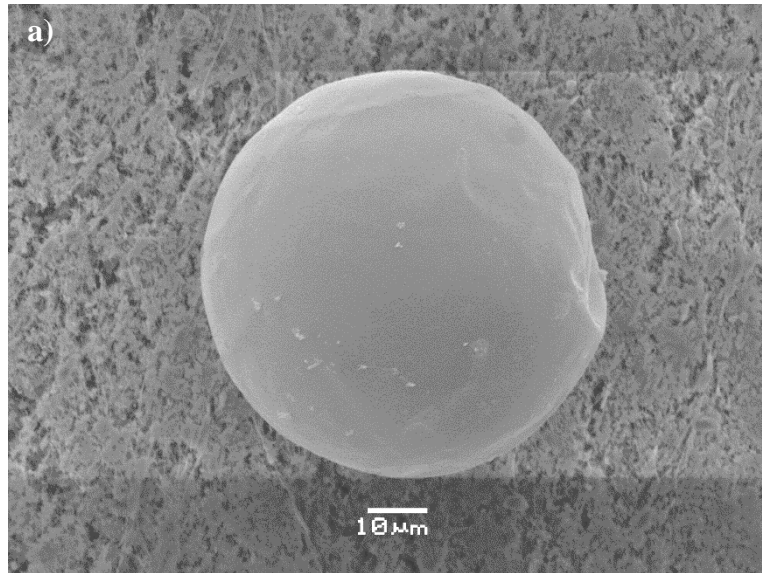


Figura 6.- Fotografías SEM de la morfología externa de huevos de *P. cf. indicus*: a) ultraestructura lisa del del corión y b) magnificación del corión.

4. DISCUSIÓN

El oxígeno disuelto es primordial en los procesos metabólicos de la mayoría de los organismos marinos (Ekau et al. 2010). En áreas de surgencia costera la ecofisiología del zooplancton se podría ver fuertemente afectada por la intensificación de las condiciones de hipoxia que actualmente están ocurriendo producto del cambio climático global.

Evaluar cómo la variabilidad ambiental podría afectar al zooplancton, es de gran importancia para comprender los potenciales cambios en la estructura del ecosistema pelágico en sistemas de surgencia costera. Las aproximaciones a nivel individual han sido consideradas para evaluar efectos en la dinámica poblacional y en la estructura comunitaria de copépodos marinos (Hidalgo & Escribano 2007, 2008; Auel & Verheye 2007). Mediante las respuestas fisiológicas ontogénicas de copépodos pelágicos asociados al SCH, en la presente investigación se evaluó: 1) experimentalmente el efecto de la hipoxia en hembras y estadios tempranos en una especie endémica (*Calanus chilensis*) y una especie cosmopolita (*Acartia tonsa*); 2) la abundancia y distribución vertical de hembras y huevos de *C. chilensis*, *A. tonsa* y *Paracalanus cf. indicus*; 3) la morfología de los huevos de las tres especies antes mencionadas y, por último se realizó 4) un modelo conceptual desarrollado a partir de los resultados experimentales e *in situ* obtenidos en este estudio.

4.1. Respuestas ontogénicas de copépodos dominantes a condiciones de hipoxia asociadas a la ZMO

Los resultados obtenidos demostraron que la sobrevivencia, potencial reproductivo y metabolismo de las hembras de *C. chilensis* no fueron significativamente afectadas en hipoxia ($0,6 - 1,9 \text{ mg O}_2 \text{ L}^{-1}$), siendo consistente con experimentos previos (Ruz et al. 2015) y con el reciente registro de *C. chilensis* (CV y adultos) habitando el interior de la ZMO ($< 10 \text{ } \mu\text{M}$, $\sim 0,22 \text{ mL L}^{-1}$) del norte del Perú (Hirche et al. 2014). En cambio, las hembras de *A. tonsa* redujeron su sobrevivencia al $\sim 50\%$ en hipoxia leve ($0,4 - 1,3 \text{ mL L}^{-1}$) y al 100% en hipoxia severa ($0,1 - 0,3 \text{ mL L}^{-1}$), mostrando que las concentraciones de oxígeno experimentales presentaron efectos subletales y letales, respectivamente. Asimismo, la producción de huevos de *A. tonsa* en el presente estudio fue similar ($24 - 0 \text{ huevos hembra}^{-1} \text{ día}^{-1}$) a valores previamente estimados en el SCH ($50 \text{ y } 3 \text{ huevos hembra}^{-1} \text{ día}^{-1}$) (Vargas et al. 2006; Aguilera et al. 2011, 2013; Ruz et al. 2015), excepto en condiciones de hipoxia severa que fue afectada

por la nula sobrevivencia de las hembras. La sobrevivencia de *A. tonsa* en condiciones de hipoxia leve (0,5 y 1,2 mL O₂ L⁻¹) fue similar a registros previos del norte de Chile (Ruz et al. 2015) y a estimaciones realizadas en sistemas de hipoxia de fondo estacional, donde el crecimiento y la sobrevivencia de *A. tonsa* y otros copépodos, se han visto significativamente disminuidas en concentraciones de hipoxia (Stalder & Marcus 1997; Marcus et al. 2004; Richmond et al. 2006). La respuesta a la hipoxia en adultos fue especie-específica e indicó que las hembras de *C. chilensis* son menos sensibles que *A. tonsa* a la hipoxia asociada a la ZMO.

El éxito de eclosión ha sido mayormente estudiado en ambientes de hipoxia de fondo estacional que en sistemas de surgencia costera, pero en ambos casos el oxígeno puede constituir un factor limitante para el metabolismo de los copépodos, lo que puede resultar en la disminución del éxito de eclosión (Lutz et al. 1994; Stalder & Marcus 1997; Choi et al. 2016; este estudio) o simplemente no generar efectos significativos en comparación con condiciones de normoxia (Richmond et al. 2006). Se observó que el éxito de eclosión de *C. chilensis* (~ 27%) y *A. tonsa* (~ 10%) no superó el 30% en hipoxia mientras que, en normoxia se registraron valores *c.a.* al 60% y 40% en cada especie, respectivamente. Resultados que fueron similares a lo previamente estimado en verano austral para *A. tonsa*, pero no para *C. chilensis* en la Bahía de Mejillones (Ruz et al. 2015), lo cual sugiere que éxito de eclosión de *C. chilensis* podría responder estacionalmente a la hipoxia al compararlo con las estimaciones realizadas durante el invierno austral de 2013 y 2014 (este estudio). Sin embargo, es importante considerar que el oxígeno no corresponde al único factor que puede afectar la ecofisiología de los copépodos, la temperatura, la calidad y cantidad de alimento asociado con la historia de vida de las hembras podría influenciar el éxito de eclosión y la sobrevivencia de estadios tempranos, principalmente al detener o reducir el crecimiento como ha sido reportado en copépodos del SCH (Torres & Escribano 2003; Vargas et al. 2006; Poulet et al. 2007).

Debido a que existe una relación inversa entre las tasas metabólicas y el tamaño en metazoos, la tasa respiración peso-específica en organismos pelágicos tiende a disminuir a medida que aumenta el peso corporal (Ikeda 1970). En este contexto, los estadios tempranos de copépodos podrían ser más sensibles a la hipoxia que los adultos debido a que un mayor metabolismo peso-específico requiere una mayor cantidad de oxígeno para suplir las demandas energéticas, además que probablemente los estadios tempranos podrían ser menos eficientes en circular bien el oxígeno disuelto si es que su corazón no se encontrara totalmente

desarrollado, como ha sido descrito en adultos (Mauchline 1998), sin embargo, esto aún se desconoce. Se ha registrado que los efectos de la hipoxia sobre las poblaciones de copépodos pueden resultar en adultos de menor tamaño debido a la disminución en las tasas de crecimiento poblacionales (Richmond et al. 2006), lo cual fue registrado en las estimaciones de crecimiento y desarrollo naupliar realizadas en *C. chilensis* y *A. tonsa*. Si bien los nauplios de ambas especies crecieron en hipoxia y en normoxia, tuvieron respuestas especie-específicas a la disponibilidad de oxígeno disuelto. La tasa de crecimiento naupliar de *C. chilensis* de N1 a N3 disminuyó significativamente en hipoxia, demostrando que los estadios tempranos de esta especie son más sensibles que los adultos. En cambio, el crecimiento naupliar de *A. tonsa* (que sólo llegó hasta N2) fue similar en ambas condiciones de oxígeno disuelto (hipoxia y normoxia). Esto sugiere que la hipoxia asociada a la ZMO podría afectar a todos los estadios de *A. tonsa* estudiados, pero influir en mayor medida sobre la sobrevivencia de estadios adultos y en la eclosión de sus huevos. La exposición de los estadios tempranos de *C. chilensis* podría extender los tiempos generacionales de esta especie y afectar el éxito reproductivo de *A. tonsa*, lo cual influye sobre sus dinámicas poblacionales desde respuestas especie-específicas a nivel ontogénico que pueden repercutir en la estructura comunitaria de los copépodos pelágicos en el SCH.

La desoxigenación contribuye a la expansión, intensificación y somerización de las de las ZMOs en los sistemas de surgencia costera generando importantes perturbaciones en el ecosistema (Stramma et al. 2010; Gilly et al. 2013). Sistemas que han mostrado tener una retroalimentación negativa entre el calentamiento global y enfriamiento costero asociado a la intensificación de los vientos favorables a la surgencia (Bakun 1990; Rykaczewski & Checkley 2008; Gutiérrez et al. 2011). Patrón que de seguir (a finales del Siglo XXI) podría adelantar la temporada de surgencia activa en ambientes estacionales (e.g. zona centro-sur de Chile) resultando en una reducción en los patrones latitudinales de la surgencia en sistemas de borde oriental (Wang et al. 2015). La intensificación de la surgencia conlleva a la compresión de la capa superior oxigenada (capa de mezcla) debido a la somerización de las ZMOs (Gilly et al. 2013). Se ha observado que la abundancia y la biomasa de copépodos asociados al SCH responden a una ventana óptima de intensidad de la surgencia, por lo cual la intensificación continua de la surgencia podría ser desfavorable para las poblaciones de copépodos pelágicos (Escribano et al. 2012), donde sólo algunas especies han desarrollado adaptaciones

metabólicas (e.g. supresión metabólica o metabolismo anaeróbico) que les permiten habitar, desovar y/o refugiarse temporalmente en el interior de las OMZs (Hidalgo 2005; Seibel 2011). Patrón que ha sido observado en algunos representantes de las familias Eucalanidae, Metrinidae y Calanidae (Hidalgo et al. 2005b; Auel & Verheye 2007; Teuber et al. 2008; Hirche et al. 2014), quienes podrían ser menos afectadas por la desoxigenación del océano si las adaptaciones suceden desde los estadios tempranos. En cambio, las poblaciones de copépodos pelágicos que no realizan migraciones verticales, como *A. tonsa* y *P. cf. indicus*, podrían verse perjudicadas ante la intensificación y somerización de las condiciones de bajo oxígeno disuelto, como consecuencia de su limitada capacidad para evitar activamente los movimientos verticales de las ZMOs.

Por otro lado, la intensidad de la hipoxia podría generar una presión de selección sobre las poblaciones de copépodos principalmente en ambientes donde los niveles de oxígeno disuelto son menores y constantes (ZMOs, $<0,5 \text{ mL L}^{-1} \sim 0,7 \text{ mg L}^{-1}$) que en ambientes de hipoxia estacional ($\sim 2 \text{ mg}\cdot\text{L}^{-1}$). Por ejemplo, especies del género *Calanus* parecen estar adaptadas a las condiciones de oxígeno propias de sus hábitats. En el SCH, *C. chilensis* sobrelleva niveles de hipoxia más intensos (Hirche et al. 2014; Ruz et al. 2015; este estudio) que *C. sinicus* y *C. pacifus* que habitan en la zona costera de los sistemas de hipoxia estacional de Quindao (China) (Wang et al. 2013) y Seattle (USA) (Keister & Tuttle 2013; Grodzins et al. 2016), respectivamente. A diferencia de *C. chilensis*, especie endémica del SCH, *A. tonsa*, es una especie cosmopolita cuyas respuestas a la hipoxia parecen ser similares en distintas regiones geográficas, a pesar de la variabilidad o la intensidad de las condiciones de hipoxia propias de cada ambiente, como se ha observado en el SCH (Ruz et al. 2015) y en sistemas de hipoxia estacional (Lutz et al. 1994; Marcus et al. 2004).

4.2. Variabilidad de la abundancia, distribución y morfología de huevos de copépodos en relación a la hipoxia de la ZMO

En el norte de Chile, la dinámica zooplanctónica se encuentra influenciada por eventos de surgencia persistentes durante el año, donde los organismos deben lidiar con un ambiente altamente heterogéneo, sujeto a cambios en temperatura, estratificación, intensidad de la surgencia y oxígeno disuelto debido a los movimientos verticales de la ZMO (Escribano et al. 1998, 2012, 2014; Hidalgo & Escribano 2008; Ruz et al. 2017). Las poblaciones de copépodos

suelen presentar reproducción continua, como ha sido observado en *C. chilensis* y *C. brachiatus*, especies que presentan tanto estadios adultos como copepoditos, nauplios y huevos durante todo el año (Hidalgo & Escribano 2008). Aunque *A. tonsa* también presenta reproducción continua, sus huevos aún no han sido registrados *in situ* durante el invierno austral, a pesar de la presencia de hembras y huevos en experimentos de producción realizados en durante la misma estación en el norte de Chile (este estudio).

Se ha observado que más del 95% de la varianza en el consumo de oxígeno en copépodos marinos depende de la biomasa y la temperatura (Ikeda et al. 2001), lo cual podría favorecer el desarrollo de estadios tempranos en las aguas superficiales, más cálidas, bien oxigenadas y ricas en alimento. En otras áreas de estudio, huevos y copepoditos de *A. tonsa* tienden a distribuirse sobre la pycnoclina (McLaren 1966; Hansen et al. 2012; Landaeta et al. 2013), mientras que los de *P. cf. indicus* usualmente se distribuyen por debajo de ella. En este contexto, el centro (DWA, por su acrónimo en inglés de depth weight average) de distribución de hembras (< 24 m) y de huevos (< 12 m) de *A. tonsa* y *P. cf. indicus* fue similar entre ambas especies, con una leve tendencia de *A. tonsa* a permanecer en aguas más someras que *P. cf. indicus*. Lo anterior, respalda la idea de que los huevos recolectados a 10 m de profundidad representan un buen índice para el stock de huevos de copépodos debido a la presencia del máximo de clorofila (Escribano 1998; Iriarte et al. 2000; Hidalgo & Escribano 2007, 2008). Sin embargo, esta profundidad podría ser una buena aproximación para copépodos pequeños, cuyas tasas de hundimiento son casi anuladas por las velocidades verticales de la surgencia, la cual ayuda a mantenerlos en aguas superficiales durante el desarrollo embrionario. No así, para especies de tamaño medio como *C. chilensis* que interactúa más seguido con la ZMO, debido a que el centro de la población de hembras y huevos de esta especie se ubica desde 10 a 27 m y desde 13 a 36 m de profundidad, respectivamente. La distribución vertical de los huevos de *C. chilensis* sugiere que las hembras podrían desovar en aguas someras sobre la ZMO y que los huevos al hundirse a una tasa de $\sim 27 \text{ m} \cdot \text{d}^{-1}$ podrían alcanzar la ZMO en pocos días, si las velocidades verticales de la surgencia no son lo suficientemente fuertes como para reflotar los huevos hacia la capa fótica. Debido a que la hipoxia no produce un efecto significativo sobre la producción de huevos de *C. chilensis* (Ruz et al. 2015, este trabajo), la exposición a la hipoxia a corto plazo como a la que se podrían encontrar sometidos los organismos en el ambiente, podría no influenciar el desove

de *C. chilensis* a diferencia de exposición más prolongada que si podría incrementar la mortalidad de los estadios tempranos.

Se ha observado que la variabilidad de la abundancia fitoplanctónica podría estar parcialmente controlada por cambios en la estratificación de la columna de agua (Peterson & Bellantoni 1987) y, por lo tanto, ser en parte responsable de la fecundidad de los copépodos. Asimismo, en el presente estudio la distribución vertical de huevos de copépodos pequeños (*A. tonsa* y *P. cf. indicus*) se correlacionó con la clorofila y la estratificación, mientras los huevos de *C. chilensis* se correlacionaron positivamente con el transporte de Ekman y la salinidad. Lo cual sugiere que la población de *C. chilensis* se relaciona fuertemente con los eventos de surgencia, como ha sido previamente reportado (Escribano 1998; Giraldo et al. 2002). La abundancia y distribución vertical de hembras y huevos es de vital importancia para mantener las poblaciones en el tiempo, principalmente en sistemas de surgencia costera donde el oxígeno puede llegar a ser un factor limitante para la ecofisiología de adultos y estadios tempranos de copépodos. Donoso & Escribano (2014) describieron la ZMO como una barrera ecológica deficiente en oxígeno que restringe al zooplancton hacia aguas someras, donde el oxígeno no sería deficiente para el crecimiento y desarrollo (Hidalgo & Escribano 2008; Ruz et al. 2015). Sin embargo, la ZMO podría actuar como una barrera para los organismos activos como nauplios, copepoditos y adultos, los cuales mediante el nado podrían evitar las condiciones de hipoxia. En cambio los huevos al ser generalmente más densos que el agua circundante se hunden hasta su eclosión (Salzen 1956; Marcus & Fuller 1986; Miller & Marcus 1994; Tang et al. 1998; Knutsen et al. 2001; Jiang et al. 2006; Wang et al. 2005; Svetlichny et al. 2010), si es que no son resuspendidos por la surgencia como fue estimado para *A. tonsa* y *P. cf. indicus*. Como ha ocurrido en otros ecosistemas y taxa, si los huevos de copépodos alcanzaran niveles de oxígeno limitantes para su sobrevivencia podrían ser afectados por mortalidad no predatoria inducida por la hipoxia (Elliot et al. 2013), suprimir su metabolismo como ha sido observado en organismos asociados a ZMOs oceánicas (Seibel 2011) o permanecer en los sedimentos en un estado de reposo hasta que existan condiciones óptimas para el desarrollo, como ha sido principalmente reportado en copépodos marinos de ambientes de agua dulce y algunas zonas costeras (Dahms 1995; Marcus 1996; Williams-Howze 1997; Katajisto 2003).

Especies del género *Centropages* y *Acartia* generalmente habitan sistemas de surgencia costera y suelen producir huevos en estado de reposo, como fue observado en *Acartia* sp. y otras especies, cuyos huevos fueron encontrados en los sedimentos de la costa central de California, indicando que estos huevos podrían sobrellevar la estación de no surgencia de ésta manera (Marcus 1995). Sin embargo, la presencia de huevos en estado de reposo no ha sido estudiada en otros sistemas de surgencia (Peterson 1998). Los huevos subitáneos y en estado de reposo podrían presentar diferencias intraespecíficas claramente distinguibles en la morfología externa, como ha sido reportado en *Paracartia latisetosa*, cuyos huevos en diapausa presentan largas espinas y los huevos subitáneos presentan espinas cortas o carecen de ellas (Belmonte & Pati 2003). Por otro lado, las diferencias pueden ser encontradas sólo a nivel ultramicroscópico, luciendo de forma similar bajo el microscopio convencional, como en el caso de *Temora longicornis* (Castellani & Lucas 2003). Durante el estudio de la morfología de los huevos de copépodos en la zona norte del SCH, se encontró que *C. chilensis* y *P. cf. indicus* presentaron sólo un tipo de huevo, mientras *A. tonsa* presentó tres tipos, los cuales se diferenciaron por el largo de las espinas que nacen como prolongaciones del corión. Estas diferencias en la morfología de huevos asociadas a *A. tonsa* en el SCH podrían ser relacionadas a la presencia de huevos en estado de reposo como ha sido observado en los sedimentos de la costa de California (Marcus 1995). Las condiciones de hipoxia y anoxia son capaces de inducir estados de dormancia o reposo en huevos subitáneos de *Acartia bifilosa* (Katajisto 2004). Asimismo, condiciones de alimento limitante (Drillet et al. 2011) u otras condiciones adversas serían capaces de inducir la producción de huevos en estado de reposo en copépodos, lo cual ha sido sugerido como estrategia de sobrevivencia a escala estacional (Chen & Marcus, 1997; Katajisto, 2004; Wu et al., 2009). Parte de las diferencias morfológicas y del éxito de eclosión de los huevos de copépodos, podrían estar dadas por la semipermeabilidad de la membrana que permite el ingreso de moléculas al interior del huevo, lo cual deja a los embriones expuestos a las condiciones del ambiente circundante (Zirbel et al. 2007). En un ambiente de surgencia semipermanente como el norte de Chile, la deficiencia de oxígeno de la ZMO podría ser un factor que gatille el estado de reposo en huevos de *A. tonsa* en la Bahía de Mejillones. Debido a lo que se conoce sobre la biología de *A. tonsa* y su capacidad de producir de huevos en estado de reposo, es probable que durante el invierno austral las mayores abundancias de huevos de *A. tonsa* se encuentren en los sedimentos en

vez de la columna de agua y por esta razón, los huevos de esta especie aún no han sido encontrado durante esta estación (Ruz et al. 2015; este estudio) Sin embargo, la presencia de huevos en estado de reposo en copépodos aún no ha sido reportado en el SCH.

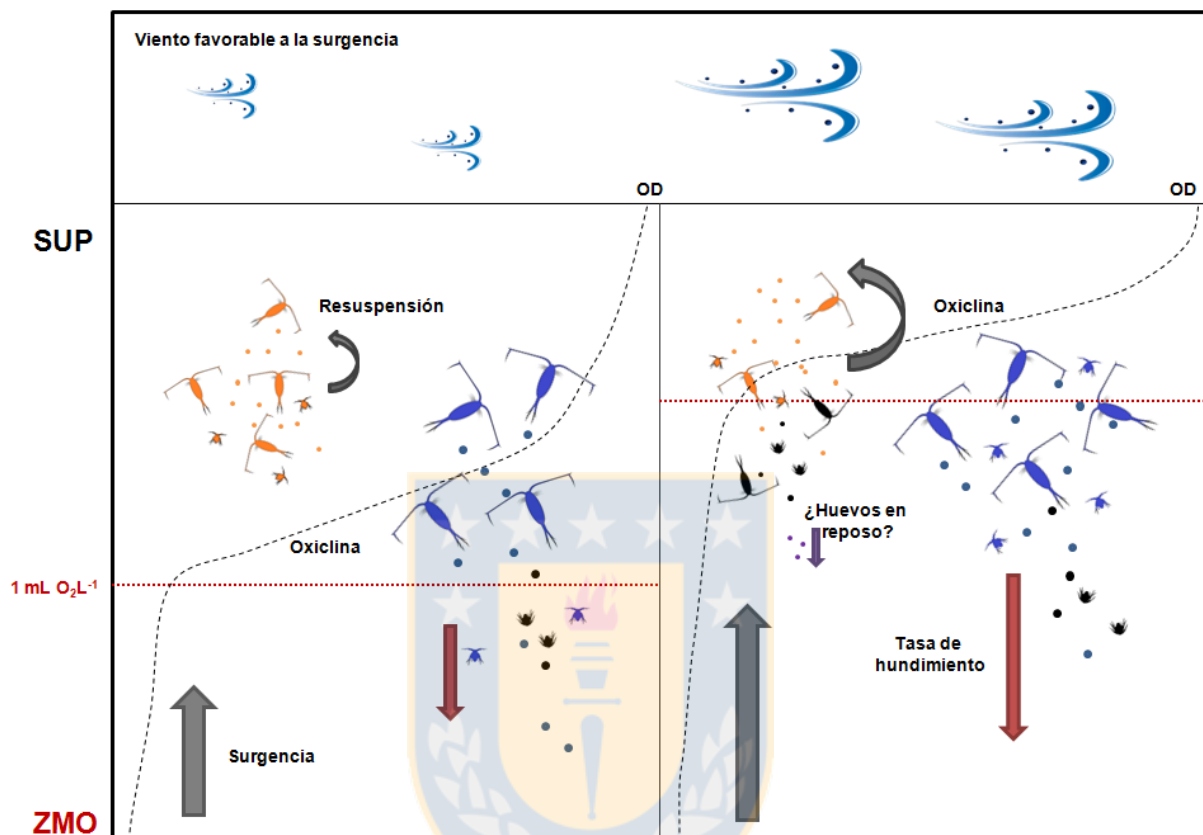


Figura 5.- Modelo conceptual de la dinámica poblacional de hembras, huevos y nauplios de copépodos pequeños (naranja) y copépodos medianos (azul) en un sistema de surgencia costera basado en la zona norte de Chile. La intensificación de vientos favorables aumenta la velocidad vertical de la surgencia (flecha gris), someriza la zona de mínimo oxígeno (ZMO) y la capa superficial oxigenada (SUP). Los huevos de los copépodos pequeños son resuspendidos por la surgencia, mientras los huevos de copépodos medianos tienden a hundirse hacia la ZMO. Se muestra el límite superior ($1 \text{ mL O}_2 \text{ L}^{-1}$) de la OMZ, perfiles típicos de oxígeno disuelto (OD), la oxiclina, los individuos que no sobreviven (negro) y la potencial presencia de huevos en estado de reposo.

El oxígeno disuelto actúa como un factor modulador de la estructura de los ecosistemas y en sistemas de surgencia costera determina la extensión del hábitat disponible para los organismos que habitan las aguas oxigenadas de la capa superficial. La intrusión de la ZMO en aguas someras podría inducir a una mayor mortalidad de copépodos como *Paracalanus cf. indicus*, *Acartia tonsa* y estadios temprano de *C. chilensis* (Yáñez et al. 2012; Ruz et al. 2015;

este estudio) (Fig. 5). Es importante realizar futuras investigaciones que permitan comprender como podrían responder otras especies de copépodos o grupos taxonómicos dominantes del zooplancton a las condiciones de hipoxia de la ZMO, para lograr obtener un mejor escenario de los potenciales cambios que podrían ocurrir en la estructura de los ecosistemas de surgencia, ante un contexto de expansión, intensificación y somerización de las ZMOs asociados al calentamiento global.

Las respuestas a la hipoxia evaluadas en este estudio, mediante aproximaciones experimental e *in situ*, permiten la extrapolación de los datos hacia potenciales respuestas a nivel poblacional, principalmente asociadas a la sobrevivencia de hembras, disminución de tamaño y tasas vitales de estadios tempranos (sobrevivencia y crecimiento) y la distribución vertical de huevos y hembras. En este contexto, la productividad de *C. chilensis*, *A. tonsa* y *P. cf indicus* en el SCH podría disminuir debido a una reducción en la biomasa por un efecto combinado de bajas concentraciones de oxígeno y temperatura (enfriamiento costero). A pesar de las limitaciones asociadas a la presente investigación relacionadas a las actividades experimentales, como estimar el crecimiento en condiciones de inanición, lo cual no permitió poder evaluar el crecimiento en estadios que presentan alimentación exógena (nauplius tardíos y copepoditos) las aproximaciones experimentales realizadas en este estudio permitieron direccionar los resultados hacia una mayor comprensión de la ecofisiología de hembras adultas y estadios tempranos de copépodos dominantes en el SCH.

Además de poder establecer que existen diferencias interespecíficas a las condiciones de hipoxia, siendo algunas especies más vulnerables que otras, se identificaron diferencias intraespecíficas donde los adultos pueden ser más o menos tolerantes que los estadios tempranos a las condiciones de hipoxia asociadas a la ZMO (Fig. 5). Las respuestas ecofisiológicas de los copépodos a la hipoxia u otras variables ambientales, influyen sobre la permanencia de sus poblaciones en el ecosistema. Debido a esto, es de gran relevancia comprender los efectos sinérgicos o antagónicos de las variables ambientales en las tasas vitales, sobrevivencia y distribución de copépodos, ya que podrían influenciar en la dinámica poblacional de copépodos en los sistemas de surgencia y principalmente, en ambientes deficientes de oxígeno como las ZMOs, que hoy en día se encuentran sujetos al cambio climático global.

5. CONCLUSIONES

Los principales resultados de la presente investigación, llevada a cabo para evaluar las respuestas ecofisiológicas ontogénicas de copépodos pelágicos ante condiciones de hipoxia asociada a la ZMO del norte de Chile fueron los siguientes:

1. Los copépodos pelágicos presentan respuestas ontogénicas a las condiciones de oxígeno disuelto presentes en el ambiente. En *Calanus chilensis*, se observó que las hembras fueron tolerantes a las condiciones de hipoxia a diferencia de los estadios tempranos. En cambio, tanto las hembras como los estadios tempranos de *Acartia tonsa* fueron susceptibles a la hipoxia asociada a la ZMO.
2. La ecofisiología de los copépodos pelágicos presenta respuestas especie-específicas a las condiciones de hipoxia. Las hembras de *C. chilensis*, especie endémica del SCH fueron más tolerantes que las de *A. tonsa*, especie cosmopolita, quienes llegaron a sufrir efectos letales a concentraciones de hipoxia severa. Los estadios tempranos de ambas especies fueron afectados negativamente por la hipoxia, al igual que la tasa de crecimiento naupliar de *C. chilensis* que fue significativamente disminuida.
3. El metabolismo de hembras de *C. chilensis* no fue afectado por condiciones de hipoxia experimentales. Su exposición a corto plazo no afectaría a la población de esta especie, no así la exposición prolongada de estadios tempranos a la hipoxia.
4. La disminución en la tasa de crecimiento de copépodos puede resultar en una consecuente reducción del tamaño final de los organismos, desde los estadios tempranos hasta llegar al estado adulto y potencialmente afectar las tramas tróficas del ambiente pelágico.
5. La distribución vertical de los huevos de las especies de copépodos dominantes en el sistema de surgencia costera del norte de Chile sería dependiente de los siguientes factores: profundidad de desove de las hembras, tasa de hundimiento de los huevos y el efecto de resuspensión generado por las velocidades verticales de la surgencia.

6. Los huevos de copépodos tienden a ser desovados en la capa superficial. Los huevos de copépodos de tamaño pequeño (*A. tonsa* y *Paracalanus* cf. *indicus*) son fuertemente resuspendidos en la columna de agua producto de la surgencia. Los huevos de copépodos medianos (*C. chilensis*) aunque sean desovados en la capa superficial se hunden y en menos de dos días ya son capaces de alcanzar la ZMO.
7. En la Bahía de Mejillones, la clorofila *a* y la estratificación fueron las variables mejor correlacionadas con las abundancias de copépodos pequeños, como *A. tonsa* y *Paracalanus* cf. *indicus*. Mientras, que el transporte de Ekman y la salinidad estuvieron mejor correlacionadas con *C. chilensis*, copépodo que se asocia a los eventos de surgencia.
8. La morfología de los huevos de copépodos puede presentar diferencias a simple vista (estereoscopio convencional) o ultramicroscópicas (Microscopía SEM). Estas diferencias fueron interespecíficas. A demás, *A. tonsa* presentó diferencias intraespecíficas, con tres tipos de huevos clasificados según el largo de sus espinas (largas, medias y cortas) como ha sido reportado para esta especie en ambientes de hipoxia de fondo estacional.
9. Respecto a las diferencias en morfología de los huevos de *A. tonsa*, es posible que esta especie presente huevos en estado de reposo (con espinas largas) en el SCH, lo cual podría ser utilizado como una adaptación para sobrevivir a la hipoxia en sistemas de surgencia costera. Sin embargo, se requiere mayor investigación al respecto.
10. Los copépodos pueden ser utilizados como sensores de cambios que ocurran en el ambiente, debido a que son capaces de responder rápidamente. Debido a esto, los copépodos podrían ser una herramienta útil para comprender el efecto de la somerización y expansión de las ZMO en el sistema pelágico en sistemas de surgencia costera.

De acuerdo a los resultados obtenidos en la presente investigación se aceptan las hipótesis propuestas.

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7. ANEXOS

Listados de Anexos

Anexo 1.- Manuscrito asociado a la tesis.

Ruz, P.M, P. Hidalgo, S. Yáñez, R. Escribano, J. E. Keister. 2015. Egg Production and Hatching Success of *Calanus chilensis* and *Acartia tonsa* in the northern Chile upwelling zone (23°S), Humboldt Current System. *Journal of Marine Systems* 148: 200-212. Doi: <http://dx.doi.org/10.1016/j.jmarsys.2015.03.007>

Anexo 2.- Manuscrito asociado a la tesis

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Anexo 1

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Egg production and hatching success of *Calanus chilensis* and *Acartia tonsa* in the northern Chile upwelling zone (23°S), Humboldt Current System



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ABSTRACT

Oxygen Minimum Zones (OMZ's) are expanding and intensifying as result of climate change, affecting Eastern Boundary Upwelling Systems. Local effects of vertical movements of OMZ's that result from changes in upwelling intensity could reduce or expand the oxygenated surface layer that most zooplanktonic species inhabit in coastal areas. Using the copepods *Calanus chilensis* and *Acartia tonsa* as model organisms, an experimental test of the impact of different dissolved oxygen (DO) concentrations (between 0.5 and 5 ml L⁻¹) on egg production and hatching success was carried out and compared with field estimations of egg production, female and egg abundance in Mejillones Bay (23°S). Abundance of *C. chilensis* was highly variability and no consistent pattern in egg production and hatching success was found across DO levels, whereas *A. tonsa* egg production had maximum values between 2.6 and 4.7 ml O₂ L⁻¹ and hatching success was positively correlated with DO ($r = 0.75$). In the field, temperature was the main factor controlling the dynamics of both species, while Chl-*a* and DO were also correlated with *C. chilensis* and *A. tonsa*, respectively. Principal Component Analysis showed that abundances of both copepods were controlled by temperature, stratification, OMZ depth, and Ekman transport, which together explained more than 70% of the total variance and were the main factors that modulated the populations of *C. chilensis* and *A. tonsa* in the upwelling zone of northern Chile (23°S). The differential responses of *C. chilensis* and *A. tonsa* to changes in DO concentrations associated with vertical movements of the OMZ suggest that *C. chilensis* may be better adapted to hypoxic conditions than *A. tonsa*, however both species are successful and persistent all year-round. We suggest that physiological responses of copepods could be used to evaluate population dynamics affected by the shoaling of OMZ's and the repercussions to trophic food webs of eastern boundary current systems.

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1. Introduction

Nowadays, one of the main focuses of study in marine sciences is to understand how climate change affects the chemistry, physics and biology of the ocean. As a result, we know that chemical transformations occur in the ocean, principally acidification, deoxygenation, and expansion of Oxygen Minimum Zones (OMZ's) (Keeling et al., 2010; Pelejero et al., 2010). Physical conditions in the oceans that are affected by

climate change include an increase in stratification due to the warming of the ocean surface (Bograd et al., 2008; Richardson, 2008) and favorable intensification of upwelling winds at eastern boundary currents, which cools coastal areas and shoals the low oxygen water of OMZ's (Bakun, 1990; Rykaczewski and Checkley, 2008). The changes in the ocean biology could be expressed as a consequence of the physico-chemistry shifts of the ocean, where plankton distribution, abundance, and phenology are important aspects of the ecosystems that can be used to understand and quantify ecological changes caused by CO₂-induced climate change (Hays et al., 2005).

The Humboldt Current System (HCS) in northern Chile has high biological productivity throughout the year. This production is driven by frequent upwelling events, which cause shoaling of the oxygen poor Equatorial Subsurface Water (ESSW) that forms the OMZ in the South Eastern Pacific, resulting in low levels of dissolved oxygen (<0.5 ml L⁻¹) near the surface (Escribano, 1998; Mann and Lazier,

Abbreviations: HCS, Humboldt Current System; DO, dissolved oxygen; OMZ, Oxygen Minimum Zone; EPR, Egg Production Rate; EPR_{exp}, Experimental Egg Production Rate; HS, Hatching Success.

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1991; Morales et al., 1999). The upper boundary of the OMZ is defined as the depth with dissolved oxygen (DO) concentrations of 1 ml L^{-1} , which in this area, is normally close to surface waters ($<50 \text{ m}$) (Fuenzalida et al., 2009; Hidalgo et al., 2005; Morales et al., 1999). Below these depths, the dissolved oxygen concentration decreases markedly to very low values ($<0.1 \text{ ml L}^{-1}$) and even to the total absence of oxygen (Ulloa et al., 2012). The upper boundary of the OMZ acts as a physical–chemical barrier to vertical distribution of most aerobic planktonic organisms in the water column (Hidalgo and Escribano, 2001; Morales et al., 1999). The OMZ is considered an Oxygen-Deficient Ecological Barrier (BEDOX) that concentrates the abundance, biomass and diversity of plankton into the oxygenated and food-rich surface water within the photic zone (Donoso and Escribano, 2014), promoting more efficient carbon flux through pelagic food webs due to higher overlap of primary production, grazers, and predators (Escribano et al., 2009; Hidalgo and Escribano, 2001; Hidalgo et al., 2010; Manríquez et al., 2009), either by direct herbivory or a heterotrophic pathway (Vargas et al., 2007).

Copepods constitute about 80% of the zooplankton biomass in the HCS (Escribano and Hidalgo, 2000; Escribano et al., 2007; Hidalgo and Escribano, 2001). About 118 species have been identified in this system; the calanoid copepods *Acartia tonsa*, *Calanus chilensis*, *Centropages brachiatus* and *Paracalanus cf. indicus* are the most abundant species (Hidalgo and Escribano, 2001; Hidalgo et al., 2010). Production and growth rates of copepods in the HCS show high temporal and spatial variability and are controlled by temperature (Escribano et al., 1998), food quality and quantity (Poulet et al., 2007; Torres and Escribano, 2003; Vargas et al., 2006), advection (Escribano, 1998), and upwelling intensity (Escribano et al., 2012). Previous studies in other systems have shown that hypoxic conditions could negatively affect *A. tonsa* egg production, hatching success and survival (Marcus et al., 2004; Richmond et al., 2006) while other copepods and their different developmental stages, like *Calanoides carinatus*, are able to tolerate low DO concentrations (Auel and Verheye, 2007). Understanding how copepod growth and reproduction respond to stress and environmental variability would greatly improve our understanding of how populations and ecosystems function (Runge and Roff, 2000).

The Antofagasta Peninsula and Mejillones Bay are characterized by cold and oxygen-poor subsurface waters due to semi-permanent upwelling throughout the year (Marín and Olivares, 1999) and an upper boundary of the OMZ located on average at 26 m depth (Hidalgo et al., 2005). In this area, year-round copepod reproduction indicates they may not be limited by food availability; the main factors affecting their life cycles and distribution are changes in temperature and DO associated with upwelling intensity (Escribano et al., 1998, 2012; Hidalgo and Escribano, 2008; Hidalgo et al., 2010). However, direct effects of the vertical displacement of the OMZ on the abundance and production rates of different copepod species remain unknown. Also, no direct measurements of the effect of different DO concentrations on production, and specifically, on copepod egg production and egg viability, are available in the HCS.

The main goal of this study was to determine whether vital rates of dominant copepods in the HCS are affected by DO concentrations that occur in the field. Vertical movements of the OMZ that result from changes in upwelling intensity could reduce or expand the oxygenated surface layer that most copepods inhabit, especially in areas where upwelling intensity and the OMZ's extension is increasing due to climate change. We hypothesize that these vertical movements would expose the copepods to lower oxygen conditions than necessary for their physiological requirements, and therefore reduce production and growth rates. To evaluate this hypothesis, several oxygen-controlled experiments were conducted to test the Egg Production Rates (EPR) and hatching success (HS) of *C. chilensis* and *A. tonsa* during spring and summer 2010. The laboratory results were complemented with a one-year time series of monthly *in situ* abundances of females and eggs, *in situ* egg production rates, and oceanographic parameters.

2. Materials and methods

2.1. Field studies

In order to study the temporal and spatial variability of zooplankton in the coastal upwelling zone of Mejillones Bay (23°S), we conducted monthly hydrographic surveys, net tows and Niskin samples during 2010. Three stations located along a coast-to-ocean transect were sampled monthly from January to December (St-1: $23^{\circ} 04.2' \text{ S}$, $70^{\circ} 25.8' \text{ W}$, station depth (z_{max}) = 60 m; St-2: $23^{\circ} 02.4' \text{ S}$, $70^{\circ} 27.0' \text{ W}$, z_{max} = 90 m; and St-3: $23^{\circ} 00.2' \text{ S}$, $70^{\circ} 28.2' \text{ W}$, z_{max} = 120 m) (Fig. 1). At each station, an autonomous oceanographic profiler CTD-O SeaBird 19 was used to obtain conductivity, temperature, density, and DO measurements.

To assess zooplankton abundance and composition, samples were obtained at each station using vertical hauls of a WP-2 net (57 cm ring diameter and 200 μm mesh) equipped with a flowmeter. The vertical hauls were conducted from 30 m to surface to ensure sampling of the upper boundary of the OMZ layer, which has been shown to have an average depth of 26 m (Hidalgo et al., 2005). Zooplankton samples were preserved in 4% buffered formalin solution for later composition analysis.

Water samples at 10 m depth were collected with a Niskin bottle (10 L) to obtain microplankton samples to estimate copepod egg abundance and Chlorophyll-*a* (Chl-*a*). This depth was chosen based on studies that showed that the Chl-*a* maximum is typically located above 15 m depth (Iriarte et al., 2000) and that changes in copepod egg abundance between 10 and 15 m is a good index of overall changes in eggs throughout the water column due to the presence of very low oxygen waters below those depths (Hidalgo and Escribano, 2007, 2008). Approximately 9.6 L of the sample was 20- μm sieved to collect microplankton samples that were preserved in 2% buffered formalin solution for later composition analysis. Chl-*a* was determined by filtering 200 ml subsamples onto GF/F filters (0.7 μm pore diameter) and analyzing by the fluorometric method (Anabalón et al., 2014; Holm-Hansen et al., 1965).

2.2. Oceanographic data

To assess the relationship between upwelling intensity and the vertical distribution of the OMZ, upwelling was estimated from Ekman transport using the equation described by Mann and Lazier (1991):

$$M_x = \tau_y / f$$

where, M_x is Ekman transport ($\text{m}^3 \text{ s}^{-1} \text{ km}^{-1}$), f is the Coriolis parameter, and τ_y is the along-shore wind stress (Pa). M_x is positive for south winds (upwelling) and negative for north winds (downwelling), and Tau (τ) was estimated as:

$$\tau_y = \rho_a * C_d * (V_y |V_y|)$$

where, ρ_a correspond to air density (1.21 kg m^{-3}), C_d is the empirical constant known as drag coefficient ($=0.0014$) and V_y represents the along-shore wind velocity (m s^{-1}). Daily wind data were taken from the Meteorological Station of Cerro Moreno (latitude–longitude: $23^{\circ} 27' \text{ S}$ – $70^{\circ} 26' \text{ W}$) maintained by the Dirección Meteorológica de Chile (<http://164.77.222.61/climatologia/>).

Water column stratification was estimated from density profiles obtained with the CTD-O as the geopotential energy anomaly (ϕ_{50}) (J m^{-3}) described by Bowden (1983) and applied in the HCS (Hidalgo and Escribano, 2007, 2008; Torres et al., 2002):

$$\phi_{50} = 1/H \int_{-H}^0 (\rho_m - \rho) g z \, dz$$

where, ρ_m is the mean density of the water column, ρ is the density at a given depth z , g is acceleration due to gravity, and $H = 50 \text{ m}$. The

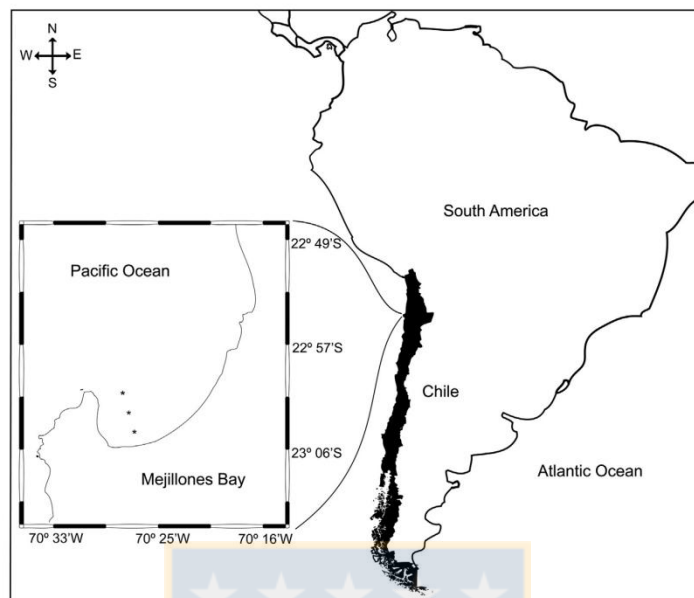


Fig. 1. South America context and study area in Mejillones Bay, northern Chile. The stations (St-1, St-2, St-3) sampled monthly during 2010 are denoted by stars: St-1 is the most southern station, closest to the coast.

geopotential energy anomaly is used to quantify the depth-averaged deficit of potential energy resulting from stratification compared to the potential energy of a totally mixed water column (Torres et al., 2002).

The depth of the upper OMZ boundary was determined as the depth where DO was $1 \text{ ml O}_2 \text{ L}^{-1}$ (Fuenzalida et al., 2009; Hidalgo et al., 2005; Morales et al., 1999). The mean and standard deviation of sea surface temperature (SST) were obtained. Vertical changes of the OMZ were also assessed using the mean and standard deviation of oceanographic variables (T, S, DO, Chl-*a*) at 10 m depth as this depth has been shown to be representative of environmental changes in the upper mixed layer that copepods inhabit (Escribano, 1998). To characterize the water column conditions where zooplanktonic samples were obtained, the average and standard deviation between 0 and 30 m depth were calculated for temperature (T_{0-30}), salinity (S_{0-30}) and dissolved oxygen (DO_{0-30}) at all sampling stations.

2.3. Egg production and hatching success experiments

Egg production and hatching success experiments were conducted on *C. chilensis* in December and on *A. tonsa* in February, March, and December. Copepods were obtained using oblique hauls from 30 to 0 m depth collected between stations St-3 and St-2. Live copepods were diluted with ambient seawater, stored in an insulated cooler, and transported to the laboratory within 2 h. Immediately after arrival at the laboratory, mature and healthy females of *C. chilensis* and *A. tonsa* were carefully sorted into 23- μm filtered seawater. In the meantime, seawater was bubbled with N_2 to create six experimental DO concentrations of 0.5, 1, 2, 3, 4 and 5 $\text{ml O}_2 \text{ L}^{-1}$, to test the range of oxygen found *in situ* across the vertical gradient. A thermoregulated bath was used to maintain the temperature at 15 °C, a value close to the annual mean temperature at 10 m depth measured by Hidalgo and Escribano (2008) during a typical non-El Niño year in Mejillones Bay. *C. chilensis* or *A. tonsa* females were carefully transferred to 1-L polycarbonate bottles filled with treatment water at a density of 10 females L^{-1} . At this

density, no crowding effects on production were expected, as suggested for *Centropages typicus* (Miralto et al., 1996). Incubations were conducted in triplicate at each of the six DO concentrations. Some experiments were repeated (also with 3 replicates). To remove food limitation and minimize egg cannibalism as factors in experiments, unlimited food ($10^3\text{--}10^4 \text{ cell mL}^{-1}$) was provided by adding concentrated *in situ* water to each bottle until a greenish color was attained (Runge and Roff, 2000; Torres and Escribano, 2003).

To avoid oxygenation, each bottle was sealed with Parafilm without bubbles before capping and incubating for 24 h. DO concentrations were measured at the beginning and the end of each incubation with an optical sensor type OXY-4 Micro Optode PreSens. Needle-type optical probes were set up using two-point calibration (0% and 100% of oxygen saturation) following the manufacturer's instructions. After 24 h, the content of each bottle was gently filtered with a 23- μm sieve attempting to leave the sieve submerged in treatment water to ensure the females and eggs remained in the water during sieving. Eggs were immediately incubated for hatching success experiments as described below; females were preserved in 4% buffered formalin solution for later measurements of the prosome length (μm) using a stereoscopic microscope equipped with an ocular micrometer ($\pm 0.01 \text{ mm}$).

Egg production rates (egg females $^{-1} \text{ day}^{-1}$) were calculated as:

$$\text{EPR}_{\text{exp}} = N_{\text{eggs}} * (24/N_{\text{females}} * t)$$

where EPR_{exp} is the experimental egg production rate, N_{eggs} corresponds to the number of eggs released during the incubation time t (in hours), and N_{females} is the number of females under observation (Runge and Roff, 2000).

Hatching success (%) was determined by calculating the percentage of eggs produced in EPR_{exp} that hatched into nauplii by 24 h after spawning. The eggs laid in each EPR_{exp} bottle were pipetted from the sieve into 250-ml polycarbonate bottles and incubated under the same DO conditions in which they had been laid. After 24 h, unhatched

eggs and nauplii were collected with a 23- μm sieve, counted and preserved for later diameter (μm) measurements. The mean of the initial and final DO concentrations was used as the concentration that females and eggs were exposed to during experiments.

2.4. Zooplankton and microplankton sample analysis

In the laboratory, all females of *C. chilensis* and *A. tonsa* were identified and counted from one half of each sample, using a stereomicroscope at 2 \times and 4 \times magnification. Their abundances were calculated as number of individuals m^{-3} of water filtered. Eggs of *C. chilensis* and *A. tonsa* from the seawater samples were identified and counted at 10 \times and 40 \times magnification. Eggs were identified using size (mean diameters of 160 μm and 81 μm for *C. chilensis* and *A. tonsa*, respectively) and morphological characteristics (e.g., spiny or smooth surface shape) according to previous eggs production experiments for both species (Ruz, unpublished data) and morphological characteristics from published data of *A. tonsa* (Hansen et al., 2010). These features allowed the eggs of *C. chilensis* and *A. tonsa* to be distinguished from eggs of other calanoid copepods found in the region. Eggs abundances were calculate as number of eggs L^{-1} .

Abundances of females and eggs were used to estimate *in situ* EPR using the approach of Edmonson (1968) in order to compare to experimental results. This method has been used to estimate EPR of other broadcast spawning calanoids, including *Clausocalanus forcatus* (Bi and Enfield, 2006), *C. chilensis* and *C. brachiatus* (Hidalgo and Escribano, 2007, 2008) and it is defined as:

$$\text{EPR} = \left(\frac{N_{\text{eggs}}}{N_{\text{females}}} \right) / D$$

where EPR is the *in situ* egg production rate (eggs female $^{-1}$ day $^{-1}$), N_{eggs} corresponds to egg abundance (number m^{-3}), N_{females} is the female abundance (number m^{-3}), and D represents the embryonic development time (day $^{-1}$) as a function of temperature described by Bělehrádek (1935) and denoted by the following equation:

$$D = a * (T - t_0)^{-b}$$

where T is the temperature ($^{\circ}\text{C}$) and a , t_0 and b are constants. Parameters a and t_0 are species-specific, while b corresponds to a fixed value for copepods ($= 2.05$). Species-specific equations of Bělehrádek embryonic development time were obtained from published data for *C. chilensis* (Escribano et al., 1998; Hidalgo and Escribano, 2007, 2008) and *A. tonsa* (Mauchline, 1998; McLaren et al., 1969), denoted by the following equations:

$$D = 947.7 * (T + 11.0)^{-2.05} \quad C. \text{ chilensis}$$

and

$$D = 489 * (T - 1.8)^{-2.05} \quad A. \text{ tonsa}$$

For both species, D values were estimated using the mean temperature over the 0–30 m layer of the water column.

2.5. Data analysis

The effects of DO and female size (as a covariable) on EPR_{exp} and HS were statistically analyzed through Analysis of Covariance (ANCOVA), used with a level of significance of $\alpha = 0.05$. A post-hoc Tukey test was done to identify significant differences between treatments (Quinn and Keough, 2002). When assumptions of parametric tests were not met, a non-parametric ANCOVA based on ranks was performed (Olejnik and Algina, 1984). Experimental data values are reported as mean \pm 1SE (Standard Error).

Non-parametric Spearman correlations were used to test statistical relationships between experimental parameters (EPR_{exp} and HS) and DO concentrations as well as relationships between *in situ* parameters (female, eggs abundance and EPR) and oceanographic parameters and indices. Principal Component Analysis (PCA) with Varimax rotation (Quinn and Keough, 2002) was used to explore patterns in *in situ* parameters of *C. chilensis* and *A. tonsa*, physical, chemical, biological variables and oceanographic indices.

Finally, to evaluate whether *C. chilensis* and *A. tonsa* demography in Mejillones Bay were consistent with the experimental results (EPR_{exp} and HS) under different oxygen levels, we made graphical comparisons between these variables and DO_{10} and the DO concentrations used in the experiments. *In situ* data values are reported as mean \pm 1SD (Standard Deviation).

3. Results

3.1. Oceanographic conditions

The water temperature throughout the whole sampling period (2010) ranged between 11.9 and 17.7 $^{\circ}\text{C}$. The monthly means suggest the presence of two periods with a transition between June and July (Fig. 2a). The first six months, the summer/autumn period, was significantly warmer (14.9 ± 0.35 $^{\circ}\text{C}$) (mean \pm 1 SD) than the second, winter/spring period (13.6 ± 0.82 $^{\circ}\text{C}$) (Two sample T-test, $T = 6.1$, $DF = 34$, $p < 0.001$).

Dissolved oxygen (DO) profiles showed a thin oxygenated surface layer above ~ 20 m, except in August and November when a deeper oxycline (> 80 m) was found at St-2 and St-3, and St-1, respectively (Fig. 2b). In the subsurface layer, oxygen-poor water prevailed during the most of the year (Fig. 2b). Coincidentally, the upper limit of the OMZ ($1 \text{ ml O}_2 \text{ L}^{-1}$) was found at an overall average depth of 21.5 m.

Relatively small salinity changes were observed during the year. However, an intrusion of high-salinity subsurface waters to the surface was observed in January, April and October (Fig. 2c), coincident with the highest values of the upwelling index (Ekman transport) (Fig. 3a). Salinity followed the trend of the upwelling, except during the end of the year when the opposite relation occurred, probably due to changes in water masses in the season (Fig. 3a and d). The upwelling index indicated that winds were upwelling favorable year-round, with intensified upwelling in spring and summer and more variable upwelling in autumn and winter. The stratification index showed the highest values from September to December, whereas the rest of the year stratification was highly variable (Fig. 3a). These indices were significantly correlated ($r = 0.57$; $p < 0.01$; $n = 12$).

Chl- a ranged from 1.3 to 106.7 mg m^{-3} with an annual average of 22.8 ± 22.6 mg m^{-3} . Two major peaks were measured in January (> 80 $\text{mg Chl-}a \text{ m}^{-3}$) and May (40 $\text{mg Chl-}a \text{ m}^{-3}$) whereas from June to December, mean values of Chl- a were close to 20 mg m^{-3} (Fig. 3e). Chl- a at 10 m depth was positively correlated with T_{10} ($r = 0.59$, $p < 0.001$), indicating that higher values of Chl- a occurred in summer and autumn when surface waters were warmer and upwelling was more intense (Fig. 3a; b; e).

During January, February, March, and December, experimental activities were carried out (white arrows in Fig. 2c). In these months, vertical profiles showed a strong thermocline (Fig. 4a) and, shallow oxycline with the OMZ upper boundary ($1 \text{ ml O}_2 \text{ L}^{-1}$) at 19, 12, 13, and 31 m for January, February, March, and December, respectively (Fig. 4b). The salinity showed well mixed subsurface water from the OMZ (Fig. 4a; c).

3.2. Egg Production Rate (EPR_{exp}) and Hatching Success (HS) experiments

Survival of *C. chilensis* females was 99.7% by the end of the experiments, with only one death in treatment 3 (Table 1). The mean Prosome Length (PL) (\pm 1 SE) of incubated females was 2476 ± 3.8 μm (range = 2143 – 2876 μm , $n = 320$) and the mean egg diameter was 160 ± 0.3 μm

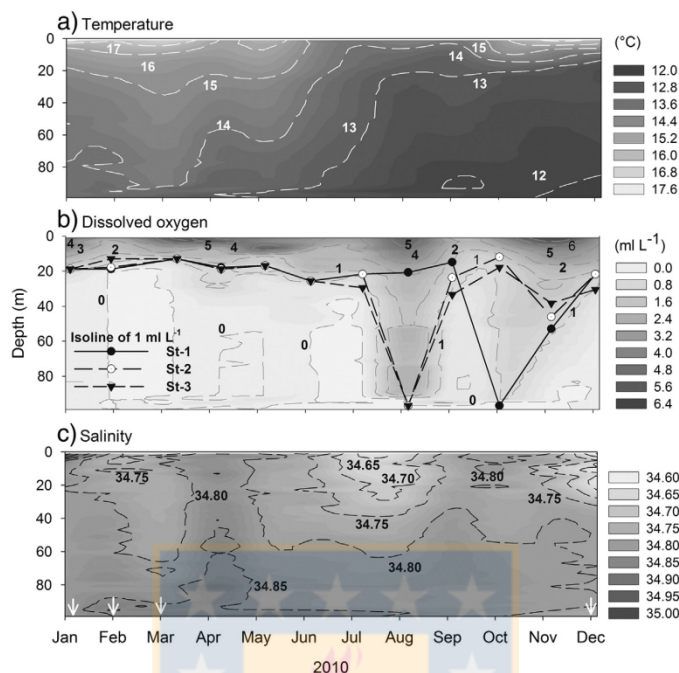


Fig. 2. Mean conditions at the three stations during 2010: a) temperature ($^{\circ}\text{C}$); b) dissolved oxygen (ml L^{-1}) with the isoline of 1 ml L^{-1} (OMZ upper limit) by station (1, 2 and 3) and; c) salinity. White arrows show the months where egg production and hatching success experiments were conducted.

(range = $143\text{--}176 \mu\text{m}$, $n = 469$). Among different oxygen levels, we were not able to identify significant differences in EPR_{exp} (ANCOVA, $F = 2.06$, $p = 0.10$) nor a significant correlation between EPR_{exp} and DO ($r = 0.24$, $p = 0.18$). However, EPR_{exp} showed high variability within and among DO concentrations (Fig. 5a). The lowest mean EPR_{exp} was measured in the $1.33 \text{ ml O}_2 \text{ L}^{-1}$ concentration, with 4 ± 1 eggs per female, while the maximum was measured at $4.30 \text{ ml O}_2 \text{ L}^{-1}$ with 41 ± 6 eggs per female (Table 1). Female size had a significant effect on EPR_{exp} (ANCOVA, $F = 7.34$, $p = 0.01$) and was positively correlated with it ($r = 0.49$, $p < 0.01$).

Hatching success (HS) of *C. chilensis* also showed no significant differences among DO levels and high variability (averaging 40.9–86.6%; Fig. 5b; Table 1) (ANCOVA, $F = 0.27$, $p = 0.93$) and, no significant correlation with DO ($r = -0.15$, $p = 0.43$). A negative correlation between HS and female size was observed ($r = -0.41$, $p = 0.03$). However, ANCOVA results show that female size had no significant effect on HS (ANCOVA, $T = -1.87$, $p = 0.08$).

Overall, *A. tonsa* survival was 85.4% at the end of the experiments. However, mortality occurred only in the lowest DO treatment ($0.91 \pm 0.12 \text{ ml O}_2 \text{ L}^{-1}$) (mean \pm SE) in which 52.4% of the females survived for 24 h (Table 2). The mean PL of incubated *A. tonsa* females was $1016 \pm 16.3 \mu\text{m}$ (range = $762\text{--}1167 \mu\text{m}$, $n = 224$) and mean egg diameter was $75 \pm 1.2 \mu\text{m}$ (range = $71\text{--}83 \mu\text{m}$, $n = 230$). Significant differences in EPR_{exp} among DO concentrations were observed (ANCOVA, $F = 6.69$, $p < 0.01$) (Fig. 5a; Table 2) without a significant correlation between these parameters ($r = 0.07$, $p = 0.73$). The lowest value of EPR_{exp} was found in the $5.00 \text{ ml O}_2 \text{ L}^{-1}$ treatment with 3 ± 2 eggs per female while the maximum was found at $4.66 \text{ ml O}_2 \text{ L}^{-1}$ with 21 ± 4 eggs per female (Table 2). In contrast to *C. chilensis*, the body size of *A. tonsa* did not affect their EPR_{exp} (ANCOVA, $F = 0.24$, $p = 0.63$). Mean HS ranged from 38.1%–73.5% (Table 2) with significantly increased HS at higher

DO (ANCOVA, $F = 3.90$, $p = 0.03$) and a significant positive correlation ($r = 0.76$, $p < 0.001$) (Fig. 5b; Table 2). HS was not affected by, nor correlated with, female size (ANCOVA, $T = -0.84$, $p = 0.42$).

3.3. In situ variability of *C. chilensis* and *A. tonsa*

In the field, *C. chilensis* females were present at all stations throughout 2010, although with high seasonal variability (Fig. 6a). Lower abundances (mean \pm 1SD) were found from January to March, June, and November to December, with a mean of 0.6 ± 0.65 (range = $0\text{--}2.4$, $n = 15$) females m^{-3} . Higher female abundances were observed in April, May, and July to October, with a mean of 45 ± 58.9 (range = $0.2\text{--}242.1$, $n = 21$) females m^{-3} . *C. chilensis* egg abundance averaged 0.4 ± 1.3 (range = $0\text{--}6.2$) eggs L^{-1} across all months, but were present only during September, October, and December. Monthly mean of *in situ C. chilensis* EPR was 30 ± 88.5 (range = $0\text{--}438$) eggs $\text{female}^{-1} \text{ day}^{-1}$ (Fig. 6a).

The non-parametric correlation analysis showed that temperature (T_{0-30}) was negatively related to *C. chilensis* eggs abundance ($r = -0.43$, $p = 0.01$) and EPR ($r = -0.39$, $p = 0.03$), but positively correlated with female abundance ($r = 0.43$, $p < 0.01$). Female abundance, egg abundance, and EPR were all negatively correlated with Chl-*a* ($r = -0.46$, $p < 0.01$; $r = -0.44$, $p < 0.01$ and; $r = -0.53$, $p < 0.01$, respectively). Female abundance was negatively, but weakly, related to upwelling ($r = -0.35$, $p = 0.03$) and stratification ($r = -0.40$, $p = 0.02$), whereas eggs ($r = 0.42$, $p = 0.01$) and EPR ($r = 0.42$, $p = 0.02$) showed a positive relationship with the stratification index.

In order to explore relationships among oceanographic conditions and *C. chilensis* biological metrics (abundances and EPR), a Principal Component Analysis (PCA) was performed. The first three principal components explained 73% of the total variance in the dataset. The

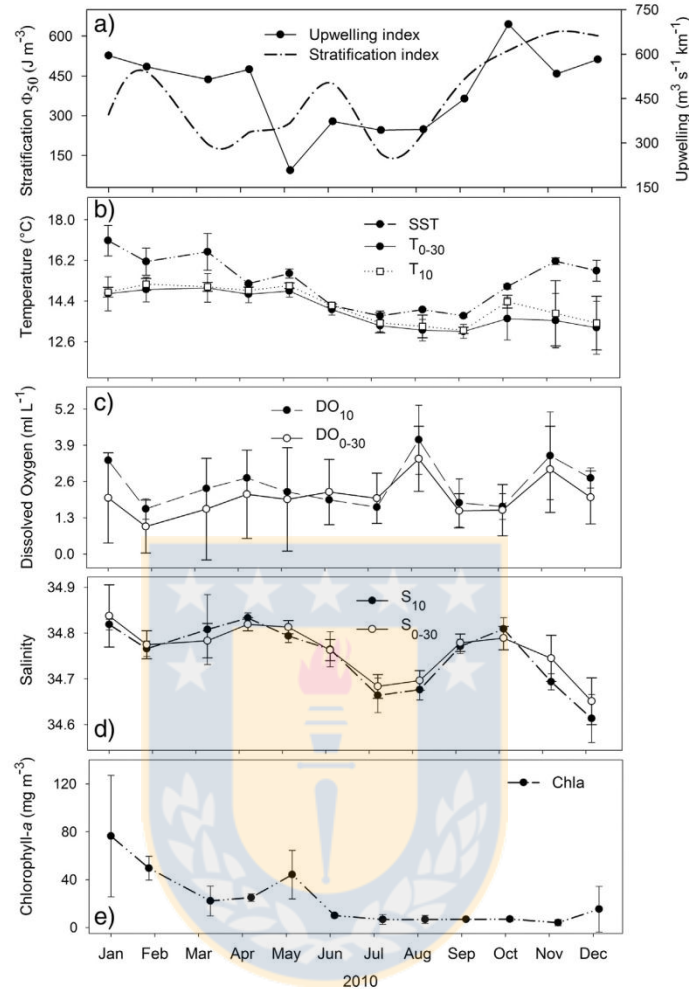


Fig. 3. Temporal variability of oceanographic conditions during 2010: a) Stratification index (J m^{-3}) and Upwelling index ($\text{m}^3 \text{s}^{-1} \text{km}^{-1}$); b) temperature ($^{\circ}\text{C}$) at 1 m (SST), at 10 m (T_{10}), and between 0–30 m depth (T_{0-30}); c) dissolved oxygen (ml L^{-1}) at 10 m depth (DO_{10}) and between 0–30 m depth (DO_{0-30}); d) Salinity at 10 m (S_{10}) and between 0–30 m depth (S_{0-30}); e) Chl-*a* (mg m^{-3}) at 10 m depth. Index values in panel a, have only one value per month; values in b, c, d and e correspond to the mean and standard deviation of the three stations.

first component (PC1) represented the temperature (T_{0-30} and T_{10}), Chl-*a*, water stratification, and female abundance and explained 29% of the variance. The second component (PC2) was related to DO (DO_{10} and DO_{0-30}) and OMZ depth, with 23% of explained variance. The third component (PC3) represented egg abundance and EPR, plus the upwelling index (Ek) with 21% of explained variance (Fig. 7a; Table 3).

A. tonsa females were also present in the field year-round, with higher abundances during spring and summer than in winter. The lowest abundance was observed in June with 2 ± 1.0 (range = 1–2, $n = 3$) females m^{-3} ; moderate abundances were found in January, February, April, May, and July to November with a mean of 45 ± 46.3 (range = 0–187, $n = 27$) females m^{-3} ; high abundances were present in March, October, and December with a mean of 258 ± 298.7 (range = 19–944, $n = 9$) females m^{-3} (Fig. 6b). The overall mean value of *A. tonsa* eggs was 9 ± 19.1 (range = 0–96) eggs L^{-1} , but eggs only were present

from January to March and October to December. Average monthly estimate of *in situ* *A. tonsa* EPR was 120 ± 289.3 (range = 0–1108) eggs female $^{-1}$ day $^{-1}$ (Fig. 6b). Non-parametric correlations between *A. tonsa* and oceanographic parameters showed that EPR increased with DO at 10 m depth (DO_{10}) ($r = 0.39$, $p = 0.02$), whereas no correlations were found with temperature, salinity, Chl-*a*, OMZ depth, or dissolved oxygen from 0 to 30 m depth (DO_{0-30}). Upwelling and stratification indices were positively correlated with egg abundance ($r = 0.44$, $p < 0.01$; $r = 0.45$, $p < 0.01$; respectively) and with EPR ($r = 0.44$, $p < 0.01$; $r = 0.46$, $p < 0.01$; respectively). However, abundance of females was not related with the indices mentioned above.

Similar to *C. chilensis* the Principal Component Analysis (PCA) of *A. tonsa* population and oceanographic variables revealed three significant components explaining 75% of the total variance. The first component (PC1) was represented by the temperature (T_{0-30} and T_{10}), Chl-*a*,

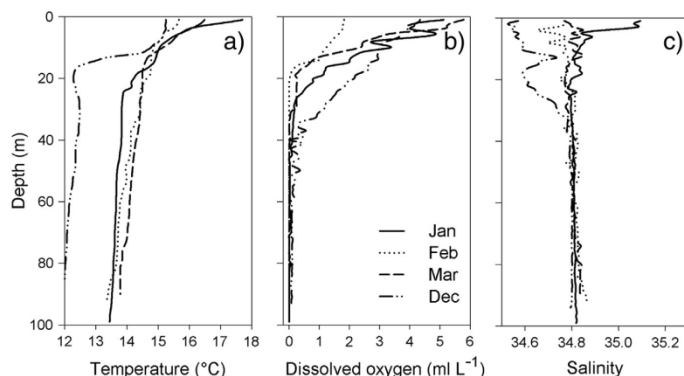


Fig. 4. Vertical profiles at St-3 during the experimental period: a) temperature (°C); b) dissolved oxygen (ml L^{-1}) and; c) salinity.

and stratification, with 28% of explained variance. The second component (PC2) showed that egg abundance and EPR of *A. tonsa* plus the upwelling index (Ek) explained 24% of the variance. Finally, the third component (PC3) was represented by DO (DO_{10} and DO_{0-30}) and OMZ depth, with 23% of explained variance (Fig. 7b; Table 3).

3.4. Comparison between field and experimental data

A comparison between *in situ* EPR and EPR_{exp} showed high *C. chilensis* EPR in the field when DO_{10} was between $\sim 1\text{--}3 \text{ ml O}_2 \text{ L}^{-1}$, but maximum EPR_{exp} were found between $\sim 3\text{--}4.5 \text{ ml O}_2 \text{ L}^{-1}$ (Fig. 8a; Table 1). In contrast, *A. tonsa* showed highest *in situ* EPR and EPR_{exp} between $\sim 2\text{--}4 \text{ ml O}_2 \text{ L}^{-1}$ (Fig. 8b; Table 2).

For *C. chilensis*, high *in situ* EPR were found between 1 to $3 \text{ ml O}_2 \text{ L}^{-1}$, whereas hatching success from experiments were not be affected by DO (Fig. 9a; Table 1). By contrast, *A. tonsa* showed the higher *in situ* EPR at mid DO concentrations in a similar range of DO where HS showed high but no the highest values (Fig. 9b; Table 2).

4. Discussion

We assessed the effect of different DO concentrations, from hypoxia to well-oxygenated waters, on experimental egg production rate (EPR_{exp}) and hatching success (HS) of two dominant copepods off northern Chile, *C. chilensis* and *A. tonsa*. The two species showed different responses to DO: *C. chilensis* EPR_{exp} and HS were not affected by DO whereas *A. tonsa* EPR_{exp} and HS were (Fig. 5). Our results only indicate the short-term effects of DO on these species since, as in many experimental studies (see Runge and Roff, 2000), we were not able to acclimate females to treatment conditions prior to spawning, but the results do indicate an interesting difference in sensitivity among species which co-occur in a region that chronically experiences low oxygen

levels. These short-term effects of DO could be relevant in the field, for example, due to DVM, vertical movements of the OMZ during upwelling, or as eggs sink through the water column.

Mejillones Bay in northern Chile presents a highly heterogeneous environment due to semi-permanent coastal upwelling all year-round, allowing the presence of physico-chemical processes that modulate copepod dynamics and community structure; as changes in upwelling intensity (Escribano et al., 2012), the presence of thermal fronts, upwelling shadows acting as retention areas (Giraldo et al., 2002; Marín et al., 1993) and shallow OMZ that could aggregate and increases copepod diversity in the food-rich photic zone (Hidalgo and Escribano, 2008; Hidalgo et al., 2010). These factors plus changes in food concentrations affect growth and development of copepods (Escribano et al., 1998; Poulet et al., 2007) and may explain the high variability in abundances and EPR (experimental and *in situ*) estimated in this study for *C. chilensis* and *A. tonsa*.

The EPR_{exp} values of *C. chilensis* were in a similar range ($\sim 0\text{--}85 \text{ eggs female}^{-1} \text{ day}^{-1}$) with previous studies where factors as temperature (Escribano et al., 1996, 1998, 2014) and food (Poulet et al., 2007) were analyzed in the HCS. Considering all data, mean EPR was slightly lower ($18.8 \pm 3.57 \text{ eggs female}^{-1} \text{ day}^{-1}$) than previous records of *C. chilensis* and other *Calanus* spp. with $\sim 30 \text{ eggs female}^{-1} \text{ day}^{-1}$ (Escribano et al., 2014; Mauchline, 1998). Regressions analyses between prosome length (PL) and the number of eggs spawned showed no significant relationship (Escribano, 1998; Escribano et al., 1996) in contrast with our results where a positive relation was found ($r = 0.49$, $p < 0.01$).

Vargas et al. (2006) found higher *A. tonsa* EPR ($50\text{--}10 \text{ eggs female}^{-1} \text{ day}^{-1}$) in central-southern Chile (36°S) than other studies on the reproductive performance of *A. tonsa* in the HCS. EPR values for *A. tonsa* range between 4 and 6 eggs $\text{female}^{-1} \text{ day}^{-1}$ (Aguilera et al., 2011) in northern and southern Chile. Aguilera et al. (2013) showed that *A. tonsa* EPR ranged from 39 and 5 eggs $\text{female}^{-1} \text{ day}^{-1}$ from coastal to

Table 1
Summary of experimental results for *Calanus chilensis* during spring/summer 2010.

T	N_e	DO_i	DO_f	DO_t	EPR_{exp}	HS	PL	$\bar{\phi}_{\text{eggs}}$	N_{females}
1	4	0.48 ± 0.06	0.50 ± 0.23	0.49 ± 0.15 (0.13–1.10)	14 ± 4	73.4 ± 5.20	2487 ± 13.25	159 ± 1.83	129
2	1	0.88 ± 0.02	1.79 ± 0.05	1.33 ± 0.04 (1.75–2.17)	4 ± 1	86.6 ± 5.09	2400 ± 46.16	162 ± 0.52	26
3	3	2.28 ± 0.13	2.80 ± 0.15	2.54 ± 0.14 (2.00–3.87)	21 ± 7	66.2 ± 11.80	2474 ± 15.47	159 ± 0.72	92 (1)
4	1	3.33 ± 0.09	3.30 ± 0.21	3.31 ± 0.15 (3.07–4.00)	35 ± 13	55.1 ± 20.41	2541 ± 41.86	165 ± 4.25	32
5	1	4.50 ± 0.01	4.09 ± 0.24	4.30 ± 0.12 (4.42–4.98)	41 ± 6	40.9 ± 13.23	2500 ± 5.70	159 ± 2.73	25
6	1	5.00 ± 0.02	4.46 ± 0.06	4.73 ± 0.04 (4.54–5.15)	7 ± 2	74.8 ± 16.23	2493 ± 23.94	162 ± 0.65	27

Incubation treatments (T) and number of experiments (N_e) run per treatment; each experiment had three replicates. This table shows mean, standard error (SE) of initial dissolved oxygen (DO_i), final (DO_f), treatment DO (DO_t) plus range (ml L^{-1}) and, SE of experimental egg production rate (EPR_{exp}) (eggs $\text{females}^{-1} \text{ day}^{-1}$), hatching success (HS) (%), prosome length (μm) (PL) and egg diameter ($\bar{\phi}_{\text{eggs}}$) (μm) of *C. chilensis*. The number of females (N_{females}) alive versus dead (value in parentheses) at the end of experiments are shown. ANCOVA test did not show significant differences among treatments for EPR_{exp} or HS.

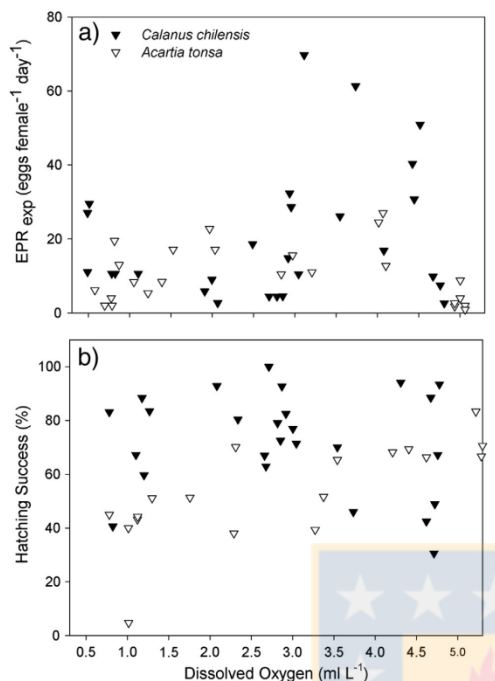


Fig. 5. Experimental variables: a) egg production rates (EPR_{exp}) (egg females⁻¹ day⁻¹) and; b) hatching success (%) at different dissolved oxygen levels (DO) (ml L⁻¹) for *Calanus chilensis* and *Acartia tonsa*.

estuarine waters (Aguilera et al., 2013). Our estimates of EPR (21–3 eggs female⁻¹ day⁻¹) were within the range of previous data for this specie in different environments of the HCS. In other seasonal environments where *A. tonsa* inhabits, also high values of EPR could be found ranging between ~100 and 20 (Marcus et al., 2004) and ~70 and 30 eggs female⁻¹ day⁻¹ (Sedlacek and Marcus, 2005) in control treatments at 20 °C, in difference to our estimates carried out at 14 °C. Considering a Q_{10} of 3 for EPR (Kjørboe and Sabatini, 1995), *A. tonsa* EPR estimated in this study mostly was within the theoretical value (~10 to 36 eggs female⁻¹ day⁻¹) estimated at 14 °C. Similar to previous studies we observed no relation between female size and egg production in *A. tonsa* (Durbin et al., 1983).

Previous *in situ* EPR estimated by Edmonson method (Edmonson, 1968) showed that *C. chilensis* in the HCS have sporadic reproductive events with values ranging between 2.7 and 403 eggs female⁻¹ day⁻¹,

mainly concentrated in spring/summer and associated with abundances of 400 eggs m⁻³ in south-central Chile (Hidalgo and Escribano, 2007), whereas in northern Chile, the reproductive pulses could take place at any time of the year with a mean value of 4.22 eggs female⁻¹ day⁻¹ and abundances up to ~2000 eggs m⁻³ (Hidalgo and Escribano, 2008). Coincidentally, our data showed high variability for *C. chilensis* and *A. tonsa* with values ranging between 0–438 eggs female⁻¹ day⁻¹ and 0–1108 eggs female⁻¹ day⁻¹, respectively. Other species have been studied using this method, EPR of *C. forcatsus* in the Gulf of Mexico was 1.93 ± 0.38 eggs female⁻¹ day⁻¹ (Bi and Enfield, 2006), showing lower values than *C. chilensis* and *A. tonsa* in the HCS. Peterson and Kimmerer (1994) suggest that the egg-ratio method could underestimate EPR due to high egg mortality rates produced by factors like predation and physiological causes that affect the embryonic development in nature (Laabir et al., 1995).

The experimental estimates of EPR were at least one order of magnitude smaller than *in situ* EPR. Analyzing both estimates by separated they are similar to previous data for *C. chilensis* and *A. tonsa* as we described above. However, the difference between these two approaches could be caused by a reduction of EPR due to female cannibalism over the eggs (Kang and Poulet, 2000), or differences in the timing and the number of eggs per clutch released (Peterson, 1988). It has been observed that EPR of *C. marshallae* changes (lower) during active to relaxation phase of the upwelling, due to a decoupling between egg production and food supply. Therefore the high heterogeneity of the upwelling system of Mejillones Bay may play a key role in the high variability of eggs abundances and *in situ* EPR.

On the other hand, high variability of HS of *C. chilensis* could be more attributed to maternal effects (Escribano et al., 1998; Poulet et al., 2007) than DO; where our results showed high values (40–87%) and variability at all experimental levels. For other copepods species such as *A. tonsa* and *Oithona colcarva* it has been observed that DO concentrations below 1 mg O₂ L⁻¹ (~0.7 ml O₂ L⁻¹) could inhibit hatching (Roman et al., 1993). We only observed a reduction in the HS of *A. tonsa* under similar DO conditions, suggesting that *A. tonsa* from the northern area of HCS could be more tolerant to hypoxia than *A. tonsa* inhabit systems with seasonal bottom hypoxia, like Chesapeake Bay (see Roman et al., 1993). The *in situ* data showed a strong seasonal pattern of EPR, and to a lesser extent female abundances, that differed between species. *C. chilensis* eggs were present only at the beginning of the spring and early summer when upwelling-favorable winds intensified (Figs. 3a and 6a). This contrasts with previous studies in which eggs were found through most of the year (Escribano, 1998; Escribano and McLaren, 1999; Hidalgo and Escribano, 2008). High abundance of *A. tonsa* eggs occurred through spring and summer when upwelling and stratification was intensified (Fig. 3a) and a shallower OMZ was present; this is the first report of the temporal distribution of *A. tonsa* eggs in Mejillones Bay. However, experimental EPR estimates of *A. tonsa* in southern-central Chile (Vargas et al., 2006) showed that this specie is able to lay eggs all year-round, with higher EPR in spring and summer than the rest of the year. Females of *C. chilensis* and *A. tonsa* were found all year-round. *C. chilensis* showed high abundances

Table 2
Summary of experimental results for *Acartia tonsa* during spring/summer 2010.

T	N _e	DO _i	DO _f	DO _t	EPR _{exp}	HS	PL	Ø _{eggs}	N _{females}
1	2	0.63 ± 0.09	1.18 ± 0.15	0.91 ± 0.12 (0.50–1.23)	6 ^{a, b, c, d, f} ± 3	38.1 ^a ± 6.8	1043 ± 9.4	79 ± 1.5	63 (30)
2	1	1.51 ± 0.02	1.83 ± 0.28	1.67 ± 0.14 (1.05–1.76)	7 ^{a, b, c, d, e, f} ± 1	53.2 ^{ab} ± 9.3	957 ± 20.4	74 ± 0.4	32
3	1	2.50 ± 0.05	2.73 ± 0.24	2.62 ± 0.12 (1.53–2.85)	19 ^{a, b, c, d, e} ± 2	52.1 ^{ab} ± 7.5	1027 ± 4.5	74 ± 0.7	37
4	1	3.50 ± 0.02	3.92 ± 0.23	3.71 ± 0.11 (2.67–4.08)	12 ^{a, b, c, d, e, f} ± 2	68.0 ^{ab} ± 0.9	1014 ± 15.4	77 ± 0.2	28
5	1	4.51 ± 0.10	4.82 ± 0.05	4.66 ± 0.03 (4.01–4.85)	21 ^{b, c, d, e} ± 4	73.5 ^b ± 5.0	1045 ± 1.7	74 ± 0.5	22
6	1	4.99 ± 0.01	5.01 ± 0.33	5.00 ± 0.17 (4.91–5.59)	3 ^{a, b, d, f} ± 2	–	1001 ± 4.9	77 ± 2.2	24

Different letter superscript denotes significant differences (pairwise comparison Tukey test; $p < 0.05$) between treatments. Incubation treatments (T) and number of experiments (N_e) run per treatment; each experiment had three replicates. This table shows mean, standard error (SE) of initial dissolved oxygen (DO_i), final (DO_f), treatment DO (DO_t) plus range (ml L⁻¹) and, SE of experimental egg production rate (EPR_{exp}) (eggs females⁻¹ day⁻¹), hatching success (HS) (%), prosome length (µm) (PL) and egg diameter (Ø_{eggs}) (µm) of *A. tonsa*. ANCOVA results show significant differences among treatments for egg production rate and hatching success. A posteriori pairwise comparison Tukey test denotes where the significant differences were found, specified with different letter superscript.

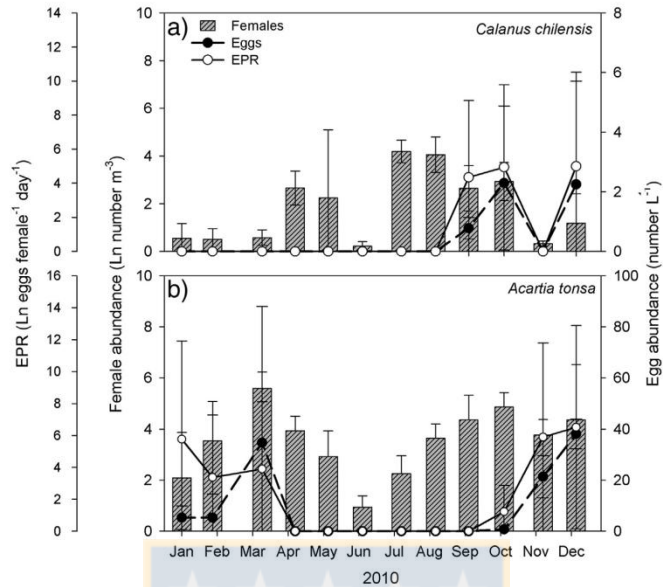


Fig. 6. Temporal changes of female abundance (Ln number m^{-3}), egg abundance (number L^{-1}) and egg production rate from *in situ* samples (EPR) (Ln eggs female⁻¹ day⁻¹) for a) *Calanus chilensis* and b) *Acartia tonsa* during 2010.

in winter and spring, in contrast with previous reports of the specie that had reported the highest female abundances during autumn and winter (Hidalgo and Escribano, 2008). Females of *A. tonsa* decreased during winter with higher abundance the rest of the year, corresponding to the first report on temporal variability of *A. tonsa* in Mejillones Bay. These differences are likely to be due to the increasing of upwelling during the last decade (2000–2008) that may be unfavorable for copepod populations (Escribano et al., 2012) plus changes in the timing of the upwelling as results of the oceanographic interannual variability of northern Chile and the influence of the Mejillones Peninsula that plays a key role on circulation and retention processes in Mejillones Bay (Letelier et al., 2012).

In the coastal upwelling zone of Mejillones Bay where food limitation potentially does not exist, copepod population dynamics are expected to be controlled mainly by temperature (Escribano et al., 2014). Copepods that inhabit these areas typically exhibit nearly continuous reproduction during the upwelling season, resulting in the coexistence of multiple generations and cohorts (Escribano et al., 2014; Hidalgo et al., 2005; Peterson, 1998). Therefore, the stage-specific copepod abundance found at any particular sampling period is at least partially affected by the specie's life cycle. For example, *C. chilensis* development is 38.1 days at 15 °C (Escribano et al., 1998), but in the field its populations have shown *ca.* 15 abundance peaks of adults in one year, with average time intervals of 20 days (Hidalgo and Escribano, 2008). As a consequence, a mismatch between the timing of a peak of adults and the cruises could lead to underestimated female abundances. However, maximal periods of female abundance of *C. chilensis* were higher than previous estimates in the bay (Escribano and McLaren, 1999; Hidalgo and Escribano, 2008) reaching values up two-fold during late winter and spring, indicating that our monthly sampling did not miss peaks of females abundances. *A. tonsa* female abundances were less variable than *C. chilensis*, possibly due to faster development associated with their smaller size, 25 days at 17 °C (McLaren et al., 1969), and so relatively higher adult production. Faster development also permits easier population maintenance within highly

advective environments, as has been shown for *A. tonsa* off southern Europe (Leandro et al., 2006).

With our index of egg production taken only from 10 m depth, we may have underestimated abundances during autumn and winter when lower stratification occurs, leading to a deeper distribution of eggs than in spring and summer. Also, advection or a mismatch between sampling times and reproductive events could influence the abundance of eggs sampled. Our data do not allow us to assess these potential sources of variability; intensive sampling (e.g., weekly) of eggs and nauplii over a broader depth range would be necessary to better estimate their association with the OMZ. However, our index likely captures the dominant patterns of variability in egg production, so allows us to make some comparisons between egg production and changes in the environment. Previous studies in the region have successfully used the abundance of eggs collected from 10 m depth as an index of variability in the full water-column egg stock (Hidalgo and Escribano, 2007, 2008), or have found similar abundances when sampling from 50 to 0 m (Escribano, 1998) and 90 to 0 m (Escribano and McLaren, 1999). In other study areas, the egg production of *A. tonsa* has been shown to occur above the pycnocline (Hansen et al., 2010, 2012; McLaren, 1966), probably to allow the eggs to spend more time in surface waters, avoiding exposure to colder temperatures and poorly oxygen waters (Hansen et al., 2012). The lack of higher reproduction at low DO levels may be a behavioral adaptation to improve the survival and development of eggs since HS is positively correlated with DO ($r = 0.76$; $p < 0.001$; Fig. 5b), besides of a potential capacity of females on suppress laying eggs under short-term unfavorable conditions, as has been reported for *Calanus* sp. (Runge and Roff, 2000). We do not know *C. chilensis* spawning depths, but other *Calanus* species, e.g., *C. finmarchicus*, have been observed to move to the surface to spawn (Miller et al., 1991).

Copepod eggs are negatively buoyant and tend to sink, with their sinking velocities depending on their size and density (Mauchline, 1998). Sinking velocities together with the embryonic development rate determine whether eggs sink into the OMZ before hatching. The sinking rate of *C. chilensis* eggs is unknown, but is likely to be on the

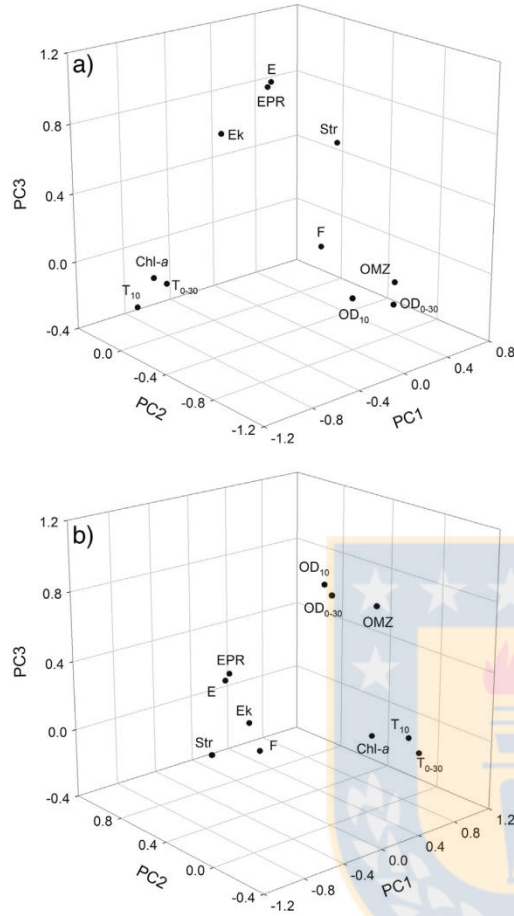


Fig. 7. Principal component analysis (PCA) applied to population parameters of a) *Calanus chilensis* and b) *Acartia tonsa*, along with oceanographic variables from the monthly time series at Mejillones Bay during 2010. The 1st three components were derived and plotted. Egg production rate (EPR), egg abundance (E), female abundance (F), temperature (T), dissolved oxygen (DO), upper boundary of the Oxygen Minimum Zone ($\sim 1 \text{ ml O}_2 \text{ L}^{-1}$) (OMZ), Chlorophyll-*a* concentration at 10 m (Chl-*a*), Ekman transport as upwelling index (Ek) and stratification index (Str). Subscript 10 corresponds to 10 m depth and 0–30 to the mean value between 0 and 30 m depth.

order of $25\text{--}35 \text{ m d}^{-1}$ based on measurements of similarly sized eggs (diameter $\sim 150 \mu\text{m}$) produced by its congener, *C. finmarchicus* (Mauchline, 1998). *C. chilensis* hatches in 1.2 days at 15°C (Escrignano et al., 1998) and, our study shows that low DO is not limiting for the hatching of its eggs during spring/summer season, if *C. chilensis* eggs reach the OMZ they would be able to hatch and potentially had a successful development. Our results are supported by recent research off northern Peru which showed that *C. chilensis* stage V and adults were abundant in hypoxic waters at concentrations between 0.11 and 1.13 ml L^{-1} (equivalent to $5 \mu\text{M}$ and $50 \mu\text{M}$, respectively); this is the only specie of Calanidae reported to be able to tolerate *in situ* hypoxia at different and non-resting developmental stages (Hirche et al., 2014). Population abundance of *C. chilensis* is linked to the OMZ depth and with upwelling (Hidalgo and Escrignano, 2008), suggesting that

Table 3

Factor loadings from Principal Component Analysis (PCA) of oceanographic and biological measurements from a monthly sampling in Mejillones Bay in 2010. The 1st three principal components (1, 2, 3) and their explained variance (%) are shown. Factors used were egg abundance (Eggs), female abundance (Females), Egg Production Rate (EPR), mean 0–30 m temperature (T_{0-30}), 10 m temperature (T_{10}), mean 0–30 m dissolved oxygen (DO_{0-30}), dissolved oxygen at 10 m depth (DO_{10}), depth of the upper limit of the Oxygen Minimum Zone (OMZ), Chlorophyll-*a* at 10 m depth (Chl-*a*), stratification index (Str) and upwelling index (Ek).

Variable component	<i>C. chilensis</i>			<i>A. tonsa</i>		
	1	2	3	1	2	3
Explained variance	29%	23%	21%	28%	24%	23%
Eggs	0.29	0.16	0.86	0.05	0.93	0.17
Females	0.55	-0.04	-0.13	-0.08	0.55	-0.12
EPR	0.25	0.14	0.84	0.04	0.89	0.22
T_{0-30}	-0.90	0.17	-0.26	0.96	-0.02	-0.15
T_{10}	-0.87	0.04	-0.06	0.90	0.03	-0.06
DO_{0-30}	0.21	-0.94	-0.14	-0.16	-0.11	0.96
DO_{10}	-0.15	-0.93	-0.02	0.14	0.17	0.91
OMZ	0.36	-0.82	-0.08	0.35	-0.09	0.82
Chl- <i>a</i>	-0.70	0.11	-0.15	0.73	0.21	-0.07
Str	0.63	-0.11	0.51	-0.78	0.36	0.04
Ek	-0.33	-0.02	0.70	0.02	0.70	-0.02

C. chilensis is better adapted to changes in DO concentrations compared with *A. tonsa* (Fig. 5a, b).

The sinking rate of *A. tonsa* eggs has been estimated between 13 and 24 m d^{-1} (Miller and Marcus, 1994). *A. tonsa* hatches between 2 and 3 days at the same temperature (McLaren et al., 1969) but, our experiments showed that the hatching success of *A. tonsa* is diminished at low DO levels, indicating that they would not hatch if they sink into the OMZ. However, in our experiments more than 40% of eggs hatch

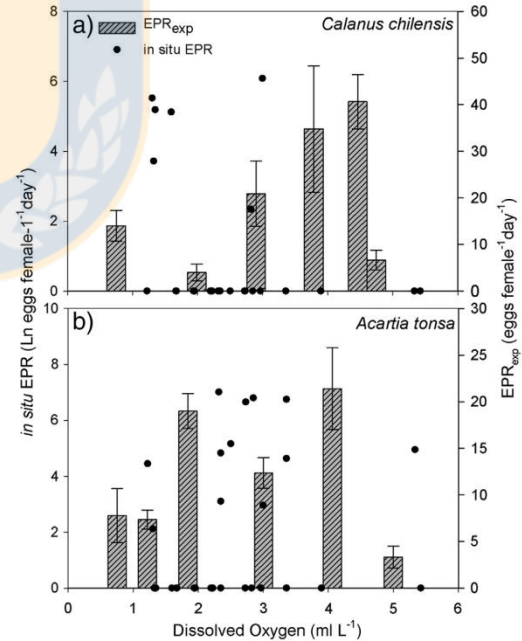


Fig. 8. *In situ* EPR ($\text{Ln egg female}^{-1} \text{ d}^{-1}$) versus dissolved oxygen at 10 m depth (DO_{10}) compared with experimental egg production rate (EPR_{exp}) ($\text{egg female}^{-1} \text{ d}^{-1}$) plotted versus experimental DO level: a) *C. chilensis* and b) *A. tonsa*.

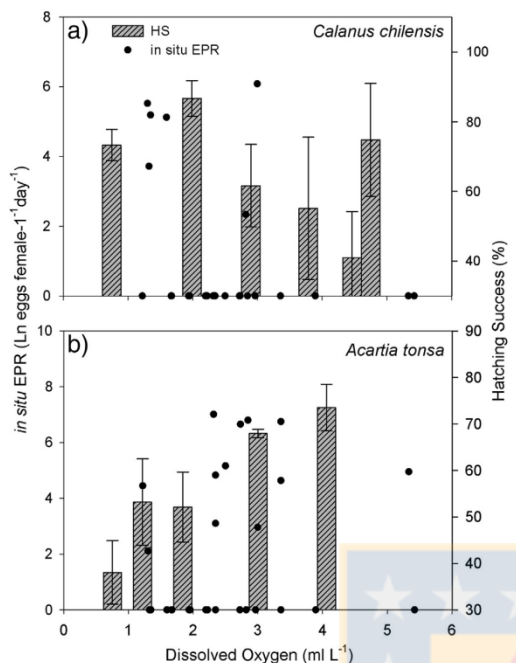


Fig. 9. In situ EPR (Ln egg female⁻¹ d⁻¹) versus dissolved oxygen at 10 m depth (DO₁₀) overlaid on experimental hatching success (HS) (%) plotted versus experimental DO level for a) *C. chilensis* and b) *A. tonsa*.

within 24 h at 15 °C, suggesting a faster development (but not quantifiable) than previously registered for *A. tonsa*. According to this, the eggs of both species would reach the OMZ before hatching in Mejillones Bay, and during the spring and summer when surface waters are warmer, eggs could hatch before encountering low DO waters as has been suggested for *A. tonsa* in Chesapeake Bay (Roman et al., 1993). An increase in the stratification allows eggs to be kept in the oxygenated shallower waters above the OMZ where successful development can occur (Hidalgo and Escribano, 2008), below which the OMZ may act as an ecological barrier deficient in oxygen (BEDOX layer) (Donoso and Escribano, 2014).

Although different developmental stages might episodically encounter food limited conditions, food has been shown not to be a limiting factor for *C. chilensis* populations in northern Chile (Escribano, 1998; Torres and Escribano, 2003) whereas for *A. tonsa*, food limitation is unknown. In central-southern HCS, where upwelling is seasonal, variability in the production of *A. tonsa* is explained by temperature and food availability, indicating that food limitation can occur there (Vargas et al., 2006). In the semi-permanent upwelling off northern HCS, dominant copepods are persistent and abundant all year round (Hidalgo and Escribano, 2001). *C. chilensis* and *C. brachiatus* are examples of persistent species characterized by continuous cohorts and reproduction (Hidalgo and Escribano, 2008). Similarly, *A. tonsa* females were highly persistent in 2010 indicating that they also might not be food limited, and others factors such as low DO could be more relevant to the population dynamics of *A. tonsa*, mainly via decreased hatching success.

Experiments in systems that experience seasonal bottom hypoxia have shown that hypoxia increases mortality and reduces growth of *A. tonsa* and other copepods (Marcus et al., 2004; Richmond et al., 2006;

Stalder and Marcus, 1997). This is consistent with our results (Fig. 5b, Table 2): *A. tonsa* populations from the Mejillones Bay and from coastal waters of Florida experienced ca. 50% mortality at concentrations of ~0.7 ml O₂ L⁻¹ (Marcus et al., 2004; Table 2 in this study). On the other hand, congeners of *C. chilensis* show different responses. Females of *C. euxinus* from the Black sea are very tolerant to hypoxia with a survival of 75% at 0.47 ml O₂ L⁻¹ and, a reduction in their respiration rates in low oxygen waters (Besiktepe et al., 2005) whereas females of *C. pacificus* from Puget Sound, Washington had high mortality (ca. 90%) at DO levels below 1 ml O₂ L⁻¹ (Keister and Grodzins, in prep.) perhaps because hypoxia in Puget Sound is less intense than in the Black Sea and the HCS (Besiktepe et al., 2005; Keister and Tuttle, 2013; Ulloa et al., 2012), guiding a local selective pressure to hypoxic conditions.

The mechanisms for adaptation to hypoxia in copepods are unknown. However, copepods that have the potential to perform DVM seem better adapted to stand strong vertical gradients in temperature and oxygen. For example, the large copepod (>4.5 mm, PL) *Eucalanus inermis* in Mejillones Bay have been described to move to deep water (>200) and probably in a lethargic mode staying within the OMZ (Hidalgo et al., 2005) or perhaps experiencing metabolic suppression as described for other organisms in OMZ systems (Siebel, 2011). By contrast, the small-sized copepod *A. tonsa* inhabit coastal waters with vertical distribution restricted to well oxygenated shallow waters above the OMZ (Escribano and Hidalgo, 2000; Escribano et al., 2009; Hidalgo et al., 2012, Hidalgo et al., 2010), whereas for *C. chilensis* (mid-sized copepod) recently has been reported to have a deeper distribution (Hirche et al., 2014) than *A. tonsa* in the HCS. *A. tonsa* seem less adapted to low oxygen waters than *C. chilensis*.

The copepod community that inhabits Mejillones Bay is affected by the persistent upwelling occurring in the region (Escribano et al., 2012; Hidalgo and Escribano, 2008). Here, changes in temperature and stratification have been reported to be the main factors influencing their populations (Escribano et al., 1998, 2014), but DO could also be important (Fig. 7; Table 3), as Elliot et al. (2012) suggest for the Gulf of Mexico. In the field, modulating factors do not act by themselves, individually, but are synergistic and diverse responses by the zooplankton community can be found. The two species in this study, *C. chilensis* and *A. tonsa*, respond differently to changes in DO, with *C. chilensis* seemingly better adapted to low oxygen than *A. tonsa*, an issue that could be balanced by the faster development of *A. tonsa*. The low DO concentrations associated with OMZ in the HCS could affect *A. tonsa* egg production and viability and be an important factor that modulates its population dynamics and structure. The negative correlations between species abundance, richness and diversity with OMZ depth (Hidalgo et al., 2010) suggest an important role of hypoxia in this ecosystem and also how the influence of the zooplankton respiration could decrease DO concentration helping to maintenance and increment the extent of the OMZ as an oxygen-deficient ecological barrier (Donoso and Escribano, 2014).

The upper depth of the OMZ in northern Chile determines the available habitat for copepods which reside in oxygenated surface waters. Deoxygenation of the surface layer could lead to an overall reduction in zooplankton biomass and to changes in species dominance through replacement of sensitive species with ones more tolerant to hypoxic conditions, or ones with faster development. Our results suggest that ecophysiological responses of dominant copepods of the HCS to environmental changes could be used to evaluate how global climate change may affect the pelagic realm in some of the most productive areas of the world, eastern boundary current systems.

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Effects of oxygen depletion on field distributions and laboratory survival of the marine copepod *Calanus pacificus*

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Copepods dominate the zooplankton, but surprisingly little is known of their tolerance to the increasing threat of hypoxia. We measured abundances of the calanoid copepod *Calanus pacificus* in relation to oxygen concentrations in the field and established its tolerance to low dissolved oxygen (DO) in the laboratory. *In situ* distributions of female *C. pacificus* were assessed with depth-stratified net sampling in Hood Canal, a seasonally hypoxic sub-estuary of Puget Sound, Washington. No clear avoidance of DO levels from supersaturated down to 2.0 mg DO L⁻¹ was observed; DO levels <2 mg L⁻¹ were rare in our sampling. In the lab, at 13°C female *C. pacificus* exhibited 100% 24-h survival at DO levels down to 1.7 mg DO L⁻¹; below 1.5 mg DO L⁻¹, survival sharply declined, with 25% survival at 1.2 mg L⁻¹ and complete mortality within 1 h at 0.9 mg L⁻¹. *Calanus pacificus* is one of the dominant copepods throughout much of the North Pacific and an important trophic link in the ecosystem. Our measurements indicate that they have a steep threshold in oxygen tolerance that is similar to those measured for other calanoid copepods and which could limit their habitat in years of severe oxygen depletion.

KEYWORDS: *Calanus pacificus*; hypoxia; survival; oxygen depletion; vertical distribution

INTRODUCTION

Globally, coastal hypoxia is increasing in geographic distribution, duration and frequency of occurrence (Doney, 2010). Hypoxia occurs naturally in some regions, while in many others anthropogenic influences including

fertilizer and sewage run-off lead to eutrophication, which through biological processes, intensifies oxygen drawdown (Howarth, 2008). As the world population has grown and temperatures have increased through global warming, the incidence of coastal hypoxia has too: nine oxygen-depleted systems were reported prior

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to 1960, with that number roughly doubling every 10 years (Diaz et al., 2003). As of 2002, 146 regions were reported to experience coastal hypoxia.

Increased hypoxia and anoxia are adversely changing the environment as shown by habitat degradation, changes in trophic-web structure, and decreased biodiversity (Howarth, 2008; Vaquer-Sunyer and Duarte, 2008). In many cases, high-value species of demersal fish and shellfish have been displaced (Diaz, 2001; Rabalais et al., 2001; Diaz et al., 2003) leaving instead less valuable pelagic species. Generally, motile fish and benthic crustaceans are absent from bottom habitats when dissolved oxygen (DO) falls below 1.5–2 mg DO L⁻¹; many less motile invertebrates die at oxygen concentrations below 1.5 mg DO L⁻¹ (Rabalais et al., 2001). While there is substantial literature on the effects of hypoxia on fisheries and benthic organisms, less is known about its effects on the underlying pelagic trophic web that supports high-value species.

In complex marine food webs, often a key trophic position is filled by one or a few species of calanoid copepod (Bollens and Frost, 1989; Schukat et al., 2013). In particular, species in the genus *Calanus* tend to play important trophic roles because of their relatively large size, lipid stores and abundance, which make them preferred diet items of many zooplanktivores (Economou, 1991; Falk-Petersen et al., 2007). As one of the most important species in marine food webs, *Calanus* have been more intensively studied than any other group of zooplankton (Marshall and Orr 1972; Vidal, 1980). Yet literature on the oxygen tolerance of *Calanus*, and indeed of any copepod, that inhabits seasonally hypoxic regions is extremely limited. Studies of oxygen tolerance have been conducted on *Acartia tonsa*, *Oithona colcarva*, *Labidocera aestiva*, *Calanoides carinatus* and *Centropages hamatus* (Roman et al., 1993; Stalder and Marcus, 1997; Auel and Verheye, 2007) and a few studies have assessed behavioral avoidance in the laboratory (e.g. Decker et al., 2003) or inferred it from field distributions (e.g. Keister et al., 2000; Keister and Tuttle, 2013). Together, the studies indicate species-specific differences in tolerances with individuals of some species surviving DO as low as 0.6 mg L⁻¹ but 100% mortality of others at 1.4 mg DO L⁻¹. Only one previous study that we are aware of has measured the oxygen tolerance of a species of *Calanus* (Ruz et al., 2015), and that was from a system with a permanent Oxygen Minimum Zone (OMZ). Overall, surprisingly little information exists on the physiological tolerances of copepods to low DO.

This study focused on female *Calanus pacificus*, the dominant *Calanus* in the North Pacific, to better understand their oxygen tolerance and behavioral responses to low oxygen concentrations in the field. We measured

vertical distributions of zooplankton and oxygen as a component of a project designed to assess the effects of seasonal hypoxia on predator–prey interactions between mesozooplankton and fish. To help explain the observed *in situ* patterns, the 24-h survival of *C. pacificus* was measured in the laboratory under controlled conditions. We hypothesized that abundances of *C. pacificus* would be positively related to oxygen concentrations in the field, and that their distributions would reflect complete avoidance of regions where oxygen was below their lethal limit.

METHOD

Study site—Sampling was conducted in Hood Canal (Fig. 1), a seasonally hypoxic sub-estuary of Puget Sound, Washington, USA. Hood Canal's deep, narrow basin is fronted by a glacial sill that inhibits circulation, which combined with high primary production, regularly leads to oxygen depletion in late summer and autumn (Gregg and Pratt, 2010). DO is routinely lowest in the southern-most reaches of Hood Canal and increases northward towards the ocean. Strong winds or tidal events can mix low oxygen waters up into the water column and may lead to displacement of the oxygen minimum layer into near-surface water (Cannon, 1975).

Field sampling—Sampling was conducted from the *R/V Clifford A. Barnes* on monthly cruises June to October in both 2012 and 2013 (10 cruises in total). Four stations were sampled on each cruise with approximate depths of (from north to south): 80 m (Dabob), 170 m (Duckabush), 120 m (Hoodsport) and 70 m (Union) (Fig. 1). Data used in this study were from the northernmost and southernmost stations (Dabob and Union). Conductivity, temperature, depth and DO were recorded using a Sea-Bird Electronics (SBE) 9 CTD array equipped with an annually-calibrated SBE 43 oxygen sensor and a WET Labs ECO-AFL/FL fluorometer. A Niskin rosette was used to collect water at 3–5 targeted water depths above and below the oxycline at each station to calibrate the CTD DO probe; oxygen was analyzed using the modified Winkler titration method (Carpenter, 1965a; Carpenter, 1965b).

Zooplankton were collected using a 0.25 m² HydroBios Multinet equipped with five 200- μ m mesh plankton nets that were opened and closed remotely to sample 4–5 discrete depth layers at each station. Depth strata sampled in each tow were chosen based on DO profiles to sample the surface mixed layer, within the oxycline, in any regions of mid-water DO minima, and one or more deep strata. Paired day and night samples

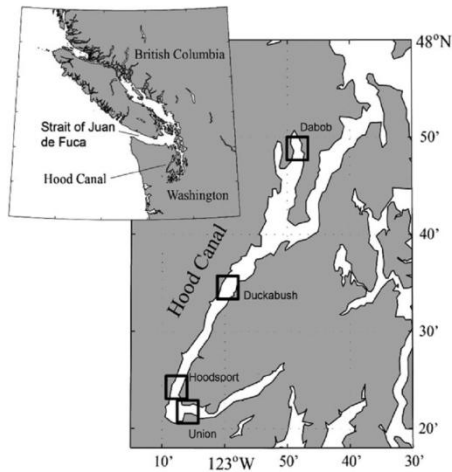


Fig. 1. Map of sampling stations in Hood Canal, Washington, USA. Data for this study were primarily analyzed from the northernmost (Dabob) and southernmost (Union) stations.

were collected at each station. The Multinet was equipped with inner and outer flow meters to measure the water volume filtered and monitor for clogging. Samples were preserved in 5% buffered formalin in seawater and returned to the lab for identification where 200–1300 (median = 445) individual zooplankton were identified, staged and counted per sample, of which the number of female *Calanus* counted ranged from 0 to 171 per sample. A total of 132 individual samples were analyzed for this study.

Laboratory Experiments—We collected zooplankton off of Shilshole Bay, Seattle by gentle vertical hauls (0.2 m s^{-1}) using a 1-m diameter ring net with $571\text{-}\mu\text{m}$ mesh equipped with a non-filtering cod end. Female *C. pacificus* were selected from the hauls and held for 24 h prior to experiments to allow recovery from capture stress. We completed three survival trials. Each trial consisted of an ambient DO control treatment ($\sim 7.0\text{--}8.0 \text{ mg DO L}^{-1}$) and one or more low-DO treatments (ranging $0.9\text{--}1.8 \text{ mg DO L}^{-1}$). Seawater for the treatments was collected from below the pycnocline in Puget Sound, held at 13°C , then filtered through a $20\text{-}\mu\text{m}$ sieve and diluted to 30.0 (typical sub-surface conditions in Puget Sound) with deionized water 24 h before experiments.

We attained target DO levels by vigorously bubbling 20-L carboys of the filtered seawater with N_2 gas. We estimated the DO level during N_2 bubbling using a Pinpoint II Oxygen Monitor (American Marine Inc.)

which had been calibrated by oxygen titrations using the modified Winkler titration method (Carpenter, 1965a; Carpenter, 1965b) on a Metrohm 765 Dosimat burette. Because the benchtop meter is not highly accurate, after bubbling each 20-L carboy to a target DO concentration, a minimum of three 125-ml biological oxygen demand (BOD) bottles were filled, then immediately fixed and sealed for Winkler titration to confirm the starting (T_0) oxygen concentration; as performed, the modified Winkler method has a total estimated error of $<0.01 \text{ mL L}^{-1}$ (Murray and Riley, 1969). Fifteen to twenty 300-mL BOD bottles per DO level were then immediately filled from the same treatment water, and one healthy female *C. pacificus* was placed in each bottle. These 300-ml bottles were placed in the dark at 13°C for 24 h. Within 1 h after introducing females and after 24 h (T_{24}), the bottles were examined to determine whether the females were alive or dead. At T_{24} , after examining for mortality, we randomly selected a minimum of three (up to six) of the 300-mL bottles and immediately fixed them for oxygen titration, which was conducted within 48 h of completing the trial.

Survival at each oxygen concentration was calculated as the percent of females in each treatment that were alive at T_{24} . The oxygen concentration estimated to be lethal to 50% of the experimental females after 24 h (the LC50) was estimated from the survival curve by fitting a linear regression using MatLab software version R2016a (The MathWorks Inc., Natick, MA).

RESULTS

Field results—As is typical in Hood Canal, oxygen was lower at Union than at Dabob, and persistently declined over the summer (Fig. 3). We sampled across a wide range of DO concentrations, from hypoxic sub-pycnocline waters at Union in autumn (minimum DO measured = 1.3 mg L^{-1}) to supersaturated surface phytoplankton blooms in early summer at Hoodsport (maximum of $15.7 \text{ mg DO L}^{-1}$). Upper 3-m temperatures ranged from 10 to 22°C ; sub-pycnocline temperatures ranged from ~ 8 to 12°C . In summer when oxygen levels were lowest, oxygen profiles showed deep water oxygen depletion typical of many estuaries (Fig. 2 left panel) which were displaced upward into the water column when dense ocean water intruded into the fjord in late summer to create mid-water oxygen minimum layers (Fig. 2 right panel).

Female *C. pacificus* were collected in 63 of the 132 plankton nets analyzed. Densities (excluding 0 values and two very high outliers) ranged from 0.2 to 157 female *C. pacificus* m^{-3} (Fig. 3). We did not find a strong relationship between female abundance and oxygen

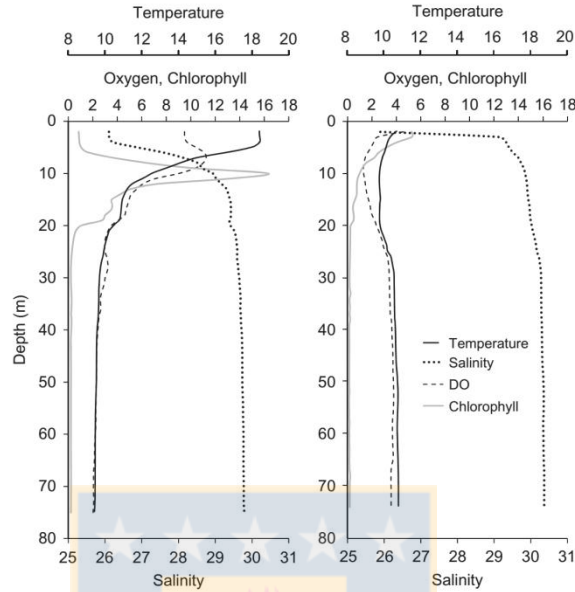


Fig. 2. CTD profiles from Union in (left panel) August 2013 and (right panel) October 2013 as examples of conditions present during the field study. Temperature ($^{\circ}\text{C}$), salinity, DO (mg L^{-1}) and fluorescence as chlorophyll ($\mu\text{g L}^{-1}$) from profiles are shown. Summary data from all net tows are given online in the Supplementary Information.

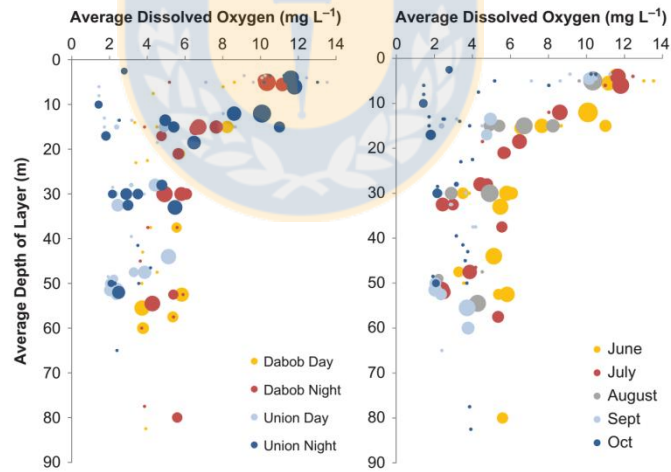


Fig. 3. Abundances of *C. pacificus* females as $\text{Log}_{10}(\text{ind. m}^{-3} + 1)$, relative to average depth of the layer sampled (y -axis) and average DO of that layer (x -axis). Abundances are shown as circles colored by (left panel) station and time or (right panel) month sampled, with the largest circle scaled to the maximum abundance (960 ind. m^{-3}) and the smallest to zero. All individual samples shown regardless of time of day collected.

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over the range of DO concentrations we sampled in the field when including zero values (Pearson $R^2 = 0.04$). *Calanus pacificus* females did show a tendency towards lower abundances with lower oxygen, and were rare in nets that sampled in depth strata where DO values were $<1.9 \text{ mg L}^{-1}$, regardless of where in the water column (deep or near surface) the low-DO layers occurred or whether the sampling was conducted during day or night. Female abundances declined from early to late summer, but did not significantly differ between stations. Day–night differences in depth distributions reflected diel migration from deeper waters during the day towards the surface and night.

Laboratory results—Over the three 24-h experimental survival trials, three ambient control DO concentrations of $\sim 7.7\text{--}8.0 \text{ mg L}^{-1}$ were tested against seven low-DO treatments that averaged $0.92\text{--}1.84 \text{ mg DO L}^{-1}$ at T_0 (Table I). Because of the difficulty in homogenizing the treatment water and maintaining constant oxygen concentrations during experimental setup, jars titrated from the five moderately low-DO treatments showed some overlap among initial and final DO concentrations. That is, the treatments that averaged $1.36\text{--}1.56 \text{ mg DO L}^{-1}$ at T_0 were not discrete from each other, whereas the lowest (0.92 mg L^{-1}) and highest (1.84 mg L^{-1}) of

the low-DO treatments were discrete from the rest. DO concentrations in the experimental jars were higher at T_0 than at T_{24} by an average of $0.26 \text{ mg DO L}^{-1}$, indicating that respiration occurred in the sealed jars during the trials. Since the oxygen concentration experienced by the females was therefore not constant, the DO concentrations shown in Fig. 4 and used in the text below as treatment levels are the average DO from all jars titrated at T_0 and T_{24} in each treatment.

All female *C. pacificus* survived 24 h in the three control treatments at 13°C , most (13 of 15; 87%) survived in the highest of the low-DO treatments ($1.70 \text{ mg DO L}^{-1}$), and all survived in the next highest treatment level in Trial 2 ($1.44 \text{ mg DO L}^{-1}$), whereas only 70% survived the same average level in Trial 3. All females died within 1 h of T_0 in the lowest oxygen concentration (0.92 mg L^{-1}). Overall, 24-h survival showed a sharp decline with decreasing oxygen concentrations between the range of $\sim 1.4\text{--}0.9 \text{ mg DO L}^{-1}$ with 100% survival at higher concentrations and 0% survival at the lowest concentration tested. Fitting a linear regression over the range of 0–100% survival between 0.9 and 1.7 mg DO L^{-1} resulted in an R^2 of 0.75 and an estimated LC_{50} of $1.3 \pm 0.1 \text{ mg L}^{-1}$.

Table I: DO and female *C. pacificus* survival data from survival experiments

	Trial 1			Trial 2			Trial 3			
	Control	LDO-1	LDO-2	Control	LDO-1	LDO-2	Control	LDO-1	LDO-2	LDO-3
Titrated DO at T_0 (mg L^{-1})	8.08	0.91	<i>1.34^b</i>	7.80	1.89	1.58	7.67	1.36	1.48	1.57
	7.89	1.04	<i>1.35^b</i>	7.72	1.86	1.57	7.68	1.50	1.45	1.54
	7.92	0.80	<i>1.37^b</i>	7.70	1.78	1.54	7.73	1.44	1.52	1.61
					1.83					1.54
Average at T_0 ^c	7.97	0.92 ^a	1.36 ^b	7.74	1.84 ^e	1.56 ^d	7.70	1.43 ^{b,e}	1.48 ^a	1.52 ^{b,d,e}
Titrated DO at T_{24} (mg L^{-1})	7.66	N/A ^a	1.07	7.31	1.56	1.29	7.44	1.23	1.26	1.42
	7.55		1.07	7.25	1.56	1.35	7.51	1.10	1.19	1.27
	7.70		1.10	7.26	1.54	1.33	7.48	1.10	1.27	1.44
								1.25	1.07	1.30
Average at T_{24}	7.64	N/A ^a	1.08	7.27	1.55	1.32	7.48	1.16	1.24	1.36
Average DO (used in Fig. 4) ± 1 Std. Dev.	7.80 \pm 0.20	0.92 \pm 0.12	1.22 \pm 0.15	7.51 \pm 0.26	1.70 \pm 0.16	1.44 \pm 0.14	7.59 \pm 0.12	1.30 \pm 0.15	1.36 \pm 0.14	1.44 \pm 0.11
<i>C. pacificus</i> : # Survived / # tested	18/18	0/18	5/18	18/18	13/15	15/15	14/14	5/20	11/20	14/20
<i>C. pacificus</i> : % Survival	100%	0%	28%	100%	87%	100%	100%	25%	55%	70%

Average DO values used as treatment levels in Fig. 4 and the text was calculated as the mean of average T_0 and average T_{24} concentrations; Std. Dev. was calculated from all individual T_0 and T_{24} titration values. LDO-1, LDO-2 and LDO-3 represent the low-DO (LDO) treatment levels in each trial. Bold numbers represent samples in which the female had died by T_{24} . Superscripts on Average at T_0 LDO values indicate overlapping ranges of oxygen concentration between treatments at T_0 .

^a100% mortality of all females within 1 h of setup eliminated the need for T_{24} titrations.

^bTitrations were not conducted at T_0 , so T_0 data (*italicized*) were estimated from T_{24} values + the average of ΔDO ($T_0\text{--}T_{24}$) from all other titrations.

^cSimilar superscripted numbers indicate treatments with overlapping range of oxygen concentrations at T_0 .

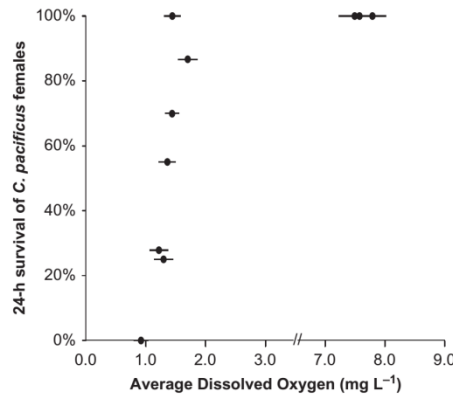


Fig. 4. The relationship between percent 24-h survival of *C. pacificus* females and DO (mg L⁻¹). See Table 1 for oxygen concentration calculations; error bars were calculated as one Std. Dev. of all T_0 and T_{24} oxygen titration values within each treatment.

DISCUSSION

We did not find evidence of avoidance of hypoxic layers, or decreased abundances of *C. pacificus* females where oxygen concentrations were low in the field. Female abundances were lowest in late summer when oxygen was lowest, but did not significantly differ between stations despite large differences in oxygen. At Union, females were abundant at oxygen levels down to 1.9 mg L⁻¹ and were present in low numbers in water column layers where oxygen concentration (averaged across the depth of the net tow) was as low as 1.4 mg L⁻¹. Although *C. pacificus* females were absent or in very low numbers at the lowest DO levels we sampled (<1.9 mg L⁻¹), a scarcity of samples at low DO prohibits drawing conclusions about whether they actively avoided those layers. Notably, some of the lowest oxygen concentrations occurred in the upper 20 m of the water column (Fig. 3), so low female abundance in those layers was not simply due to avoidance of deep water where oxygen depletion typically occurs in estuaries.

To test whether the observed field distributions reflected physiological tolerance for low oxygen, as opposed to an inability to sense and move to avoid stressful oxygen levels, we tested the survival of female *C. pacificus* in response to DO in the lab. Our results suggest there is a tight range in DO concentration that defines a rapid decline in 24-h survival of *C. pacificus* females; from 100% survival at ~1.5 mg DO L⁻¹ to 0% survival at 0.9 mg DO L⁻¹ at 13°C. Thus, the laboratory results suggest that at ≤13°C, females would not need to avoid concentrations of 1.5 mg L⁻¹ to survive

periods shorter than 24 h. Based on the laboratory results, females would be expected to avoid concentrations <1.5 mg L⁻¹, particularly in regions with warmer temperatures and hence higher BOD (Brown et al., 2004), but we did not observe oxygen concentrations in the field lower than 6 mg L⁻¹ where temperature was ≥13°C. We were not able to assess whether females collected from low oxygen layers were alive at the time of collection, so there is the possibility that some collected from deep hypoxic water may have been sinking carcasses.

The oxygen survival thresholds we found for Hood Canal *C. pacificus* were similar to those reported for the copepods *A. tonsa* and *O. colcarva* which also occupy seasonally hypoxic regions. Stalder and Marcus (1997) reported a rapid decline in survival of Florida Gulf Coast *A. tonsa* between 1.4 (100% survival) and 0.9 mg DO L⁻¹ (0–10% survival) at 20°C. Roman et al. (1993) reported that complete mortality of Chesapeake Bay *A. tonsa* occurred at ≤1.7 mg L⁻¹ and significant mortality of *O. colcarva* at <2 mg L⁻¹ at 20°C, but that ~15% of *O. colcarva* survived concentrations as low as 0.6 mg L⁻¹. It is clear that inter-specific and even inter-population differences in oxygen tolerance exist, but among species that inhabit seasonally hypoxic regions, thresholds at which decreased survival and complete mortality occur appear to be within the fairly small range of ~1–2 mg DO L⁻¹.

These values are in stark contrast with those reported for copepods that are adapted to life in the low oxygen environments of permanent OMZs. While the diversity of copepods that can inhabit the core of OMZs is low compared to oxygenated surface waters (Wishner et al., 2008), some copepods such as *Lucicutia grandis*, *Eucalamus elongatus* and *Spinocalanus antarcticus* can be abundant in <0.05 mL L⁻¹ (<0.07 mg L⁻¹) deep in the Arabian Sea OMZ (Wishner et al. 2000, 2008). On the other hand, copepods such as *Subeucalanus subtenius* suffer high mortality in 5% O₂ saturation (0.6 mg L⁻¹ at 10°C) despite inhabiting the Eastern Tropical Pacific, so are restricted to near surface waters above the OMZ (Cass and Daly, 2014).

The measured survival of *C. pacificus* females in response to oxygen was similar to that found for the closely related *C. carinatus* in the Benguela upwelling system (Auel and Verheye, 2007). These authors reported 100% survival of female and stage CV *C. carinatus* at 1.6 mg DO L⁻¹, a sharp decline in survival between ~1.0 and 0.5 mg L⁻¹ and complete mortality at 0.2 mg L⁻¹. The lower oxygen concentration at which complete mortality occurred may again reflect the difference between copepods that inhabit regions with permanent OMZs versus seasonally hypoxic regions.

Ruz et al. (2015; in review) reported that >85% of *Calanus chilensis* females collected from the Humboldt OMZ region survive 24-h at DO concentrations between 0.6 and 2.0 mg L⁻¹, but that females collected within low DO water have lower respiration and other measures of metabolism than those collected from above the OMZ. Despite occupying an OMZ region, *C. carinatus* seems not to have evolved tolerance to oxygen levels found in the core of the OMZ (<0.8 mg L⁻¹), as some other copepods (e.g. *Pleuromamma robusta* and *Aetideopsis carinata*) in the Benguela System have (Auel and Verheye 2007).

Calanus, like other large zooplankton, migrate vertically to avoid visual predation near the surface during the day (Frost, 1988; Dagg et al., 1998) or during diapause (Dahms, 1995). Fish are generally more sensitive to low DO than zooplankton (Miller et al., 2002) and may exhibit physiological responses or avoidance of DO levels <3–5 mg L⁻¹ (Breitburg et al., 2001; Vaquer-Sunyer and Duarte, 2008; Ekau et al., 2010). Since *C. pacificus* are able to tolerate much lower oxygen levels than many fish, depths where DO is <3 mg L⁻¹, but above their tolerance threshold of ~1.5 mg DO L⁻¹, may serve as additional predation refuges. In coastal upwelling areas, some species of *Calanus* have been observed to migrate into OMZs. For example, *C. chilensis* was observed in high densities (60% of the population) in DO ranging from 0.92 to 1.38 mg L⁻¹, likely to avoid predation by a local anchovy population in the Humboldt upwelling system (Hirche et al., 2014). Off the Santa Barbara Basin, *C. pacificus* was found to migrate to just above the OMZ for increased protection from predators while in the fifth copepodid (CV) stage, then enter a diapause state for the winter (Osgood and Checkley, 1997). Alldredge et al. (1984) observed high abundance of diapausing stage CV *C. pacificus* in very low oxygen (0.2 ml L⁻¹) deep waters of the Santa Barbara Basin. The ability to tolerate more severe conditions than predators may be an important trait that permits survival of populations.

CONCLUSIONS

We examined distributions of adult female *C. pacificus* in relation to oxygen in a seasonally hypoxic sub-estuary of Puget Sound, Washington, and conducted laboratory experiments to quantitatively define their physiological tolerance to DO in an effort to contribute to the understanding of the effects of hypoxia on marine trophic interactions. We found little evidence of avoidance of hypoxia in the field at oxygen concentrations ≥1.9 mg L⁻¹. Laboratory results confirm that the copepods are

tolerant of oxygen levels as low as ~1.5 mg L⁻¹ over a 24-h period at 13°C, but suffer complete mortality at 0.9 mg L⁻¹. *Calanus pacificus* is one of the dominant *Calanus* species of the Northeast Pacific (Star and Mullin, 1981). By gaining a thorough understanding of the physiological limits and behavioral responses of this important species to environmental stressors, we will be better able to predict changes in marine ecosystems in the face of climate change.

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