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Efectos de la radiación UVB en Chile centro-sur (36°S) en embriones de anchoveta *Engraulis ringens* (Jenyns 1842) y sardina común *Strangomera bentincki* (Norman 1936)

PAULINA VÁSQUEZ ROJAS
CONCEPCIÓN-CHILE
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Profesora Guía: Alejandra Llanos Rivera
Profesor Co-Guía: Leonardo Castro Cifuentes
Depto. de Oceanografía, Facultad de Cs. Naturales y Oceanográficas
Universidad de Concepción

Universidad de Concepción
Dirección de Postgrado

La Tesis de *"Magíster en Ciencias con Mención en Oceanografía"* titulada *"EFECTOS DE LA RADIACIÓN UVB EN CHILE CENTRO-SUR (36°S) EN EMBRIONES DE ANCHOVETA* ENGRAULIS RINGENS *(JENYNS 1842) Y SARDINA COMÚN* STRANGOMERA BENTINCKI (NORMAN 1936)" de la Srta. PAULINA VÁSQUEZ ROJAS y realizada bajo la Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, ha sido aprobada por la siguiente Comisión de Evaluación:

Dra. Alejandra Llanos-Rivera
Profesora Guía
Universidad de Concepción ______________________

Dr. Leonardo Castro Cifuentes
Profesor Co-Guía
Universidad de Concepción ______________________

Dra. Camila Fernández
Miembro Comité de Tesis
Universidad de Concepción ______________________

Dra. Laura Farías
Directora
Programa de Postgrados en Oceanografía
Universidad de Concepción ______________________

A Julia y Hernán, mis padres.

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Curriculum Vitae

Paulina Vásquez Rojas

Nacida el 18 de Diciembre, 1985, en Santiago de Chile

2004-2007: Licenciado en Biología, Universidad de Concepción, Chile.
2008: Biólogo con Mención en Biodiversidad y Conservación, Universidad de Concepción, Chile.
2010-2014: Magíster en Ciencias con mención Oceanografía, Universidad Concepción, Chile.

PUBLICACIONES

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ÁREAS DE INVESTIGACIÓN
Principal: Oceanografía biológica
Secundaria: Desarrollo embrionario de peces pelágicos y efectos medioambientales.

EXPERIENCIA DOCENTE
- Ayudante cursos de pregrado:
Biología III, Universidad de Concepción, Chile, 2008.
Zoología General II, Universidad de Concepción, Chile, 2008.
Fundamentos Investigación Científica, Universidad de Concepción, Chile, 2010.

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RESUMEN

Efectos de la radiación UVB en Chile centro-sur (36°S) en embriones de anchoveta *Engraulis ringens* (Jenyns 1842) y sardina común *Strangomera bentincki* (Norman 1936).

Autora: Paulina Vásquez Rojas

Profesora Guía: Alejandra Llanos Rivera

Profesor Co-Guía: Leonardo Castro Cifuentes

Los niveles de radiación ultravioleta B (UVB) que inciden en la Tierra han aumentado debido a la reducción del ozono estratosférico, producto de la actividad humana. El incremento en la UVB ha provocado efectos perjudiciales en embriones de peces marinos planctónicos, los cuales son vulnerables debido a que habitan las capas más superficiales de la columna de agua. Los efectos perjudiciales sobre los embriones pueden ser letales y subletales, los últimos se pueden manifestar con la presencia de malformaciones morfológicas, con un menor éxito de eclosión y con la pérdida de boyantez positiva en los embriones. Mediciones de los niveles actuales de UVB en Chile centro-sur (36°S) indican que los embriones de peces planctónicos que se encuentran en esta zona están expuestos a niveles de UVB que para otras especies de peces son nocivos (0,05-4,04 W m^{-2} UVB). En este contexto, la anchoveta *Engraulis ringens* Jenyns 1842 y la sardina común *Strangomera bentincki* (Norman 1936) desovan embriones planctónicos, los cuales poseen una elevada tasa de mortalidad natural. Estas características los hacen buenos modelos para estudiar los efectos de los niveles actuales de radiación UVB en Chile centro-sur (36°S) sobre embriones de peces nativos, lo cual constituye el objetivo de este estudio.

Para esto, embriones de anchoveta y sardina común fueron expuestos a una dosis diaria de 0,1 W m^{-2} de UVB correspondiente al promedio anual de UVB en Chile centro-sur. Luego de la irradiación en los embriones se evaluó la mortalidad, la presencia de malformaciones morfológicas, el éxito de eclosión y se cuantificó el efecto sobre la boyantez. Finalmente, se determinó si la exposición de embriones de estas dos especies a los niveles de UVB registrados desde Julio del 2011 a Enero del 2012 en el ambiente natural pueden haber inducido efectos letales o subletales, teniendo en consideración la distribución vertical descrita

por primera vez en este estudio para ambas especies, así como las características oceanográficas en la zona de estudio durante el periodo antes mencionado.

A través de la experimentación en laboratorio se estableció que los niveles de UVB de la zona centro-sur de Chile (36°S), provocan efectos letales y subletales en embriones de peces marinos planctónicos tales como los embriones de anchoveta y sardina común, y además de esto se determinó que en terreno existe una parte de la población de las especies evaluadas que puede experimentar los efectos nocivos observados luego de la exposición a UVB.

ABSTRACT

UV radiation effects on the embryos of the anchoveta *Engraulis ringens* (Jenyns 1842) and common sardine *Strangomera bentincki* (Norman 1936) off Central Chile (36°S)

The levels of ultraviolet B radiation (UVB) that reaches the Earth have increased due to stratospheric ozone depletion as a result of human activity. Increased UVB cause detrimental effects on planktonic marine fish embryos, which are vulnerable because they inhabit the upper layers of the water column. Adverse effects on the embryos can be lethal and sublethal. Sublethal effects manifest in embryos with the presence of morphological malformations, with lower hatching success and loss of positive buoyancy. Measurements of current levels of UVB in south-central Chile (36°S) indicate that planktonic fish embryos found in this area are exposed to UVB levels which for other fish species are harmful (0.05-4.04 W m^{-2} UVB). In this context, anchoveta *Engraulis ringens* Jenyns 1842 and common sardine *Strangomera bentinck*i (Norman 1936) spawn planktonic embryos which have a high rate of natural mortality. These characteristics make them suitable models to study the effects of current levels of UVB radiation in south-central Chile (36°S) on native fish embryos, which is the main goal of this study.

For this, embryos of anchovy and common sardine were exposed to a daily dose of 0.1 W m^{-2} UVB which correspond to the annual average of UVB in south-central Chile. After irradiate the embryos the mortality, the presence of morphological defects and the effect on buoyancy were assessed. Finally, we determined whether exposure of embryos of anchoveta and common sardine to the UVB levels recorded from July 2011 to January 2012 may have induced lethal or sublethal effects as observed in the experiments, taking into account the vertical distribution described for the first time in this study for both species and the oceanographic features in the study area during the period mentioned above.

With the performed laboratory experiments it was established that UVB levels off south-central Chile (36° S) can cause both lethal and sublethal effects on planktonic marine fish embryos, such as anchoveta and common sardine embryos, and in the it field was determined that there is a part of the population of the tested species that can suffer adverse effects observed in the experiments after the exposure to UVB.

1.- INTRODUCCIÓN

La radiación solar que llega a la Tierra está constituida por la radiación visible (VIS 400 a 700 nm) y la radiación ultravioleta (UVR 280 a 400 nm). En la actualidad, los niveles de UVR que inciden en la superficie terrestre han aumentado ya que están directamente relacionados con el ozono estratosférico, el principal responsable de su absorción y que ha reducido sus concentraciones como consecuencia de la actividad humana (Farman *et al.* 1985, Whitehead *et al.* 2004). Esto ha provocado el incremento de los niveles de UVR en la región Antártica y en latitudes medias del Hemisferio Sur (Madronich 1994, Vernet *et al.* 2009).

El incremento en los niveles de UVR que inciden en la superficie terrestre ha desencadenado efectos perjudiciales para una amplia variedad de organismos (Häder *et al.* 2007) entre los cuales destacan los embriones de peces marinos planctónicos por su particular vulnerabilidad, dado que habitan las capas más superficiales de la columna de agua en donde la penetración de la UVR es mayor que en profundidad (Zagarese & Williamson 2004). La vulnerabilidad de los embriones de peces a la UVR está determinada además por factores externos e internos:

- Factores externos: determinan la transparencia de la columna de agua y en consecuencia la profundidad a la que penetra la UVR desde la superficie. Incluyen características de la columna de agua tales como la mezcla, la estratificación y sus propiedades ópticas. Estas últimas, dependen de la presencia de compuestos que absorban parte del espectro UV como el carbono orgánico disuelto (COD) y la clorofila-*a* (Laurion *et al.* 1997, Kuhn *et al.* 1999, Lesser *et al.* 2001, Vernet *et al.* 2009).
- Factores internos: características propias de la especie que conllevan a la protección y reparación del daño en tejidos y ADN provocados por la exposición a UVR (Lesser *et al.* 2001, Williamson *et al.* 2001, Browman 2003). Estas características incluyen la estacionalidad y ubicación del desove (latitud, distancia de la costa), la distribución vertical de los huevos y la presencia de pigmentos protectores como carotenoides y melanina cuticular en los embriones (Chioccara *et al.* 1980, Plack *et al.* 1981, Dethlefsen *et al.* 2001).

Experimentalmente, los efectos nocivos evidenciados en embriones de peces planctónicos se han presentado con la exposición a longitudes de onda de entre 280 y 320 nm, pertenecientes al espectro UVB. Asimismo, esta porción del espectro UV es la cual ha acrecentado más sus niveles de penetración en la columna de agua en comparación a longitudes de ondas más largas correspondientes al VIS (400 a 700 nm) y a la radiación UVA (400 a 320 nm). Esta última porción del espectro UV ha sido relacionada con mecanismos de reparación del daño provocado por la exposición a UVB (Lesser *et al.* 2001).

La exposición a UVB provoca efectos letales y subletales en los embriones (Tabla 1). Los efectos letales conducen a un incremento en la tasa mortalidad, la cual se acentúa si los embriones son expuestos en estadios de desarrollo previos a la gastrulación (Kouwenberg *et al.*1999, Kuhn *et al.*2000, Steeger *et al.*2001, Zagarese & Williamson 2000, Browman 2003). Los efectos subletales pueden presentarse como una disminución en las tasas de desarrollo embrionario y de crecimiento larval, con la presencia de malformaciones morfológicas en embriones y larvas (Hunter *et al.*1981, Lesser *et al.* 2001) y con la pérdida de boyantez positiva en los embriones (Dethlefsen *et al.*1996a, Dethlefsen *et al.* 2001).

La pérdida de boyantez positiva es especialmente relevante ya que produce que los huevos cambien su distribución vertical, la cual determina: i) la extensión y dirección en la cual los huevos pueden ser transportados, ii) la alteración de la tasa de desarrollo y iii) la mortalidad, dependiendo de la coincidencia de los embriones con depredadores, y de las condiciones fisicoquímicas y de alimentación en las cuales eclosionen las futuras larvas luego de la alteración de boyantez de los huevos (Applegate & Ley 1988, Mangor-Jensen & Waiwood 1995, Vetter *et al.*1999, Ospina-Álvarez *et al*. 2012).

Estudios que evalúen los niveles actuales de la UVR en el Pacífico Suroriental son escasos. No obstante, Hernández *et al*. (2006) documentan una variación estacional en la UVR en la zona de Chile Centro-Sur (36°S), con altos niveles de radiación durante el verano (~35 W m^{-2} UVA y ~6 W m^{-2} UVB). Sin embargo, según lo reportado por Rain *et al.* (2012), se ha registrado más bien un aumento en los niveles de radiación UVB sin que exista un aumento proporcional de la UVA. Igualmente, mediciones realizadas por la Dirección Meteorológica de Chile de irradiación máxima UVB en Chile Centro-Sur (Fig.

1) permiten inferir que los huevos de peces planctónicos que se encuentran en esta zona están expuestos a niveles promedio de radiación máxima UVB que para otras especies son nocivos a letales (0,05-4,04 W m^{-2} UVB) (Tabla 1).

La anchoveta *Engraulis ringens* (Jenyns 1842) y la sardina común *Strangomera bentincki* (Norman 1936) desovan huevos planctónicos en Chile Centro-Sur (36°S) durante la estación reproductiva que comienza en julio y que se extiende hasta diciembre-enero (Cubillos *et al.* 1999). Los huevos de ambas especies se distribuyen en los primeros 20 m de la columna de agua, incrementando su abundancia hacia la superficie (Giraud 2011). En la anchoveta se ha descrito una disminución en la calidad bioquímica del huevo a medida que la estación reproductiva avanza (Castro *et al.* 2002), por lo tanto los embriones presentan una menor calidad al finalizar el periodo reproductivo. Dado que la anchoveta y la sardina común comparten similitudes en las características reproductivas se podría esperar lo mismo para esta última. Otra característica relevante es que ambas especies tienen una elevada tasa de mortalidad natural (Cubillos *et al.* 1999) la que podría aumentar si los embriones son expuestos a niveles de radiación UVB que para otras especies son dañinos, más aún teniendo en cuenta que ambas especies carecen de pigmentos hasta la organogénesis temprana y que sus huevos son de corion trasparente (Herrera *et al.* 1987, Artüz 1999).

El conjunto de las características antes mencionadas hace que los embriones de anchoveta y sardina común constituyan sujetos de estudio para evaluar los efectos letales y subletales de los niveles actuales de radiación UVB en embriones de peces planctónicos de la zona Centro-Sur de Chile (36°S).

Tabla 1. Efectos de la exposición experimental a niveles de radiación ultravioleta B (UVB) registrados en terreno en embriones de especies de peces planctónicos marinos en distintas regiones del mundo.

Especie	Zona de Estudio	Irradiancia UVB (W/m^2)	Efectos	Referencia
Limanda limanda, Pleuronectes platessa	54ºN 6ºE	3,63	Aumento mortalidad, Pérdida boyantez, malformaciones	Dethlefsen *et al.* 1996a
Limanda limanda, Pleuronectes platessa	54ºN 6ºE	0,98 3,63	Pérdida de boyantez malformaciones Aumento mortalidad, Pérdida boyantez, malformaciones	Dethlefsen *et al.* 2001
Pleuronectes platessa	45°N 68°W	0,21 0,47	Aumento mortalidad. Aumento mortalidad y pérdida de boyantez.	Steeger *et al.* 2001
Gadus morhua	44°N 67°W	0,05	Aumento mortalidad, Menor longitud en la eclosión	Lesser *et al.*2001
Gadus morhua	49°N 59°W	4,04	Aumento mortalidad.	Kouwenberg *et al.*1999

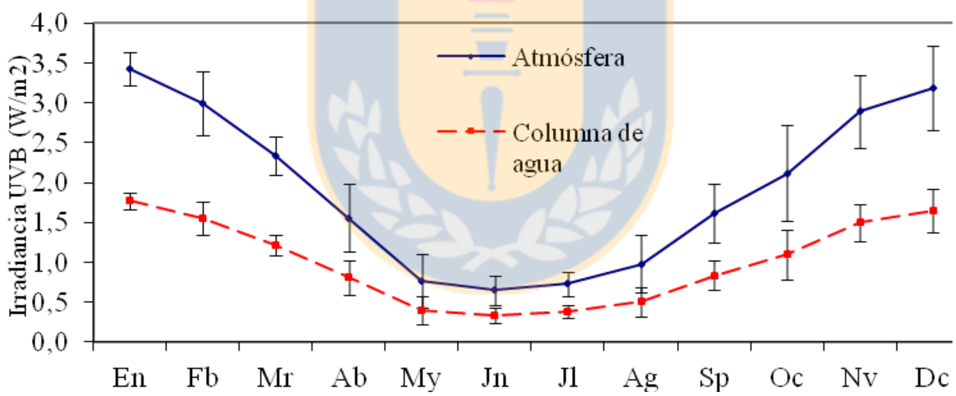

Fig. 1. Irradiancia máxima de UVB en ($W\ m^{-2}$) en Chile centro-sur (36°S) durante el año 2009. La línea azul indica la incidencia de UVB en la atmósfera y la línea roja la incidencia de la radiación UVB en la porción superficial de la columna de agua (0-5 m) teniendo en cuenta la reflexión (51,80%) (Rain *et al.* 2012).

2.- HIPÓTESIS Y OBJETIVOS

Hipótesis

1. La exposición de embriones de anchoveta y sardina común a los niveles actuales de radiación ultravioleta B existentes en Chile centro-sur (36°S) provoca efectos letales en ambas especies.
2. La exposición de embriones de anchoveta y sardina común a los niveles actuales de radiación ultravioleta B existentes en Chile Centro-Sur (36°S) provoca que éstos manifiesten efectos subletales.

Objetivo general

Caracterizar los efectos de los niveles actuales de radiación UVB en Chile centro-sur (36°S) sobre embriones de anchoveta y sardina común.

Objetivos específicos

1. Establecer la presencia de efectos letales en embriones de anchoveta y sardina común expuestos a niveles actuales de UVB registrados en Chile centro-sur.
2. Determinar si hay una disminución en el porcentaje de eclosión de larvas de anchoveta y sardina común desde embriones expuestos a los niveles actuales de UVB registrados en Chile centro-sur.
3. Evaluar la frecuencia y tipos de malformaciones morfológicas en embriones de anchoveta y sardina común expuestos a niveles actuales de UVB registrados en Chile centro-sur.
4. Establecer la influencia sobre la boyantez de embriones de anchoveta y sardina común que tiene la exposición de éstos a los niveles actuales de UVB registrados en Chile centro-sur.
5. Evaluar diferencias en los efectos letales y subletales en embriones de anchoveta y sardina común expuestos en distintos estados de desarrollo embrionario a los niveles actuales de UVB registrados en Chile centro-sur.

6. Describir la distribución vertical de embriones de anchoveta y sardina común en Chile centro-sur y de acuerdo a esto, establecer los niveles de radiación UVB a los que se encuentran expuestos en la columna de agua.

3.- MATERIALES Y MÉTODOS

3.1. Obtención de embriones de anchoveta y sardina común

Los embriones de anchoveta y sardina común fueron obtenidos desde muestras recolectadas durante el año 2011 y principios del 2012 en estaciones de muestreo ubicadas en la Bahía de Concepción y en el Golfo de Arauco (Fig. 2). El muestreo se realizó con red Bongo (60 cm ancho de boca, 300 µm de trama, equipada con flujómetro General Oceanics) mediante lances oblicuos desde 20 m de profundidad hasta la superficie con una velocidad de arrastre menor a 2 nudos.

Las muestras fueron mantenidas a 12°C y trasladadas al laboratorio en donde se separaron los embriones de anchoveta *Engraulis ringens* y sardina común *Strangomera bentincki* utilizando una lupa estereoscópica equipada luz fría (Zeiss Stemi SV 11). La separación de embriones se realizó por estado de desarrollo, según el criterio de Krautz *et al.* (2010) en el cual se distinguen: Estado I (embrión no formado), Estado II (embrión temprano: el embrión abarca hasta la mitad del corión) y Estado III (embrión tardío: el embrión abarca más de la mitad del corión).

3.2. Exposición de los embriones a radiación solar artificial

La dosis experimental de irradiación corresponde al promedio anual de irradiación UVB del año 2009 (Dirección Meteorológica de Chile, 2009) para el área de Chile centro-sur (36°S): 1,97 W m^{-2} UVB. Considerando los valores de reflexión (51,80%) y atenuación (10% en los primeros 5 m) de la UVB en la zona de estudio (Rain 2012), los embriones estarían expuestos a 0,1 W m^{-2} en los primeros 5 m de la columna de agua. Dado que la exposición máxima se produce durante 2 horas la dosis de irradiación diaria escogida fue de 720 J m^{-2} para el UVB y de 235.6 J m^{-2} para el UVA.

Los embriones obtenidos fueron expuestos en una cámara de irradiación artificial (UV irradiation chamber BS-03, Dr. Grobel Germany) a tres tratamientos: 1) VIS+UVA+UVB, 2) VIS+UVB y 3) Control; los que se detallan en la Tabla 2.

Tabla 2. Diferentes tratamientos utilizados en los experimentos de irradiación solar artificial en los cuales fueron expuestos embriones de anchoveta y sardina común.

Tratamiento	Filtro	Dosis diaria (J m^{-2})	Irradiación en cámara
VIS+UVA+UVB	Ninguno	720 UVB	Sí
VIS+UVB	UVA	235,6 UVA ;720 UVB	Sí
Control	VIS, UVA, UVB	0	Sí

Cada tratamiento consideró al menos 30 embriones de anchoveta y de sardina común en el mismo estado de desarrollo, los cuales fueron instalados en 60 mL de agua de mar filtrada e incubados a 12°C en oscuridad. El número de réplicas (mínimo 2) de cada tratamiento varió según la abundancia de embriones en las muestras de plancton. La dosis de UVB (720 J m^{-2}) se aplicó diariamente durante tres días.

3.3. Cuantificación de sobrevivencia, malformaciones y éxito de eclosión

Luego de la exposición a radiación solar artificial los embriones se monitorearon cada 12 horas hasta la eclosión de la larva, utilizando una lupa estereoscópica (Zeiss Stemi SV 11) con luz fría. En estos monitoreos y en cada uno de los tratamientos se cuantificaron los siguientes efectos letales/subletales:

Sobrevivencia: para cuantificar la sobrevivencia se contabilizó el número de embriones vivos y embriones muertos, determinándose con estos valores el porcentaje de embriones sobrevivientes.

Malformaciones: la frecuencia de malformaciones se evaluó mediante el análisis detallado de cada embrión, contabilizándose el número de embriones cuya morfología se desviaba de la definida para cada especie (anchoveta: Moser & Ahlstrom (1985); sardina común: Sepúlveda *et al.* (2000)). Cada una de las malformaciones observadas fue fotografiada para su posterior descripción.

Éxito de eclosión: para determinar el éxito de eclosión una vez transcurrido el desarrollo embrionario se contabilizó el número de embriones y de larvas presentes en las réplicas de cada uno de los tratamientos.

3.4. Medición de boyantez

Para medir cambios en la boyantez se aplicaron dos tratamientos de irradiación: 1) VIS+UVA+UVB (Irradiados) y 2) Control (Sin irradiar), considerando como mínimo 7 embriones por tratamiento. Luego de la irradiación, los embriones fueron trasladados a un sistema de columnas de gradiente de densidad de acuerdo al protocolo de Coombs *et al.* (1981). Las columnas (una para cada tratamiento) fueron monitoreadas cada 6 horas, aumentando la frecuencia de monitoreo cuando los embriones estaban próximos a la eclosión.

3.5. Medición de la distribución vertical de embriones

Se determinó el número de embriones en los distintos estratos de la columna de agua, para ello, desde Julio 2011 hasta Enero 2012 se obtuvieron muestras durante el día desde las estaciones ubicadas en la Bahía de Concepción (Fig. 2) con una red Tucker trawl (0,5 m^2 área de boca, 300 µm de trama, equipada con flujómetro General Oceanics) y los siguientes estratos fueron considerados: 0-5, 5-10, 10-15, 15-20, 20-30, 30-40 y 40-50 m. Las muestras fueron fijadas en formalina al 5% y en laboratorio se separaron los embriones según especie y estado de desarrollo. Luego se calculó la abundancia de cada estado desarrollo en cada estrato de la columna de agua. Además, en cada estación de muestreo se realizaron perfiles hidrográficos con un CTD Seabird 19plus para la construcción de perfiles de densidad.

3.6. Análisis estadístico

Los datos expresados en porcentaje fueron transformados (arco-seno). Para determinar diferencias de sobrevivencia entre los distintos tratamientos se utilizó un Análisis de Covarianza (ANCOVA). Las diferencias en los efectos subletales entre tratamientos se determinaron con un Análisis de Varianza (ANOVA). Cualquier diferencia significativa de los distintos tratamientos fue determinada utilizando una prueba *a posteriori* de comparaciones múltiples de Tukey HSD. En el caso de los experimentos de boyantez se aplicó una prueba t de Student.

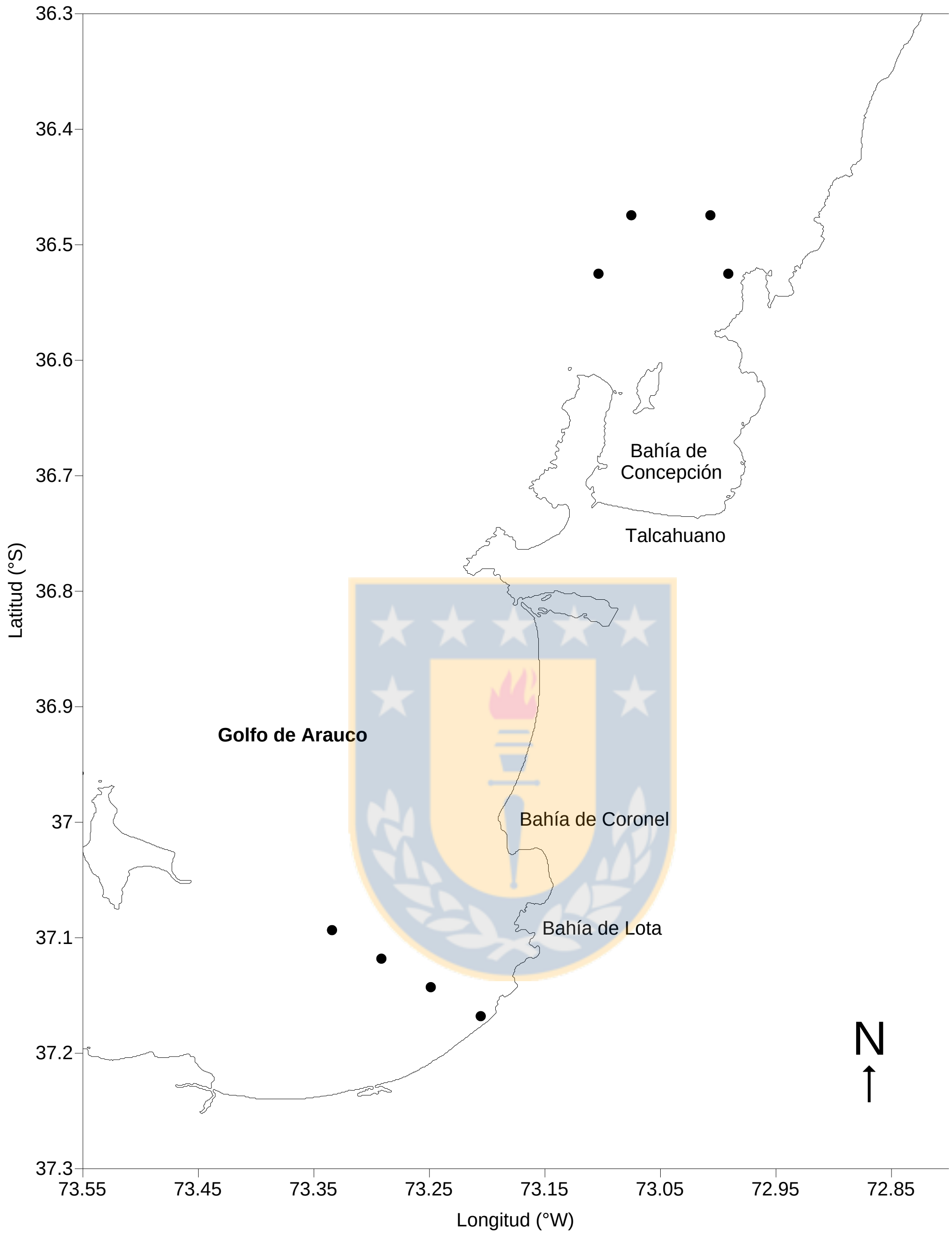

Fig. 2. Área de estudio ubicada en la Zona centro-sur de Chile (36°). Los puntos en zonas aledañas al Golfo de Arauco y a la Bahía de Concepción indican estaciones en las cuales se obtuvieron muestras planctónicas mensualmente desde Julio del 2011 hasta Enero del 2012.

4.- RESULTADOS

4.1. Capítulo 1. Artículo científico sometido a publicación en revista Marine and Freshwater Research.

Artículo científico sometido a publicación en Revista Marine and Freshwater Research (Código: MF14038): "UV radiation effects on the embryos of the anchoveta *Engraulis ringens* and common sardine *Strangomera bentincki* off Central Chile (36°S)"

EFECTOS DE LA RADIACIÓN UV EN EMBRIONES DE ANCHOVETA *ENGRAULIS RINGENS* (JENYNS 1842) Y SARDINA COMÚN *STRANGOMERA BENTINCKI* (NORMAN 1936) EN CHILE CENTRAL (36°S).

P. Vásquez, A. Llanos-Rivera, L. R. Castro & C. Fernández.

Resumen

Se ha propuesto que los niveles actuales de radiación UVB pueden causar efectos letales o subletales en embriones de peces que se ubican en las capas más superficiales de la columna de agua. Los niveles de UVB observados en Chile Central (36°S 73°W) indican que los embriones de peces planctónicos pueden estar expuestos a niveles de radiación UVB nocivos. Desde Julio 2011 a Enero 2012 embriones de anchoveta *Engraulis ringens* y sardina común *Strangomera bentincki* fueron utilizados para probar experimentalmente si los niveles de UVB en Chile Central producen efectos letales o subletales en embriones de peces epipelágicos. Simultáneamente, se determinó si los embriones pueden estar expuestos a niveles nocivos de UVB en terreno. Los resultados experimentales indican que la radiación UVB puede provocar una disminución en el éxito de eclosión, cambios en la boyantez y malformaciones embrionarias. Estos resultados, junto con la distribución vertical observada de los embriones y los niveles de radiación UVB en terreno durante el final de la primavera sugieren que los embriones de ambas especies pueden estar sometidos a efectos letales o subletales producto de la exposición a UVB.

Title

UV radiation effects on the embryos of the anchoveta *Engraulis ringens* and common sardine *Strangomera bentincki* off Central Chile (36°S).

P. Vásquez, A. Llanos-Rivera, L. R. Castro & C. Fernández.

Keywords

UV radiation, fish embryos, hatching, survival, malformations, vertical distribution, embryo buoyancy, natural mortality

Abstract

It has been proposed that current levels of UVB radiation could cause lethal or sublethal effects on fish embryos located in the upper layers of the water column. Observed levels of UVB off Central Chile (36°S 73°W) indicate that planktonic fish embryos could be exposed to harmful UVB radiation. From July 2011 to January 2012 embryos from anchoveta *Engraulis ringens* and common sardine *Strangomera bentincki* were used to test experimentally whether the UVB levels in Central Chile produce lethal or sublethal effects in epipelagic fish embryos. Simultaneously it was determined if the embryos could be exposed to harmful UVB levels in the field. Our experimental results show that UVB may cause a decrease in hatching success, changes in buoyancy and embryonic malformations. These results, along with the observed vertical distribution of embryos and UVB radiation levels in the field during late spring suggest lethal and sublethal effects may be occurring in the embryos of both species.

Introduction

Levels of ultraviolet radiation (UVR 280-400 nm) reaching the Earth have increased due to the reduction in stratospheric ozone concentrations (Farman *et al.* 1985; Whitehead *et al.* 2004) especially in mid-latitudes of the Southern Hemisphere (Madronich 1994; Vernet *et al.* 2009). The increased levels of UVR have triggered adverse effects on several taxa (Häder *et al.* 2007), therefore, of particular concern at any latitude is that ozone depletion may result in dramatic effects on the health and eventual mortality of planktonic life-history phases of marine organisms. Embryos of marine fishes are particularly vulnerable to this portion of the solar radiation (Llabrés *et al.* 2012), because they inhabit the surface layer of the water column where UVR impact is higher than in deeper layers (Zagarese and Williamson 2004).

The vulnerability of fish embryos to UVR is determined by external and internal factors. External factors affect the transparency of the water column and include features such as mixing and stratification, and also the optical properties of water which depend on the presence of compounds that absorb part of the UV spectrum such as dissolved organic carbon (DOC) and chlorophyll-a (Laurion *et al.* 1997; Kuhn *et al.* 1999; Lesser *et al.* 2001; Vernet *et al.* 2009). Internal factors are features of the species that lead to the protection and repair of tissues and DNA damage caused by UVR exposure (Lesser *et al.* 2001; Williamson *et al.* 2001; Browman *et al.* 2003). These features include timing and spawning location (latitude, distance from the coast), vertical distribution of the embryos and the presence of protective pigments in embryos (Chioccara *et al.* 1980; Plack *et al.* 1981; Dethlefsen *et al.* 2001).

The harmful effects of UVR on planktonic fish embryos have been determined experimentally by exposure to the wavelengths of the UVB spectrum (280-320 nm) which penetrate deeper into the water column than the longer wavelengths of Visible (VIS 400-700 nm) and UVA radiation (400-320 nm) (Lesser *et al.* 2001). The lethal effects lead to higher mortality rates (Kouwenberg *et al.* 1999), and the sublethal effects may lead to decreases in embryonic and larval growth rates, morphological malformations in embryos and larvae, which affect escape and prey consumption by the larvae (Table 1) (Hunter *et al.* 1981; Lesser *et al.* 2001; Fukunishi *et al.* 2013). It can also lead to the loss of positive buoyancy in embryos (Dethlefsen *et al.* 1996a; Dethlefsen *et al.* 2001). The loss of positive buoyancy is particularly important because it produces changes in the vertical distribution of embryos which may determine the extent and direction in which the embryos can be transported, the development rate and exerts an influence on mortality (Applegate and Ley 1988; Mangor-Jensen and Waiwood 1995; Vetter *et al.* 1999; Ospina-Álvarez *et al.* 2012).

Studies assessing current levels of UVR off South-Central Chile (36°S) are scarce. Hernández *et al.* (2006) document a seasonal variation in the UVR with high levels of radiation during summer (~35 W m^{-2} UVA, ~6 W m^{-2} UVB) and Rain *et al.* (2012) reported an increase in UVB radiation levels without a proportional increase in the UVA. Measurements carried out in South-Central Chile by the Chilean Meteorological Office allow us to infer that planktonic fish embryos found at this latitude are exposed to high levels of UVB (0.05 to 4.04 W m^{-2}) that are harmful or lethal to fish embryos (Kouwenberg *et al.* 1999; Dethlefsen *et al.* 1996a; Lesser *et al.* 2001; Steeger *et al.* 2001).

The anchoveta *Engraulis ringens* (Jenyns 1842) and the common sardine *Strangomera bentincki* (Norman 1936) spawn planktonic embryos off South-Central Chile (36° S) during the reproductive season that begins in July and extends through to December or January (Cubillos *et al.* 1999). The embryos of both species are distributed in the upper 20 m of the water column, increasing their abundance towards the surface (Giraud 2011). Another significant characteristic is that both species have a high natural mortality rates (Cubillos *et al.* 1999) that could increase if embryos are exposed to high UVB radiation levels (proved harmful to other fish species) especially considering that their early stages lack pigments until early organogenesis and their embryos have a transparent chorion (Herrera *et al.* 1987; Artüz 1999).

In this study we assessed the lethal and sublethal effects of current levels of UVB radiation on planktonic fish embryos of anchoveta and common sardine off South-Central Chile (36°S). First, we experimentally determined the UVB effects on embryo development by identifying and quantifying malformations; we estimated changes in developmental characteristics and also determined changes in embryo buoyancy at different irradiation treatments. Finally, using the observed vertical distribution of embryos in a coastal area off Concepción, we estimated the fraction of the embryos present in the water column potentially exposed to harmful levels UVB during the spawning season.

Methods

Embryo sampling

Embryos of anchoveta and common sardine were obtained from plankton samples collected in short cruises during July 2011 to January 2012 in an area off South-Central Chile (36°S) (Fig. 1). Sampling was performed with a regular Bongo net (60 cm mouth diameter, 300 µm mesh, equipped with a General Oceanics flowmeter) by gentle oblique trawling (<2 knots) from 20 m to surface. Samples were maintained at 12°C and quickly transferred to laboratory where sorting of anchoveta and common sardine embryos was performed using a stereomicroscope equipped with a cold light (Zeiss Stemi SV 11). The sorting of the embryos was performed by stage of development, according to the classification criteria used by Krautz *et al.* (2010) in which are distinguished: Stage I (without embryo), Stage II (early embryo: embryo covers half of the chorion) and Stage III (late embryo: the embryo covers more than half of the chorion).

Ultraviolet exposure

The embryos obtained were exposed in a UV irradiation chamber BS-03 (Dr. Grobel, Germany) to three irradiation treatments (Table 2): 1) VIS+UVA+UVB: embryos were exposed to VIS, UVA and UVB irradiation, 2) VIS+UVB: embryos were exposed to VIS and UVB irradiation and 3) Control (dark): this treatment did not receive any irradiation. An UVA light filter was utilized in treatment 2 and in treatment 3 a filter for all types of irradiation was used.

The chosen irradiation dose corresponds to the annual average of the daily maximum UVB irradiation reported by the Chilean Meteorological Office in 2009 for the South-Central Chile area (36°S): 1.97 W m^{-2} UVB. Rain *et al.* (2012) found that in the study area the reflection of UVB had a value of 51.80% and that attenuation of this wave length results in only 10% of the UVB that reaches the surface penetrating to 5 m deep. Therefore considering reflection and attenuation the embryos would be exposed to 0.1 W m^{-2} UVB in the first 5 m of the water column. As the maximum exposure to solar radiation lasts for about 2 h daily (Chilean Meteorological Office 2009) the chosen daily dose was estimated as equivalent to 720 J m^{-2} for UVB and to 235.6 J m^{-2} for UVA.

In all treatments a minimum of 30 embryos, at the same stage of development, of each species were placed in culture plates with 6 wells of 10 ml of filtered seawater each (five embryos per well) and maintained at 12°C, the average temperature from the field at the collecting sites, in the dark in an FTC90E incubator. The number of replicates for each treatment depended on the embryo abundance in planktonic samples, with a minimum of thirty individuals. Since the embryos of both species complete embryonic development after three days at 12°C (Sepúlveda *et al.* 2000) the daily dose was applied over three, two and one days for embryos in stages I, II and III, respectively.

Survival, malformations and hatching success

After the artificial sunlight exposure, all treatments were checked every 12 h until hatching using a stereomicroscope equipped with a cold light; the observation did not extend for more than 10 min. In each control the number of live and dead embryos were counted (% survival), and when the embryonic development finished the number of embryos and larvae were quantified to determine the hatching success in each treatment. To assess the presence of malformations a detailed analysis of each embryo was performed and the number of embryos whose morphology deviated from that described for each species was quantified (for the anchoveta Moser and Ahlstrom (1985), and for the common sardine Sepúlveda *et al.* (2000)). Each observed malformation was photographed for further description. The artificial irradiation experiments described above were carried out at three embryonic development stages (I, II and III), in order to detect differences in the presence of lethal and sublethal effects in the embryos according to the stage of development at which they were exposed.

Buoyancy experiments

Two experimental conditions of irradiation were set to measure changes in embryo buoyancy: 1) VIS+UVA+UVB (irradiated embryos) and 2) Control (dark). Each experimental condition considered seven embryos. After exposure the embryos were transferred to a density-gradient column system (Coombs 1981), one for each treatment. The salinity gradients were constructed by gently filling the columns with ten solutions of seawater with different salinities in the range of 29 to 38 psu, a normal salinity range at the studied area. After filling the columns they were maintained at 12°C throughout the experimental period. In each column, a set of four glass spheres of known density were added (1.0221 g cm^{-3}, 1.0237 g cm^{-3}, 1.0254 g cm^{-3}, 1.0273 g cm^{-3}), with each spheres settling at a depth in the column corresponding to its neutral buoyancy. The position of each sphere in the column and their density were utilized to construct a calibration curve in which the x-axis was height in the column (cm) and the y-axis was the sphere density (g cm^{-3}). This curve was then utilized to determine the density of the embryos. The system was monitored every 6 h, but the frequency was increased when the embryos were close to hatching.

Vertical distribution in the natural environment

To determine what proportion of embryos are exposed in the first 5 m of the water column to the experimental UVB irradiation, stations located off Concepcion Bay (Fig. 1) were sampled during the hours when the sun was at its zenith. From July 2011 to January 2012 seven depth strata (0-5, 5-10, 10-15, 15-20, 20-30, 30-40 and 40-50 m) were sampled with a Tucker trawl plankton net (0.5 m^2 mouth area, 300 µm mesh, equipped with a General Oceanics flowmeter). The embryo abundance of each species was calculated in each stratum of the water column (abundances expressed in individuals*1000 m^{-3}).

Statistical analysis

Survival, morphological malformations and hatching success of the embryos in our experiments where expressed as a percentage, for this reason the data were then arc-sin transformed. Differences among survival in the different treatments were determined using an Analysis of Covariance (ANCOVA) at a significance level of 0.05%. Differences in sublethal effects among treatments were determined using an analysis of variance (ANOVA) at a significance level of 0.05%. ANCOVA and ANOVA were performed after verifying that variances were homogeneous. To compare among treatments Tukey–Kramer (HSD) multiple comparisons test were used.

Results

The abundance and developmental stage of common sardine and anchoveta embryos varied in our collection dates throughout the sampling period. Accordingly, 12 experiments with embryos at stage I, 6 experiments at stage II and 7 experiments at stage III were carried out for anchoveta. With the common sardine 6 experiments with embryos at stage I, 6 experiments with embryos at stage II and 6 experiments with embryos at stage III were conducted.

Survival among treatments

The survival of embryos of anchoveta and common sardine (Fig. 2) showed significant differences between irradiation treatments (ANCOVA: anchoveta $F_{(2)}=8.8$ $P=0.001$; common sardine $F_{(2)}=6.4$ $P=0.002$) with the higher mortality in the treatment VIS+UVA+UVB. Differences were also found depending on the day of irradiation with the fourth day of irradiation presenting the highest mortality (ANCOVA: anchoveta $F_{(1)}$=716.9 P <0.01; common sardine $F_{(1)}$=92.0 P<0.1).

In both species analyzed, the embryos irradiated at stage I, progressively decrease in survival as embryonic development proceeded, a trend observed in all irradiation treatments. Twenty four hours after the second dose of irradiation (second day of irradiation) in both species a lower survival rate was observed in the treatment VIS+UVA+UVB with the lowest values at the fourth day of irradiation (anchoveta: 25% and common sardine 5%; Fig. 2). In contrast, the embryos in conditions VIS+UVB and Control had a similar levels of survival; higher than 55% for all the days of irradiation (Fig. 2).

In anchoveta embryos irradiated for the first time at stage II, their survival declined in all treatments over the period of exposure, with an average of 15% decline per day. The lowest survival was observed in the treatment VIS+UVA+UVB, which on the last day of irradiation had a final survival of less than 25%. Embryos of common sardine showed higher survival in treatment VIS+UVB with values close to 90% between days of irradiation. In treatments VIS+UVA+UVB and Control the survival was similar with values of 60% and 80% between consecutive days of irradiation during the stage II period (Fig. 2).

The survival of anchoveta embryos irradiated for the first time at stage III dropped from 90% on the first day of irradiation to 50% on the third day of irradiation in treatments VIS+UVA+ UVB and VIS+UVB, while the Control treatment maintained a survival higher than 90% over all the days of irradiation. Common sardine embryos irradiated first at stage III showed a survival near 95% between treatments over the entire exposure period.

In anchoveta there were no differences (ANCOVA, $F_{(2)}$=1.7 P=0.19) in survival of irradiated embryos among different stages of development. In common sardine however, differences in survival occurred among stages of development at which we applied the irradiation (ANCOVA, $F_{(2)}$=6.4 P=0.002) with stage I having the highest overall mortality.

The survival rates between anchoveta and common sardine embryos were different at the three stages of development evaluated (Stage I ANOVA $F_{(1)}$=6.2 P=0.01, Stage II ANOVA $F_{(1)}$ = 54.6 P<0.01 and Stage III ANOVA $F_{(1)}$ =84.1 P<0.01) with the common sardine embryo exhibiting the highest rates of survival in stage II and III experiments. However, the common sardine embryos irradiated at stage I appeared to be more sensitive to UVR than anchoveta, with the final survival rates at the end of the experiment being lower than for anchoveta.

Malformations

The observed types of malformations were similar in both species (Fig. 3) and the most frequent were the presence of dead tissue at all stages of development, blisters on the yolk in the earliest stages and twisting of the notochord at different levels of intensity in embryos with an embryonic development more advanced than stage II. Incomplete hatching of larvae in later stages of development was also observed.

Considering the pooled data there were no significant differences in the frequency of malformations between species (ANOVA, $F_{(2)}=0.4$ $P=0.511$). Anchoveta embryos first irradiated at stages I and II had malformations frequencies of about 40% in the VIS+UVA+UVB treatment and of less than 20% in Control treatment, in embryos first irradiated at stage III VIS+UVB treatment had the highest malformation frequency and the lowest in Control treatment. In the case of common sardine embryos, the presence of malformations was higher in embryos first irradiated at stage I in treatment VIS+UVB+UVA, whereas for treatments VIS+UVB and Control the embryos irradiated at this stage of development had a malformation frequency of less than 15%. In common sardine embryos first irradiated at stages II and III the frequency of malformations was similar in treatments VIS+UVB+UVA and VIS+UVB (~ 40%) but lower in the Control treatment (<20%).

The frequency of malformations was different among irradiation treatments (ANOVA, anchoveta $F_{(2)}=10.7$ $P<0.01$; common sardine $F_{(2)}=5.01$ $P=0.016$) (Fig. 4) for both species. For anchoveta, the difference in the frequency of malformations occurred at the Control treatment (<20%), which was lower than that of the other treatments (VIS+UVA+UVB, Tukey N=53 P<0.001; VIS+UVB, Tukey N=53 P<0.001). In the latter treatments the frequency of malformations exceeded 35%, regardless of the stage of development at which embryos were irradiated for the first time. For common sardine, the lowest malformation rates also occurred at the Control treatment never exceeding 20% regardless of the stage of development at which embryos were irradiated for the first time.

In both species, within each irradiation treatment, there were no statistical differences in the frequency of malformations of the embryos irradiated at different stages of development (ANOVA: anchoveta $F_{(2)}$=1397.6 P =0.12; common sardine $F_{(2)}$=75.6 P=0.85; Fig. 4).

Hatching success

Interspecific differences in hatching success were observed, with a higher values in common sardine embryos in all treatments and stages of development (ANOVA, $F_{(2)}$=48.9 P=0.01). In both species there were also significant differences in hatching success among embryos exposed to the different irradiation treatments (ANOVA anchoveta $F_{(2)}$=3.2, P=0.048; common sardine $F_{(2)}$=2007.6 P<0.01; Fig. 5). These differences among treatments were mainly explained by the differences between the Control versus the VIS+UVA+UVB treatment, with embryos in the Control exhibiting higher hatching success (anchoveta Tukey N= 69 P=0.038; common sardine P<0.01).

For anchoveta, there were no differences in hatching success among embryos irradiated at the different stages of development (ANOVA, $F_{(2)}$=1.9 P=0.16), however it can be seen that the minimum hatching success (<10%) occurred in embryos irradiated at stages I and II in treatment VIS+UVA+UVB, and the maximum hatching success occurred in embryos first irradiated at stage III, in the Control treatment (~35%).

In common sardine there were differences in hatching success among irradiated embryos at the different stages of development (ANOVA, $F_{(2)}$=9.07 P=0.001), particularly between embryos irradiated at stage III which showed higher hatching success compared to embryos irradiated at stages I (Tukey N=22 P=0.03) and II (Tukey N=22 P=0.001). The lowest hatching success occurred in embryos first irradiated at stages I and II in the treatment VIS+UVA+UVB with values below 30%, while embryos in the Control treatment at stage III had the higher hatching success with values higher than 90%.

Buoyancy changes

According to the availability of embryos in the plankton a total of five embryo buoyancy experiments with anchoveta and two with common sardine were performed; in these experiments seven embryos per treatment of each species were utilized (Fig. 6 and 7).

There were differences between the mean buoyancy of embryos exposed to the irradiation treatment VIS+UVB+UVA versus those of the Control treatment (dark). These differences were characterized by a reduction of positive buoyancy in those embryos exposed to irradiation compared to those of the Control treatment. For anchoveta (Fig. 6), these differences occurred in three out of five experiments: December 1 (t-test, t_{58}=3.10, P <0.05), December 15 (t-test, t_{63}=3.68, P<0.05) and January 13 (test-t, t_{25}=3.68, P<0.05). For common sardine embryos (Fig. 7) there were also differences in the buoyancy of irradiated embryos versus the Control embryos in the two experiments carried out: December 15 (t Test t_{66}=4.41, P<0.05) and December 20 (t test, t_{58}=3.48, P<0.05). A clear pattern of recovery of buoyancy values in embryos of either of the species evaluated.

Considering the buoyancy data from all the dates pooled, the mean density difference between VIS+UVB+UVA just irradiated embryos and those in the Control was about 1 sigma-t unit in both species (Fig. 6 and 7). As development proceeded this difference in density decreased to an average of 0.5 sigma-t units through most of development up to few hours just before hatching when the difference increased to about 1 sigma-t unit.

Environmental conditions, embryo abundance and their vertical distribution in the field

The vertical section of density (sigma-t) throughout the sampling season reveal the normal change in oceanographic conditions expressed as a deeper mixed layer with lower seawater densities at the surface resulting from the rain and higher river discharges in winter to a shallower mixed layer and the raise of salty and colder subsurface waters in spring and summer as a result of wind driven coastal upwelling (Fig. 8a). The UVB radiation level at the sea surface at noon during the main spawning season of both species in the area gradually increased from July (0.0025 W m^{-3}) to November (0.0097 W m^{-3}) (Fig. 8b). During the same period, the abundance of anchovy embryos at the surface layer (0-5 m) decreased from July (~38000 individuals*1000 m^{-3}) through November (13000 individuals * 1000 m^{-3}). Common sardine embryo abundance in this surface layer, instead, was more variable across the season with maximum abundances in August and October (~500000 individuals*1000 m^{-3}) and a none present in September and November. Accordingly, our data indicates that a low number of anchovy embryos were exposed to high UVB radiation levels in the sea surface layer in October and November and also that only during November a low proportion of common sardine embryos may have been exposed to possible damage from UV radiation.

The vertical distribution of embryos at different stages of development for both species was variable over the sampling period (Fig. 9). In July the anchoveta embryos were concentrated in the 0 to 20 m depth strata, independent of stage of development considered. In August the vertical distribution was deeper, especially for embryos at stage I of which 50% were found in the strata 30 to 50 m. In October, less than 10% of stage I embryos were in the shallower layer (0-5 m), about 50% of the stage III embryos were distributed in the first 15 m of water column, whereas more than 70% of the embryos at stages II and III were distributed below 20 m. In contrast to previous months, in November, most stage I and II embryos were distributed closer to the surface with about 50% of the embryos at stage I within the 5-10 m, and the remaining 50% distributed in the 10 to 15 m strata.

Common sardine embryos followed a different distribution pattern. In July and August more than 50% of the embryos at stage I were located within the first 5 m of the water column. In July also, most embryos at stages II and III were located between 0 and 20 m. In August, besides that 50% of stage I embryos at the surface layer, another 30% of them occurred at the second near surface layer (5-10 m). In October, 90% of common sardine embryos at all stages of development were distributed below 30 m. In November 100% of the stage III embryos were distributed between 10 and 15 m.

Discussion

Anchoveta is the most important fisheries resource and a key species in the pelagic trophic web along the Humboldt Current system and, along with the common sardine, the most abundant spawning fish during winter and spring off Central Chile. In this study, laboratory experiments were carried out to tests whether average UVB radiation levels observed in Central Chile (36 °S) could cause deleterious effects on embryos of these two species. Our results show that the levels of UVB used caused both lethal and sublethal effects on the planktonic embryos of the anchoveta and the common sardine. In addition, from field data it was determined that planktonic embryos occurring in the shallower layer (0-5 m) may experience the adverse effects observed in laboratory after exposure to UVB.

The survival of anchoveta and common sardine embryos declined progressively after the second day of irradiation in experiments that began with embryos at stage I of development, and also they showed a higher rate of decay in the treatment that included both UVA and UVB. This decrease in the survival was more pronounced than when the embryos irradiated were at stages II and III. The higher vulnerability to UVR of stage I embryos probably results because this stage occurs prior to the closing of the blastopore, a period in which the embryos undergo processes of cell division and differentiation. This cellular process can be altered by inducing an epiboly disruption (Strähle and Jesuthasan 1993; Kouwenberg *et al*. 1999; Steeger *et al.* 1999) which leads to a decrease in the survival of the embryos. Both species have equal sensitivity when they are irradiated with UVR at stages prior to the blastopore closure. However, at more advanced stages of embryonic development (stages II and III) common sardine is more resistant to UVR suggesting that the response to UVR exposure at later stages of embryonic development could be species-specific. Survival rates determined by other authors in experiments with other fish species of similar embryo characteristics (eg. transparent chorion, lack of pigments and shallow vertical distributions) have shown similar or higher decreases in survival rates. For example, embryos of the flatfish *Pleuronectes platessa* irradiated at stage I exhibited a similar decrease in survival after the first day of irradiation (Steeger *et al*. 2001), while the Atlantic cod *Gadus morhua* exhibited a higher decrease in survival after the first day of irradiation (about 50%) (Lesser *et al.* 2001).

The effect of exposure of fish embryos to UVA radiation is still matter of debate. Some authors (Sancar and Sancar 1988; Dong *et al*. 2007) have identified the involvement of UVA in the photo-repair processes following exposure to UVB so that, embryos exposed to UVB and UVA exhibit higher survival rates than those exposed only to UVB. Other authors (Vetter *et al*. 1999; Lesser *et al*. 2001) point to UVA as a cause of DNA damage in pelagic fish embryos, given that embryos irradiated with UVA and UVB have lower survival than those irradiated only with UVB. In this study treatment VIS+UVA+UVB and separately the VIS+UVB treatment were included in order to infer the effect of UVA radiation. This results indicated that exposure to UVA increased the rate of embryo mortality in anchoveta and common sardine coinciding with Lesser *et al.* (2001) and Vetter *et al.* (1999) reports. Note that the experiments in which the UVA has participated in photo-repair have been performed under a lower dose of UVA than that observed in the field (Lesser *et al.* 2001), accordingly those results do not necessarily resemble environmental conditions and hence the effect on fish embryos in their habitat, especially in areas with high levels of UVA and UVB. Thus, it is likely that the net effect of UVA radiation, deleterious or not might depend on the dose at which embryos are exposed in each experiment or natural condition.

The type of malformations observed after UVR exposure in both species are the same as those described for a variety of planktonic fish embryos collected from the marine environment in areas free of pollution, and whose presence has been linked to abrupt changes in some physico-chemical characteristics of water or endogenous factors (eg. maternal effects) (Cameron *et al.* 1992; Dethlefsen *et al.* 1996b; Schreck *et al.* 2001). Malformations for both species evaluated in this study from samples obtained in the natural environment off Central Chile (36°S) have already been described and quantified. Llanos-Rivera *et al.* (2013) determined that for anchoveta embryos the frequency of malformations was higher in spring-summer (5%) than in winter (4%), and Vásquez *et al.* (2010) determined that the level of malformations was lower in winter (3%) and higher in spring-summer months (6%) for common sardine embryos. In the present study, the highest radiation levels off South-Central Chile (36°S) occurred during late spring and summer, and hence, it is not possible to discount the potential influence of UVR in the presence of these malformations in both species. The type of UVR derived malformations described in our study are consistent with those reported in others studies (Vásquez *et al.* 2010; Llanos-Rivera *et al.* 2013) i.e. the most common malformations were those that prevent the closure of the blastopore and the presence of embryos with a twisted notochord. Future studies focusing on these coincidences may shed light on the effects of the UVR, particularly in these apparently very critical stages of the embryo development.

The experimental design carried out in our study considered that the same embryos were used to assess lethal and sublethal effects. The fact that for both species, the embryos regardless of the stage of development at which they received their first irradiation had an average survival higher than 50%, does not imply that they successfully hatched. The hatching success for anchoveta quantified in these experiments was low; even in the Control condition (non-irradiated), and below the percentage value reported by Krautz *et al*. (2013) for this species under the same experimental conditions. For the common sardine the hatching success was higher than for the anchoveta and hatching in the Control condition was higher than 60%. It was noted that several embryos, especially in the case of the anchoveta, survived through to complete embryonic development, but nevertheless, these survivors could not hatch. At this point of embryonic development the chorion begins to degrade and without any apparent malformation the embryos died inside the embryo, a phenomenon recorded in this study as "delayed or prevented from hatching". The hatching process is caused by biochemical mechanisms such as digestion of the chorion by choriolitics enzymes (De Gaspar *et al.* 1999) and by physical mechanisms in which the movement of the embryo results in the destruction of the chorion (Von Westernhagen 1988). The presence of malformations and hatching success are directly related because sometimes embryos with a certain types of morphological malformations lose the mobility needed for hatching. Observations carried out in this study indicated that in embryos that have certain types of malformation in the notochord delayed hatching was frequent. This phenomenon was also observed by Dethlefsen *et al*. (2001) after exposing embryos of *Limanda limanda* and *Pleuronectes platessa* to UVB light indicating that delayed hatching is an indirect effect of UVR exposure. However, we also observed embryos without apparent malformation with delayed hatching, suggesting that the enzymatic process involved in the chorion degradation could be altered by UVR exposure.

This is the first study to describe the buoyancy of anchoveta and common sardine over their embryonic development; and it is also the first direct evaluation of changes in buoyancy of an embryo after receiving UVR. First, it should be noted that the pattern of changes in buoyancy as the embryonic development advanced, in untreated embryos, coincides with the pattern described for other species of clupeiforms such as the european anchovy *Engraulis encrasicolus* and the european pilchard *Sardina pilchardus* (Coombs *et* *al.* 2004). This pattern indicates that embryos have positive buoyancy at the beginning of embryonic development through to the mid-point in development where a slight decrease in buoyancy occurs; then the embryos become denser at pre-hatching stages. In terms of the effects of UVR, the results showed a loss of buoyancy after embryos are irradiated with a dose of UVR corresponding to the field levels in the study area. The precise basis for the change in buoyancy in fish embryos after irradiation with UVR has yet to be explained. Apparently, buoyancy can be modulated by the addition of lipids or by an influx of water (Craik and Harvey 1987) permitted by the semi-permeability of the chorion and changes of density in the perivitelline space (Dethlefsen *et al*. 2001). In this context, the participation of aquaporins have also been mentioned, these are proteins that allow the passage of water through the chorion to decrease the density of the perivitelline fluid and also allow the passage of free amino acids which are derived from the proteolysis of embryonic yolk protein, inducing an increase in the density of the perivitelline fluid (Fabra *et al.* 2005).

There are a few studies dealing with the experimental quantification of changes in embryo buoyancy (Dethlefsen *et al.* 2001; Steeger *et al.* 2001) after UVR exposure. In these studies the effect has been identified indirectly, e.g. trough of the observation that irradiated embryos, although alive, were at the bottom of the incubation containers where experiments were performed. From these results it has been hypothesized that the loss of buoyancy on fish embryos as *Pleuronectes platessa* and *Limanda limanda* is severe. In our study, using a density gradient columns methodology and knowing the density profiles in the field water column (Fig. 8a), an increase of 1 sigma-t unit in the buoyancy was quantified. For example, stage I embryos of anchovy and common sardine which have an initial density of 24.7±0.8 and 24.8±0.6 sigma-t, respectively, are placed in the upper layers of the water column. If these embryos received UVB levels such as those used in this study, they would experience an increase of 1 sigma-t in density, therefore, to achieve the neutral buoyancy the embryos would sink at least 10 m in the water column, modifying their vertical distribution.

Changes in vertical distribution due to loss of buoyancy induced by UVR exposure produces that embryos change their location in the water column, and the implications of this change are varied. On the one hand it may reduce the chances of dispersion of the embryos, since the change in the vertical distribution can affect the transport of embryos to nursery areas (Sundby 1983; Huret *et al*. 2007; Ospina-Álvarez *et al.* 2012). Some authors have linked the loss of buoyancy as a way to mitigate the effects of UVR since the embryo avoids exposure by sinking to greater depths in the water column; or by choosing deeper areas for spawning (Huff *et al.* 2004; Fukunishi *et al.* 2010). However, in the case of the anchoveta it has been determined that at higher latitudes in the South Hemisphere (41°S), this species spawns mainly in the upper layers (Giraud 2011), despite the presence of stronger UVR irradiation, thus embryos in this areas could receive higher doses of UVB than those used in this study.

The approach of this research was to evaluate experimentally the UVR effects in fish embryo and extrapolate the results to the natural environment. During the period that we carried out this study, anchoveta embryos were most abundant in July and their lowest abundance was recorded during November, the month in which solar radiation was highest, reaching the levels of irradiation used in our experiments. By incorporating this information into the analysis of the vertical distribution of the embryos in November, we quantified that a fraction of at least 20% of the embryos at stages II and III may have been irradiated with a dose of UVR that might result in lethal or sublethal effects i.e. mortality, malformations, delay at hatching and buoyancy loss. Moreover, the effects of UVR on anchoveta embryos could increase towards the end of the spawning season (December-January) as for this species a decrease in the biochemical quality of embryos as the spawning season progresses has been described (Castro *et al*. 2002; Llanos-Rivera and Castro 2004). Therefore, anchoveta embryos would be provided with less biochemical resources in months with more UVR, which might make them more vulnerable to the effects of UVR since they would have fewer resources to allocate to the photo-repair processes. For common sardine, although abundances increased as the UVB radiation did, it is unlikely that this species received a dose of harmful radiation in the field, since during November the embryos were distributed below 15 m where levels of solar radiation are low due to the reflection and attenuation (Rain 2012) in the study area.

Conclusions

The effects of UVR observed in this study directly or indirectly caused a decrease in survival in anchoveta and common sardine, two heavily exploited species that have high natural mortality rates. The effects of current levels of UVR radiation should be taken into account as an additional cause of natural mortality for embryos of these species at this latitude.

Until now, the presence of malformations in the natural environment have not been associated with the exposure to UVR, so the results of this study suggest that the UV radiation should be added to the list of environmental factors that could cause malformations in planktonic embryos in areas with high levels of solar radiation such as in the southern areas of the Southern Hemisphere.

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Figure Legends

Fig. 1 Study area located off Central Chile (36°). Points at the Gulf of Arauco and Conception Bay are the plankton sampling stations assessed during July 2011 to January 2012

Fig. 2 Survival (%) of anchoveta *Engraulis ringens* (left panel) and common sardine *Strangomera bentincki* (right panel) embryos exposed to three treatments of irradiation applied at different stages of development (I, II and III). Each symbol is the mean survival of several experiments measured after 12 h after the irradiance was applied. Vertical lines are standard deviations.

Fig. 3 Different types of morphological malformations observed in anchoveta (top panel) and common sardine (bottom panel) embryos in UVR experiments. Anchoveta: twisted notochord (a, b), dead tissue (b, c), delayed hatching of embryos (d, f) and other types of hatching problems (e, g). Common sardine: dead tissue (a, d, g), blisters in the yolk (b), twisted notochord (e, f) and delayed hatching of embryos (g)

Fig. 4 Frequency (%) of malformations of anchoveta *Engraulis ringens* and common sardine *Strangomera bentincki* embryos exposed to three irradiation treatments in which the first dose was applied at three different stages of development (I, II and III). Bars are the mean of several experiments and lines are standard deviations

Fig. 5 Hatching success (%) of anchoveta *Engraulis ringens* and common sardine *Strangomera bentincki* embryos exposed to three irradiation treatments in which the first dose was applied at three different stages of development (I, II and III). Bars are the mean of experiments and lines are standard deviations

Fig. 6 Mean buoyancy (density in sigma-t units) of the anchoveta *Engraulis ringens* during its embryonic development (in hours) in five experiments performed during December 2011 and January 2012. Symbols represent mean buoyancy of seven embryos exposed to two treatments: ■: VIS+UVA+UVB and ●: maintained as controls (dark). Lower right graph shows the mean differences of density data (sigma-t units) from all dates pooled between embryos irradiated at stage I with VIS+UVA+UVB versus embryos exposed to a control treatment (dark). Lines across symbols are standard deviations

Fig. 7 Mean buoyancy (density in sigma-t units) of the common sardine *Strangomera bentincki* during its embryonic development (in hours) in five experiments performed during December 2011 and January 2012. Symbols represent mean buoyancy of seven embryos exposed to two treatments: ■: VIS+UVA+UVB and ●: maintained as controls (dark). Lower graph shows the mean differences of density data (sigma-t units) from all dates pooled between embryos irradiated at stage I with VIS+UVA+UVB versus embryos exposed to a control treatment (dark). Lines across symbols are standard deviations

Fig. 8 *a*: UVB radiation (continuous line) at the seawater surface (Rain 2012) during the dates sampled during the present study and mean abundance (Individuals*1000m^{-3}) of anchoveta (black) and common sardine (gray) embryos within the first 5m of the water column from July to November 2011. Dotted line indicates the UVB dose applied in the experiments of this study. *b*: Vertical section of density (sigma-t) of the water column throughout the study period at the stations in which embryos of anchoveta and common sardine were collected. Dotted vertical lines indicate sampling days. The white point indicate an embryo that receives de UVB dose used in this study and the grey point is the place where the embryo will sink after increasing 1 sigma-t in buoyancy as a result of the UVB exposure

Fig. 9 Abundance (%) of anchoveta *Engraulis ringens* and common sardine *Strangomera bentincki* embryos at different stages of development (I, II and III) in the different strata of the water column at the area studied from July to November 2011

Table Leyends

Table 1. Effects of experimental exposure to levels of ultraviolet B (UVB) radiation registered in the natural environment in planktonic fish embryos of different regions of the world.

Table 2. Different treatments used in the experiments of artificial sunlight irradiation in which embryos of anchoveta and common sardine were exposed.

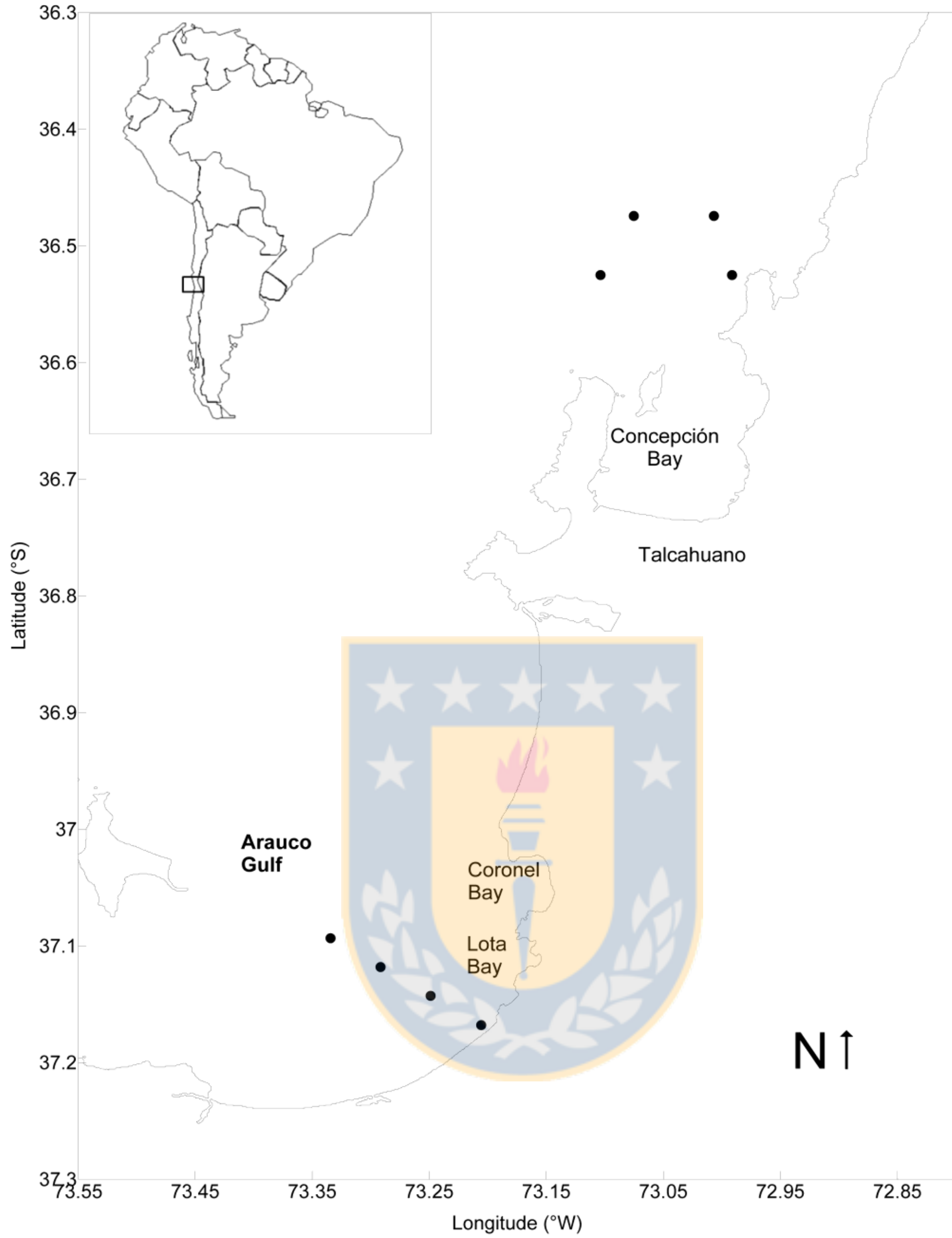
36.3
36.4
36.5
36.6
36.7
36.8
36.9
37
37.1
37.2
37.3
Latitude (°S)
73.55
73.45
73.35
73.25
73.15
73.05
72.95
72.85
Longitude (°W)
Concepción
Bay
Talcahuano
Arauco
Gulf
Coronel
Bay
Lota
Bay
N

Fig. 1

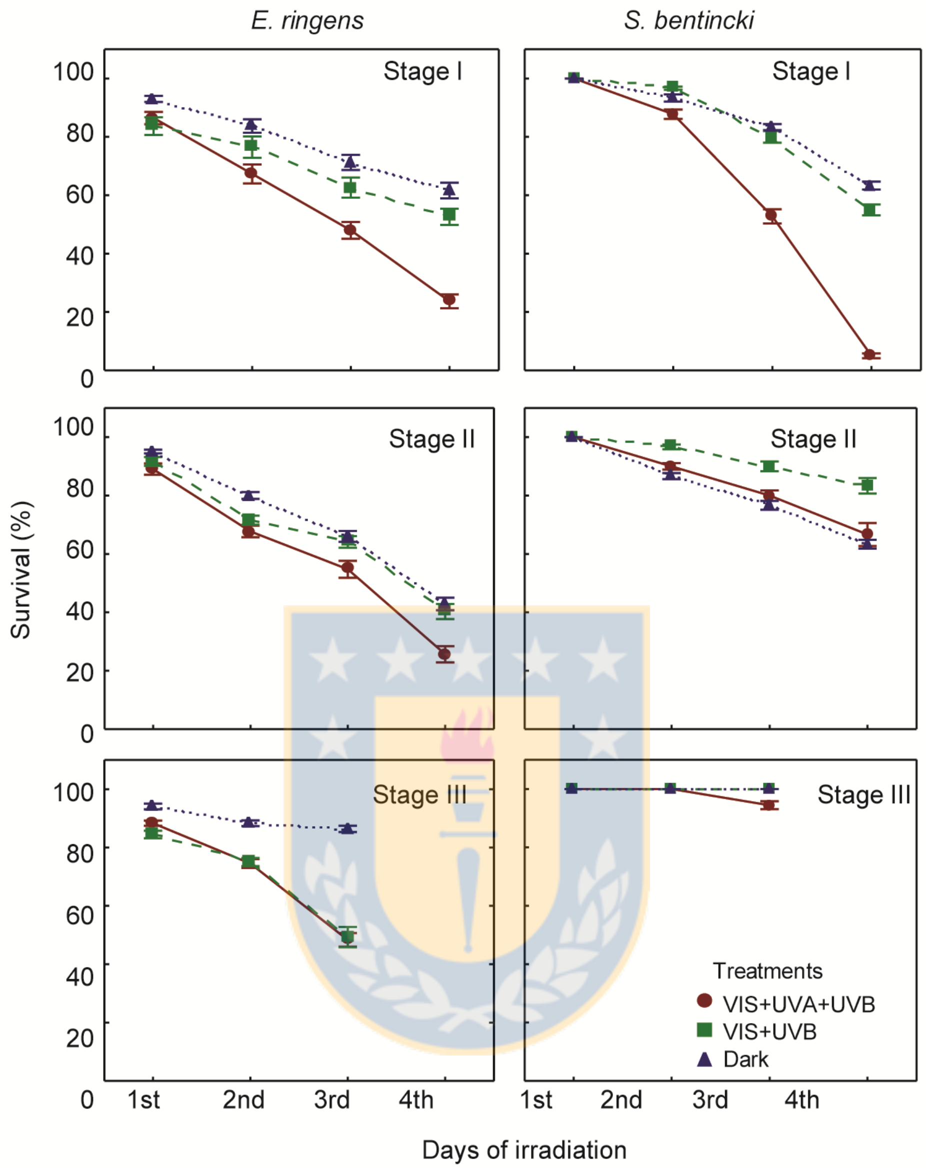
E. ringens
S. bentincki
Stage I
Stage II
Stage III
Survival (%)
0
20
40
60
80
100
1st
2nd
3rd
4th
Days of irradiation
Treatments
VIS+UVA+UVB
VIS+UVB
Dark

Fig. 2

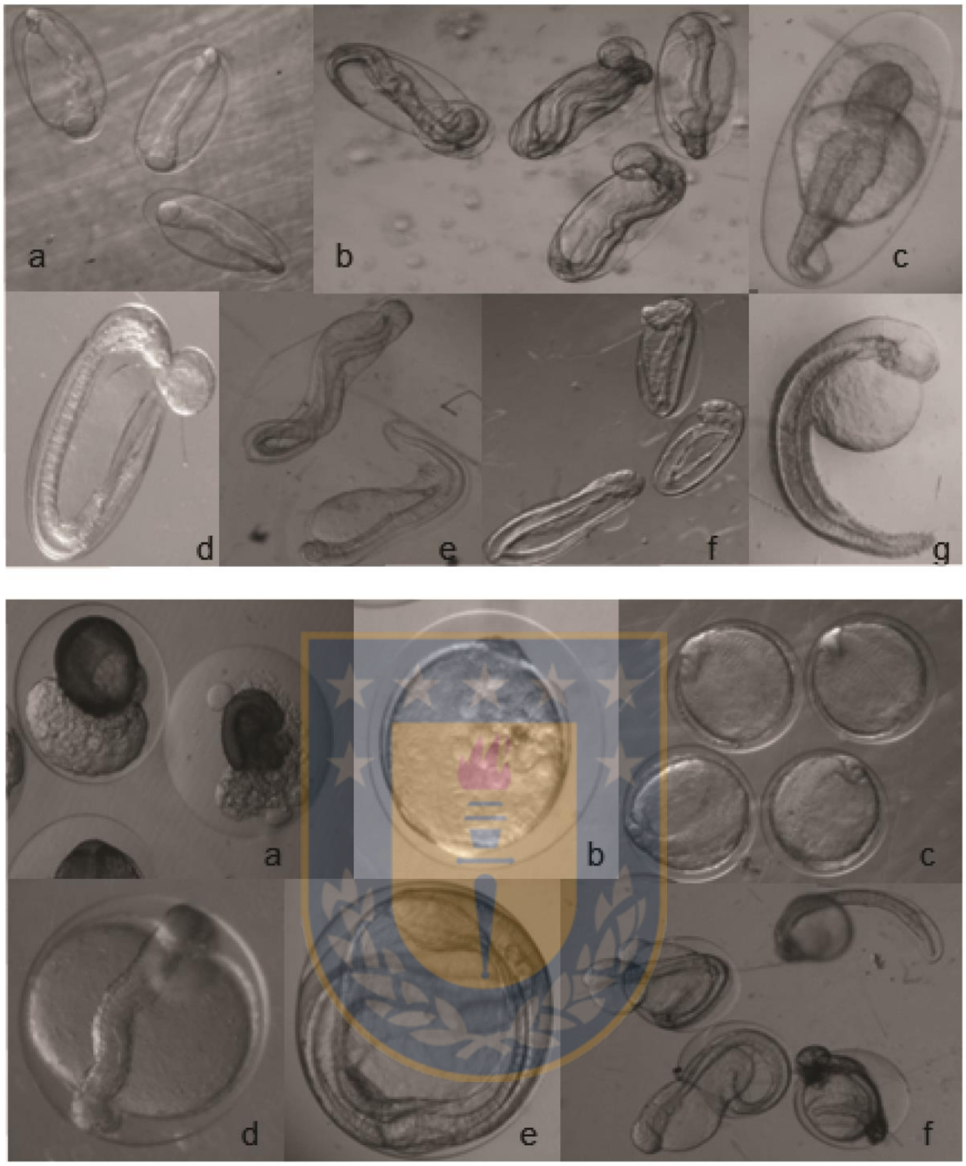

Fig. 3

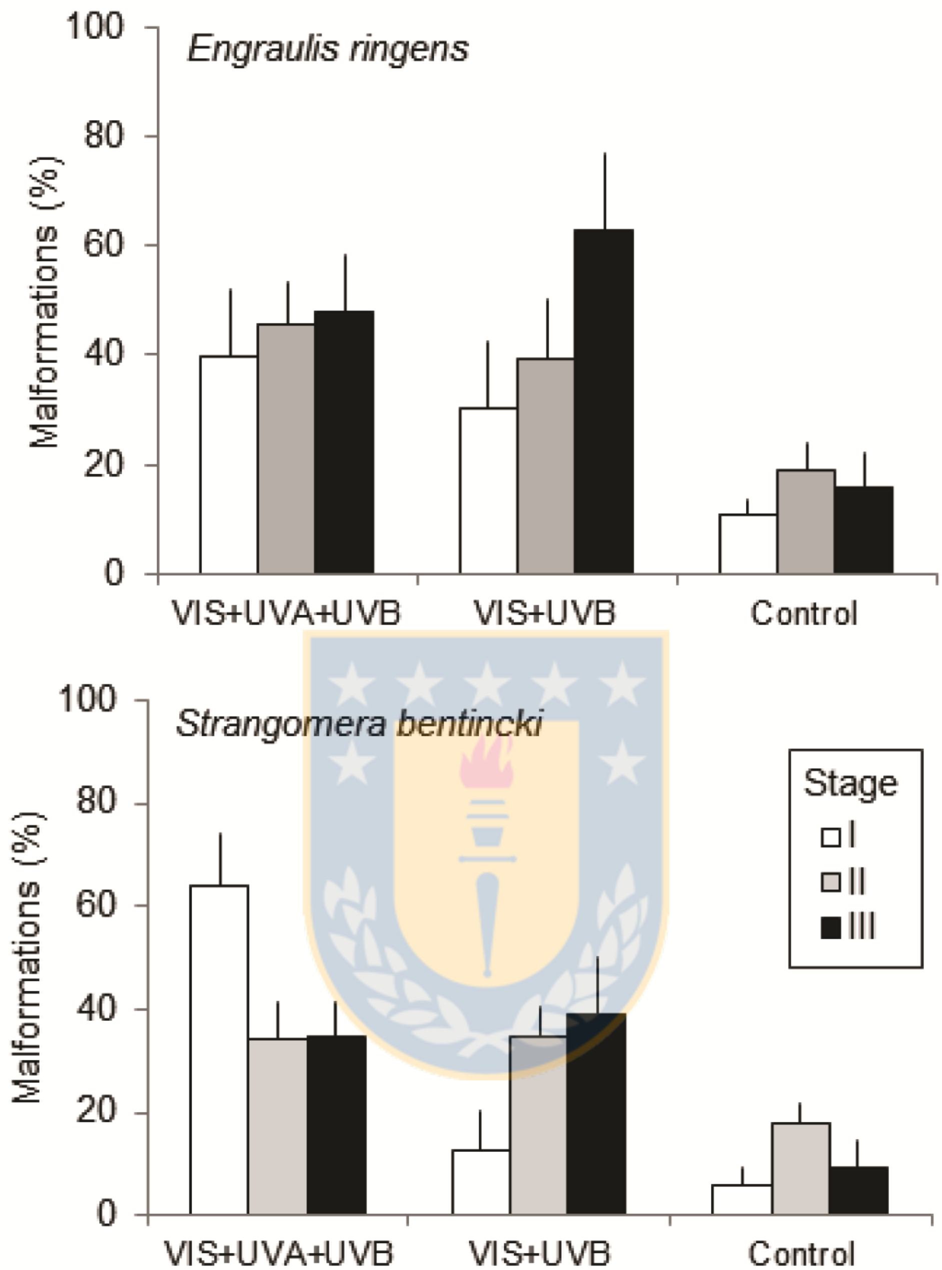
Engraulis ringens
Malformations (%)
100
80
60
40
20
0
VIS+UVA+UVB
VIS+UVB
Control
Strangomera bentincki
Stage
I
II
III

Fig. 4

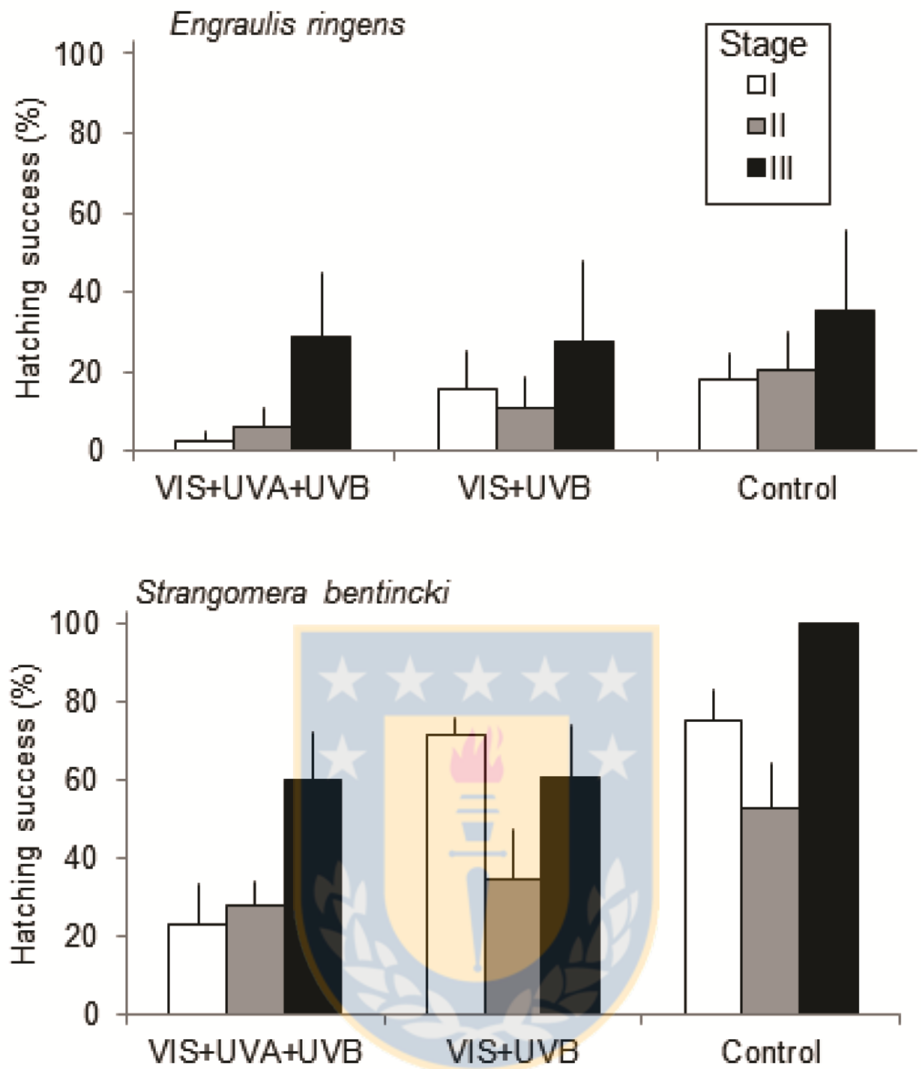
Engraulis ringens
Stage
I
II
III
Hatching success (%)
100
80
60
40
20
0
VIS+UVA+UVB
VIS+UVB
Control
Strangomera bentincki
Hatching success (%)
100
80
60
40
20
0
VIS+UVA+UVB
VIS+UVB
Control

Fig. 5

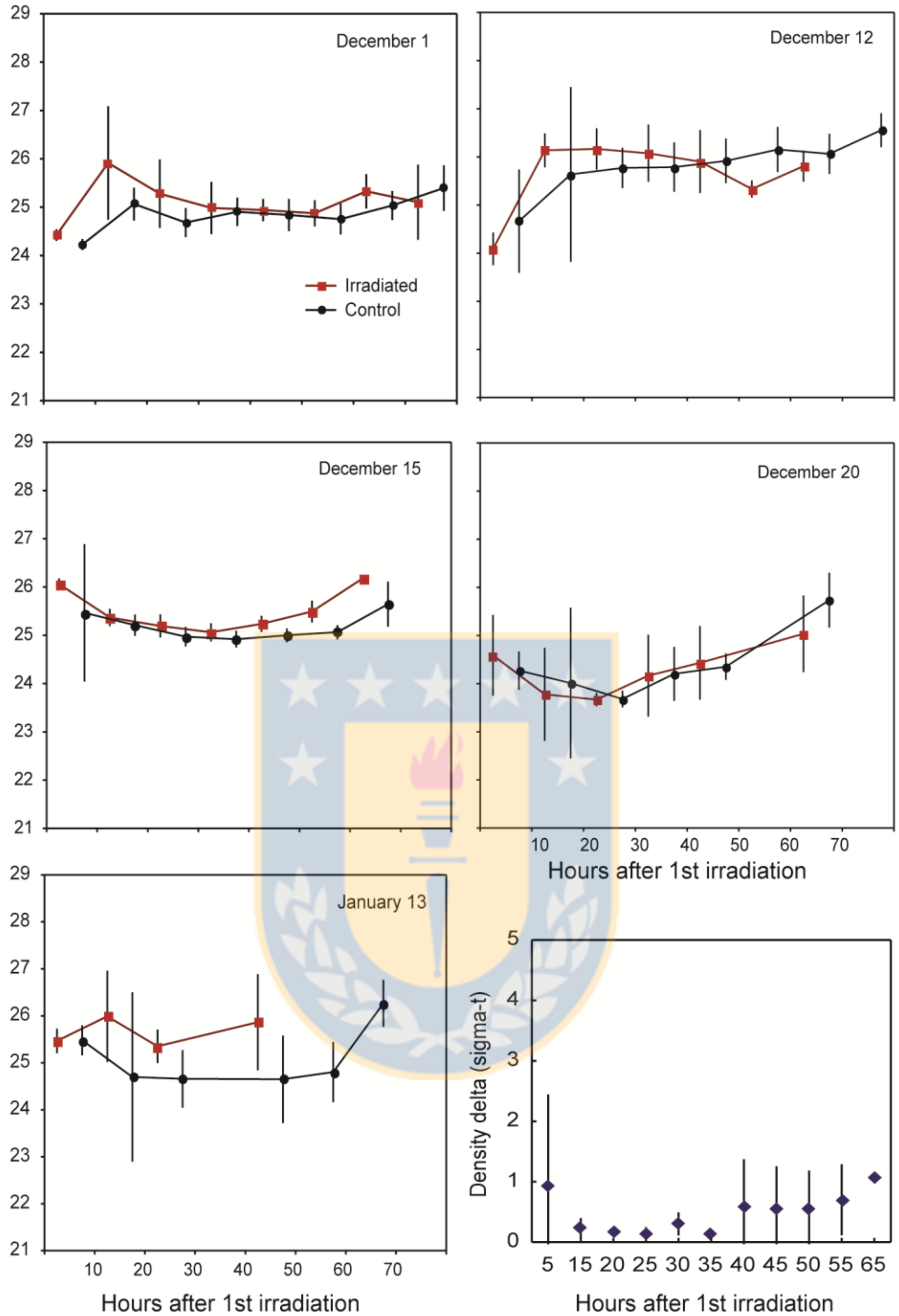
December 1
Irradiated
Control
December 12
December 15
December 20
January 13
Hours after 1st irradiation
Hours after 1st irradiation
Density delta (sigma-t)
Hours after 1st irradiation
29
28
27
26
25
24
23
22
21
10
20
30
40
50
60
70
5
4
3
2
1
0
5 15 20 25 30 35 40 45 50 55 65

Fig. 6

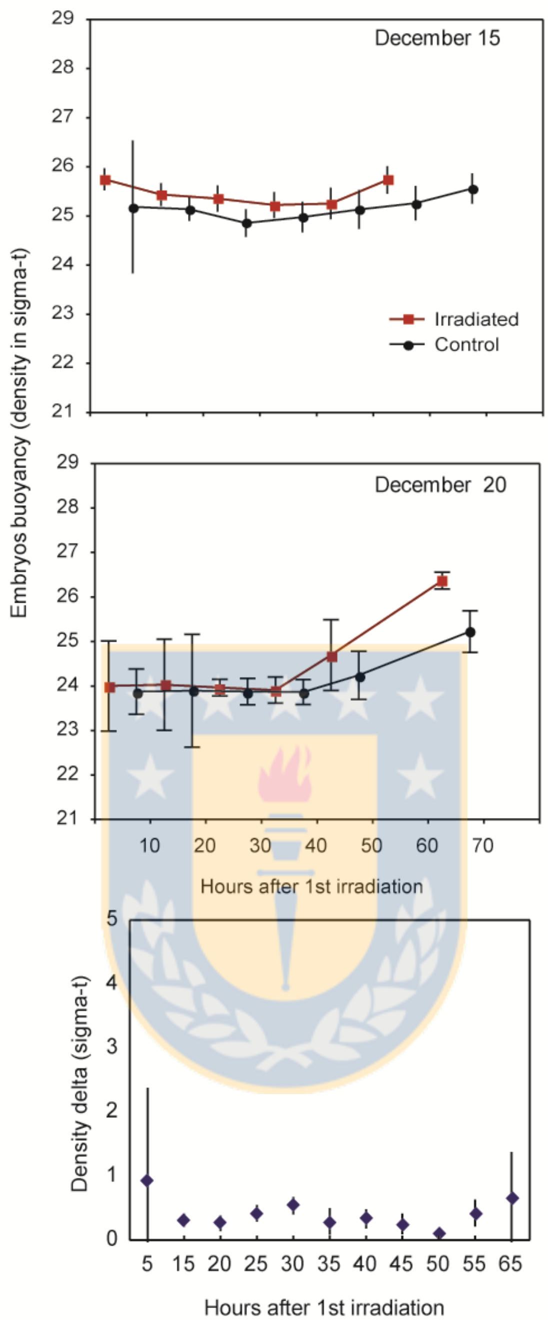
December 15
Irradiated
Control
Embryos buoyancy (density in sigma-t)
December 20
Hours after 1st irradiation
Density delta (sigma-t)
Hours after 1st irradiation

Fig. 7

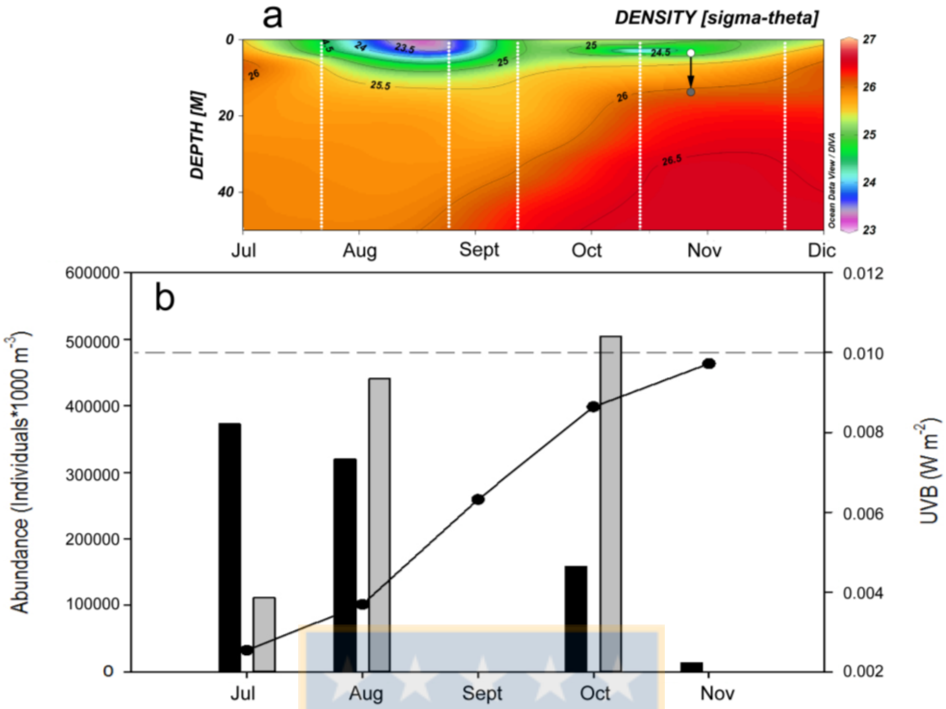
a
DENSITY [sigma-theta]
DEPTH [M]
0
20
40
Jul
Aug
Sept
Oct
Nov
Dic
Ocean Data View / DIVA
27
26
25
24
23
b
Abundance (Individuals*1000 m-3)
600000
500000
400000
300000
200000
100000
0
UVB (W m-2)
0.012
0.010
0.008
0.006
0.004
0.002
Jul
Aug
Sept
Oct
Nov

Fig. 8

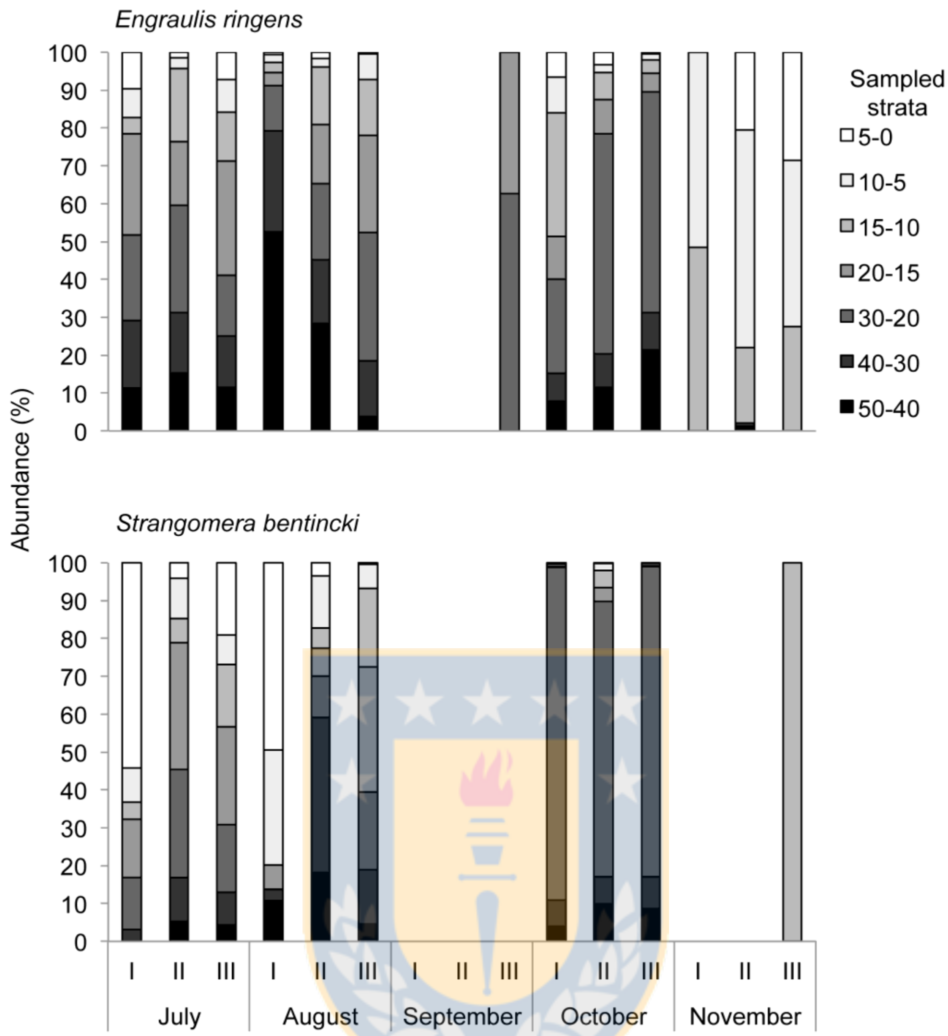
Engraulis ringens
Strangomera bentincki
Abundance (%)
Sampled strata
5-0
10-5
15-10
20-15
30-20
40-30
50-40
July
August
September
October
November

Fig. 9

Table 1.

Species	Study area	UVB (W m^{-2})	Effects	Authors
Limanda limanda, Pleuronectes platessa	54ºN 6ºE	3.63	Increased mortality. Buoyancy loss. Malformations.	Dethlefsen *et al.* 1996
Limanda limanda. Pleuronectes platessa	54ºN 6ºE	0.98-3.63	Increased mortality. Buoyancy loss. Malformations.	Dethlefsen *et al.* 2001
Pleuronectes platessa	45°N 68°W	0.21-0.47	Increased mortality. Buoyancy loss.	Steeger *et al.* 2001
Gadus morhua	44°N 67°W	0.05	Increased mortality. Lower hatching length.	Lesser *et al.* 2001
Gadus morhua	49°N 59°W	4.04	Increased mortality.	Kouwenberg *et al.*1999

Table 2.

Treatment	Filter	Daily dose (J m^{-2})	Irradiation at camera
VIS+UVA+UVB	-	720 UVB	+
VIS+UVB	UVA	235.6 UVA ; 720 UVB	+
Control	VIS, UVA, UVB	0	+

5.- DISCUSIÓN

La radiación solar que llega a la Tierra es de suma relevancia para los procesos físicos, químicos y biológicos que en ésta se llevan a cabo, no obstante y producto de la actividad humana, parte del espectro de esta radiación ha sido demostrada dañina para organismos con distintos niveles de organización, como los embriones de peces marinos planctónicos. Tal es el caso de la anchoveta y la sardina común, especies que en este estudio fueron utilizadas para demostrar a través de la evidencia experimental y de terreno que los niveles actuales de radiación UVB de la zona centro-sur de Chile (36°S) pueden causar efectos letales y subletales en parte de la biota marina.

En los experimentos se demostró que en ambas especies la sobrevivencia fue menor cuando los embriones fueron irradiados en etapas de desarrollo previas a la gastrulación (Estado I), y con una mayor tasa de decaimiento en el tratamiento que incluye UVA y UVB. La vulnerabilidad de estos estados tempranos está dada por la intensidad de los procesos de división y diferenciación celular que están experimentando, los que son alterados luego de la exposición a niveles altos de UVR (Strähle & Jesuthasan 1993, Kouwenberg *et al.* 1999, Steeger *et al*. 2001). En los estados de desarrollo embrionario más avanzados (estado II y III), la sardina común es más resistente a la UVR, lo que indicaría que la respuesta a la exposición a esta radiación sería especie-específica en etapas más avanzadas del desarrollo embrionario. Asimismo, los resultados indican que la exposición a UVA aumentaría la tasa de mortalidad de los embriones de anchoveta y sardina común coincidiendo con lo registrado por Vetter *et al*. (1999) y Lesser *et al*. (2001), descartando en estos experimentos la participación de la UVA en procesos de fotorreparación.

Luego de la exposición a UVR, en ambas especies se observó la presencia de malformaciones morfológicas, cuyo tipo y frecuencia son consistentes con las descritas para una variedad de embriones de peces planctónicos, incluyendo a la anchoveta y a la sardina común (Vásquez *et al.* 2010, Llanos-Rivera *et al.* 2013). La presencia de las malformaciones en estudios previos se ha relacionado a cambios abruptos en algunas de las características físico-químicas del agua así como a factores endógenos (Cameron *et al*. 1992, Dethlefsen *et al*. 1996b, Schreck *et al.* 2001), sin vincularse nunca a la exposición a UVR, cuya influencia no se debería descartar dados los presentes resultados.

El éxito de eclosión en general fue menor en anchoveta, especie en la que fue posible observar a algunos embriones que sobrevivieron hasta completar el desarrollo embrionario, y a pesar de que carecían de malformaciones no lograron eclosionar, fenómeno registrado como "retraso o impedimento de la eclosión". Asimismo, en algunos embriones con malformaciones en la notocorda el retraso o impedimento de la eclosión fue frecuente, ya que pierden movilidad imposibilitando que el corion se destruya por mecanismos físicos (Von Westernhagen 1988).

Es importante destacar que este es el primer estudio que describe la boyantez en anchoveta y sardina común a lo largo del desarrollo embrionario, y que el patrón coincide con el descrito para otras especies de clupeiformes como la anchoa europea *Engraulis encrasicolus* y la sardina europea *Sardina pilchardus* (Coombs *et al.* 2004). Con respecto al objetivo de este estudio, los resultados evidencian una pérdida de boyantez luego de que los embriones son irradiados con una dosis de UVR correspondiente a la que se da en terreno en el área de estudio. Este cambio de densidad en los embriones estaría modulado por la incorporación de lípidos o por un influjo de agua permitido por acuaporinas (Craik & Harvey 1987, Dethlefsen *et al*. 2001, Fabra *et al.* 2005).

La información obtenida en laboratorio y terreno permite afirmar que los embriones pueden experimentar un incremento en densidad que los haría descender en la columna de agua para alcanzar la boyantez neutral, modificando así su distribución vertical. Los cambios en la distribución vertical podrían reducir las posibilidades de dispersión de los embriones hacia áreas de retención y crianza (Sundby 1983, Huret *et al.* 2007, Ospina-Álvarez *et al*. 2012). No obstante, el área de Chile central (36°S) al ser una zona de surgencia activa durante el periodo de Octubre a Marzo, hace que se generen procesos de advección de las capas superficiales a través de la capa de Ekman (~20 m de profundidad) (Sobarzo & Djurfeldt). De esta manera, si los embriones descienden en la columna de agua hasta áreas próximas a la picnoclina se encontrarán con menores velocidades horizontales de las corrientes lo que podría disminuir las posibilidades de ser advectados lejos de la costa (Castro *et al.* 1993, Landaeta & Castro 2012).

Es importante destacar que la pérdida de ozono no sólo se restringe a las zonas más centrales y australes del Hemisferio Sur en las cuales existen factores que atenúan la UVR durante el año, tales como la cobertura de nubes, el ángulo solar durante los meses de otoño e invierno, la escorrentía y el influjo de los ríos, entre otros (Laurion *et al.* 1997). Así, la variabilidad latitudinal de los niveles de UVR está determinada por factores como la estación del año y las condiciones atmosféricas lo que sugiere que los niveles de UVR en latitudes bajas (23°) pueden ser incluso mayores (Vernet *et al*. 2009). Por lo tanto, es necesario determinar si a latitudes más bajas los niveles de UVR son capaces de producir efectos nocivos en diferentes componentes de la biota marina, sobre todo en especies planctónicas que estarían más expuestas a la radiación solar.

El análisis de la distribución vertical durante el periodo de estudio indica que durante Noviembre al menos un 20% de los embriones de anchoveta en estado II y III puede haber sido irradiado con una dosis de UVR que pudiera traducirse en un efecto letal o subletal. Más aún, los efectos de la UVR en los embriones de esta especie podrían incrementarse hacia finales de la época de desove cuando la calidad bioquímica de los embriones es menor (Diciembre-Enero) (Castro *et al.* 2002, Llanos-Rivera & Castro 2004), por lo que dispondrían de menos recursos bioquímicos para destinar, por ejemplo a procesos de fotorreparación en los meses con mayores niveles de radiación solar.

Los efectos de la UVR observados en este estudio de manera directa o indirecta provocan una disminución en la sobrevivencia de anchoveta y sardina común, dos especies altamente explotadas que poseen una alta tasa de mortalidad natural. Por esta razón es imperativo tener en cuenta los efectos de los niveles de radiación UVR actuales como una causa más de mortalidad natural para los embriones de estas especies en estas latitudes.

No obstante, uno de los efectos clasificados como subletales podría en alguna medida aumentar la tasa de sobrevivencia de los embriones como es el caso de la pérdida de boyantez la que podría aumentar la retención en zonas costeras.

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