UNIVERSIDAD DE LAS PALMAS
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TESIS DOCTORAL

CONTRIBUCIÓN A LA BIOLOGÍA DE *Sarda chiliensis chiliensis* (CUVIER, 1832), PARA EL DESARROLLO DE LA ACUICULTURA DE PECES MARINOS EN ZONAS ÁRIDAS DEL NORTE DE CHILE

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UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
ESCUELA DE DOCTORADO

Dr. DANIEL MONTERO VÍTORES, COORDINADOR DEL PROGRAMA EN ACUICULTURA SOSTENIBLE Y ECOSISTEMAS MARINOS DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA, INFORMA QUE:

La Comisión Académica del Programa de Doctorado, en su sesión de fecha ……../…..…./…… tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada CONTRIBUCIÓN A LA BIOLOGÍA DE *Sarda chiliensis chiliensis* (CUVIER, 1832), PARA EL DESARROLLO DE LA ACUICULTURA DE PECES MARINOS EN ZONAS ÁRIDAS DEL NORTE DE CHILE presentada por el doctorando Renzo Gerardo Pepe Victoriano y dirigida por el Doctor Rafael Ginés Ruiz y el Codirector Doctor Germán Merino Araneda.

Y para que así conste, y a efectos de lo previsto en el Arto 11 del Reglamento de Estudios de Doctorado (BOULPGC 7/10/2016) de la Universidad de Las Palmas de Gran Canaria, firmo la presente en Las Palmas de Gran Canaria, a …….. de …….. de 2022.

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
ESCUELA DE DOCTORADO

Programa de doctorado en ACUICULTURA SOSTENIBLE Y ECOSISTEMAS MARINOS DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

Título de la Tesis CONTRIBUCIÓN A LA BIOLOGÍA DE *Sarda chiliensis chiliensis* (CUVIER, 1832), PARA EL DESARROLLO DE LA ACUICULTURA DE PECES MARINOS EN ZONAS ÁRIDAS DEL NORTE DE CHILE.

Tesis Doctoral presentada por Don: Renzo Gerardo Pepe Victoriano

Dirigida por el Dr. Rafael Ginés Ruíz

Codirigida por el Dr. Germán Merino Araneda

Las Palmas de Gran Canaria, a 11 de abril de 2022

_____	_____	_____
Director	Codirector	Doctorando

Epígrafe

"La única y verdadera esperanza del hombre… es el mar"

Jacque Cousteau

Dedicatoria:

"A mis hijos Renzo, Piera, Franco, Tamara, Alessandra y Giulia,
a mi madre Yolanda,
a mis nietos Renzo y Luca,
a mi querida compañera, mujer y amiga, Lorena Cornejo Ponce,
y a los que han partido, mi padre Nicolás, mi hermana Lidia y
la madre de mis hijos, Andrea."

Agradecimientos

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En primer lugar agradezco a mi director de tesis doctoral Dr. Rafael Ginés Ruíz de la Universidad de Las Palmas de Gran Canaria, España, por la confianza que me dio al aceptar dirigir mi investigación.

También, merece un reconocimiento especial, mi colega, amigo y co-director de esta tesis doctoral, Dr. Germán Merino Araneda de la Universidad Católica del Norte de Coquimbo, Chile, por la ayuda recibida en todo el proceso de publicación de mis trabajos, cuyos consejos y aportes, me dieron la visión oportuna y precisa de los trabajos investigativos.

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Por último, agradecer a cada uno de las personas que quedarán en el anonimato en este escrito, pero que solo yo podré reconocer de una u otra manera el apoyo que me brindaron en el proceso del programa de doctorado.

Tesis Doctoral por Compendio de Publicaciones

El Reglamento de estudios de doctorado de la Universidad de Las Palmas de Gran Canaria, aprobado en Consejo de Gobierno de 26 de febrero de 2019 (Boletín Oficial de la ULPGC de 04/03/2019), establece en el artículo 12 los requisitos generales que debe cumplir una tesis doctoral por compendio de publicaciones.

La **tesis por compendio de publicaciones** es un formato especial en el que la tesis queda constituida por el conjunto de trabajos publicados por el doctorando en el marco del Plan de Investigación de su tesis doctoral.

Requisitos para la presentación de una tesis por compendio:

a) Un mínimo de **tres publicaciones**, con unidad temática, **indexadas** en el Journal Citations Reports, Arts and Humanities Citation Index o equivalentes, de las que el doctorando sea el primer autor o autor principal. Al menos una de ellas deberá haber sido publicada en una revista cuyo índice de impacto la situé dentro de la primera mitad en orden decreciente de índice de impacto entre las revistas del área.
b) Para acreditar la condición de **autor principal**, esta deberá ser reconocida por el resto de los autores de las publicaciones presentadas como núcleo de la tesis doctoral, al mismo tiempo que estos deberán renunciar a utilizar estas publicaciones como núcleo principal de otras tesis doctorales, sin perjuicio de que dichas publicaciones puedan ser presentadas como méritos complementarios en las tesis doctorales que pudieran presentar los otros autores de dichas publicaciones.
c) En áreas de especial incidencia tecnológica dos de estas publicaciones podrán ser sustituidas por patentes en explotación o publicaciones en congresos reconocidos por la ANEP en sus baremos para la obtención de sexenios.

d) Que en las publicaciones o patentes conste la ULPGC a través de la filiación del director o del doctorando.

Las tesis doctorales presentadas como compendio de publicaciones deberán ajustarse al formato establecido en los apartados del 1 al 3, del artículo 11 del presente Reglamento y contener los apartados siguientes:

a) Una introducción en la que se presenten los objetivos de la tesis, los trabajos publicados y la justificación de la unidad temática de la tesis.
b) Una copia de los trabajos publicados.
c) Las conclusiones finales.
d) En el caso de que lo dispuesto en los apartados a y c se haya redactado en una lengua diferente del español, deberá incluirse un resumen en español según el artículo 10 del presente reglamento, de una extensión de entre 5 y 20 paginas, en el que se incluyan los objetivos y las conclusiones.

Expuesto lo anterior, esta tesis doctoral cumple con lo establecido en el artículo mencionado anteriormente, ya que se presentan tres publicaciones, considerando que todas estas el doctorando se encuentra como primer autor y además están publicadas en revistas científicas del ámbito de conocimiento del programa de doctorado. Las tres publicaciones cuentan con los indicios de calidad que exige el programa de doctorado, esto es:

Que, los tres artículos están indexados en Journal Citations Reports.

A continuación, se detallan los indicios de calidad de las publicaciones:

1. Renzo Pepe-Victoriano, Héctor Aravena-Ambrosetti and Germán E. Merino. 2021. **Breeding of a Wild Population of South Pacific Bonito *Sarda chiliensis chiliensis* (Cuvier 1832) Broodstock under Laboratory Conditions in Pisagua, Northern Chile**. Animals,12, 24.
   https://doi.org/10.3390/ani12010024
   Factor de impacto: 2.70
   Cuartil Q1

2. Renzo Pepe-Victoriano, Loreto Miranda, Aurelio Ortega and Germán E. Merino. 2021. **First natural spawning of wild-caught premature south pacific bonito (*Sarda chiliensis chiliensis*, Cuvier 1832) conditioned in recirculating aquaculture system and a descriptive characterization of their eggs embryonic development.** Aquaculture Report, 19, 100563
   https://doi.org/10.1016/j.aqrep.2020.100563
   Factor de impacto: 3.14
   Cuartil Q1

3. Renzo Pepe-Victoriano, Loreto Miranda, Aurelio Ortega and Germán E. Merino. 2021. **Descriptive morphology and allometric growth of the larval development of *Sarda chiliensis chiliensis* (Cuvier, 1832) in a hatchery in northern Chile**. Aquaculture Report, 19, 100576
   https://doi.org/10.1016/j.aqrep.2020.100576
   Factor de impacto: 3.14
   Cuartil Q1

La unidad temática de las publicaciones que integran esta tesis por compendio, en conformidad con lo establecido en el artículo 12 del Reglamento de estudios de doctorado, se manifiesta, que todas versan sobre la biología de *Sarda chiliensis chiliensis*.

Finalmente, la estructura de esta tesis doctoral cumple lo establecido en el articuló 12.2 del Reglamento de estudios de doctorado,

a) una introducción en la que se presenta los objetivos de la tesis, la presentación de los trabajos publicados y la justificación de la unidad temática de la tesis;
b) una copia de los trabajos publicados (publicaciones);
c) las conclusiones finales.

CAPÍTULO 1

Antecedentes General de Sarda chiliensis chiliensis

Introducción

La Acuicultura de peces marinos ha llamado considerablemente la atención de los países, principalmente latinoamericano, entregando un auspicioso panorama en las últimas décadas. Lo dicho anteriormente radica debido al desarrollo y optimización de la tecnología de cultivo en jaulas, así como también a las mejoras de los métodos de producción de juveniles, lo que ha multiplicado los cultivos de orientación comercial, debido al número de especies. A esto se suma el gran valor que posee el mercado mundial con los productos extraidos de especies marinas, particularmente de aquellas de carne blanca.

Los avances científicos a nivel mundial en los últimos 50 años, han permitido mejorar en gran medida los conocimientos acerca del funcionamiento de los ecosistemas acuáticos, así como la conciencia mundial de la necesidad de gestionarlos de forma sostenible (FAO, 2020).

Las ventajas que posee Chile comparativamente son numerosas en áreas de la acuicultura, respecto a otros países, particularmente, para el cultivo de especies marinas. Muchas universidades y empresas privadas a través de diferentes proyectos, proponen impulsar continuamente la cría de peces nativos marinos, con el objetivo de contribuir a nuevas oportunidades de negocio con un alto impacto potencial en términos económicos, orientado principalmente a la exportación de productos de alto valor, como también a generar oportunidades para fortalecer la actividad productiva del sector pesquero artesanal chileno, que se ha visto seriamente deteriorado en los últimos años, producto del colapso de las principales pesquerías. Desde esta perspectiva las diferentes investigaciones plasmadas en tres artículos científicos proponen un fuerte potencial de impacto social, que permitirá a entidades gubernamentales de nuestro territorio poder diversificar la acuicultura chilena.

El objetivo de estas investigaciones fueron:

a) Articulo 1: Cultivar una población de Bonito del Pacífico Sur, *Sarda chiliensis chiliensis,* capturada en estado salvaje, para establecer un primer plantel de potenciales reproductores en condiciones de laboratorio en el norte de Chile.
b) Artículo 2: Realizar un estudio actualizado para describir el desarrollo embrionario durante la incubación, en condiciones de acuicultura.
c) Artículo 3: Describir el desarrollo morfológico y los patrones de crecimiento alométrico de la serie de estadios de las larvas de *Sarda chiliensis chiliensis* cultivadas en estanques para la definición de los criterios iniciales del cultivo larval.

La metodología de las investigaciones planteadas, están basada en antecedentes generales de la especie, además de información relativa al cultivo de peces marinos de características similares, como lo es para *Sarda sarda* principalmente realizada en España, el cual a través de proyectos con atunes han podido desarrollar y conocer aún más esta especie. Un aporte importante consiste en la experiencia que posee Chile en la acuicultura, que brinda conocimientos avanzados para esta área, el cual facilita nuevas metodologías de trabajo en el desarrollo de nuevas especies a cultivar.

De esta forma, estas publicaciones científicas, contribuirán a impulsar y fortalecer la diversificación de la acuicultura en la zona norte de Chile mediante el cultivo de una especie nativa como lo es *Sarda chiliensis chiliensis.*

Descripción de la especie

Sarda chiliensis chiliensis se caracteriza por poseer un cuerpo esbelto y alargado, algo comprimido y moderadamente robusto. Dientes de la mandíbula superior de 18 a 30, en mandíbula inferior de 14 a 25. Primera aleta dorsal con 17 a 19 espinas, aleta anal presenta de 12 a 15 radios y aleta pectoral de 22 a 26 radios

(Arana, 2012). Cuerpo de color azul oscuro con brillos metálicos y con 5 a 9 franjas oscuras que se orientan de dorsal a ventral en forma oblicua. Al costado y en el vientre son plateados y tiene aletas dorsales, levemente separadas. El cuerpo está cubierto por pequeñas escamas de tipo cicloide, poco visibles, excepto en la región de la cabeza y el corselete. Por detrás de la segunda aleta dorsal y anal presenta pequeñas aletillas. El pedúnculo caudal es angosto, con dos pequeñas quillas a cada lado y además otra quilla mediana de mayor tamaño entre ellas a cada lado. Estos especímenes puede alcanzar un peso de 5,5 kg y una longitud de 1 m. Se caracteriza por tener una boca grande, con dientes cónicos, así como por sus ojos grandes y redondos (Mann, 1954). Los bonitos se desplazan en grandes cardúmenes y necesitan nadar continuamente dado que carecen de vejiga natatoria. (Chirichigno, 1980).

Figura 1.- Ejemplar de *Sarda chiliensis chiliensis* (Pepe-Victoriano *et al.,* 2021)

El género *Sarda*, es miembro de la familia de *Scombridae*, es un pez de aguas templadas y tropicales, especie epipelágica nerítica, o sea, que vive en profundidades medias y nada formando grandes cardúmenes que se acercan a la costa sólo en primavera (Mann, 1954) actualmente ha tenido una variada aceptación en el mercado mundial.

Nombres locales

Chile	: Bonito, Mono
España	: Bonito
Colombia	: Bonito
México	: Bonito
Perú	: Aguadito, Bonito, Cerrajón, Chaucha, Chauchilla, Monillo, Monito, Mono
Suiza	: Chilensk Boni
USA	: Pacific bonito
Rusia	: Chilijskaya pelamida, Vostochnaya pelamida

Distribución

Sarda chiliensis se encuentra solo en el pacífico este (Collette & Chao, 1975). Su alcance geográfico es separada en un Norte y en un Sur por una población tropical de *Sarda orientalis,* al norte la subespecie *Sarda Chiliensis lineolata* (Girard 1858) se encuentra a partir de las costas de Alaska (60°16´N,145° 32´W) hasta el sur de Cabo San Lucas en la punta de Baja California (22 °20´N, 112 ° 27´O) y en las islas Revillagigeo. Al sur la subespecie *Sarda chiliensis chiliensis* esta presente de Mancora, Perú, justo al sur del Golfo de Guayaquil hacia el sur, llegando hasta Talcahuano, Chile (Yoshida, 1980).

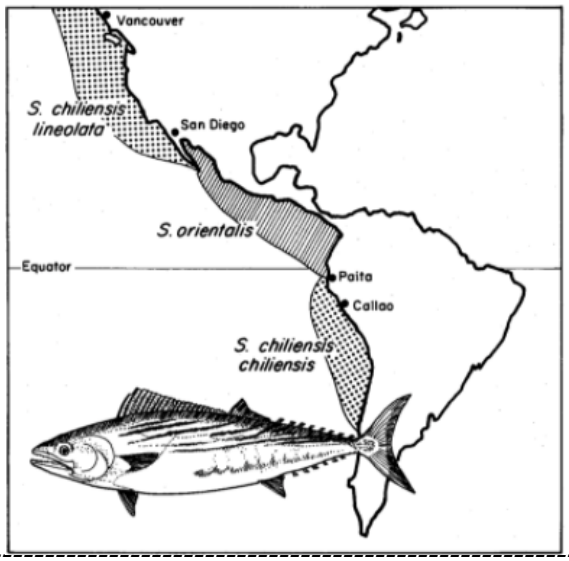

Figura 2.- Mapa de distribución *Sarda chiliensis chiliensis* (extraído de www.fao.org).

De este género se reconocen cuatro especies: *Sarda australis, Sarda chiliensis*, *Sarda orientalis* y *Sarda sarda (Collette & Chao,* 1975).

Ecología Trófica

Los peces representan importantes depredadores en los ecosistemas acuáticos, ya que son capaces de alimentarse de la mayoría de las comunidades y de los recursos disponibles en el sistema (Vander Zanden & Vadeboncoeur, 2002). Debido a que son organismos móviles con tiempos generacionales y tamaños corporales relativamente grandes, los peces suelen conectar a través de su alimentación zonas litorales, bentónicas y pelágicas (Jeppesen *et al*., 1997; Schindler & Scheurell, 2002; Pace *et al*., 2004) e incluso comunidades espacialmente distantes (Dolson *et al*., 2009; Massol *et al*., 2011). Asimismo, a través de la alimentación son capaces de provocar fuertes efectos en cascada sobre niveles tróficos inferiores (Carpenter & Kitchel, 1993; Lövgren & Persson, 2002). De esta manera, pueden afectar directa o indirectamente la mayoría de los componentes del ecosistema, acoplarlos y promover cambios en los ciclos de nutrientes y la dinámica energética (Polis *et al*., 1996; Motta & Uieda, 2005; Knight *et al*., 2005; 2006). En este contexto resulta fundamental conocer el rol de los peces en los ecosistemas acuáticos, particularmente considerando que muchas especies se están perdiendo en un alto número de ecosistemas (Olden *et al*., 2007). La caracterización de los hábitos alimentarios de la comunidad ictícola representa un abordaje básico para avanzar en la comprensión de su papel en la estructura y funcionamiento de la diversidad biológica (Winemiller, 1990).

El estudio de la ecología trófica es necesario para entender la biología y la ecología de los organismos, y el alimento es uno de los factores más importantes e influyentes (Wöhler & Sánchez 1994).

El estudio del contenido estomacal es una forma común de investigación de las cadenas alimentarias de una comunidad biológica marina (Berg, 1979), que permite obtener información valiosa acerca del rol de las especies dentro de un ecosistema (Heupel & Bennett, 1998), a través del conocimiento del tipo de especies

que consumen, de sus preferencias y del efecto de sus hábitos alimentarios sobre el resto de la comunidad.

Alimentación

En Chile, las primeras investigaciones sobre la alimentación de peces se efectuaron en especies demersales, pelágicas, submareales, intermareales, etc., donde en su gran mayoría han sido dirigidas al conocimiento de las especies que son importantes en las pesquerías (Silva & Stuardo, 1985; Flores & Rojas, 1987; García & Chong, 2002; Medina & Arancibia, 2002).

Magnuson & Heytz (1971) examinaron los hábitos alimentarios de los peces escómbridos, señalando que existe selectividad, en término del tamaño del alimento. Sugieren que los predadores más grandes han reducido su capacidad para capturar presas pequeñas (crustáceos), esto debido a una relativamente grande abertura entre las branquispinas. Entre los escómbridos del mismo tamaño, *Sarda chiliensis lineolata* y *Sarda orientalis* tienen la más grande abertura de branquias (1,8 – 3,3 mm).

Un estudio realizado a un total de 1.498 estómagos de *Sarda chiliensis lineolata,* fue efectuado por Pinkas (1971), mostrando claramente que la anchoveta del norte *Engraulis mordax*, fue el mayor componente (75,9 % en volumen) seguido por el calamar común, *Loligo opalescens* (18,0 % en volumen). El resto estuvo constituido por peces varios y unos pocos crustáceos.

Un estudio realizado por el instituto del mar del Perú (2007) concluye que la alimentación de *Sarda chiliensis chiliensis* en la zona del Callao-Perú, la ingesta de pejerrey, y en una menor proporción la anchoveta, se encuentran en ejemplares menores a 36 cm; a diferencia de los de mayor talla quienes se alimentaron fundamentalmente de anchovetas, durante el periodo de otoño-primavera.

Recientes estudios sobre la alimentación de *Sarda chiliensis chiliensis* realizadas en el norte de chile y sur del Perú, fueron los realizados por Medina & Araya (2019) y Pepe-Victoriano *et al*., (2022) el cual este último, analizó un total de

1404 estómagos encontrando una predominancia de *Pleuroncodes monodon* y *Engraulis ringens,* concluyendo que podría ser una especie generalista.

Pesca y Producción

En California, el bonito del pacífico oriental es capturado comercialmente por los barcos pesqueros, pero mayormente por pesca de barcos privados, (Yoshida, 1980). A mediados de los años sesenta, la pesquería chilena del bonito, entre Iquique y Antofagasta se expandió, en una actividad casi totalmente artesanal con redes de mallas y de cerco flotante, pero es una forma pequeña para la operación industrial de bonito, donde Chile está especializado en la pesca de atún (Yoshida, 1980). Los desembarques de la subespecie del norte (*S. c. lineolata*) en California y México han fluctuado mucho en los últimos 50 años a partir de menos de 1.000 toneladas métricas a cerca de 14.000 t. en los años setenta, el ranking en el puesto número 13 (4.003 t un valor de $ 1.222.000 dólares) en el total de desembarques de California de 1976. Los desembarques del Perú de *Sarda chiliensis chiliensis*, aumentó de casi cero, en 1940 a un máximo de 110.000 toneladas anuales en los años sesenta, a partir de entonces fue disminuyendo hasta llegar a las 40.000 toneladas a mediados de los años setenta (Yoshida, 1980). Las captura en el mundo, para la especie se redujo entre 10.219 t en 1976 y 15.936 t en 1981, alcanzando 21.308 t en 1977 (Collette & Nauen, 1983).

En Chile, la captura de *Sarda chiliensis chiliensis* se realiza principalmente en el norte del país, en las regiones de Arica y Parinacota, Tarapacá y Antofagasta, abarcado casi el 95% de la captura total en el año 2020 (Fig. 3a) (SERNAPESCA, 2020). Durante el mismo año solo en los meses de enero y diciembre no existe captura de esta especie (Fig. 3b).

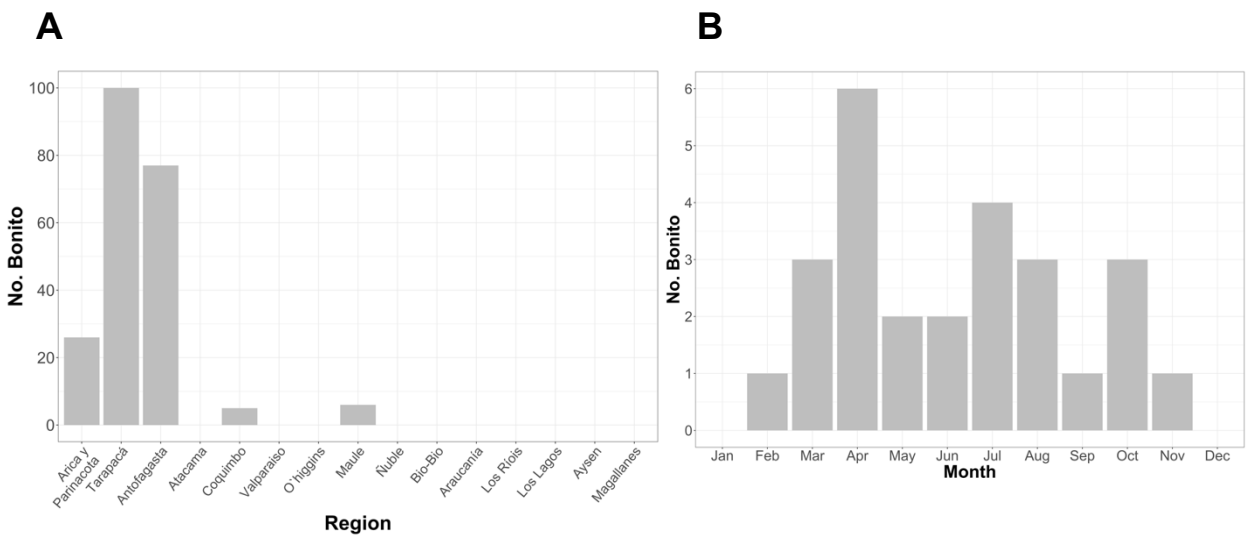

Figura 3.- Captura de *Sarda chiliensis chiliensis* en Chile.

A: Captura por región B: Captura por mes

(extraído de SERNAPESCA, 2020)

Captura y Transporte de Peces Marinos

Al comenzar un cultivo de peces se necesita de ejemplares para adaptarlos al cautiverio como potenciales reproductores, los cuales deben ser capturados del medio donde viven y así asegurar la continuidad del cultivo (Muñoz et al., 2012), en general, estos cultivos empiezan con la captura de juveniles (Botero & Ospina, 2002; Papandroulakis *et al.,* 2004; Belmonte *et al.,* 2007; Grignon, 2010).

El cultivo de peces marinos nativos en Chile es un tema de la actualidad, dirigido principalmente a la investigación, donde se han capturado juveniles silvestres de *Merluccius australis* (merluza del sur), *Cilus gilberti* (corvina), *Eleginops maclovinus* (róbalo), *Seriolella violácea* (Cojinoba) *Seriola lalandi* (Palometa), *Paralichthys adspersus* (lenguado chileno), *Oplegnathus insignis* (San Pedro), *Medialuna ancietae* (pez acha) y recientemente *Sarda chiliensis chiliensis* (Silva & Flores, 1989; Cortes *et al.,* 2001; Bustos & Landaeta, 2005; Pepe-Victoriano *et al*., 2022a).

El transporte de los juveniles (potenciales reproductores) deben tener características particulares para cada especie, pero uno de los patrones generales

serian: baja densidad, peces pequeños, flujo de agua continuos en la embarcación y oxígeno permanente en el trasporte terrestre. Cumpliendo con estos requisitos podemos asegurar un transporte con un alto porcentaje de sobrevivencia (Pepe-Victoriano *et al*., 2022b)

Hábitat y Biología

Especie epipelágica nerítica, de migración nictimeral. En aguas costeras el bonito habita la capa superficial mas temperada y salina del agua subantártica, en las aguas oceánicas se encuentra asociado al agua subtropical superficial, caracterizada por altas temperatura y salinidad. A diferencia de los adultos, los juveniles se encuentran generalmente mas cerca de la costa. Durante los fenómenos climáticos que incrementan la temperatura del mar, se generan las condiciones apropiadas para que se presente en abundancia y más arriba de su promedio. Forma cardúmenes por tamaño.

Características de reproducción

Sarda chiliensis chiliensis alcanza su madurez sexual aproximadamente a los dos años de edad (Collette & Nauen, 1983).

En el hemisferio sur, el desove se produce en las aguas cercanas a la costa entre septiembre y diciembre. En el hemisferio norte, el desove se inicia a principios de marzo, las poblaciones del sur avanzan hacia el norte en los meses siguientes en función del aumento de la temperatura. La evidencia sugiere que incluso a un año de edad *S. chiliensis lineolata* se puede encontrar en las zonas de aguas frías influenciada por los vertidos térmicos (Collette & Nauen, 1983). En su mayoría, los bonitos maduran a principios de temporada y tienden a vivir más en la costa, en comparación con los peces más jóvenes. El desove es en lotes, y el número de huevos que aporta en una temporada por una muestra de 3 kg se ha estimado en alrededor de varios millones. La fecundidad aumenta exponencialmente con el tamaño (Collette & Nauen, 1983).

Las cuatro especies de *Sarda* son usualmente heterosexuales, no existiendo dimorfismo sexual entre machos y hembras. Tras investigaciones de Mgnuson & Prescott (1966) para *S. chiliensis lineolata* en cautiverio, observaron un dimorfismo sexual a través de las características de comportamiento del bonito, constatando que unos eran "bamboleantes" ("wobblers") y otros "seguidores" ("followers"). Determinando que los "bamboleantes" eran hembras y los "seguidores" eran machos, sin embargo, Vildoso (1960) en *S. chiliensis chiliensis* encuentra casos de hermafrodismo, en aguas peruanas.

En las costas de Chile las hembras de *S. chiliensis chiliensis* alcanzan inicialmente la madurez sexual a una longitud de 51 cm (Barret, 1971). Para los bonitos del Perú, Vildoso (1960) determinó que la talla del primer desove está entre 47 y 53 cm Kuo (1970) estableció que en la población del Hemisferio Norte, la hembra alcanza la madurez sexual a 51 cm y que a esta longitud el pez tiene 5 años aproximadamente.

Aunque algunos patrones de comportamiento como la expulsión de gametos durante una natación en círculo hayan sido observados sólo en *S. chiliensis lineolata*, se espera que las otras especies de *Sarda* tengan el mismo comportamiento (Magnuson & Prescott, 1966).

La época de desove de *S. chiliensis chiliensis* en aguas chilenas comienza en septiembre y tiene su máximo en octubre y noviembre, finalizando antes de abril (Barret, 1971). En el Perú es muy similar, con el máximo del desove extendido desde octubre hasta febrero (Vildoso, 1960).

La mayoría de las observaciones tienden a apoyar a Klawe (1961), quien, basado en las capturas de larvas y juveniles de *S. chiliensis*, ha establecido que el desove de esta especie tiene lugar en la estación más cálida frente a Baja California, Perú, y Norte de Chile.

Composición Nutricional

A lo largo del mundo ha sido ampliamente reconocido que la pesca y la acuicultura nos entregan productos que constituyen alimento importante para el

mundo entero, principalmente por su valioso aporte de nutrientes en la dieta humana (Chukwu & Mohammed, 2009) y se les considera como uno de los alimentos más completos por la calidad y cantidad de nutrimentos que aporta (Izquierdo *et al*., 2000). Es sabido que conforma una fuente muy alta de proteína, minerales y vitaminas esenciales de gran valor biológico (Astorga *et al*., 2007; Fuentes *et al*.,2009; Özden 2010a). Además, por su escaso contenido en grasas y calidad de ácidos grasos, muchos estudios reportan los efectos favorables del consumo de estos organismos para una mejor salud (Castro-González *et al*., 2007; Perea *et al*., 2008; Özden 2010b).

El bonito, se encuentra dentro del grupo de pescado azul, por lo que su contenido graso es elevado si lo comparamos con otros pescados, como los llamados magros. Posee unos 6 g de grasa por cada 100 g de porción comestible. Su grasa es rica en ácidos grasos omega-3, que contribuyen a disminuir los niveles de colesterol y de triglicéridos en la sangre, además de hacer la sangre más fluida, lo que rebaja el riesgo de formación de coágulos o trombos (Martin, 2018).

Además, esta especie, al igual que los otros peces, es una buena fuente de proteínas de muy alto valor biológico y posee cantidades variadas de vitaminas y minerales. Entre las vitaminas, se encuentran las del grupo B, como la B2 (más abundante en pescados azules) y la B9, aunque su contenido es menos relevante si se compara con otros alimentos ricos en estos nutrientes (hígado, levadura de cerveza, cereales integrales, legumbres). Respecto a otros pescados, el bonito tiene un contenido sobresaliente de vitamina B3 y B12, esta última muy superior a muchos pescados y carnes (Martin, 2018) (Tabla 1).

Tabla 1.- Composición nutricional por 100 g de porción comestible (extraído de Martin, 2018)

Energía (kcl)	138
Proteínas (gr)	21
Grasas (gr)	6
Hidratos de carbono (gr)	0
Hierro (mg)	1
Magnesio (mg)	28
Yodo (mg)	10
Calcio (mg)	35
Zinc (gr)	1
Colesterol (mg)	38
B2 o riboflavina (mg)	0,2
B3 o niacina (mg)	17,8
B9 o ácido fólico (mc g)	15
B12 (mcg)	5
Vitamina A (mcg)	40
Vitamina D (mcg)	20
Ácidos grasos saturados (gr)	1,2
Ácidos Grasos monoinsaturados (gr)	1,6
Ácidos grasos poliinsaturados (gr)	1,4

Medidas de regulación

Para esta especie no existen medidas de regulación (veda, tamaño mínimo de extracción y cuota de captura), excepto, que no se puede destinar para harina de pescado.

Sistema de pesca

Esta especie es importante para la pesca recreativa con anzuelo y sedal que opera desde embarcaciones privadas, muelles, embarcaderos, y desde la costa. También se captura con redes de cerco por la flota industrial y red de enmalle por el sector artesanal.

Utilización para consumo humano

La carne del bonito es rica en aceites y posee una moderada cantidad de grasa, que se reduce al 6% en este pez, mientras que en el atún alcanza el 10%. Esta carne es cotizada principalmente en el mercado asiático, donde se comercializa en mayor medida como congelado y conserva. En Perú además se consume asado y al horno (Frimodt, 1995).

En las pescaderías se suele presentar entero y eviscerado en rodajas. Se llama ventrescas a las piezas que se sacan de la zona ventral del pez. Se puede comercializar congelado en rodajas, aunque no es muy habitual ya que el pescado azul tiene un duración más corta congelado. Se puede encontrar descongelado en pescaderías, que lo venden luego al corte. Cuando se compra en rodajas, tiene poco desperdicio, sólo sobra la piel y la espina, que pueden suponer el 15-25% del peso. Un bonito entero presenta mucho desperdicio, ya que la cabeza es grande y en ese caso la parte comestible es sólo del 58%.

Posible acumulación de tóxicos

Son peces carnívoros, por lo que pueden acumular tóxicos como por ejemplo metales pesados. Por ello, se recomienda que las embarazadas y los niños no hagan un consumo abusivo de este tipo de pescados, aconsejando un consumo de tan sólo una vez por semana.

CAPÍTULO 2

Captura, Transporte y Acondicionamiento de Reproductores de Peces Marinos

Introducción

Existen dos fuentes principales para la obtención de los reproductores, los peces cultivados o criados y los provenientes del medio natural con captura de ejemplares silvestres juveniles o adultos (Silva & Oliva, 2010), estos últimos (adultos) pueden sufrir daños dependiendo del arte de pesca, la manipulación y el traslado a los centros de cultivo, lo que se traduce en incertidumbre e inestabilidad de las capturas y el estrés para los peces, con consecuencias negativas, tales como inmunosupresión, enfermedades por patógenos primarios y/o secundarios, atresia gonadal, calidad de huevos, entre otros. Por lo que generalmente la captura de juveniles es lo ideal para el comienzo en el acondicionamiento de futuros reproductores de especies potencialmente interesantes para la acuicultura nacional (Muñoz *et al.*, 2012).

En el caso de los peces pelágicos, el aumento de la demanda de atunes ha incrementado el esfuerzo de la pesca, de tal manera que algunos de estas especies están en peligro de sobreexplotación, ejemplo de ésto es el atún de aleta amarilla *Thunnus albacares* y la albacora *Thunnus alalunga*, del pacífico sur (Skillman, 1975). Como consecuencia de lo anterior, la atención se ha dirigido a recursos atuneros relativamente subutilizados, como *Katsuwonus pelamis* (barrilete). Además, algunos de los atunes pequeño como los bonitos, del género *Sarda*, también han tenido una mayor atención (Yoshida, 1980).

Sin embargo, las pesquerías de bonito del Pacífico Sur están siguiendo la misma tendencia de declive de las poblaciones silvestres, y a pesar de los años de explotación pesquera, sus antecedentes relacionados con sus etapas embrionarias de desarrollo temprano son lo suficientemente escasos como para poder establecer planes de gestión de la especie en su medio natural o para generar información de base para su cría en sistemas de cultivo acuícola.

Es sabido que el éxito en la producción acuícola de cualquier especie implica el conocimiento de las características morfofisiológicas y de comportamiento de la especie en cultivo, siendo relevante el estudio entre el desarrollo embrionario y los primeros estadios juveniles (Gisbert *et al*., 2002, 2004; Botta *et al*., 2010; Azfar

Ismail *et al*., 2019; Syafiq *et al*., 2020; Yoshinori *et al*., 2020). La descripción del desarrollo embrionario en los peces permite numerosas ventajas, como herramientas para la correcta gestión de los recursos acuícolas y pesqueros (Conklin *et al*., 2004; Pepe-Victoriano *et al*., 2012; Marancik *et al*., 2020), para la identificación precisa de los ciclos vitales (Oka *et al*., 2020), y para el reconocimiento de embriones y larvas en entornos naturales (Davis *et al*., 2020). Todo lo anterior permitirá una mejor evaluación de las condiciones ambientales de desove de los reproductores (Pepe-Victoriano *et al*., 2013), así como generar artificialmente ambientes que puedan conducir a una crianza saludable de las larvas y, en consecuencia, a la cría de juveniles con excelentes índices de producción (Alves & Moura, 1992; Celik & Sukran, 2019).

Captura y Transporte

El estrés de la captura y la manipulación de los ejemplares pueden afectar los parámetros sanguíneos, el hipotálamo y la pituitaria, con las correspondientes afectaciones de los niveles de gonadotropina, esteroides y el cortisol en sangre. (Harmon, 2009). En cuanto al sistema reproductivo se puede presentar: atresia de los ovocitos, huevos de menor tamaño, y en consecuencia disminución en los parámetros reproductivos (fertilización, eclosión y viabilidad de las larvas). De igual manera se pueden presentar ciertas afectaciones físicas, como descamaciones y pérdida del mucus, lo cual incrementa la probabilidad de infecciones que pueden llegar a provocar la muerte. Entre los aspectos importantes a tener en cuenta antes y durante la captura y la manipulación de los ejemplares están (Álvarez – Lajonchère & Hernández, 2001; Harmon, 2009)

No se deben capturar muchos ejemplares a la vez, ya que la interacción entre ellos pueden causarles laceraciones. Emplear bolsas oscuras, sin nudos, suaves al tacto y con orificios en el extremo para desocupar el agua. No se deben colocar los animales con el vientre hacia arriba, a no ser que sea necesario y sólo bajo sedación, uso de técnicas de captura eficientes empleo de artificios para manipular los ejemplares individualmente. En lo posible no se deben manipular a

los animales en seco, uso de anestésicos efectivos y seguros. Asegurar condiciones de tranquilidad, pocas personas y silencio durante el manejo de los animales.

Ortega y de la Gándara (2007a) lograron capturar reproductores de bonito del Atlántico (*Sarda sarda*). Los ejemplares fueron capturados mediante un paño de red que se dispone en forma perpendicular a la costa. Los bonitos, una vez capturados, son inmediatamente depositados en un estanque circular de 130 cm de diámetro y 75 cm de profundidad, dotado con una bomba para recircular el agua y un difusor de oxígeno, de tal forma que se mantenga siempre por sobre el 90% de saturación (Ortega y de la Gándara, 2007b). Al llegar a puerto, los ejemplares son trasladados rápidamente a las instalaciones de acondicionamiento.

Acondicionamiento de Reproductores

Los antecedentes acerca del cultivo de esta especie son escasos. De acuerdo a los antecedentes entregados por McFarlane *et al*., (2000) indican que es posible mantener reproductores de bonito del Pacifico (*Sarda chiliensis*) en cautiverio en estanques de 4,3 m^3, a temperaturas de 20,4 ± 0,3°C y con un fotoperiodo de 16L:8O. Los ejemplares se alimentaron cuatro veces a la semana con una dieta consistente primariamente de peces y calamares.

Ortega & de la Gándara (2007a) lograron mantener reproductores de bonito del Atlántico (*Sarda sarda*). Los ejemplares de bonito fueron mantenidos en dos tanques de 20 m^3 de capacidad, redondos y de color oscuro. Como parte del manejo de los ejemplares, estos se desinfectaron mediante baños de formol (150 ppm) y agua oxigenada (200 ppm) durante 1 hora, los que fueron administrados cada dos días durante la primera semana de cautividad (Ortega & de la Gándara, 2007a). La alimentación se entregó al tercer día de cautividad y para facilitar el aprendizaje alimentario se introdujo en los estanques un ejemplar domesticado de *Seriola*. Se realizaron 8 capturas donde los ejemplares presentaron pesos que fluctuaron entre los 1.050 g y los 3.200 g, las mortalidades variaron entre

un 75 y 20%. La temperatura al momento de la captura fluctuó entre los 17,2 y los 15,5°C.

Los resultados obtenidos por Ortega & de la Gándara (2007a) indican que los ejemplares mostraron un buena adaptación a la cautividad, aceptando distintas especies de peces como alimento y mostrando una elevada tasa de ingestión (7-8% peso/día). Estos autores entregáron indicaciones para el manejo de esta especie el cual fue aplicado a los reproductores de bonito del Pacífico (*Sarda chiliensis chiliensis*). Así, su experiencia indica que las mallas con las cuales se realiza la pesca deben ser de caucho o con lonas de plástico para evitar la pérdida de escamas y del mucus protector. Del mismo modo, debe evitarse el uso de mallas de nylon y tocarlos con las manos, ya que esto aumenta la mortalidad. Por otro lado, los mejores resultados se obtienen cuando las capturas se hacen a la menor temperatura posible, ya que pescas realizadas a temperaturas entre 23 y 24°C entregaron mortalidades del 100%, mientras que las mejores supervivencias se obtuvieron en capturas realizadas a temperatura del agua de mar de 15°C.

Otro de los factores a considerar para el mejor manejo de los ejemplares tiene relación con el peso de los mismos, es así como los ejemplares de más de 3 kg son más difíciles de capturar y manejar con el arte de pesca, lo que también influiría en la mayor mortalidad de estas capturas. Finalmente, la densidad del transporte tambien juega un rol determinante y que podría explicar las altas mortalidades observadas en las algunas de las capturas, ya que aumenta la colisión entre ellos.

Primer Artículo:

Breeding of a Wild Population of South Pacific Bonito *Sarda chiliensis chiliensis* (Cuvier 1832) Broodstock under Laboratory Conditions in Pisagua, Northern Chile.

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Breeding of a Wild Population of South Pacific Bonito *Sarda chiliensis chiliensis* (Cuvier 1832) Broodstock under Laboratory Conditions in Pisagua, Northern Chile

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Simple Summary: Knowing the biology of fish is fundamental to advance in the culture of wild marine fish species. This is why it is important to form an initial population of broodstock to obtain eggs, larvae, and juveniles of this species for aquaculture research. Therefore, in this research, 24 specimens of "bonito" were captured, transported, and conditioned, and after 14 months in captivity, the fish spawned spontaneously. The eggs were collected and deposited in incubators at 20 °C. By the third day, these eggs had hatched. The newly hatched larvae, as well as the eggs, were characterized during their first morphological changes, which explains that the capture, transport, and conditioning processes were successfully carried out in this research.

Abstract: The wild population of South Pacific bonito *Sarda chiliensis chiliensis*, which has a wide distribution in northern Chile, is considered of importance in Chilean aquaculture. The biological feasibility of cultivation of any marine species begins with the establishment of an initial broodstock population to obtain eggs, larvae, and juveniles. In this work, 22 South Pacific bonito fishing campaigns were carried out in Pisagua, Chile, between spring in November 2011 and the summer in January 2012. At least 74 specimens were obtained of which 24 survived the capture and transport processes. Fish were stocked in a recirculating land-based aquaculture system, and at 14 months under captivity, fish began spawning. Eggs were collected, to describe some stages of development, and were placed in incubators at 20 °C and on the third-day eggs hatched. Larvae reached a total length between 1.435 and 1.7 mm, which were accurately characterized during their first morphological changes. This is the first work that describes the capture, transport, and acclimatization in captivity of a breeding population of wild Pacific bonito in Chile.

Keywords: wild-caught broodstock; RAS; spawning; egg incubation; larval culture

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1. Introduction

The demand for tuna has steadily increased from 0.6 million tones in 1950 to 4 million tones in 2007, leading to the overexploitation of some tuna species, such as yellowfin *Thunnus albacares*, and albacore *Thunnus alalunga* in the Pacific south [1–3]. Consequently, the fisheries have focused on relatively underutilized tuna resources, such as skipjack tuna *Katsuwonus pelamis* [4–6]. Some small tunas, such as the bonito of the genus *Sarda*,

have also been the object of fishing, and among their species, some already show indices of overexploitation, and several researchers are calling for the establishment of fisheries management strategies [7–9].

The species *Sarda chiliensis* is a temperate epipelagic schooling fish distributed along the Pacific coast and separated by a tropical zone into two subspecies: the northern subspecies, *Sarda chiliensis lineolata* (Girard, 1858), and the southern subspecies, *Sarda chiliensis chiliensis* (Cuvier, 1832). The northern subspecies is distributed from the coast of Alaska (60°16′ N) to the south to Cabo San Lucas, at the tip of Baja California (22°20′ N) [10]. The geographic range for the southern subspecies ranges from Mancora, Peru (south of the Gulf of Guayaquil) to Talcahuano, in southern Chile.

The management (capture, transport, and conditioning) of broodstock in tanks and the development of a culture technology for this species is a decisive and critical step to improve the sustainable diversification of aquaculture. The present study reviews the technology applied to complete and close the biological cycle of the South Pacific Bonito. These studies and trials have the benefit of improving adaptation to confinement and domestication of a species with a high commercial value for human consumption. In addition, the benefits of the knowledge derived from closing its life cycle imply the improvement of cultivation techniques through the management and control of environmental and biological parameters.

Currently, little biological background, for accurate identification of life cycles [11], and the recognition of embryos and larvae in natural environments [12], types and amount of feed [13,14], reproduction [15], larval development [16], and growth rates [17] is available specifically for the *Sarda chiliensis chiliensis*. Scientific aquaculture studies on the species are rare, which is concerning because of the importance that this resource might have for the diversification of Chilean aquaculture.

South Pacific bonito has a wide distribution in northern Chile and is a candidate species for diversifying Chilean aquaculture. In aquaculture, it is relevant to determine the biological feasibility to rear a new species through the establishment of a first broodstock population to obtain eggs, larvae, and juveniles for research purposes. The two strategies practiced to establish a broodstock are from farmed fish or through the capture of juvenile or adult wild fish [18–21]. The capture of adult wild fish is complex as several reports indicate injuries caused by fishing gear, handling, and the conditions of transfer to aquaculture facilities. The consequences of an inadequate capture and transfer process will generate uncertainty and instability concerning the viability of the fish as a valuable broodstock. Depending on the degree of stress [22] to which the wild-caught adult fish were subjected, some negative consequences could be expected, such as immunosuppression, primary and secondary pathogenic diseases, gonadal atresia, and decreased egg quality, among others. Due to the sensitivity of wild adult fish, capture strategies recommend using juveniles to initiate the conditioning of future breeders of potentially interesting species for aquaculture [23].

The purpose of this research was to rear a wild-caught population of South Pacific Bonito, *Sarda chiliensis chiliensis* to establish a first breeding stock under laboratory conditions in northern Chile.

2. Materials and Methods

According to previous capture experiences, it has been observed that South Pacific bonito (Figure 1) are very sensitive to manipulation. Therefore, care was taken to maintain minimal contact with the fish skin during all steps involved in the capture and transfer to the land-based RAS facility. The RAS consisted of passing the culture water through a closed circuit, in which it was subjected to filtering and disinfection treatment. This water processing was carried out continuously to eliminate built-up contamination coming from fish waste, food remains, and others. It should be noted that only one experience of ova and larvae culture, corresponding to one spawning, was carried out in the present research

for rearing feasibility purposes. For other observed spawnings, the date of spawning, the amount of egg, and the size of eggs were recorded.

Figure 1. Specimen of South Pacific Bonito (*Sarda chiliensis chiliensis*).

2.1. Catch and Transportation of South Pacific Bonito

A total of 22 fishing campaigns were conducted for South Pacific bonito during the Chilean late spring and summer months, between November 2011 and January 2012. The specimens were caught in the sector of Pisagua in northern Chile (19°36′22.57″ S, 70°12′09.96″ W). A fishing gear called "chispa", which consists of a fishing line with a barbless hook and a lure, was dragged by a boat at speeds of 2 to 3 knots [24]. Once the fish had taken the hook, it was brought on board and transferred untouched into a 1 m^3 black fiberglass transport tank. This tank was supplied with continuously flowing oxygenated seawater using a 0.5-hp pump (PedrolloTM) and a 9-m^3 compressed oxygen gas cylinder that diffused the gas through a ceramic diffuser (Figure 2). Fish larger than 1 kg and smaller than 1 kg were differentiated at the time of their capture, weighing them in a container with water and using a digital balance V-1026 (MoccoTM). For each campaign, 4 or 5 specimens were captured, which were kept in the transport tank on board the vessel at a density that would allow for adequate survival as suggested for *Thunnus albacares* [24]. Sea transport time fluctuated between 1 and 3 h per campaign, depending on the distance from the coast to the capture site and how fast specimens could be captured. The fishes were transferred from the transport tank, located on the vessel board, to another transport tank mounted on a vehicle using trays with black lids to maintain minimal contact with the fish skin. It is worth mentioning that the vehicle-mounted tank had the same design features as the one onboard the vessel. During the fish transport, both the temperature and dissolved oxygen were monitored every one hour using a model YSI 55 oxygen meter. The transport time from land to the culture unit in the La Capilla sector was 5 h.

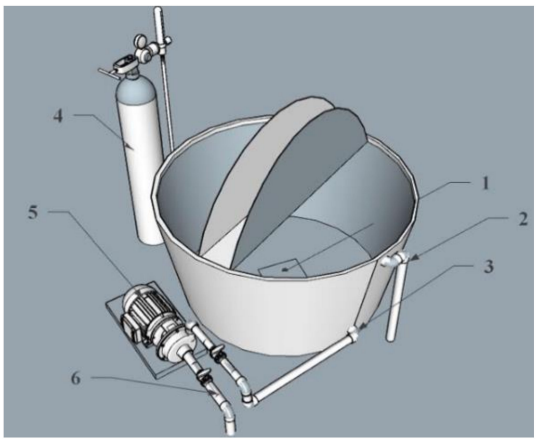

Figure 2. Scheme of the fish transport tank that was installed onboard the fishing vessel. 1. Oxygen outlet; 2. Water outlet from the tank; 3. Water inlet to the tank; 4. oxygen tank; 5. Pump; 6. seawater intake pipe.

2.2. Land-Based Recirculating Aquacultural System (RAS) for Rearing of South Pacific Bonito

A land-based recirculating aquaculture system (RAS) was used for rearing a South Pacific bonito broodstock founding population from a captured wild stock (Figure 3). Seawater was continuously recirculated with two 1.5-hp pumps (ReggioTM, model SM150) and treated physically for solids removal and biologically for ammonia removal before re- into the rearing tank. Recirculated seawater was treated for the removal of suspended solids in a linear sequence with a fiberglass settling tank (5000 L), a Hayword sand filter (Blupools, model S360T2), and an Azud ring filter (Azud S.A., modular 100 model). The removal treatment of the culture water of the ammonia excreted by the fishes was carried out using a biofilter (5000 L) containing 3 m^3 of biomedia (Figure 3). The seawater was aerated within the rearing tank with three diffuser stones (SweetwaterTM, model AS23L) 25 cm long.

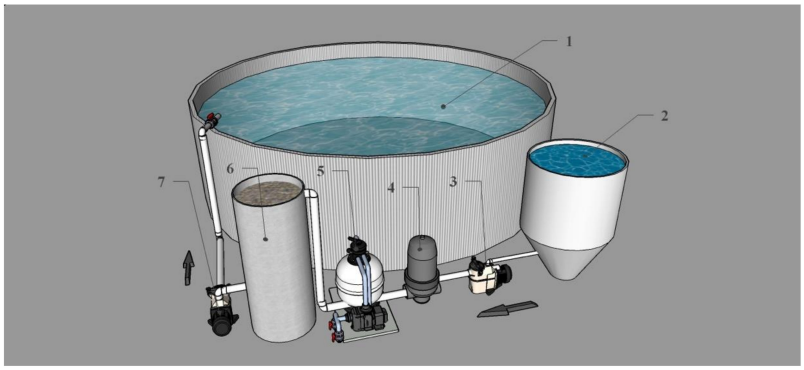

Figure 3. Schematic of the land-based RAS for conditioning South Pacific bonito broodstock: 1 Broodstock rearing tank (75 m^3); 2 Make-up seawater storage tank (5 m^3); 3 1.5-hp pump for RAS make-up water; 4 Sand filter; 5 Ring filter; 6 Biofiltration tank (5 m^3); 7 1.5-hp pump for seawater recirculation.

A 75-m^3 cylindrical metallic tank made of steel sheets, corrugated and hot-dip galvanized, and joined with high strength bolts, mounted on a cement floor was used as a rearing tank. The joints were sealed with a flexible asphalt tape that was cured at room temperature. A 1 mm thick plastic liner cover (ASTM D751) was used to contain the seawater in the culture tank. The rearing tank had a diameter of 7.4 m and a water column of 1.76 m. The rearing tank was covered with black mesh to provide 80% shade. The RAS was operated with natural photo and thermo-periods.

2.3. Reception and Adaptation to Captive Rearing of South Pacific Bonito

The 75 m^3 rearing tank was stocked, seven months before South Pacific bonito's arrival, with four specimens of yellowtail kingfish (YTK) *Seriola lalandi*. The YTKs were obtained from a local farming facility and had an average weight of 800 g. The YTK were used first to provide ammonia for the biofilter start-up and then to facilitate the process of adaptation to the captivity of the South Pacific bonito.

Sarda chiliensis chiliensis were continuously observed during the first 24 h after being sown in the breeding tank. In this way, it was possible to record survival after capture from the wild and detect any change in their general external health during their initial adaptation to captive conditions. This procedure was carried out for each of the fishing campaigns. The fish's records were made visually because it is not advisable to have physical contact with the animals due to their sensitivity. All dead fish were immediately removed, from the rearing tank, during the initial fish adaptation process to the aquaculture facilities.

Fish were fed once a day between 400 to 500 g of a fresh diet consisting of sea silverside *Odontesthes regia* and between 300 and 400 g twice a day of a dry formulated commercial feed for marine fish breeders (Skretting, NOVA ME 2000, and protein percentage 52%). Fresh and dry feeds were offered to South Pacific bonito from Monday to Saturday as recommended for other scombrids [24–27]. A formalin bath, with a concentration of 1:6000, was carried out in February 2012 for each one of the wild-caught fish to eliminate external parasites, particularly those that could be lodged in the gills.

2.4. RAS Water Quality Monitoring

The dissolved oxygen and temperature levels were recorded three times a day using a YSI model 55 oxygen-meter, both in the rearing tank and at the makeup seawater. Ammonium, nitrite, nitrate, and pH were measured in the rearing tank twice a day using a Hanna table spectrophotometer (model HI-83225).

3. Results

3.1. Catch and Transportation of South Pacific Bonito

A total of 74 South Pacific bonito were caught across 22 campaigns between November 2011 and January 2012 and of which 50 potential broodstock fish did not survive under the conditions used during their tank transport on the vessel. The injuries caused by the catch and the transfer of the selected fish were minimal. Consequently, no treatment with a therapeutic solution was needed to prevent a possible increase in mortalities by management.

The fish survival range for the overall 22 campaigns was between 0% and 100%. There was no correlation between the number of fish transported in the tank (from 1 up to 8 fish/m^3) and the fish survival. For instance, transport densities of 1 fish/m^3 resulted in survival rates from 0% up to 100%; 2 fish/m^3 with survivals between 0% to 60%; 3 fish/m^3 between 33.3% up to 66.7%; 4 fish/m^3 between 25% and 75%; 5 fish/m^3 between 20% and 60%; of 5 fish/m^3 between 20% and 60%; of 6 fish/m^3 between 0% and 33.3% (Table 1). A total of 42% of the fish caught were discarded immediately because of the physical damage caused by the fishing gear, mainly considerable injuries to the mouth and gills.

The survivals of the captured fish increased as seasonality transited from late spring to summer. Survival rates for the November, December, and January campaigns were 18.1 ± 14.3%, 29.5 ± 15.3%, and 75.4 ± 17.5%, respectively (Table 1). During the South Pacific bonito fishing campaigns, the seawater temperature ranged between 17.1 and 18.2 °C.

The fish transport density in a vehicle from the dock to the land-based RAS for the cultivation and conditioning of breeding stock did not exceed 4 to 5 fish per tank. The fish transport from the disembarkation dock to the land-based recirculating aquacultural system (La Capilla, Arica) lasted approximately 5 h. There were no mortalities during this transport.

3.2. Reception and Adaptation of South Pacific Bonito

Live *Sarda chiliensis chiliensis* that were transferred into the rearing tank were not weighed to minimize handling stress and to cause unnecessary injuries. By the fourth week, it was observed that the first groups of stocked South Pacific bonito began accepting the commercially formulated feed. Fresh and dry daily feed accounted for up to 5.4% of their body weight, assuming an estimated average weight of 1 kg for the 24 stocked fish by the end of the fisheries campaign.

Forty-five days after the last fishing campaign, only two fish (8.3%) died within the broodstock rearing tank. At the end of the 13th month in captivity and one month before the first natural spawning, the accumulated mortality was 11 individuals (45.8%) (Table 2). All 11 dead fish had a compressed abdominal section, and post mortem examination of the fish stomach area showed an empty stomach.

Table 1. Weight of wild-caught fish, transport density, and survival of South Pacific bonito in Pisagua.

Capture Date	Number of Fishing Campaign	Total Fish Caught	Ocean Temperature °C	Arrival Temperature °C	Weight of Fish Caught		Fish Density in Vessel Transport Tank Fish/m^3	Mortality in Capture Tank	Fish Placed into Conditioning Tank		Survival (%)
					Fish > 1 kg	Fish < 1 kg			Fish > 1 kg	Fish < 1 kg	
November 2011	1	6	17.1	21.2	4	2	6	6	0	0	0.0
	2	0	17.1	-	-	-	-	-	-	-	-
	3	4	17.3	21.0	2	2	4	3	0	1	25.0
	4	2	17.2	21.2	2	0	2	2	0	0	-
	5	3	17.4	21.3	1	2	3	2	0	1	33.3
	6	4	17.4	21.4	2	2	4	3	0	1	25.0
	7	4	17.6	21.4	2	2	4	3	0	1	25.0
December 2011	8	4	17.5	21.4	2	2	4	3	0	1	25.0
	9	5	17.5	21.3	4	1	5	4	0	1	20.0
	10	2	17.6	21.4	0	2	2	1	0	1	50.0
	11	3	17.8	21.3	1	2	3	2	0	1	33.3
	12	6	17.7	21.4	3	3	6	4	0	2	33.3
	13	8	17.7	21.2	5	3	8	6	0	2	25.0
	14	7	17.9	21.3	3	4	7	5	0	2	28.6
	15	1	17.8	21.1	1	0	1	1	0	0	0.0
	16	2	17.8	21.4	0	2	2	1	0	1	50.0
	17	0	17.8								
January 2012	18	3	18.0	21.2	1	2	3	1	0	2	66.7
	19	0	17.9	-	-	-	-	-	-	-	-
	20	4	18.2	21.3	1	3	4	1	0	3	75.0
	21	5	18.1	21.2	2	3	5	2	0	3	60.0
	22	1	18.1	21.2	0	1	1	0	0	1	100.0

Table 2. Data show the successful campaigns fisheries of South Pacific bonito capture and then stocked in the rearing tank. Cumulative wild captured, stocked fish, fish mortality, and fish population in the rearing tank, during the conditioning and spawning period are shown. Wild-caught South Pacific bonito were fed once daily in the morning with a fresh diet, and twice in the afternoon with a commercial diet. Feeding was offered as soon as the captured fish were stocked in the rearing tank, and continued during the conditioning and spawning period.

		Fish Captured per Month												Conditioning Period												Spawning	
Diet Type	**Number of Fisheries Campaign (See Table 1 for Corresponding Dates)**	**November 2010 Later Spring**				**December 2010 Summer**				**January 2011 Summer**				**February to December 2012**												**January and February 2013**	
		wk1	**wk2**	**wk3**	**wk4**	**wk1**	**wk2**	**wk3**	**wk4**	**wk1**	**wk2**	**wk3**	**wk4**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**
Fresh diet once daily																										
	3	1																								
	5		1																							
	6			1																						
	7				1																					
	8 and 9					2																				
Dry diet twice daily	10						1																			
	11 and 12							3																		
	13, 14, and 16								5																	
	17 and 18									2																
	19 and 20										3															
	21											3														
	22												1													
	Cumulative wild stocked fish	1	2	3	4	6	7	10	15	17	20	23	24	0	0	0	0	0	0	0	0	0	0	0	0	0
	Fish mortality in the rearing tank	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	4	3	1	2	1	0	0	0	0
	Fish population in the rearing tank	1	2	3	4	6	7	10	15	17	20	23	24	24	22	22	22	18	15	14	12	11	11	11	11	11

3.3. RAS Water Quality Monitoring

The average temperature and average dissolved oxygen conditions of the water in the rearing tank between January 2012 and December 2012 were 18.80 °C (SD 2.306) and 6.10 mg L^{-1} (SD 0.361) (Figure 4).

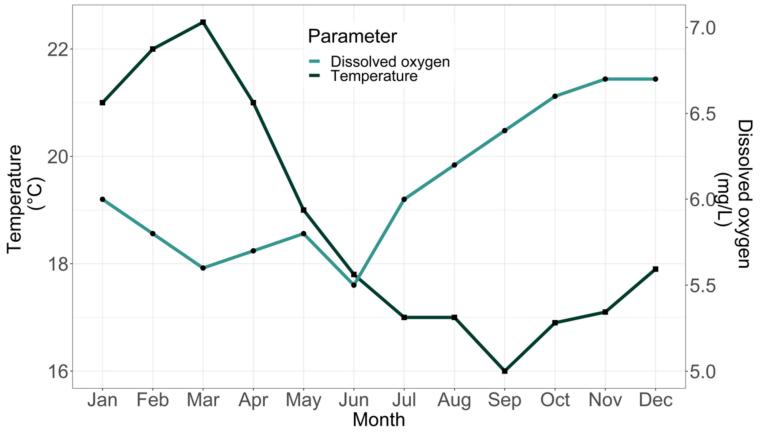

Figure 4. Monthly seawater temperature (°C) and dissolved oxygen concentration (mg L^{-1}) in the rearing broodstock tank.

The means of ammonium, nitrite, nitrate, and pH in the rearing pond between January 2012 and December 2012 were 0.025 mg L^{-1} (SD 0.996); 0.018 mg L^{-1} (SD 0.888); 35 mg L^{-1} (SD 0.326), and 6.8.

3.4. South Pacific Bonito Spawning between January and March 2013

Pacific bonito reached sexual maturity almost 12 months after the last fishing campaign and began to spawn spontaneously in early January 2013 (summer in the southern hemisphere). The spawning lasted until early march of the same year, and at least three weekly spawnings were recorded. The largest number of eggs observed during the spawning period was in February 2013.

According to the history of egg collection, spawning occurred during the course of the morning, which is supported by the fact that the eggs collected, and analyzed between 14 and 16 h, were in states of four blastomeres (110 to 120 min post-fertilization, MPF) to advanced morula (300 to 320 MPF). For all the eggs measured between the neurological and metameric states, it was observed that the vertical diameter of the egg (DHV) with 1.469 ± 0.016 mm was less than the horizontal diameter of the egg (DHH) with 1.622 ± 0.018 mm.

4. Discussion

The control of the reproduction of various species of fish has been a definitive step towards achieving production on a commercial scale and the development of fish farming [24,26]. Most of these species were captured to resolve biological feasibility issues for their cultivation in captivity [28]. As one of the most interesting stages of life is that related to the conditioning of potential broodstock animals that can be subjected to the techniques of reproductive control as the first step of domestication, as in the case applied in this research for South Pacific bonito. This study reports a successful attempt to capture and transport wild *Sarda chiliensis chiliensis* to a rearing land-based RAS research facility where they were maintained and eventually spawned naturally.

4.1. Capture and Transportation of South Pacific Bonito

The transport of live wild fish is a critical step towards establishing a captive brood fish population, and it is quite difficult to handle fish that have not yet reached their reproductive stage. The acquisition of breeding populations of South Pacific bonito from the natural environment requires special care, which is not usually required for the other development stages, such as the capture of juveniles reported by Flores and Rendíc, [28]. To establish a broodstock population, the native marine fish capture strategy should be designed in such a way that minimal animal handling is required, ensuring the well-being of the specimens caught in their new growing environment [29]. In particular, Scombrids fish must swim constantly since they present negative buoyancy and ram-type ventilation [26]. Capture and transport protocols for the Atlantic bonito *Sarda sarda*, which has been described as a notoriously difficult fish to transport due to its limitations against nitrogen accumulation and dissolved oxygen depletion in the water, have been reported for up to 25 h by road and air [30]. In the case of the South Pacific bonito, its capture was made by fishing them with hooks without barbs to minimize damage to the mouth and gills, as described for *Thunnus albacares* [24], *Euthynnus affinis*, and *Cybiosarda elegans* [26]. This fishing protocol could result in less handling stress and thus positively affect the extension of time observed for acclimatization and conditioning under captive conditions.

Water temperature is an important factor regarding the transport of live fish, and low temperatures are generally suggested to decrease fish metabolism and stress [31]. It was not possible in the 22 fisheries campaigns to control the water temperature in the fish transport tank since the protocol used required a constant flow of fresh seawater. Even though Pacific bonito survival seemed not to be affected by the temperature during their transport. It was observed that overall catches survivability increased as catches progressed from late spring towards summer, with a survival range between 66.7% and 100% for the last catch period on January 2012 compared with catches on November 2011 (average 18.1 $\pm$ 14.3%) and December 2011 (average 29.5 $\pm$ 15.3%) (Table 1). It was assumed that the increase in the survival of the fish as more catches were made was due to an increasingly better practice of the protocols associated with their handling, managing, and transportation.

Captures of South Pacific bonito in this study between November 2011 and January 2012 show the importance of fish size and transport density in ensuring better survival during transport from the sea to the rearing tank. It was observed that in those catches where the fish exceeded 1 Kg, none of the animals survived during their transport on the boat (Table 1). The lack of survival in fish weighing more than 1 kg, could be attributed to the stress experienced by the fish at the time of capture, as well as the handling and transportation procedures. In contrast, fish smaller than 1 kg were more docile during capture and handling, which resulted in better survival at the end of their transportation and maybe ensured a better adaptation to the captive culture conditions. Similarly, Wexler et al. [24] recommended capturing wild fish smaller than 1 kg and a lower transportation density, which improved survival for *Thunnus albacares*. Bar et al. [26] also reported that *E. affinis* and *C. elegans* weighing less than 1 kg survive the transport, whereas larger fish did not survive. It has been described that physiological disturbances occur in fish during capture, transport, and handling, which reflect some degrees of stress, like (a) primary blood changes (i.e., increased blood levels of ACTH, catecholamine, and cortico-steroids); (b) secondary physiological changes (i.e., changes in oxygen consumption rate, ammonia, and carbon dioxide excretion), and (c) tertiary changes affecting production indexes (i.e., growth rates, survivability) [32]. Meka and McCormick [33] reported that wild specimens of rainbow trout (*Oncorhynchus mykiss*) showed high levels of lactate and cortisol in the plasma, which are recognized indicators of stress after handling and transport. Huax & Sjöbeck [32] found that physiological parameters affected by the capture of wild *Perca fluviatilis* were recovered and stabilized within two to four days after capture. Burke et al. [34] tried unsuccessfully to determine the causes of mortality after 2 or 3 days of capture and delayed mortality up to 20 days in captivity of wild captured *Katsuwonus pelamis*. Davis [35] reported that effects due to capture and handling could lead to reduced growth and delayed mortality

and recommended a direct approach of stress conditions was to measure reflex responses after physical stimulation in free-swimming fish. Stressors may be acute (short-term) or also chronic (long-term), and their strength can range from mild to severe, which can be gauged by the induced stress response and its outcomes [36].

Improvements to the catch and transport protocols should be implemented to minimize exposure to stress and increase the survival of wild fish. Suggested methods to reduce stress include: (a) to induce a metabolism reduction by lowering the transport water temperature a few degrees in comparison to the temperature that is registered in the oceanic water for open-water transport broodstock tanks [37]; (b) to apply anesthetics or an injection with a tranquilizing solution [38]; (c) to transport fish under low density or load mass [26]; (d) to design appropriate transport devices for pelagic ram ventilatory fish [26]. In particular, when using wild-caught fish as a broodstock base, it will be necessary to consider all the above recommendations along with the appropriate size of the transport tank, its relationship to the size of the fish, and the water quality requirements during transport [24]. Even though the fish transportation density plays a determining role in fish survivability, that condition itself cannot explain the registered mortalities suffered by *Sarda chiliensis chiliensis* larger than 1 kg. Nevertheless, our transportation density was higher than that reported by Ortega and de la Gándara [39]. During fish transportation, the likelihood of a collision among the fishes could increase significantly, especially when they became disoriented and began to swim against the other fish. Erratic swimming of captured fish had already been reported for *Sarda sarda*, and a way to avoid this problem could be the use of the pipe transport method described by Bar et al. [26].

4.2. Reception and Adaptation of South Pacific Bonito

It is not clear in this research whether or not the presence of yellowtail kingfish *Seriola lalandi*, a shoal fish already adapted to the land-based RAS, facilitated the adaptation to the rearing tank and the feeding learning process of the South Pacific bonito. However, it has been already reported that the presence of domesticated shoal fishes provides a better adaptation of wild fish to rearing conditions and an increase in the foraging efficiency, which has been discussed elsewhere [40–43]. To avoid starvation of wild captured fish and to assure success in their adaptation to the rearing facility, it has been suggested to feed them with fresh dead fish [26] or even live foods [24]. In this research, the Pacific bonitos were fed to an approximate 5.4% of their body mass per day, with a fresh and a dry diet, and it was observed that they began to feed on the commercial diet for marine fish broodstocks (Skretting brand, NOVA ME 2000) approximately four weeks after their introduction to the rearing tank. Yazawa et al. [27] were feeding Eastern little tuna *Euthynnus affinis* with defrosted feed at 5% to 10% of their body weight per day, with fourteen of the 32 initial fish surviving the captivity conditions after one year of rearing in a 70 m^3, with most mortalities attributed to fish colliding with the tank walls. The daily feed offered to South Pacific bonito in our research was less than the 10% feeding rate offered to condition wild mackerel tuna *Euthynnus affinis* and leaping bonito *Cybiosarda elegans*. Bar et al. [26] reported early mortalities for those fish that refused to feed. In our case, we did not observe initial mortalities when conditioning Pacific bonito, which could imply that our fish were properly fed.

Between November 2011 and February 2012, there were no mortalities in the fish stocked in the rearing tank. However, in the year 2012, after about 45 days of the last fishing campaign, a couple of fish died in march and ten more between June and October (Table 2). The late mortalities recorded allow inferring that all fish that survived transport did not present late mortality and adapted adequately to the culture conditions [12]. The causes of the mortalities were unknown, however, one common feature among the dead fish was the compression of the abdominal section. Right after a fish post mortem examination, it was observed that the stomachs of the fish were empty. The mortality of the breeding stock observed during conditioning was within normal ranges and consistent with that reported for *Graus nigra* [23] and *Dissostichus eleginoides* [44]. The latter authors reported that the

highest mortalities for *Dissostichus eleginoides* in the conditioning period occurred in the first rearing months, which was not the case observed here for South Pacific bonito and requires further research to explain it. Bar et al. [26] also reported mortalities of leaping bonito and mackerel tuna after being reared for 6 and 11 months, respectively, and the causes of their mortalities were unknown.

To our knowledge, the mortality of wild conditioned broodstock of *Sarda chiliensis chiliensis* in captivity, observed in our research, would constitute the first report for this novel aquaculture species. In this context, to attribute this reported mortality to the conditioning of bonitos in captivity deserves several hypotheses: first, that although all the fish stocked into the rearing tank were weighing less than one kilogram per fish, as the months of conditioning passed some of these fish made a difference in size which allowed a possible hierarchy of almost 50% of the fish, with favored to more aggressive fish to successfully obtain its feed; second hypothesis that could explain the mortality of the fish would be the that they are visual for fed capture since the water of the rearing tank gradually increased its turbidity, mainly with microalgae, which could have prevented a clear visibility of the fish of the offered feed; a third hypothesis to explain such mortality, is the point of no return in adult fish, perhaps due to the non-feeding of some fish that are the consequence of the hierarchy and/or the water turbidity, which would considerable affect their energy requirements to grow, mature sexually and adapt to captivity; a fourth hypothesis, could be a long term chronic level of stress that the fish could have had in the rearing tank due to endogenous and exogenous conditions, which could present physiological changes caused by confinement, starvation, lighting, and rearing tank size, as reported by Aiyelari et al. [45] for *Clarias gariepinus*. Wang et al. [46] reported a high concentration of cortisol and low concentration of lysozyme for *Perca fluviatilis*, caused by possible adverse conditions present in the rearing tank that produces stress in the fish. It is also worth proposing that post-spawning mortality occurs in several other fish species such as *Perca fluviatilis* [47] and *Psetta maxima* [48], although in our research, spontaneous spawning occurred three months after the last registered mortality.

Ortega and de la Gándara [39] suggested that wild fish taken into captivity must be conditioned as fast as possible to respond early to feed, primarily with fresh food, which will lead the specimens to recover more quickly from the stress of the catch and sharply improve their external appearance. The supply of primarily fresh or frozen diets has generally occurred for the conditioning of wild-caught breeding stock as described by Silva and Oliva [19] for breeding stock of *Paralichthys adspersus* and Muñoz et al. [23] for *Graus nigra*. In our case, fresh diets were provided to South Pacific bonito as a complement to a dry formulated feed, which was appropriate for the species as the fish completed maturation and natural spawning in the rearing tank. The fish showed an adequate adaptation to the feeding regime based on fresh sea bream (*Odontesthes regia*) supplemented with a dry broodstock formulated feed (brand Skretting, NOVA ME 2000). The diet during the conditioning period of the broodstock was most likely adequate as the abundant spawning indicated that the completion of gonadal maturation and the production of good quality eggs.

4.3. South Pacific Bonito Spawning between January and March 2013

Sarda chiliensis lineolata has been described as a rapidly growing species able to reach 51 cm fork length and up to 1.8 Kg in the first year [17]. Males can mature and spawn at 1 year old, and a few females will spawn at 2 years old, but most will do at 3 years with 69 cm fork length [17]. Most surviving *Sarda chiliensis chiliensis* in this research were less than 1 kg at the time of their stock at the rearing tank and could be within their first year of life. Consequently, most of our fish might have been reaching their second year of life while being conditioned in the rearing tank, which might explain the natural spawning observed at the beginning of the year 2013, as it was reported for *Sarda sarda* [49].

The surviving *Sarda chiliensis chiliensis* grew and matured in the rearing tank, and the latter was evidenced by spontaneous spawning that began in January 2013 in captivity.

At the beginning of the spawning period, there were 11 Pacific bonito in the rearing tank, which means a 54% survival rate after one year in captivity, which is similar to the reported survival for *Sarda sarda* [39]. The eggs analyzed in the different spawns released naturally by the *Sarda chiliensis chiliensis* specimens maintained dissimilar characteristics with *Sarda sarda* (e.g., egg diameter, number of oily drops from the egg, and hatching).

It seems that the 75 m^3 land-based RAS tank size and the general rearing conditions were appropriate for conditioning *Sarda chiliensis chiliensis* as it was for Eastern little tuna *Euthynnus affinis* in a 70 m^3 land-based open flow rearing tank [27]. However, in our case, the fish had multiple and continuous natural spawning events without the need to administrate GnRHa to induce a spawning as it was required for *E. affinis*.

4.4. Present and Future of the South Pacific Bonito in Chilean Aquaculture

Scombids are a pelagic fish family with high aquaculture potential due to their rapid growth and high commercial value. These fish are the subject of major fisheries all over the world. The majority of research efforts have been focused on growing species of the genus *Thunnus*, primarily bluefin tuna. Other species in the family, however, should be considered. Under conditions controlled in captivity, the Pacific bonito, similar to the Atlantic bonito, is a species with a rapid growth reaching up to 1.8 Kg during the first year of life, and males sexually mature at 1 year of life and females at 3 years [17]. The Pacific bonito is a gonochoric species with an asynchronous development of the gonad, and sexes cannot be distinguished using external anatomy [17].

Fishing for wild South Pacific bonito (capture and transport) to establish a suitable broodstock population to develop a rearing technology will constitute the first step to incorporate this novel species towards the diversification and sustainability of Chilean marine aquaculture. This species was able to adapt to the farming conditions in a land-based RAS and started breeding in captivity a year after their capture from the wild. In addition, several spontaneous spawning occurred during the whole summer season [50,51]. These studies and trials have the benefit of improved adaptation to confinement and the domestication of a species with a high commercial value for human consumption and whose life cycle is likely to be fully managed in captivity. Besides, the knowledge benefits derived from closing their life cycle provide the possibility of establishing spawning broodstocks to generate larvae, which will involve the development of cultivation protocols to define methods of species management and requirements for the control of environmental and biological parameters in captivity.

The establishment of this first stock of South Pacific bonito allows other breeders to investigate the biological feasibility of using it as a surrogate species. The South Pacific bonito is a species that is phylogenetically close to the bluefin tuna but with smaller body size and a briefer generation time. The technology to cultivate Pacific bonito should be based on the already widely applied commercial cultures of tuna and marine fish worldwide. In the case of South Pacific bonito, one should expect to be able to adapt the technology used in the cultivation of yellowtail kingfish (*Seriola lalandi*), which is already commercially available near Caldera city in Chile.

5. Conclusions

- An initial population of *Sarda chiliensis chiliensis* was established from a wild-caught stock, an important step in improving the diversification and sustainability of Chilean aquaculture.
- All the fish caught and transported were specimens weighing less than one kilogram, which allowed a survival rate of over 63%.
- The wild broodstock was conditioned in a 75 m^3 rearing tank under a seawater recirculation system, which allowed the first spawning of *Sarda chiliensis chiliensis* in Chile.
- A small population of pelagic fish, *Seriola lalandi*, was used for the process of adaptation to captivity and food learning of *Sarda chiliensis chiliensis*.

- A protocol was established for the capture of potential wild *Sarda chiliensis chiliensis* broodstock and their transport for 5 h to a recirculating aquaculture system on land.

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Institutional Review Board Statement: The Universidad de Tarapacá Comité Ético Científico stated that all ethical and biosafety conditions are observed in its work with laboratory animals, the spawning of the cultured fish indicates that their maintenance in the culture pond was successful, without stress or environmental mistreatment since in the environment they were in they were similar to their natural environment.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available for privacy reasons.

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CAPÍTULO 3:

Desove y Desarrollo embrionario de Peces Marinos

Introducción

Se ha realizado un gran esfuerzo en acondicionar y mantener reproductores en cautiverio con vistas a producir la mejor calidad y cantidad de huevos, aunque todavía falta una estandarización de métodos de varias especies, que permitan asegurar determinadas cantidades de huevos de calidad (Pepe-Victoriano *et al*., 2021a) De todas formas, parece que la fase de larva en algunas especies es la que más problemas tiene, y aunque se han realizado pruebas larvales y de producción de juveniles en varias especies, éstas requieren de continuidad y repetición, así como investigaciones encaminadas a superar la baja supervivencia larval y/o el lento crecimiento de los juveniles.

Desove de Reproductores

Ortega & de la Gándara (2007b) acondicionaron ejemplares adultos de bonito para obtener desoves. Durante la primera estación reproductora no se obtuvieron puestas, sin embargo al llegar la segunda estación reproductora, los ejemplares mostraron el comportamiento típico del cortejo sexual. Los ejemplares presentaron un peso promedio de 3 kg y la densidad era de alrededor de 1 kg/m^3. Los ejemplares mantenidos en los estanques realizaron 3 puestas (cada 3 días) y se obtuvo un total de 301.000 huevos. Estos autores también capturaron bonitos del medio y recién pescados, se procedió a extraerles los gametos por masaje abdominal, procediendo a continuación a fecundarlos. La mayoría de los machos expulsó esperma tras una ligera presión abdominal, mientras que de las hembras exploradas, menos del 20% produjo óvulos viables. El número total de huevos fecundados obtenidos por esta vía fue de 127.000 (6 hembras).

Características de los huevos

El desarrollo embrionario y larvario de la especie *Sarda chiliensis chiliensis* fueron descritos por McFarlane *et al*., (2000). Estos autores lograron obtener huevos fecundados de ejemplares mantenidos en cautiverio, usando una red de plancton

de 2 m y de 800µm de malla. El diámetro de los huevos colectados fue de 1,58 ± 0,05 mm y presentaron sobre un 95% de huevos fertilizados.

Los huevos fertilizados se mantuvieron a una densidad de 50 – 100 por litro en estanques cilíndricos negros de 380 L. La entrada de agua fue a través de una barra horizontal spray en la superficie del estanque y la salida del agua fue a través de un drenaje central en el fondo del estanque. El sistema utilizado fue semi-abierto y el agua filtrada se adicionó al reservorio a una tasa de 2,5 L/min. Los estanques fueron alumbrados con lámparas fluorescentes de 40 W, con un fotoperiodo de 12L:12º, pero con luz indirecta de la sala (24 h). Los estanques recibieron aireación continua desde una línea de aire central, sin difusor y la temperatura se mantuvo entre los 22,1 ± 0,9°C y los 26,1 ± 0,2°C.

Durante el desarrollo larval McFarlane *et al*., (2000) aplicaron la técnica del agua verde, donde se usó una combinación de fitoplancton (*Nannochloropsis*, *Chaetoceros* e *Isochrysis* spp) y pasta algal criopreservada para mantener un ambiente turbio en los estanques. Inmediatamente después de la incubación, se adicionaron copépodos (*Euterpina actifrons*) y rotíferos (*Brachionus plicatis*). Nauplius de Artemia, los bonitos recién eclosionados y larvas de serrano (*Fundulus grandis*) fueron presentados subsecuentemente en forma solapada como aliemento vivo.

Los juveniles de bonito se transfirieron a un estanque de engorde de 4 m de diámetro y 0,5 m de profundidad. El sistema de circulación fue abierto, con agua de mar filtrada adicionada a una tasa de 10 L/min. Se utilizaron bombas sumergibles para producir una corriente circular fuerte en el estanque y la temperatura se mantuvo a 22 ± 1°C.

McFarlane *et al*., (2000) describieron 5 estados de desarrollo larvario. El estado larvario que alcanza al día 12 de cultivo (L4-L5) presenta un marcado canibalismo asociado a un incremento en la talla y de la actividad natatoria. Los juveniles se obtienen al día 29 de cultivo, ya han adquirido las caracteristicas de los adultos y frecuentemente exiben un comportamiento de cardumen, en estos ejemplares el canibalismo desaparece.

El desarrollo embrionario y larvario de *Sarda sarda* ha sido descrito por Ortega y de la Gándara (2007b). Estos autores obtuvieron huevos fecundados desde ejemplares de dos años de vida y mantenidos en cautiverio, con una tasa de fecundación del 16,0 ± 2,94%. Los huevos tenían un diámetro de 1.048 ± 103 µm y un peso seco de 122,5 ± 3 µg. Estos autores también obtuvieron huevos fecundados desde ejemplares recién capturados. Los huevos presentaron un diámetro de 1.118,1 ± 49,21 µm y un peso seco de 80 ± 1 µg.

El desarrollo embrionario a 20°C es rápido. Las larvas comienzan a eclosionar a las 48 h post fecundación, completándose el 100% de los huevos unas 6 horas después. La tasa de eclosión obtenida fue del 60%.

La secuencia de alimentación entregada por Ortega & de la Gándara (2007b) se muestra en la Tabla 2.

Tabla 2: Secuencia alimentaria entregada durante el desarrollo larva de *S. sarda*.

Alimento	Día de Cultivo
Rotífero	2-12
Nauplius de Artemia	8-25
Larvas de denton recien eclosionadas	15-20
Artemia congelada y Pescado congelado	20-30
Pienso seco	día 25 en adelante

Artículo 2

First natural spawning of wild-caught premature south pacific bonito (*Sarda chiliensis chiliensis*, Cuvier 1832) conditioned in recirculating aquaculture system and a descriptive characterization of their eggs embryonic development.

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First natural spawning of wild-caught premature south pacific bonito (*Sarda chiliensis chiliensis*, Cuvier 1832) conditioned in recirculating aquaculture system and a descriptive characterization of their eggs embryonic development

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ABSTRACT

This investigation revealed the first biological bases of embryonic development until the hatching of South Pacific bonito *Sarda chiliensis chiliensis* eggs. The characteristics and stages of embryonic development were described until the hatching stage. The eggs were obtained by natural spawning from wild-caught broodstocks that were conditioned to captivity for more than 1 year in a 75 m^3 marine land-based aquaculture recirculating system in Chile. The water temperature range in the broodstock RAS, during the natural spawns events, was between 18.6 °C and 19.8 °C in December 2012, between 20.4 °C and 21.2 °C in January 2013, between 21.5 °C and 22.4 °C in February 2013, and between 21.90 °C and 22.87 °C in march. Incubators' water temperature was kept similar, through daily water exchanges, to the temperature recorded at the broodstock tank to lessen thermal stress which could affect the embryonic development. Thirty-one embryonic stages were characterized in 71.83 h until eggs hatching. Five periods of embryonic were distinguished: morula, blastula, gastrula, neurula, and metamery. Translucent telolecitic eggs hatched on the third day of incubation, and which was comparatively longer than reported for Atlantic bonito *Sarda sarda* and the eastern Pacific bonito *Sarda chiliensis lineolata*. The morphometric data with the most significant variability between the stages of embryonic development were: the length and height of the head, and the length and height of the eye. These are the first embryonic development studies conducted with eggs naturally spawned from wild-caught *Sarda chiliensis chiliensis*.

1. Introduction

South Pacific bonito *Sarda chiliensis chiliensis* is a marine finfish, commonly referred as "small tunas", with high commercial value at international markets (Collette and Nauen, 1983; Baibbat et al., 2019), in part due to worldwide overfishing on species such as yellowfin tuna *Thunnus albacares*, albacore *T. Alalunga*, and longtail tuna *Thunnus tonggol* (Yoshida, 1980; Yokoi et al., 2019; Griffiths Shane et al., 2020). However, South Pacific bonito's fisheries are following the same wild stocks declination trend, and despite the years of fishing exploitation, its antecedents related to its early development embrionary stages are scarce enough to be able to establish management plans for the species in its natural environment or to generate background information for its rearing in aquaculture farming systems.

It is known that success in aquaculture production for any given species involves knowledge of the morphophysiological and behavioral characteristics of the species in cultivation, being relevant the study between the embryonic development and early juvenile stages (Gisbert et al., 2002, 2004; Botta et al., 2010; Azfar Ismail et al., 2019; Syafiq et al., 2020; Yoshinori et al., 2020). The description of embryonic

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development in fish allows numerous advantages, like tools to correctly manage aquaculture and fishery resources (Conklin et al., 2004; Pepe--Victoriano et al., 2012; Marancik et al., 2020), for accurate identification of life cycles (Oka et al., 2020), and for the recognition of embryos and larvae in natural environments (Davis Aaron et al., 2020). All of the above will allow a better evaluation of the spawning environmental conditions of the brood fish (Pepe-Victoriano et al., 2013) as well as to artificially generate environments that may lead to a healthy rearing of larvae, and consequently to juveniles farming with excellent production indexes (Alves and Moura, 1992; Celik and Sukran, 2019).

The South Pacific bonito is a fishing resource that, due to its commercial value, is being considered as a candidate for marine aquaculture in the north of Chile. However, there is little information regarding its biology of embryonic and larvae development (Miranda et al., 2014). In the northern zone of Chile, aquacultural technological studies are being carried out to determine the feasibility of cultivating the South Pacific bonito. Therefore, it is a necessity to establish a first-time stock of broodfish from wild-captured juvenile or adult fish that can be conditioned to spawn in captivity (Silva and Oliva, 2010; Stieglitz et al., 2017; Flores and Rendíc, 2011). The development of studies aimed at determining the biological viability of a candidate species for aquaculture should characterize both the reproductive strategy in captivity and the embryonic and larval development under reproductive conditions (Gomathi et al., 2020; Aydin et al., 2020). In this way, it will then be possible to ensure the availability of juveniles to carry out subsequent studies aimed at determining the technological feasibility of aquaculture. Consequently, it is necessary to generate information on embryonic development in *Sarda chiliensis chiliensis*, as a biological basis to elaborate protocols for a captive breeding technique for this targeted species and which in turns helps in broodstock management and development and standardization of larval rearing techniques. Marine teleost fish hatch from pelagic eggs and most of their organ systems begin to form at the end of the embryonic period or in the vitelline stage. Such organ systems reach their functionality at the end of the vitelline stage, which can last from a few days to a week, depending on the temperature and the reabsorption of the yolk (Gisbert et al., 2004; Betti et al., 2006; Betti, 2011).

There are no studies or protocols for wild-capturing and captivity-conditioning of medium-sized South Pacific bonito broodstocks, nor are the growing conditions for embryonic development of *Sarda chiliensis chiliensis* known. However, there are some protocols for capturing medium-sized fish for other *Scombrids* species (Bar et al., 2015). There are also embryonic descriptions made within the same genus, like *Sarda chiliensis lineolata* (McFarlane, 2000) and *Sarda sarda* (Ortega, 2015). The present work on *Sarda chiliensis chiliensis* aims to carry out an accurate study to describe the development of the embryo from fertilization of the egg to hatching under aquaculture conditions, through the wild-capture of adult animals and their subsequent maintenance in a 75 m^3 commercial pilot system. In this way, a protocol for the conditioning of wild-caught broodstock and a description of embryonic development during egg incubation could be generated, which can subsequently be used as a baseline for studies towards the biological and technical feasibility of a South Pacific bonito land-based aquaculture phase.

2. Materials and methods

The study was carried out south of the city of Arica, at La Capilla beach (Latitude 18°32`13.71`` S and Longitude 70°19`33.81`` W), in the Arica and Parinacota Region (Chile).

2.1. Wild-caught fish and transportation

Twenty-two campaigns were conducted to capture wild specimens of South Pacific bonitos between November 2011 and January 2012 in the Pisagua sector, in northern Chile (19°36`22.57``S, 70°12`09.96``W). South Pacific bonito *Sarda chiliensis chiliensis* was captured by fishing them with barbless hooks to minimize damage to the mouth and gills as described for *Thunnus albacares* (Wexler et al., 2003), *Euthynnus affinis*, and *Cybiosarda elegans* (Bar et al., 2015). Between 1 and 8 *Sarda chiliensis chiliensis* were transported in each marine campaign carried out. The temperature range of the seawater during the South Pacific bonito fishing campaigns was between 17.1 and 18.2 °C.

Based on the authors' previous capture experiences, wild *Sarda chiliensis chiliensis* is very sensitive to manipulation and tends to shed its scales when they are captured, like many other fish species taken from the wild to reunite a breeding population. Therefore, meticulous care was exercised to minimize the handling of the fish and to prevent any possible damage to their skin during all steps related to capturing, transporting, and subsequently transferring them to our land-based RAS facility. Therefore, live fishes that were transferred to the land-based conditioning tank were unweighed to minimize stress due to excessive handling and cause unnecessary injuries, as suggested by Bar et al. (2015) for medium-sized Scombrids. Therefore, the length and weight of the fishes captured alive was estimated using the length to weight relationship of the fish that did not survive in the catches, and also compared with the length to weight relationship reported by:

Campbells and Collins (1975) for *Sarda chiliensis lineolata* (fish ranged between 23 and 79 total length; N = 3000 fish) (Table 1):

$$W = 0.009376*L^{3}\cdot 08{,}962 \quad (1)$$

Barret (1971) for *Sarda chiliensis chiliensis* (fish ranged between 40 and 73 total length; N = 595 fish) (Table 1):

$$W = 0.0118*L^{3.09} \quad (2)$$

and Medina and Araya (2019) for *Sarda chiliensis chiliensis* (fish ranged between 33.8 and 65.1 total length; N = 81 fish) (Table 1)

$$W = 0.0164*L^{2.9398} \quad (3)$$

where W as live weight (g) and L as total length (cm)

All fish, at the time of capture, were weighed in a container of water using a V-1026 digital scale (Mocco™) (fish measured on the fishing boat). All caught fish that were larger than 1 kg were discarded, and only smaller than 1 kg were transported to La Capilla land-based RAS. From a three-year previous experience fishing South Pacific bonito, we learned that all fish larger than 1 kg never survived the maritime transport towards the land-based RAS facilities. Therefore only fish less than 1 kg were this time selected for transporting. Each of the live fish landings, and their subsequent transport to the land-based recirculating

Table 1
The fish rearing biomass was estimated based on the length-weight relationships, and thus the daily feeding rate for the delivery of commercial feed was determined.

Current work fish total length	Campbells and Collins, 1975 Eq. 1 (a)	Barret, 1971 Eq. 2 (b)	Medina and Araya, 2019 Eq. 3	Estimated fish biomass Nx[(a + b)/2]	Feeding rate
TL (cm)	W (kg)	W (kg)	W (kg)	B (kg)	%BW/d
36	0.60	o.r.	0.62	14.47	5.5
37	0.66	o.r.	0.67	15.75	5.1
38	0.71	o.r.	0.72	17.11	4.7
39	0.77	o.r.	0.78	18.54	4.3
40	0.84	1.05	0.84	22.65	3.5
41	0.90	1.14	0.90	24.45	3.3
42	0.97	1.22	0.97	26.34	3.0
43	1.04	1.32	1.04	28.32	2.8
44	1.12	1.41	1.11	30.41	2.6
45	1.20	1.51	1.19	32.60	2.5

o.r., out of range for a given length-weight relationship; W, fish estimated weight; %BW, percentage of fish biomass weight; N, fish number was 24 at initial stocking of the rearing tank.

aquacultural system (RAS), lasted approximately 5 h. During transport, temperature and dissolved oxygen were monitored every 1 h with an oximeter (YSI model 55).

2.2. Wild-caught fish reception and adaptation to the RAS

The land-based RAS components were one 75 m^3 fish conditioning tank, two 1.5 hp pumps (Reggio, model SM 150), one head tank (polyethylene container with a capacity of 10 m^3), one sand filter (Hayword model S360T2), and finally to a ring filter plus biofilter (Azud modular model 100). Twenty-one South Pacific bonito weighing less than 1 kg were stocked in a single 75 m^3 conditioning tank with walls made of corrugated steel plates and covered with a black PVC geomembrane liner. This tank was used for the adaptation of the fish to the conditions of captivity.

Water quality parameters were measured in the fish conditioning tank to describe environmental conditions during the rearing of South Pacific bonito's broodstocks. Dissolved oxygen and temperature levels were recorded three times a day (08:00; 14:00 and 20:00 h) through a YSI model 55 brand oximeter. The pH, ammonium, nitrite, and nitrate were measured twice a day (11:00 and 17:00) using a benchtop spectrophotometer (Hanna model HI-83,225).

Seven months before the arrival of the wild-caught South Pacific bonito, the broodstock conditioning tank was stocked with four 0.8 kg specimens of Yellowtail Kingfish (YTK) *Seriola lalandi* that we already had in our farming facilities. The *S. lalandi* were first used to start-up the RAS biofilter component. Later, upon arrival of the South Pacific bonito, *S. lalandi* specimens guided and facilitated *Sarda chiliensis chiliensis* in its process of adaptation to captivity. As we expected, the presence of YTK, a shoal fish that were already adapted to the confinement, facilitated the feeding learning process of the South Pacific bonito. Social behavior is naturally developed in school fish, and several reports indicate its rapid adaptation to rearing conditions and weaning with moist or manufactured commercial diets (Brown and Laland, 2001; Rodewald et al., 2011; Klefoth et al., 2013). During the first three weeks of captivity, the fish were fed a fresh diet consisting of sea silverside (*Odontesthes regia*), which was daily delivered to them from Monday to Saturday in quantities between 400 and 500 g. South Pacific bonito were weaned from a fresh fish-based diet to a commercially formulated feed (Skretting Nova me 2000) in the first four weeks of being stoked in the conditioning tank. By the fourth week, the South Pacific bonitos began accepting the commercially formulated feed, which was delivered from Monday to Saturday twice a day at a rate between 300 and 400 g per feeding.

The total length of the wild-caught reared fish was visually estimated and backed-up with monthly descriptive photographic records (Fig. 1). This procedure was monthly performed, along with a make-up water protocol of up to 75 % of the rearing tank volume. Monthly water exchanges were carried out to keep the nitrate concentration less than 200 mg L^{-1} ± 4.33. In RAS, the low water rate replacement leads to the accumulation of nitrate, a byproduct of nitrification, and no safe levels for nitrate have been established for marine brood fish (Fernandes et al., 2007; Rodrigues et al., 2011; van Bussel et al., 2012; Orellana et al., 2014; Yang et al., 2019). The water depth of the conditioning tank was reduced to about 50 cm during the water renewal process. At this time, visual inspection of the external health status of the fish and the estimation of the growth in total length was made, which was also accompanied by a photographic record. The estimated growth in weight of the fishes was obtained from the growth curves of Campbells and Collins (1975) and Barret (1971). Next, we proceed to calculate the feed ration that would be delivered to the South Pacific bonitos during 30 days, and so on for the following months (Table 1). The described methodology was taken from the work of Bart et al. (2015) for the conditioning to captivity of medium-sized wild-caught mackerel tuna (*Euthynnus effinis*) and leaping bonito (*Cybiosarda elegans*).

Fig. 1. During the seawater exchange of the culture tank, the volume of water is appreciably reduced. This necessary action gives a perfect visualization of the fishes and allowed sufficiently a visual estimation with a reference element of their total length. In this proper way, the W-L relationship models were used to reasonably estimate the biomass of the population and properly adjust the monthly feeding.

2.3. Spawning and incubation of eggs

Wild-caught South Pacific bonito's broodstock spawned spontaneously after 14 months of conditioning in captivity in a land-based system. The buoyant eggs were collected from the surface of the rearing tank using a plankton net of 300 μm mesh size. Afterward, the eggs were separated in the laboratory according to their embryonic development. Next, they were poured into three incubators (acrylic aquarium of 300 × 150 × 200 mm) under quiet water conditions for approximately 15 min to eliminate all the eggs that settled on the bottom during that time. Buoyant eggs were considered fertilized (viable) and settled eggs non-viable. Following that, aeration was provided to each of the three incubators through airstones diffusers (model ASI-3), at a rate of about 0.1 L min^{-1}. This protocol for eggs handling was performed every time a spawning occurred to recover viable eggs. Natural spontaneous spawning happened during December in the year 2012, and February and March in the year 2013.

Gentle aeration of the incubator water allowed the eggs to remain evenly distributed in the water column. Incubators were kept in a warm room, and the incubation density was between 2000 and 2500 eggs per L^{-1}, as suggested by other authors (Pepe-Victoriano et al., 2012; Ibarra-Castro et al., 2012a). Under the described incubation conditions, the stages of embryonic development were observed and monitored until hatching of the eggs. The incubator water was changed by up to 70 % of its volume daily. The water utilized for the replacements was obtained from the RAS and was filtered at 1 μm, strongly aerated, and kept in the same incubator room. In this way, it was ensured that the salinity and pH of the water in the incubators were similar to that of the broodstock tank. The water temperature in the incubators was also similar to the temperature recorded in the RAS. It was decided to maintain this water quality protocol for incubation since it is yet unknown for South Pacific bonito what is the salinity, pH, and temperature range that favors its embryonic development.

2.4. Egg sampling for morphological characterization of the embryo

Eggs at the optic vesicle stage were collected from the broodstock tank and used for the rearing embryonic development studies until the hatching of the larvae was observed. During each day of incubation, subsamples of 10 eggs were taken at random by the hour from each incubator and observed under a Japan Optical Co. Stereoscopic microscope (model XTL-2310) to examine the embryonic development. The characteristics of fertilized eggs were documented. Simultaneously, with each egg sample, both temperature and hydrogen potential (pH) were recorded through a Hanna Instruments Waterproof pH Tester (model HI 98,127). This protocol was followed as recommended by Schultz (2003), who studied the period of embryonic development of *Sarda chiliensis*.

2.5. Morphometric characterization of the embryo

Viable eggs were first photographed through a Samsung digital camera (model ST66) and then from the stereoscope microscope using a microscopy camera (model Moticam 1000). All eggs were fixed in 5% formalin right after they were photographed. The characteristics of embryonic development through hatching were documented.

Forty viable eggs were selected to establish the egg size of *Sarda chiliensis chiliensis*: 20 eggs in the neurulation period and 20 eggs in the metameric period. There were measured the horizontal egg diameter (HED), which corresponded to the horizontal egg diameter measured from the equatorial line; and the vertical egg diameter (VED), which corresponded to the vertical egg diameter measured from the sagittal axis of the embryo.

Morphometric observations were performed to embryonic development periods identified as post-fertilization hours (HPF). Measurements were made to horizontal egg diameter (HED); vertical egg diameter (VED); length and height of the yolk sac (LY and HY); length and height of the eye (LE and HE), measured from the horizontal and vertical middle line of the eye; and, the diameter of the horizontal oil drop (DHOD) and diameter of vertical oil drop (DVOD). The time required for each stage of embryonic development from fertilized egg throughout hatching was calculated and recorded.

2.6. Statistical analisys for neurulation and metameric embryos

A descriptive and comparative statistic was performed for two development groups: neurulation and metameric embryos. Significant embryological differences were examined between morphometric measurements within the stage and between stages. Data were first analyzed for normality, and when they were rejected, then the comparison was performed using a mean test; else, a *t*-test was used. A 95 % confidence level was used for both statistical analyzes. Furthermore, a multivariate technique was required for the main components, employing the logic of retaining the components according to Tables 2 and 3. The software utilized was SPSS 16.0.

3. Results

3.1. Wild-Caught fish and its transportation

A total of 74 wild *Sarda chiliensis chiliensis* were caught across 22 campaigns between November 2011 and January 2012. A total of 31 of the wild-caught fishes were discarded immediately because of the visible damage caused by the fishing gear, mainly considerable injuries to the mouth and gills. And 19 fish died due to handling conditions utilized during their transport at a density up to 8 fish m^{-3} (fish < 1 kg) on the fishing vessel, which lasted between 40 and 60 min. The fish survival range of the 22 campaigns was between 0% and 100 % (from 1 up to 8 fish m^{-3}) transported in the tank. The temperature range of the seawater during the South Pacific bonito fishing campaigns was between 17.1 °C and 18.2 °C.

The fish transport density did not exceed three fish m^{-3} (fish < 1 kg) during their transport in a 1 m^3 tank on a vehicle from the dock to the land-based RAS. The land transport of the fishes, from the place of disembarkation to the land-based recirculating aquacultural system (RAS), lasted approximately 5 h. Finally, 24 potential broodstock fish survived the fishing gear, the handling conditions utilized during their transport on the fishing vessel, and the vehicle land transport to the RAS facilities.

Table 2
Summary of the hypothesis test of median for periods of neurulation and metamerism of *Sarda chiliensis chiliensis*.

Null Hypothesis	Test	Significance	Decision
The "head length" Medians were the Same among the State categories	Test of Medians of independent samples	0.015[1,2]	Reject the null hypothesis

Table 3
Matrix of components for the different morphometric characters measured in embryos of *Sarda chiliensis chiliensis*.

	Component	
	1	2
VED	0.939	−0.092
HED	0.817	−0.310
Head height	0.896	−0.294
Head length	0.916	−0.020
Oil drop height	0.220	0.885
Oil drop length	0.740	0.409
Eye height	0.917	−0.053
Eye length	0.872	0.199

In previous experiences of catching other marine fish, it had been determined that up to 8 fish could be successfully transported in 1 m^3 of water volume. However, when putting the freshly caught bonitos into the transport tank, they became extremely agitated and swam very swiftly, causing them to repeatedly hit the tank walls and each other. Under these maritime transport conditions, survival was between 0% and 100 %. This fish transport protocol should undoubtedly be improved for the Bonitos to reduce the conditions that cause mortalities, either by increasing the volume of the maritime transport tank, generating some type of restriction on the mobility of the fish without harming their well-being and health, the seasonal effect of the catch, size of the fish caught, water flows during transport, dissolved oxygen levels, condition of stress due to catch, among others.

3.2. Wild-caught fish adaptation to the RAS

It was observed by the fourth week, that the first groups of stocked *Sarda chiliensis chiliensis* began accepting the commercially formulated feed. The fish were fed with fresh and dry daily feed to approximate 5.4 % of their body weight per day, assuming an estimated average weight according to Table 1 for the 24 stocked fish by the end of the fisheries campaign. During the conditioning period, 13 more fishes died between March and October 2012. Therefore for the spawning period, we had 11 potential broodstocks, which represent 45.8 % of the initially stocked fish.

The average temperature and average dissolved oxygen conditions of the water in the conditioning tank between November 2011 and March 2013 were 19.46 °C ± 2.07 and 5.96 mg L^{-1} ± 0.39 (Fig. 2). On the other hand, an average for pH was 7.84 ± 0.17, ammonium 0.02 mg L^{-1} ± 0.007, and nitrate 0.55 mg L^{-1} ± 0.032.

3.3. Spawning and incubation of eggs

Wild-caught South Pacific bonito spawned almost 12 months after their stocking in the conditioning rearing tank. Fish spontaneous spawning, without application of hormonal inducers, began from the beginning of January 2013 (summer in the southern hemisphere). Spawning lasted until early March (late summer) of the same year, and at least three spawns were recorded weekly. The highest number of eggs observed during the spawning period was in February 2013. The estimated total length of the stocked fish (November 2011 to January 2012) in the conditioning tank was just about 36 cm, and by the end of March 2013, it was approximately 45 cm, corresponding to estimated growth in weight from 0.6 kg to 1.2 kg, respectively (Table 1).

The water temperature range in the broodstock RAS, during the natural spawns events, was between 18.6 °C and 19.8 °C in December 2012, between 20.4 °C and 21.2 °C in January 2013, between 21.5 °C and 22.4 °C in February 2013, and between 21.90 °C and 22.87 °C in

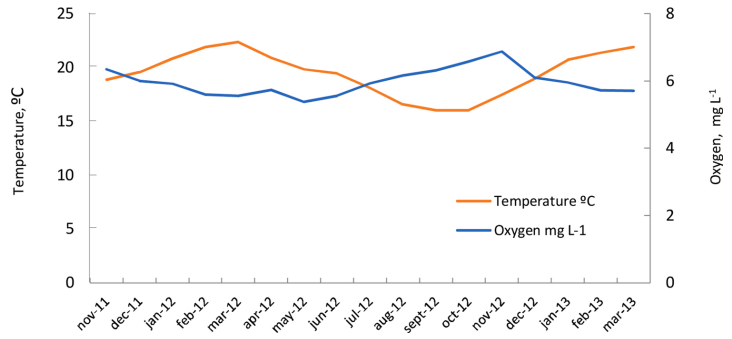


Fig. 2. Monthly temperature (ºC) and oxygen (mg L^{-1}) recordings in marine land-based RAS in northern Chile.

march. The incubators' water temperature was kept similar, through daily water exchanges, to the temperature recorded at the broodstock tank. This procedure allows for lessening any possible thermal stress, which could affect embryonic development.

According to the egg collection story, South Pacific bonitos' spawning must have occurred early in the morning. The eggs collected from *S. chiliensis chiliensis*, after 14–16 hours of incubation, were in stages of four blastomeres (110–120 MPF) and an advanced morula (300–320 MPF).

3.4. Morphological characterization of the embryo

Viable or fertilized eggs produced by *Sarda chiliensis chiliensis* were spherical, translucent, pelagic, non-adhesive, and of the telolecitic type due to the accumulation of yolk in the plant pole. They presented a discoidal meroblastic division during the first stages of development.

Details of the stages of embryonic development of *Sarda chiliensis chiliensis* are given in Table 4. There were 31 characterized stages of embryonic development that were recorded during 71.83 h (3 days) of incubation (Table 4). The time to stage of embryonic development relationship was obtained at 20.40 °C and 22.87 °C, which corresponds to the spawning periods of January and March 2013, having an average of 21.71 °C ± 0.88.

3.5. Morphometric characterization of neurulation and metameric embryos

The vertical egg diameter (VED) was 1.469 mm ± 0.240 mm, and the horizontal egg diameter (HED) was 1.622 mm ± 0.236 mm. It was considered as egg size, the average of the HEDs of all the eggs measured in the state of neurulation and metamerism, because it had the greatest length in comparison to VEDs. These fertilized eggs had between 1 and 6 oil drops with a height between 0.209 and 0.353 mm and an average of 0.283 ± 0.048. The length of the oil drop varied between 0.215 and 0.423 mm and an average of 0.331 ± 0.050.

3.6. Statistical analisys for neurulation and metameric embryos

When the *Sarda chiliensis chiliensis* embryos were compared in their stages of neurulation and metamerism, significant statistical differences ($0.05 < p$) were found for the variables high-head, high-eye, and length of the eye among the eggs of the different spawnings incubated and studied in this experience, revealing that between both stages the characters analyzed were highly variable. Consequently, we proceeded to compare the stages through the means test, rejecting the null hypothesis of equality, since significant statistical differences were found ($p < 0.05$) (Table 2). As a result of the methodology of the main components, in which a set of morphometric variables (VED, HED, head-height, head-length, oil drop diameter, eye-length, and eye-height) were analyzed, it was obtained that the first component explained only 60 % of the variability and the second component 15 % (Table 3). As in the neurulation and metamerism stages, the development of the analyzed characters of the main components among themselves and for each stage was highly variable among them.

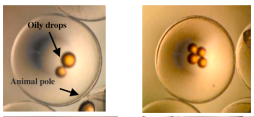
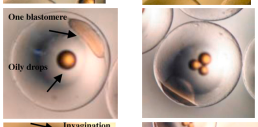
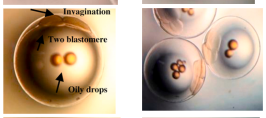
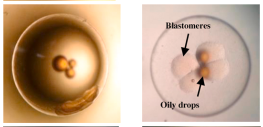
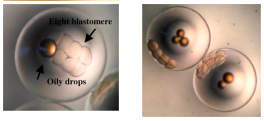
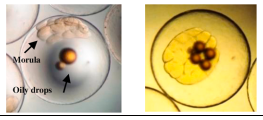
4. Discussion

Chilean aquaculture in the northern regions is becoming a genuine alternative throughout farming native marine finfish species like South Pacific bonito (*Sarda chiliensis chiliensis*). The success, of any aquacultural farming activity, depends upon knowing certain morphophysiological, and behavioral characteristics of the targeted species. And this comes highlighting the relevancy to study the early larvae living days to apply rearing protocols as well as to detect any malformations which could end with a rearing with a low survival index. The references for the preliminary stages of the biological development for *Sarda chiliensis chiliensis* are scarce. Therefore, it is necessary to establish the biological feasibility of the cultivation of this species to subsequently advance towards the technical and the economic feasibility to promote any aquaculture entrepreneurship with South Pacific bonito. As an example, fisheries data from Campbells and Collins (1975) seems to be useful for aquaculture purposes since reports that Pacific bonito *S. c. lineolata* ranged from 23 to 79 cm total length grows rapidly during their first three years of life with a slower growth from three to six years old. The present study aims to describe the sequential events of embryonic development of *Sarda chiliensis chiliensis* eggs naturally spawned from wild-caught South Pacific bonito reared under captive conditions in a 75 m^3 marine land-based RAS. At the time of the natural spawning, there were 11 fishes in the conditioning tank. In this investigation, it was unpossible to verify which of the conditioned fishes contributed to the pool of eggs that were naturally spawned. However, it is relevant to establish methods to identify which fishes are spawning, and which ones provide the most significant percentage of viable eggs. For instance, in a land-based culture facility for research on yellowfin tuna, *Thunnus albacares*, at the Achotines Laboratory in the Republic of Panama, a genetic monitoring of the captive broodstock and their spawned eggs is performed to determine which fish are contributing with gametes in each of the spontaneous natural spawning (Margulies et al., 2016).

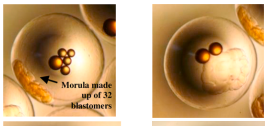
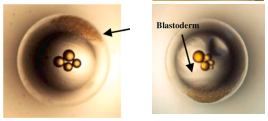
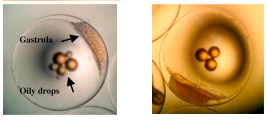
The form of aquaculture that is directly linked to capture fisheries operations is termed capture-based aquaculture (CBA), which involves the capture of live hydrobiological species from the wild and its subsequent use in aquaculture (FAO, 2011). In most cases, capture-based aquaculture represents the necessary first step in the move towards hatchery-based aquaculture (HBA) for the development of fully

Table 4
Morphological characterization of the different development periods observed in *Sarda chiliensis chiliensis*, note that MPF corresponds to minutes post fertilization. Average water incubation temperature was 21.71 °C ± 0.88 for spawning periods of January and March 2013.

a

DEVELOPMENT PERIOD	DESCRIPTIÓN	TIME (Minutes post fertilization; MPF)	FIGURES
Egg just fertilized or zygote	Translucent egg with yellow iridescence towards the perivitelline space. Oily drops range from 1–6 orange-yellow color. With the passage of time there is a conspicuous accumulation of yellowish cytoplasm towards the animal pole, to form the blastodisk.	1	
One blastomere zygote	Once the blastodisk was developed, it gives rise to the first blastomere, the presence of a telolecite egg was observed, which coincides with the literature for fish in general, with a large amount of translucent yolk. The blastomere, on the other hand, is discolored yellow-orange and occurs perpendicular to oil droplets ranging from 1–6 drops.	70 to 80	
Egg of two blatomeres (beginning of segmentation)	The segmentation corresponds to the partial or discoidal meroblastic type, which occurs only in the animal pole, coinciding with the type of telolecite egg. The division begins with an invagination in the central part of the blastomere, the process tends to lengthen the dividing cell, when the latter is finished, and the blastomeres are compacted.	90–100	
Egg of four blastomeres	The division of the blastomeres is symmetrical, the daughter cells are very similar in size, after 20 min in this stage there was already an egg of 4 cells. It was observed that when manipulating the egg the oily drops move inside the egg. The same process of elongation of the blastomeres in division is observed.	110 to 120	
Early morula. Egg of eight blastomeres	During this stage, it was observed that the blastomeres lose their symmetry with respect to the previous stages, although the difference is not marked. It can be noted that the mitotic division is of an exponential type. With each consecutive division it was observed that the blastomeres decreased in size.	130 to 140	
Morula intermedia Sixteen blastomere egg	It was observed the formation of a cell layer of 16 cells, of 4 × 4 blastomeres, which tend together to form a semicircular morula. The cells that are towards the corners are smaller than the adjacent ones. While the shape of each blastomer tends to a more squared than oval shape	150 to 160	

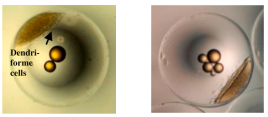
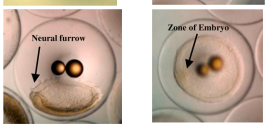
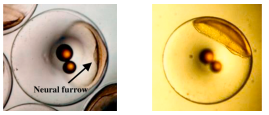
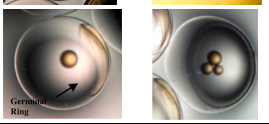
b

DEVELOPMENT PERIOD	**DESCRIPTION**	**TIME (Minutes post fertilization; MPF)**	**FIGURES**
Thirty-two blastomere egg	In this stage it can be seeing the formation of a morula with 2 cellular layers, of 16 cells each, which together do not exceed the area covered by the egg formed by a single blastomere. The shape of blastomeres becomes irregular.	180 to 200	
Advanced morula	There was a large association of small cells of irregular shape to circular and flattened dorso-ventrally, concentrated in a single cell pole that covers approximately one fifth of the surface of the egg. The blastoderm began to cover the yolk due to epibolia, given by the presence of a new cell group in the periphery.	300 to 320	
Early blastula	Two cell types were observed, a predominant one that makes up the blastoderm, which was surrounded by a layer of flat cells, irregular to dendriform, translucent and larger. The cellular set had the appearance of a hat. The eggs were observed with 1–6 oily drops.	460 to 480	

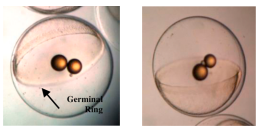
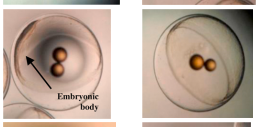
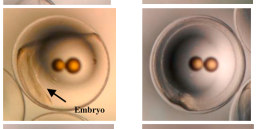
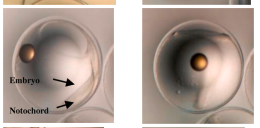
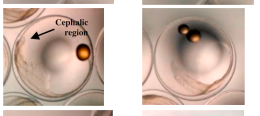
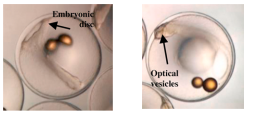
(*continued on next page*)

Table 4 (*continued*)

b

DEVELOPMENT PERIOD	DESCRIPTION	TIME (Minutes post fertilization; MPF)	FIGURES
Advanced blastula	The hat shape is lost, due to an extension of the egg cell mass towards the equatorial pole. Peripheral dendriform cells took the form of a translucent border.	660 to 680	
Early gastrula	The development zone of the embryo can already be seen with the beginning of the formation of the neural furrow.	860 to 880	
Gastrula	The blastoderm had covered approximately 1/4 of the yolk.	1080–1100	
Late gastrula	The blastoderm had covered approximately 1/4 to 1/3 of the yolk. The germinal ring begins to form.	1300–1320	

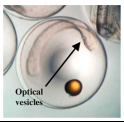
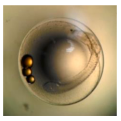
c

DEVELOPMENT PERIOD	DESCRIPTION	TIME (Minutes post fertilization; MPF)	FIGURES
Neurulation	The blastoderm had covered approximately 1/3 to 1/2 of the yolk and the germ ring was well defined. The formation of a thin line in the blastoderm was observed, where the formation of the embryonic body was distinguished.	1560 to 1580	
Neurulation	The blastoderm had covered approximately 2/3 of the yolk, the formation of the embryonic body or embryo outline shows a widening of what will be the cephalic region.	1680 to 1700	
Neurulation	It was clearly observed the body of the embryo that acquires greater thickening, especially on the cephalic region. The development of the embryo occurs towards head-tail direction, where the back part of the body develops as the blastopore closes. The formation of the notochord was observed.	1800 to 1820	
Neurulation	The formation of rudimentary optical vesicles begins, and the presence of the notochord was observed.	1980 to 200	
Neurulation	There was a greater development of the cephalic region, still incipient, and presence of the neural tube in formation.	2160 to 2180	
Neurulation	The embryonic disc was almost closed and the embryo outline almost finished. There was a cephalic region of greater development, which highlights the formation of optical vesicles; it was also seeing the formation of a protoencephalon and incipient formation of somites. There was no pigmentation.	2340 to 2360	
Neurulation		2520 to 2540	

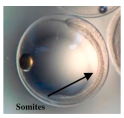
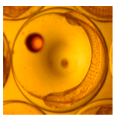
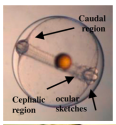
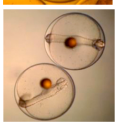
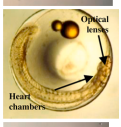
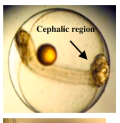
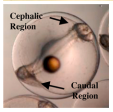
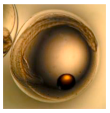
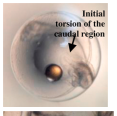
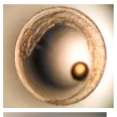
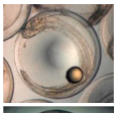
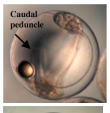
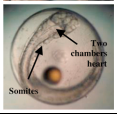
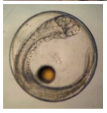
(*continued on next page*)

Table 4 (*continued*)

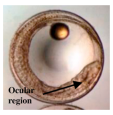
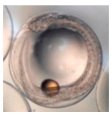
c

DEVELOPMENT PERIOD	DESCRIPTION	TIME (Minutes post fertilization; MPF)	FIGURES
	Disk completely closed, the embryo had a length that went from pole to pole, **somites** were observed along the embryo, and the caudal region was flattened anteroposteriorly, without further development. There was no twisting of the sagittal axis. **Notocorda** and **neural tube** completely formed and optical vesicles without pigmentation.		 

d

DEVELOPMENT PERIOD	DESCRIPTION	TIME (Minutes post fertilization; MPF)	FIGURES
Neurulation	A thickening of the body was observed in the dorso-ventral direction. Presence of well-defined somites. Punctate chromatophores appear on the flanks of the embryo. The cephalic region is differentiated by the presence of ocular sketches of a caudal region that is flattened dorso-ventrally.	2700 to 2720	 
Neurulation	The caudal region thickens back ventrally in relation to the previous stage. There was a greater development of the cephalic region, and the perivitelline space was more noticeable. **Somitos** more marked. No apparent pattern was observed in the chromatophore distribution.	2880 to 2900	 
Metamerie	Greater development of the cephalic region that expands laterally from the optical vesicles, where the optical lenses are distinguished. Presence of a heart of two chambers, which beats. Sketch of closed mouth. Small torsion of the caudal region, less than 45° in relation to the sagittal axis. Body appearance of the most opaque and granular embryo.	3060–3080	 
Metamerie	Characteristic of this stage is the tail twisted at 45 degrees with respect to the sagittal axis, it is an unpigmented embryo, although the presence of pinpoint chromatophores in the caudal region and lower flanks is observed. Reduction in the number of oil drops ranging from 1 to 2.	3240 to 3260	 
Metamerie	The embryo has a length of two thirds of the egg, more abundant punctate chromatophores were observed in the caudal region, extending along the flanks without reaching the cephalic region. There is a thickening of trunk and caudal region. A sketch of the heart that is already beating.	3420–3440	 
Metamerie	Embryo moves, a conduit was observed between the notochord and the heart that could correspond to the digestive tract. More defined somites. In the terminal caudal region, a narrow section corresponding to the caudal peduncle was observed. Embryos were observed with only an oily drop.	3600 to 3620	 
Metamerie	Like the previous stage, the embryos possess a **single oily drop**. The cephalic region has a greater development, where the **eyes** stand out because of their size; there is a greater development of the somites in this area. **Embryonic fin** was well differentiated from the rest of the embryo.	3750 to 3770	 

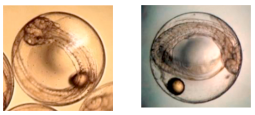
e

DEVELOPMENT PERIOD	DESCRIPTION	TIME (Minutes post fertilization; MPF)	FIGURES
Metamerie	In this stage, the length of the embryo makes the tail and face confront each other. There is a marked development of the face.	3930 to 3950	 

(*continued on next page*)

Table 4 (*continued*)

e

DEVELOPMENT PERIOD	DESCRIPTION	TIME (Minutes post fertilization; MPF)	FIGURES
Pre-hatching	This embryo contorts its trunk so that the caudal region is parallel to the height of the face. This stage is the last before larvae eclosion. It was observed a heart constituted by two cameras, the larger one looks towards the face.	4110 to 4120	
Hatch	At the time of hatching, it was observed that the larva breaks the egg with the head. Make a series of contortions in an "S" shape and in a spiral. The process does not take more than 5 min, and then the larva is out of the shell.	4290 to 4300	
Hatching larvae	It was observed that the larvae hatch aided by the head which is used to break the egg membrane. From the beginning of the process until its end, approximately 20 min passed.	4320	
Post hatch larvae	Presence of a single embryonic fin, which extended from the cephalic region to the rostral region. A non-functional and closed mouth was observed. The yolk sac covered two thirds of the total length of the larva, acquiring an inverted pear shape, where the oily drop was located at the posterior end.	1 min after hatch	

closed-cycle aquaculture, as in the case of yellowtails (*Seriola spp.*) and flatfishes (*Paralichthys spp.*) (Conkling et al., 2004; Ottolenghi et al., 2004; Silva and Oliva, 2010). In some cases, there is no transition and continues as CBA, as in the case of eels (*Anguilla spp.*) and tunas (*Thunnus spp.*) (Ottolenghi et al., 2004; Dalsgaard et al., 2013). And sometimes, even when HBA is achieved and reaches commercial production levels, as in the case of the bay scallop *Argopecten purpuratus*, it not fully substitutes CBA (von Brand et al., 2016). The results obtained during the conditioning of the premature CBA wild-caught South Pacific bonito (fish with less than 1 kg in live weight) in a 75 m^3 marine recirculating aquacultural system, their spawning and the embryonic development of the egg until hatching are discussed below.

4.1. Wild-caught premature fish and transportation

The 22 campaigns allowed the capture of 74 fish, of which 31 were immediately discarded, and only 43 were carried from the capture zone to the landing area. During the 40–60 min of maritime transportation, a mortality of 19 fish was recorded. None of the fish died during the land transport, which lasted approximately 5 h. The dimensions and volume of water in the transportation tank on the boat and the land system were the same. The primary differences between marine and land transportation were: in the marine, there was a permanent open flow-through water circulation, between 1 and 6 fish were carried, and the fish just experienced the stress of the capture; for land, the water was stagnant, pure oxygen was provided, survivors fish were transported at a density between 1 and 3 fish, and fish had the stress due to a transfer from one tank to another.

Water temperature represents an influential factor regarding the transportation of live fish, and low temperatures are generally suggested to decrease fish metabolism and stress (Vollmann-Schipper, 1978). In none of the 22 fisheries campaigns were possible to control the water temperature in the fish transport tank, since the protocol implemented required a constant flow of fresh seawater. South Pacific bonito survival seemed not to be affected by the temperature during the fish transport. However, overall captures survivability increased as catches progressed from late spring towards summer, with a survival range between 66.7 % and 100 % for the last catch period in January 2012 compared with catches on November 2011 (average 18.1 ± 14.3 %) and December 2011 (average 29.5 ± 15.3 %).

Based on previous experiences of the authors with South Pacific Bonito, a total mortality of fish greater than 1 kg has been observed. In contrast, a fish smaller than 1 kg was easier to catch and handling, which resulted in obtaining live fish at the end of their transportation to be adapted to the captive rearing conditions. In the same way, Wexler et al. (2003) recommended capturing wild fish smaller than 1 kg to ensure a lower transport density, which improved survival for *Thunnus albacares*. Bar et al. (2015) also reported that *E. affinis* and *C. elegans* weighing less than 1 kg survived the transport, whereas larger fish did not survive.

Mortality rates (19 out of 43 fish) for South Pacific bonito weighing less than 1 kg were comparatively higher than those reported for *Sarda sarda* (Ortega and de la Gándara, 2007b). This mortality may be due to the increase in stress due to the aggressive struggle that the fish undergo at the catch time, and the stresses associated with their handling and transport. Fish density throughout the time of transportation could as well represents a determining role in fish survival. However, this condition cannot explain by itself the mortality observed in fish smaller than 1 kg during the catches of *Sarda chiliensis chiliensis*. Davis (2010) mentioned that capture and handling techniques may contribute to diminish fish growth and delayed mortality, and recommend a direct approach of stress conditions to measure reflex responses after physical stimulation in free-swimming fish. Improvements to the catch and transportation protocols should be implemented to minimize exposition to stress and increase the survival of wild-caught South Pacific Bonito. Suggested methods to reduce stress include: a) to induce a metabolism reduction, by lowering the transport water temperature a few degrees in comparison to the temperature that is registered in the seawater for flow trough wild-caught broodstock tanks (Harmon, 2009); b) to apply anesthetics or an injection with a tranquilizing solution (Williams et al., 2004); c) to transport fish under low density or load mass (Bar et al., 2015); d) to design an appropriate transportation device for pelagic ram ventilatory fish (Bar et al., 2015). Expressly, when wild-caught fish are

going to be used as a foundational broodstock, it will be necessary to consider all the previous recommendations along with the appropriate size of the fish and the water quality requirements during transportation (Wexler et al., 2003).

4.2. Wild-caught fish adaptation to the RAS

To prevent starvation of wild-caught fish, and to assure success in their adaptation to captivity conditions, it has been suggested to feed them with raw fish (Bar et al., 2015) or even live foods (Wexler et al., 2003). In this research, the South Pacific bonitos were fed to approximate 5.4 % of their body mass per day with a raw and manufactured commercial diet. And it was observed that the fish began to feed on the commercial diet for marine fish broodstocks (Skretting brand, NOVA ME 2000) approximately four weeks after their introduction to the rearing tank.

For *Sarda chiliensis chiliensis*, the weight-length relationship was found to be consistent with those reported by Campbells and Collins (1975) and Barrett (1971) (Table 1), since both curves reported a total length similar to the weight ratio for the range of approximate size of fish wild-caught in this investigation (Table 1). Currently, an investigation by Medina and Araya (2019) reported a remarkably consistent weight-length relationship with the present study, further confirming our results regarding the estimation of length for the calculation of population biomass and thus estimating the food rations delivered monthly to fish.

Between November 2011 and February 2012, there were no mortalities in the fish stocked in the rearing tank. However in the year 2012, after about 45 days of the last fishing campaign, a couple of fish died in March, and eleven more between June and October. These mortalities, which occurred several months after stocking the fish, allow us to infer all the fish that survived the transport adapted adequately to the culture conditions. On the other hand, the presence of some type of chronic long-term stress in the land-based RAS could be a possible cause that explains the death of 13 specimens during their conditioning rearing time (Davis, 2010).

Ortega and de la Gándara (2007b) suggested that wild-caught fish taken into captivity must be conditioned as fast as possible to respond early to feed, primarily with raw feed, which will lead the specimens to recover more rapidly from the stress of the catch and sharply improve their external appearance. The supply of fresh or frozen diets has primarily occurred for the conditioning of wild-caught premature stocks, as described by Silva and Oliva (2010) for *Paralichthys adspersus*, Muñoz et al. (2012) for *Graus nigra*, and Bar et al. (2015) for *E. affinis* and *C. elegans*. The diet during the conditioning period of the broodstock was most likely adequate due to the abundant spawning events resulted from the completion of gonadal maturation and the production of good quality eggs.

4.3. South pacific bonito broodstock spawning

Sarda chiliensis lineolata has been described as a rapidly growing species able to reach 51 cm fork length and up to 1.8 kg in the first year (Lewis, 2008). Males can mature and spawn at 1-year old, and a few females will spawn at 2-years old, but most will do at 3-years old with 69 cm fork length (Black, 1978; Lewis, 2008). Most wild-caught surviving *Sarda chiliensis chiliensis* in this research was less than 1 kg at the time of their stock at the conditioning tank, and therefore maybe they were within their first year of life. Most wild-caught South Pacific bonitos might have been reaching their second year of life while being conditioned in the rearing tank, which might explain the natural spawning observed at the beginning of the year 2013. A similar result was reported for *Sarda sarda* (Ortega, 2015).

In northern Chile, it has been reported that wild-stocks of South Pacific bonito undergoes a seasonal reproductive cycle typical of temperate zone fishes, as spawning begins in September (spring) and ends before April (late summer) with no reproductive activity in the other half of the year (Barrett, 1971; Goldberg. and Mussiett, 1984). Wild-caught South Pacific bonito naturally spawned in our RAS from the beginning of January 2013 (summer) and lasted until early March 2013 (late summer), and at least three spawns were weekly recorded. The water temperature in the broodstock RAS was between 18.6 °C and 19.8 °C in December 2012, between 20.4 °C and 21.2 °C in January 2013, between 21.5 °C and 22.4 °C in February 2013, and between 21.90 °C and 22.87 °C in march.

Black (1978) analyzed the egg diameters in the ovary of wild spawning Pacific bonito, *Sarda chiliensis lineolata*, and found that this fish spawned more than once each season, however, the exact frequency could not be determined. Atlantic bonito *Sarda sarda* is reported as multiple spawners with asynchronous oocytes at different levels of development present at the same time in the ovary (Macías et al., 2005). Hence we expect that South Pacific bonito is also an asynchronous spawner with a long reproductive season, as described for other small tuna species. Therefore for aquaculture production planning purposes, there is a need now to determine the potential fecundity (number of oocytes to be liberated by a spawning female), which is reported to be related to the size (weight and length) (Hajjej et al., 2017), and age of the female (Ibarra-Castro et al., 2012b).

At the beginning of the spawning period, there were 11 South Pacific bonito in the rearing tank, which means a 45.8 % survival rate after one year in captivity, which is similar to the reported survival for *Sarda sarda* (Ortega and de la Gándara, 2007b). The estimated total length of the stocked fish (November 2011 to January 2012) in the conditioning tank was approximately 36 cm, and by the end of March 2013, it was approximately 45.0 cm (Table 1). Barrett (1971) found that female *Sarda chiliensis* of approximately 51 cm (fork length) were mature, and Goldberg. and Mussiett (1984) reported from northern Chile that the smallest mature female had a total length of 48.5 cm. The total estimated length of the wild-caught South Pacific bonitos farmed in La Capilla for March 2013 is very close to the lower range reported by Goldberg. and Mussiett (1984) for natural spawning off the coast of northern Chile.

4.4. Morphological characterization of the embryo

These are the first egg embryonic development studies conducted with eggs obtained from wild-caught *Sarda chiliensis chiliensis* broodstock conditioned in aquaculture land-based systems in Chile. The incubators' water temperature was kept similar, through daily water exchanges, to the temperature recorded at the broodstock tank (as described in 4.3) because we do not know what represents the appropriate temperature for egg culture.

For the Atlantic bonito *Sarda sarda*, under conditions of captivity, an egg of smaller diameter was obtained with a reported average of 1.301 mm ± 0.036, and with many oily drops that ranged between 1 and 12 (Ortega, 2015). When comparing the average egg size of *Sarda chiliensis chiliensis* with the reported data for the egg sizes of *Sarda sarda* and *Sarda chiliensis lineolata*, it was observed that there is more similarity with the egg sizes of *Sarda chiliensis lineolata* (Table 5).

Ortega and de La Gandara (2007a) reported that the first cell divisions for *Sarda sarda* eggs begin 90 min after fertilization (MPF). Our methodology did not allow us to determine when the first cell divisions began because the eggs we obtained come from natural spawning. During the incubation period, it was observed that after 60 h of embryonic development, all eggs possessed a single oily drop, assuming the drops tended to fuse, probably to facilitate buoyancy. During all this time, the embryo underwent the following stages of development: Morula, Blastula, Gastrula, Neurula, Metamery, Organogenesis, and Hatching.

Sarda sarda eggs hatched after 48 h of incubation at 21 °C, developing the first divisions between 1:30 and 1:45 h after fertilization. And 20 min later, the first eggs were observed in the state of four cells, and so on: Morula status was registered after 5 h, 24 h after embryo formation,

Table 5
Egg sizes and the differences between each species with respect to *Sarda chiliensis chiliensis* in millimeters.

Species	Egg size (mm)	Difference (mm)	Researcher
Sarda sarda	1.293	0.329	Ortega (2015)
Sarda chiliensis lineolate	1.580	0.042	McFarlane (2000)
Sarda chiliensis chiliensis	1.622	0.000	Miranda et al (2014)

and a few hours later the segmentation began (Ortega and de la Gándara, 2007a). On the other hand, McFarlane et al. (2000) indicated that *Sarda chiliensis lineolata* kept in captivity at the Bay Aquarium (Monterey, USA), the diameter of the eggs captured were 1.58 mm ± 0.05, which began to hatch within 48 h of spawning which coincided with *Sarda sarda*. In our case, *Sarda chiliensis chiliensis* required 3 days of embryonic development before egg hatching, and the size of the egg was similar to the size of the egg generated by the wild populations of Pacific bonito (1.52–1.68 mm) (Ambrose, 1996; McFarlane et al., 2000). In our research with *Sarda chiliensis chiliensis*, the estimated time elapsed from egg fertilization to hatching was 72 h (71.8 h), which is consistent with Schultz (2003), who points out that *Sarda chiliensis* required 3 days to undergo the embryonic period.

It has been described that *Sarda chiliensis chiliensis* has an intermediate and closer egg size to *Sarda chiliensis lineolata* than to *Sarda sarda* (McFarlane et al., 2000). It is striking that, however, *Sarda chiliensis chiliensis* has a greater similarity with *Sarda sarda* in the first moments of embryonic development. The eggs analyzed from the different spawns released naturally by the *Sarda chiliensis chiliensis* in the conditioning tank had distinctive characteristics when compared with *Sarda sarda* (e.g., egg diameter, egg number of oily drops, and hatching time). It was observed that the beginning of the somito-genesis in *Sarda chiliensis chiliensis* occurs before the blastoderm completely covers the vitelline cell, at approximately 36 HPF, which has already been described in the development of other teleost species (Botta et al., 2010).

Concerning the fertilized eggs of *Sarda chiliensis chiliensis* it was observed: in 70 min (1.17 h) the presence of a first blastomere; at 80 min (1.33 h) the first division; and at 2 h, the formation of an egg with four blastomeres, which coincides with *Sarda sarda* embryonic time development. A morula of 16 cells was observed in 160 min (2.67 h) and an advanced morula in 320 min (5.33 h), which also coincides with the period of 5 h observed by Ortega and de la Gándara (2007a). There was additionally a similarity to the period in which the outline of the embryo in development appears, with 24 h for *Sarda sarda*, and with 28 h for *Sarda chiliensis chiliensis*. Among the three species, the eggs of *Sarda chiliensis chiliensis* remain the ones that take the longest to hatch, with almost 72 h when they are incubated at the same temperatures at which the broodstock are grown (see 4.3).

The development of the embryo of *Sarda chiliensis chiliensis* was remarkably similar to that described for *Sarda sarda* in its early stages (Viñas et al., 2010). However, the time of hatching and the diameter of the egg would be differentiating criteria between these two species. The hatching period indicated for *Sarda sarda* and *Sarda chiliensis lineolata* was 48 h, concordant with what was exposed by Civera-Cerecedo et al. (2004), have in common that both correspond to species belonging to the Northern Hemisphere and that inhabit warm waters associated with the western edge of the continents. On the other hand, *Sarda chiliensis chiliensis* produces a larger egg size and would be associated with colder waters coming from the Humboldt Current from the western continental side, but in the Southern Hemisphere. Low temperatures will likely cope with the more prolonged period required for hatching (Civera-Cerecedo et al., 2004). Pepin (1991) and Pauly and Pullin (1988), suggest that the time used by fish eggs for their embryonic development and hatching was directly related to their size and inversely related to temperature. Hence, it seems that similar species with larger eggs should spend more time completing their embryonic development and hatching, consequently this situation should be investigated to try to explain why *Sarda ch. chiliensis* takes one more day to hatch.

4.5. Morphometric characterization of the embryo

The difference in the morphometric characters among embryonic stages would be caused by the development of the cephalic region. The most significant coefficients of variability found in this investigation were for high-head, high-eye, eye-length, and head-length. The high-head had the most significant coefficient of variability among the measured characters. On the other hand, the morphometric head-length character revealed a non-normal distribution of variables. Therefore, the observed transitions between the distinct stages would be primarily given by these four morphometric variables, and it would be unnecessary to investigate the other variables. The descriptions used for the embryos development stages agreed with Miranda et al. (2014), who reported that the coefficients of variability are relatively high for variables such as high-head, high-eye, eye-length, and head-length.

It was established that South Pacific bonito, both the head and eye dimensions are the criteria to define the stages in embryos. With the *t*-test, the existence of significant differences was pointed out, which was to be expected for the stages of larval development. The most marked differences in embryonic development were in the stages of neurulation and metamerism.

5. Conclusion

From an aquaculture point of view, the results obtained in the present study allow us to conclude that wild-caught stocks of medium-size South Pacific bonito *Sarda chiliensis chiliensis* can be captured, transported, and conditioned for breeding under captivity conditions and spawn naturally after 13 months of rearing in a 75 m^3 land-based RAS. Wild-caught South Pacific bonito, at the time of capture, were accustomed to eating live prey, and the weaning onto the farm-made moist feed and manufactured commercial feed was a successful aspect for our aquaculture operations. The capture-based aquaculture strategy to gather premature South Pacific bonito in this research represented a necessary first step to set-up a breeding stock, which was successful in the move towards hatchery-based aquaculture (HBA) for the development of fully closed-cycle aquaculture for this species. The successful spawning of wild-caught *Sarda chiliensis chiliensis* in captivity indicates that controlled reproduction for mass production of larvaes can be achieved, allowing the development of hatchery culture technology to a commercial scale without depending on wild fish.

Sarda chiliensis chiliensis spawned eggs are pelagic, singles non-adhesive, and can be screened out from the rearing tank overflow seawater managing a typical hatchery buoyant egg collector. The incubation period of *Sarda chiliensis chiliensis* eggs required almost 3-days to undergo an embryological development through yolk-sac larvae without mouth at hatching. These marine fish larvae will require a hatchery facility with a live-feed production system as described for other marine finfish to feed their early stages. The specific information generated here will help to properly recognize the distinct stages of embryo development and standardize the appropriate protocols for collecting and incubating eggs to produce state-of-the-art marine hatchery yolk sac larvae.

Studies are suggested from now on to refine egg rearing protocols towards increasing the survival in the yolk-sac and first-feeding larval stages, which will be the key to developing a successful start *Sarda chiliensis chiliensis* aquaculture.

Author statement

We hereby confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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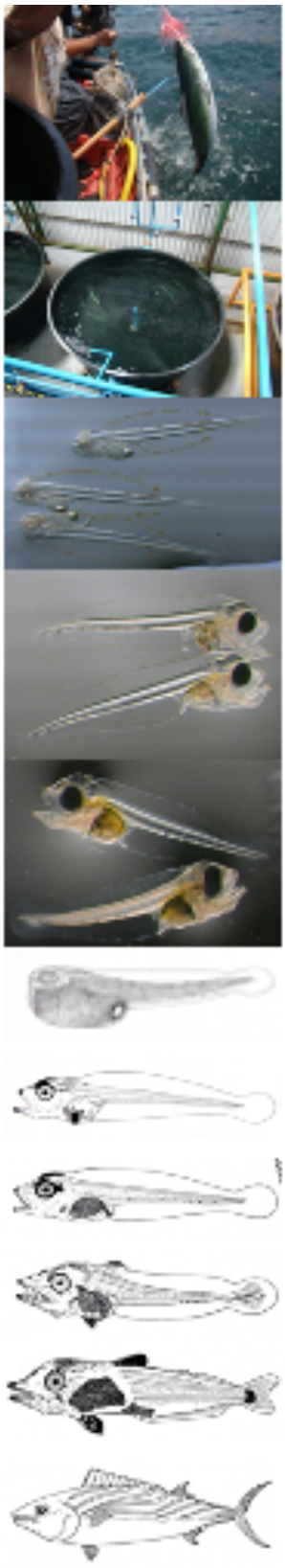

CAPÍTULO 4:

Desarrollo Larvario y Cultivo Larval de Peces Marinos

Introducción

Procedimientos adecuados en la reproducción y crianza, permiten entregar crías a gran escala (millones de larvas por batch) para los sistemas de engorde en sus diferentes niveles (extensivo, semi-intensivo e intensivo) y también para los otros sistemas como estanques, jaulas, canales, raceway y otros (Escárcega, 2020). En efecto, resulta posible asegurar nuevas cadenas de valor con especies que presentan valores nutricionales altos y una importante demanda en el mercado. Esto es justamente el objetivo central de las investigaciones publicadas, entregar conocimiento y una nueva forma de elevar las producciones actuales de peces marinos a nivel comercial.

Actualmente existe la oportunidad de generar mas producción de larvas de peces marinos en estadio de alimentación (cuando han agotado sus reservas e inician su alimentación natural) esto permite un perfeccionamiento de tecnologías para el cultivo larval y la crianza, capaces de garantizar la necesidad de larvas y juveniles que se requiere en los sistemas de engorde.

Hace muchas décadas, China ha venido desarrollando modelos productivos como el sistema acoplado de desove e incubación (Escárcega, 2020) el cuál entrega con eficacia al vasto potencial biológico de diferentes especies de peces de importancia comercial, aumentando la productividad en los desoves, incubación y la producción larvas.

De la misma forma, para la producción de larvas a mayor escala, se ha venido desarrollado cultivos larvales en sistemas abiertos como por ejemplo en estanques, que facilitan el manejo de una gran cantidad de larvas, con sobrevivencia, que alcanzan hasta un 60% para el periodo larval en estadio de alimentación a larvas de 3 cm (Horváth, Tamás & Coche, 1986). Para los peces marinos, países como Tailandia y Australia producen esta alternativa con gran éxito en la producción comercial de larvas de robalo del Indo pacífico, *Lates calcarifer* (Kungvankij *et al*., 1985).

Cultivo Larval

Existen factores biológicos (p. ej. canibalismo, calidad de la progenie, dispersión de tallas, densidad), físicos (p. ej. temperatura e iluminación) y químicos (p. ej. oxígeno y pH) que pueden afectar a las larvas de peces marinos durante su desarrollo; sin embargo, existen otros factores de manejo tales como el tipo de alimento suministrado, la calidad de los nutrientes, frecuencia alimenticia, entre otros, que también han mostrado tener gran influencia en la supervivencia y crecimiento de las larvas (Civera-Cerecedo *et al*., 2004).

El comienzo de la alimentación exógena es un proceso muy importante que se caracteriza a menudo por altas mortalidades, cuya disminución es uno de los objetivos primordiales en el desarrollo de la tecnología de producción de juveniles, pues asegura una sobrevivencia adecuada al final del período larval.

Como las larvas comen unos pocos organismos cada vez, éstos deben estar disponibles en todo momento. Las larvas no tiene las aletas bien desarrolladas y son muy pequeñas en los primeros días por lo que no pueden nadar mucho y el volumen que puede ser revisado por ellas es pequeño y los intentos fallidos son de más del 90 % en los primeros días, el aprovechamiento del alimento disponible es extremadamente bajo. Por lo que los organismos del alimento, deben estar en densidades altas.

En el ambiente natural, la alimentación de las larvas de peces marinos se compone de complejas redes tróficas que van cambiando en función del crecimiento. La alimentación se basa en diatomeas, dinoflagelados, flagelados, tintinados, ciliados, cladóceros, copépodos, huevos de bivalvos, quetognatos, lamelibranquios, gasterópodos, poliquetos, decápodos, otras larvas de peces, entre muchos otros organismos.

Es por esto que, para que las larvas aseguren su supervivencia deberán seleccionar una presa de tamaño adecuado y de movimiento lento; además que esta presa una vez ingerida sea fácil de digerir y que cubra sus requerimientos nutricionales mínimos. Sin embargo, su uso conlleva a su vez ciertas desventajas, entre las cuales destacan:

a) Las variaciones en su calidad nutricional (García-Ortega *et al*., 2000).
b) Alto costo de producción, instalaciones adicionales y requerimiento adicional de mano de obra (Yúfera *et al*., 2000).
c) Pueden llegar a ser deficientes en macro y micronutrientes. Un ejemplo son las artemias, las cuales se caracterizan por ser deficientes en ácidos grasos poliinsaturados (PUFA´s, por sus siglas en inglés) principalmente de la familia omega-3 (p. ej DHA y EPA) y no logran satisfacer los requerimientos nutricionales de la mayoría de las larvas de peces marinos (Yúfera *et al*., 2000).
d) Alta mortalidad observada en el destete en los peces marinos (transición del alimento vivo a alimento formulado) (Dantagnan *et al*., 2006).

Todas estas desventajas han llevado a la industria acuícola a tener un gran interés en el desarrollo de alimentos formulados, comúnmente llamados como microdietas compuestas para aquellos empleados en las etapas larvarias, los cuales puedan sustituir al alimento vivo desde el inicio de la alimentación exógena, o que al menos reduzca al máximo la dependencia de los larvicultivos por los dichos alimentos, pero sin afectar u obtener menores tasas de crecimiento o supervivencia que los observados al emplear los alimentos vivos (Zambonino-Infante & Cahu, 2001).

Desarrollo morfológico y crecimiento alométrico de los peces marinos

Durante el período larval, los peces marinos presentan una serie de cambios morfológicos, conductuales, metabólicos y digestivos con el fin de transformarse en organismos juveniles (Osse *et al*., 1997), al conjunto de estos cambios se le conoce como proceso metamórfico. Para la mayoría de los teleósteos es posible observar 4 episodios de desarrollo larvario; alimentación endógena, preflexión, flexión y postflexión, corroborado en los estudios del crecimiento alométrico en el lenguado de California (*Paralichthys californicus*) reportado por Gisbert *et al*., (2002).

El período de alimentación endógena, comienza desde la eclosión y termina al empezar la alimentación exógena, el de preflexión se caracteriza por el inicio de la alimentación exógena y la etapa de preflexión del notocordio, así también el período de flexión se caracteriza por la curvatura hacia arriba de la punta del notocordio y postflexión por la completa flexión de la punta del notocordio (Chen *et al*., 2006; Gisbert *et al*., 2002; Martínez-Lagos & Gracia-López 2009).

El desarrollo morfológico de órganos de importancia vital para el organismo es seguido por el desarrollo de estructuras con menor prioridad (Osse *et al*., 1997), observándose de esta manera que las tasas de crecimiento de diferentes zonas corporales u órganos son diferentes, a ésta característica se le conoce como alometría y es comúnmente observada en las larvas de peces marinos (Fuiman, 1983).

El crecimiento alométrico en larvas de peces marinos, se considera una respuesta de adaptación para contrarrestar las presiones ambientales, aumentando las probabilidades de supervivencia y el crecimiento durante este período crítico del organismo (Fuiman, 1983). De esta manera, en estudios realizados en el pez lisa (*Chelon labrosus*), se observó que el crecimiento alométrico positivo de la cabeza para este organismo, estuvo relacionado con el desarrollo de los sistemas vitales principales tales como el sistema nervioso, alimenticio, respiratorio y sensorial (Khemis *et al*., 2012). Datos similares tuvieron estudios reportados por Wold *et al*., (2008) y Martínez-Lagos, (2009) en cabrilla sardinera (*Mycteroperca rosacea*) y bacalao común (*Gadus morhua*) respectivamente.

El estudio del desarrollo morfológico y crecimiento alométrico ha tomado gran importancia en el campo de la acuicultura, ya que conocer la morfología larvaria normal de los peces marinos ayudaría a entender tendencias en relación con la ecología de las especies en diferentes etapas de desarrollo. Así también, esta información podría ser usada para evaluar las condiciones de cultivo que produzcan juveniles y adultos de peces marinos de alta calidad (Pepe-Victoriano *et al*., 2021b).

Artículo 3

Descriptive morphology and allometric growth of the larval development of *Sarda chiliensis chiliensis* (Cuvier, 1832) in a hatchery in northern Chile.

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Descriptive morphology and allometric growth of the larval development of *Sarda chiliensis chiliensis* (Cuvier, 1832) in a hatchery in northern Chile

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ABSTRACT

This research describes for the first time, and necessary for its practical application in aquaculture, the morpho-physiological development, the allometric growth patterns, and behavioral characteristics of the early stages of larval development of the South Pacific bonito (*Sarda chiliensis chiliensis*). Bouyant eggs, from natural spawning, were collected from the conditioning rearing tank where wild-broodstocks were stocked for over a year in a recirculation system. The viable eggs were distributed among three aquariums to investigate the morphological and morphometric characteristics of the larval development periods that were unknown before this investigation. We described that the larval development periods extended to 519 h for the pre-juvenile stages and from 519 to 591 h for the juvenile stage. The total reabsorption of the oil globule occurred between 81 and 108 h, and this description corresponds to a differentiating characteristic of *S. chiliensis chiliensis* since it would now be one of the three *Sarda* species that presents a single oil globule in the larval period. Allometric growth patterns showed that the eyes, mouth, and head developed faster than other bodily characters. Therefore, the larvae are prioritizing for a fully-functional mouth when they enter the exogenous feeding stage. It was also observed that the visual development of the larvae was closely related to the height and length of the head and with the height and length of the eyes. Finally, and in accordance with the morphological and morphometric characteristics of the observed larvae, in this research six post-embryonic stages were described: larval stages 1 to 4, pre-juvenile and juvenile.

1. Introduction

The South Pacific Bonito (*Sarda chiliensis chiliensis*) is a neritic epipelagic species of the Scombridae family that lives in shoals (Chero et al., 2016). This species is distributed from Máncora, Peru, just south of the Gulf of Guayaquil to Talcahuano, Chile (Collette and Nauen, 1983). It is a species that lives in areas of upwelling currents with thermal ranges that oscillate between 15 °C and 22 °C (Samamé, 1993). Regarding the ontogeny of this species, as regards eating habits, they are mainly carnivorous fish, and they feed on *euphausiids*, anchovy *Engraulis ringens*, mackerel *Trachurus murphyi*, munida *Pleuroncodes monodon*, and the giant squid *Dosidicus gigas* (Collette and Nauen, 1983; Blasković et al., 2011). Regarding the growth of tunids in general it is fast, as Ortega (2015) has reported for *Sarda sarda* and *Thunnus thynus*. Cannibalism in the larval stages has also been observed in the larval behavior of these species. And morphologically, they have been described with several oily drops in embryonic development (Ortega and de la Gándara, 2007, 2009; Ortega, 2015).

South Pacific bonito could be a new candidate with the potential for diversifying Chilean aquaculture because of its market prospects and the feasibility of capturing wild broodstock to condition them in captivity for the production of larvae in a hatchery, especially in the extreme north of the country (Goldberg and Mussiett, 1984; Gálvez and Castillo, 2015; Wurmann and Bastos, 2017). Both males and females have rapid growth in the first years of life until reaching their gonad maturity, which is estimated to occur in the third year of age (Samame, 1997). These fish reach a size of 33.2 cm fork length and a weight of 500 g in the first year of life (Samame, 1997). For Peru, it has been estimated that

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the size for the first spawning is between 47 cm and 53 cm of fork length when the fish are about two years old (Yoshida, 1980). The first studies on reproductive aspects of *S. chiliensis* were carried out in Peru with Schweigger (1947) (cited by Gálvez and Castillo, 2015), who reported that spawnings occurred in the wild between September and March. Therefore, when females reach maturity, they have asynchronous spawning that will facilitate the bio-programming of the annual production of a fish farm.

Despite the number of years that the wild population of the South Pacific bonito has been exploited by commercial and artisanal fisheries (Food and Agriculture Organization (FAO, 1974; Collette and Nauen, 1983), there is little knowledge related to its morphophysiological development, growth patterns, and behavioral characteristics corresponding to the first stages of larval development. Morphogenesis and differentiation are rapid and complex processes that occur during early ontogeny in a short time. Information related to the morphological development of larval stages in fish is paramount to define management protocols in aquaculture, in particular for the development of larviculture protocols (Gozlan et al., 1999; Koumoundouros et al., 1999; Gisbert et al., 2002; Papadakis et al., 2018; Kupren et al., 2019; Lv et al., 2019).

In 2008, a land-based recirculation system was implemented for the conditioning of wild-caught *S. chiliensis chiliensis* broodstocks in the "La Capilla" sector, 10 km from the city of Arica. In this stage, several natural spawning occurred after a year of rearing the fish in captivity. The real feasibility of obtaining viable eggs will allow the development of the scientific and technological bases for the hatchery cultivation of South Pacific bonito larvae and juveniles. Therefore, the present study aims to describe the morphological development and allometric growth patterns of the staging series for hatchery-reared *S. chiliensis chiliensis* larvae for the definition of the initial criteria for the larviculture of this species.

2. Materials and methods

The research was carried out in the 'La Capilla' sector (Latitude 18.5167 S, and Longitude 70.3333 W), 10 km south of the city of Arica, Chile, where a land-based aquaculture recirculating system was built to work with wild-caught South Pacific bonito *S. chiliensis chiliensis* (Fig. 1). All fish, at the time of capture, were weighed in a container of water using a V-1026 digital scale (MoccoTM) (fish measured on the fishing boat). All caught fish that were larger than 1 Kg were discarded, and only smaller than 1 Kg were transported to La Capilla land-based RAS. From a three-year previous experience fishing South Pacific bonito, we learned that all fish larger than 1 Kg never survived the maritime transport towards the land-based RAS facilities. Therefore only fish less than 1 Kg were this time selected for transporting. A total of 74 fish were caught in the 22 campaigns carried out between November 2011 and January 2012. Any fish that presented some damage due to fishing gear or greater than 1 Kg was released into the sea. Finally, 24 fish smaller than 1 Kg were selected, which were transported and stocked in the 75 m^3 conditioning tank at an estimated density of 0.3 Kg/m^3. The conditioning tank was manufactured with corrugated steel plates and then covered with a black PVC geomembrane.

We have learned from previous captures that wild *Sarda chiliensis chiliensis* is extremely sensitive to handling and typically tends to shed its scales when captured. Therefore wild-caught fish were stocked in the land conditioning tank without being weighed or measured. In this proper way, the considerable stress associated with excessive handling, which could undoubtedly cause unnecessary injury, was minimized. Wild fish handling procedures were followed as suggested for medium-sized scombrids (Bar et al., 2015). Consequently, the size and weight of the fish caught alive was estimated using the length-weight relationship of the fish that did not survive the maritime transport, and we also compared it with the length-weight relationship reported by Medina and Araya (2019) for *Sarda chiliensis chiliensis* (total length of fish ranged from 33.8–65.1 cm; N = 81 fish) (Table 1).

$$W = 0.0164 * L^{2.9398} \quad (1)$$

The total length of the wild-caught reared fish was visually estimated and backed-up with monthly descriptive photographic records. Next, the estimated growth in weight of the fishes was obtained from Eq. 1. Next, we proceed to calculate the feed ration that would be delivered to the South Pacific bonitos during 30 days, and so on for the following months (Table 1). The described methodology was taken from the work of Bart et al. (2015) for the conditioning to captivity of medium-sized wild-caught mackerel tuna (*Euthynnus effinis*) and leaping bonito (*Cybiosarda elegans*). All South Pacific bonito were then fed twice a day at a rate of 300–400 g with a dry formulated feed 'Nova Me 2000' (protein percentage 52 %) manufactured by Skretting for marine fish breeders. A natural photoperiod was maintained during the broodstock conditioning period.

Fish were captured between November 2011 and January 2012, and a spontaneous spawning happened in January 2013. Buoyant eggs were manually collected from the culture tank using 300-μm-mesh plankton net. The collected eggs were then separated according to their embryonic development and distributed to three aquariums with continuous aeration. All aquariums were placed inside a climate-controlled room.

Larval rearing tanks were cleaned once a day by siphoning the

Table 1
The fish rearing biomass was estimated based on the length-weight relationships, and thus the daily feeding rate for the delivery of commercial feed was determined.

Current work estimated fish total length	Medina and Araya, 2019 Eq. 1	Estimated fish biomass NxW	Feeding rate
TL (cm)	W (kg)	B (kg)	%BW/d
36	0.62	14.8	5.5
37	0.67	16.0	5.1
38	0.72	17.4	4.7
39	0.78	18.7	4.3
40	0.84	20.2	3.5
41	0.90	21.7	3.3
42	0.97	23.3	3.0
43	1.04	25.0	2.8
44	1.11	26.7	2.6
45	1.19	28.5	2.5

W, fish estimated weight; %BW, percentage of fish biomass weight; N, fish number was 24 at initial stocking of the rearing tank.

Fig. 1. Specimen of South Pacific Bonito (*Sarda chiliensis chiliensis*).

bottom to remove particulate material and dead larvae. The temperature and dissolved oxygen levels were recorded three times a day using a YSI model 55 dissolved oxygen meter. Total ammonia as nitrogen and nitrate were daily measured with a compact Hanna nutrient analysis photometer model HI-83225, to ensure the proper maintenance of suitable water quality conditions for larval culture. The temperature and pH were simultaneously measured with the aid of HI 98127 testers (Hanna). The water temperature, dissolved oxygen concentration, and pH were 23.8 ± 1.4 °C, 5.8 ± 0.4 mg/L, and 7.84 ± 0.1 pH, respectively. Fish were exposed to a natural photoperiod.

2.1. Larval feeding

Exogenous feeding of the larvae began two days post-hatch (dph). The first feeding was primarily with rotifers (*Brachionus plicatilis*) enriched for 2 h with *Chlorella sp.*, at a concentration of 30,000–40,000 cells ml^{-1}. Rotifers were offered as live-food three times per day to maintain a concentration of 5 rotifers ml^{-1}. Exogenous feeding coincides with the complete pigmentation of the eyes, a situation that has been typically described for other fish larvae (Peña and Dumas, 2007; Blaxter, 1986).

In previous experiences of the rearing of larvae of bonito from the South Pacific, cannibalism was observed from 3 dph. Then, from 5 dph, the rotifers were gradually replaced by day 7, with brine shrimp nauplii (*Artemia franciscana*) enriched with baker's yeast and microalgae. The live diet was administered to the larvae three times a day to maintain a concentration of 10 nauplii ml^{-1}. Therefore, the feeding protocol was modified to include the delivery of brine shrimp from 4 dph (Fig. 2).

The fish larvae were fed up to 25 dph with a granulated micro pellet with a size between 840 and 1410 microns (Othoime, Reed Mariculture). It has 51 % protein, 11 % lipids, and packed in bags of 1 Kg. *Mugil cephalus* larvae were offered as live-feed along with the micropellets from 16 dph until 23 dph (Fig. 2). At the end of the delivery of *Mugil cephalus* larvae, we began to add a fish marine pellet (Nova me 2000) manufactured by Skretting. By 25 dph, the micro-pellet feeding was interrupted, and only the marine fish pellet continued to be offered to the South Pacific bonito larvae.

Mugil cephalus larvae were captured near the mouth of the local Lluta river and transferred in a 12-liter container twice a day to the larval rearing laboratory. Subsequently, *Mugil cephalus* larvae were transferred to a beaker, filled with seawater from the rearing system, and immediately poured into the larvae rearing aquariums. Once the *Mugil cephalus* larvae were consumed by the South Pacific bonito larvae, properly feeding was continued with a pellet-based diet (Nova Me 2000), which was offered to satiety 3 times a day.

2.2. Morphological characteristic of larval developmental stages

To describe and analyze the larval stages of development of the South Pacific bonito, about 6–8 individuals were carefully extracted from the aquariums every two hours during the day (between 08:00 and 20:00 h), and every four hours at night (between 20:00 and 08:00 h). Allometric larvae measurements were taken along lines parallel or perpendicular to the horizontal axis of the body (Gisbert et al., 2002; Kupren et al., 2019). Dead or abnormal specimens were intentionally excluded from the study.

The morphological larvae development observations were made using a Japan Optical Co. stereoscopic microscope (model XTL-2310), and the pictures were obtained using a Samsung model ST66 digital camera and a microscopy camera model Moticam 1000. The stages of morphological development were described using the system developed by Ortega and de la Gándara (2007) for *S. sarda.*

2.3. Morphometric characteristic of larval development stages

The volume of the yolk sac (Vsv) and the volume of the oil globule (Vgo) of the larvae were calculated considering the yolk sac as an ellipse and the oil globule as a sphere, using the following formulas (Cetta and Capuzzo, 1982; Heming and Buddington, 1988; Aristizabal, 2006):

For volume of the yolk sac (Vsv): $\mathbf{Vsv = \pi/6\ LH^2}$ (2)

For volume of the oil globule (Vgo): $\mathbf{Vgo = 4/3\ \pi r^3}$ (3)

where, L represents the major axis of the ellipse, H represents the minor axis (vertical) of the ellipse, and r is the radius of the sphere, all in millimeters.

Fifteen morphometric characteristics were considered for each specimen sampled (Fig. 3). Morphometric observations were made during the development periods identified in this research. The characteristics for Fig. 3A were: (1) distance between the basal part of the yolk sac and the upper part of it (yolk height, YH); (2) distance between the anterior distal end of the yolk sac and the posterior distal end of the yolk sac (yolk length, YL); (3) height of the oil droplet with the larva of the anteroposterior lateral position (oil globule height, OGH); (4) diameter of the oil droplet with the larva of the anteroposterior lateral position (oil globule length, OGL); and (5) the distance from the most distal end of the snout to the most distal end of the tail fin (total length TL). The characteristics for Fig. 3B were: (1) head height measured at the operculum level (HH); (2) distance from the anterior tip of the head to the posterior part of the operculum (head length, HL); (3) Height of the body at the level of the anus, without considering the dorsal fin (Pre-anal height, PH); (4) distance between the front end of the snout to the anus (pre-anal length, PL); (5) diameter of the eye with the larva of lateral anterior-posterior position (eye length, EL); (6) eye height with the larva in the anterior lateral position (EH); (7) length of the lower jaw (snout length, SL); (8) distance from the back of the head to the base of the tail fin (NL, notochord length); and (9) the distance from the most distal end of the snout to the most distal end of the tail fin (total length TL).

2.4. Allometric growth

The allometric growth of 9 morphometric characters was calculated from the first feeding (day 2) to stage 5. Allometric growth was calculated with the power function:

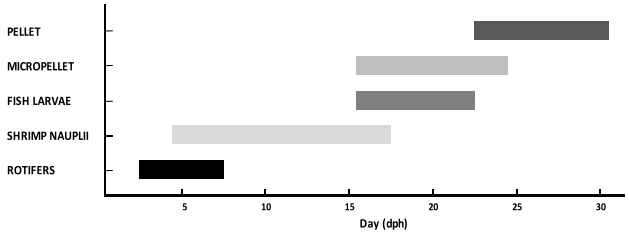


Fig. 2. Feeding protocol for *Sarda chiliensis chiliensis* larvae.

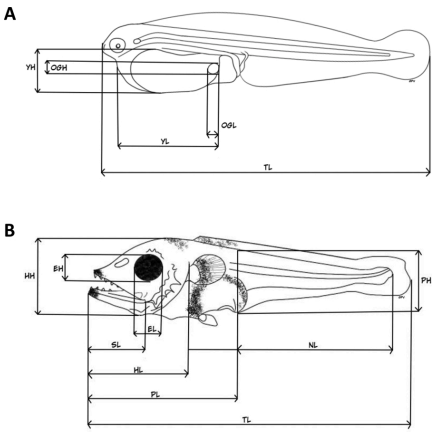


Fig. 3. Morphometric characters measured in *Sarda chiliensis chiliensis.*
A: YH, yolk height; YL, yolk length; OGH, oil globule height; OGL, oil globule length; TL, total length
B: HH, head height; HL, head length; PH, Pre-anal height; PL, pre-anal length; EL, eye length; EH, eye height; SL, snout length; NL, notochord length; TL, total length

$$Y = aX^b \quad (4)$$

where, *Y* was the dependent variable (morphometric measured character), and *X* was the independent variable (TL), *a* was the intercept and *b* was the growth coefficient (Fuiman, 1983). If $b = 1$, the growth was isometric, if $b > 1$ the allometric growth was positive, and if $b < 1$ the allometric growth was negative (Fuiman, 1983).

2.5. Larvae behavior

No specific experiments were done to test swimming ability of larvae and only general. Observations were made in the rearing tanks during day-light conditions.

2.6. Statistical analyses

A multivariate analysis was carried out, due to the considerable number of variables, to define the morphological development from hatchin g to the absorption of the yolk sac, using the principal components technique. The number of components retained was considered as a measurement criterion. On the other hand, a model was sought that related the larvae length parameter with time, for which it was resorted to: fit a curve, find the goodness-of-fit statistics, analyze the model using an ANOVA, and finally be able to determine the specific significance of each parameter of the regression equation based on the t-statistic. All statistical analyzes were performed with the R software. Water temperatures were plotted according to the time they were measured, thus generating a temperature-time graph, to determine whether the temperature of the culture water of the study groups could influence the results.

3. Results

3.1. Larval feeding

Fish larvae were fed rotifers in small additions to adjust the feeding rate according to consumption. The food supply begins with brine shrimp nauplii, followed by microgranules, and finally with *Mugil cephalus* larvae (Stuart and Drawbridge, 2013; Muñoz et al., 2012; Pepe-Victoriano et al., 2012). As a result of observations of cannibalism (in previous experiences) and considering that the nauplius brine shrimp were small, it was decided to incorporate *Mugil cephalus* larvae into the diet, whose size in total length was between 3 and 4 mm. From the 16 dph, the piscivorous feeding phase began, which consisted of the introduction of *Mugil cephalus* larvae, whose size was between 10 and 20 mm at a density of 20 larvae per liter.

South Pacific bonito larvae are visual feeders. Their hunting behavior was characterized by twisting the entire body into a sinusoidal position, followed by rapid bursts towards the detected prey. The larval feeding protocol for the culture of South Pacific bonito is shown in Fig. 2.

3.2. Morphological characteristic of larval developmental stages

The hatching of the eggs occurs at approximately 72 h of incubation, resulting in larvae that were completely translucent under the stereoscope. When hatching, the larvae are 4.12 ± 0.03 mm in total length. The head was tilted downward, and the hatching gland was on the underside of the head surface. The mouth was open, and the eyes and gills operculum was absent. No fins were shown, except for the large primordial fin bordering the notochord. The primordial fin was wider in the dorsal part of the body and narrowed in the caudal portion. The larvae displayed a large yolk sac that contains an oil globule in the posterior portion. The larval growth, from the hatching of the egg to the pre-juvenile stage, can be seen in Fig. 4.

Moderate growth, in the first stages of larval development, was associated with the internal development of mandibular and ocular structures. Nevertheless, when the larvae reach 10 mm of total length at about 500 h post-hatch, the growth rate accelerates exponentially (Fig. 4). The stages of larval, pre-juvenile, and juvenile development proposed for *Sarda chiliensis chiliensis* in this study, are observed in Fig. 5. The volume of the yolk sac, oil globule, and their reduction during larval development, can be seen in Fig. 6A and B.

3.3. Morphometric characteristic of larval development stages

A model was elaborated for the variables 'total larval length/hours post-hatch', which corresponded to a third-order descriptive-explanatory model (total larval length $= 4 + 0.003\ h + 1.97E^{-0.7}\ h^3$) of larval growth. The individual parameters were all statistically significant ($p < 0.05$), which were subjected to a goodness of fit test that resulted in the highest adjustment capacity of the model.

As a global model, the ANOVA for the third-order polynomial regression computed the same data, which was corroborated by the goodness of fit statistics—correlation of determination and adjusted correlation of determination, which were all greater than 93 %; furthermore, the estimation error was low.

The correlation between the morphometric measurements of larvae was analyzed employing a matrix of components (Table 2). The analysis showed that these measures were highly correlated. The number of parameters could not be reduced, since only one of the components showed a linear combination for the original variables. Considering that the natural state of the measured variables was irreducible, it was not possible to obtain additional information by segregating this group of variables.

3.4. Allometric growth

From hatching to larval stage 5, growth in total length (TL) was related to age (dph) by a potential function (Fig. 7). During this period, measured body proportions and growth rates changed variably. Growth of head height (Fig. 7A) and head length (Fig. 7B) were analyzed at stages 3, 4, and 5 during larval development. From the beginning of the third stage of development and up to 300 dph, the growth in head height

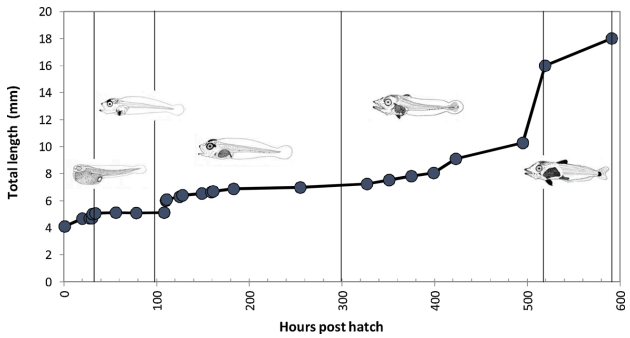


Fig. 4. South Pacific bonito total length growth from hatching until the pre-youth stage.

was allometrically positive (b = 1.476). On the other hand, the head length for the same state maintained a negative allometric growth (b = -0.031). From the fourth larval stage (300–519 dph) and the fifth larval stage (519–591 dph), both height and head length growth were allometrically positive.

Growth analysis of belly height (Fig. 7C) and belly length (Fig. 7D) were performed for larval development stages 3, 4, and 5. For the larval stage 3, the belly height presented a positive allometric growth (b = 1.157). On the contrary, and for the same state of development, the belly length showed a slight negative allometric growth (b = -0.009). For the other two stages, the growth was allometrically positive (Fig. 7C and D).

Growth of eye height (Fig. 7E) and total length (Fig. 7F) were also analyzed in the same stages of larval development, as shown in the previous graphs. In larval stage 3, the eye height had a slight positive allometric growth (b = 0.201), and the eye length had a negative allometric growth (b = -1.565). For the growth of the other larval states in eye height and eye length (Fig. 7E and F), they had positive allometric growth.

3.5. Larvae behaviour

Day 1 and 2 dph: Once the eggs hatched, the larvae remained at the top of the water column, distributed along the entire surface of the aquarium. As the hours passed, the yolk sac began to be absorbed, and with it, the buoyancy of the larvae decreased, and as a consequence, they move with the water column. From the second day after hatching, most of the larvae had a smaller yolk sac, and they began to swim and capture their first live prey (Rotifers), thus starting the exogenous feeding stage.

Day 3 and 4 dph: During the first 3 days post-hatch, the larvae had periods of rest and swimming. Between 3 and 4 dph, most of the larvae actively swam and maintained their position in the water column.

Day 5 and 6 dph: the larvae changed from a total anguilliform swimming style, characterized by a high amplitude flexion over a large part of their body, to a partial anguilliform style, in which only the caudal region flexed, but the rest of the body remained relatively rigid.

Day 7 and 9 dph: the larvae began to feed more consistently and aggressively, showing a moderate degree of cannibalism.

Day 10 dph and onwards: the larvae were very voracious, using rapid and coordinated movements to capture prey. Cannibalism was significant, with the largest larvae preying on the smallest, and cannibalism was even observed among larvae of the same size.

The average temperature recorded during the investigation period was 23.75 +/- 1.45 °C.

4. Discussion

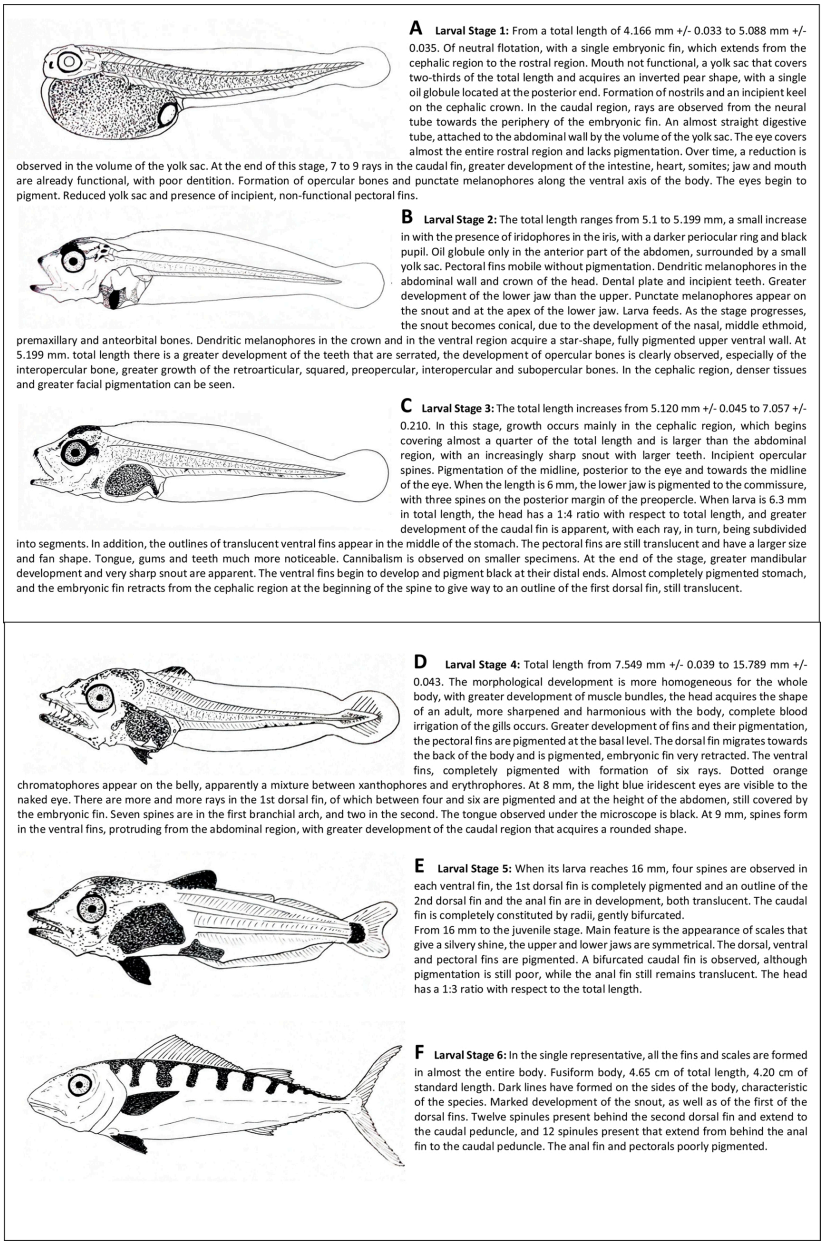
As with most teleost species, the functional hatching systems of the South Pacific Bonito larvae were still incomplete and undeveloped. Consequently, the growth, development, and differentiation of newborn larvae resulted in changes in body shape, morphology, metabolism, swimming skills, and behavior (Gisbert et al., 2002). This transformation in juveniles occurred in a relatively short time to achieve metamorphosis, which could be influenced by various factors such as the physiological capacity of each species and the cultivation conditions to which they were subjected.

4.1. Feeding habits

According to Avilés-Quevedo and Castelló-Orvay (2004), eating habits are determined by water temperature, digestion times, and food availability, which are factors that affect the growth rate of marine fish larvae in the same or different species. Exogenous feeding, as defined by the quantity of live-feed consumed, increased with voracity, rapid mobility, and cannibalism being characteristic behaviors for this group of fish, as reported by Gisbert et al. (2002) and Ortega (2015).

The survival and development in each larval stage depend on the availability and abundance of food. Consequently, survival-related organs develop preferentially and exhibit an allometric growth pattern (Gagnat et al., 2016). Houde (1974) notes that the time from hatching to eyes pigmentation presumably represented the time in which the larvae can only feed on their yolk reserves and not from exogenous sources. The onset of exogenous feeding was observable for *S. chiliensis chiliensis* from 53 h of age (~2.2 days), where the larvae had completely pigmented eyes and began to prey on rotifers (eyes began to pigment gradually after 34 h), although they still had a remnant yolk sac surrounding the single oil globule. On the second day post-hatching, the larvae presented a developed digestive tract, which implies the presence of a functional mouth for food consumption (McFarlane et al., 2000). In Fig. 6, it can be seen that there is a significant consumption of yolk between 28 and 30 h, which could have been triggered by eye pigmentation.

Rotifers are essential in the cultivation and feeding of a significant number of marine fish larvae (Sorgeloos and Léger, 1992). Rotifers have a small size, a smooth and constant movement in the water column, a prolific capacity for mass production in culture, ease of handling in terms of assimilation of nutritional enrichment diets, and tolerance to a wide range of changes in the rearing environment. These characteristics make the rotifer an optimal organism as the first food for marine fish larvae (Hirayama, 1985). Numerous studies have revealed the importance of DHA and, particularly, the requirement of a high DHA / EPA ratio to obtain high growth rates, stress resistance, and pigmentation in fish larvae (Sorgeloos and Léger, 1992). In such cases, the use and



A **Larval Stage 1:** From a total length of 4.166 mm +/- 0.033 to 5.088 mm +/- 0.035. Of neutral flotation, with a single embryonic fin, which extends from the cephalic region to the rostral region. Mouth not functional, a yolk sac that covers two-thirds of the total length and acquires an inverted pear shape, with a single oil globule located at the posterior end. Formation of nostrils and an incipient keel on the cephalic crown. In the caudal region, rays are observed from the neural tube towards the periphery of the embryonic fin. An almost straight digestive tube, attached to the abdominal wall by the volume of the yolk sac. The eye covers almost the entire rostral region and lacks pigmentation. Over time, a reduction is observed in the volume of the yolk sac. At the end of this stage, 7 to 9 rays in the caudal fin, greater development of the intestine, heart, somites; jaw and mouth are already functional, with poor dentition. Formation of opercular bones and punctate melanophores along the ventral axis of the body. The eyes begin to pigment. Reduced yolk sac and presence of incipient, non-functional pectoral fins.

B **Larval Stage 2:** The total length ranges from 5.1 to 5.199 mm, a small increase in with the presence of iridophores in the iris, with a darker perioocular ring and black pupil. Oil globule only in the anterior part of the abdomen, surrounded by a small yolk sac. Pectoral fins mobile without pigmentation. Dendritic melanophores in the abdominal wall and crown of the head. Dental plate and incipient teeth. Greater development of the lower jaw than the upper. Punctate melanophores appear on the snout and at the apex of the lower jaw. Larva feeds. As the stage progresses, the snout becomes conical, due to the development of the nasal, middle ethmoid, premaxillary and anteorbital bones. Dendritic melanophores in the crown and in the ventral region acquire a star-shape, fully pigmented upper ventral wall. At 5.199 mm. total length there is a greater development of the teeth that are serrated, the development of opercular bones is clearly observed, especially of the interopercular bone, greater growth of the retroarticular, squared, preopercular, interopercular and subopercular bones. In the cephalic region, denser tissues and greater facial pigmentation can be seen.

C **Larval Stage 3:** The total length increases from 5.120 mm +/- 0.045 to 7.057 +/- 0.210. In this stage, growth occurs mainly in the cephalic region, which begins covering almost a quarter of the total length and is larger than the abdominal region, with an increasingly sharp snout with larger teeth. Incipient opercular spines. Pigmentation of the midline, posterior to the eye and towards the midline of the eye. When the length is 6 mm, the lower jaw is pigmented to the commissure, with three spines on the posterior margin of the preopercle. When larva is 6.3 mm in total length, the head has a 1:4 ratio with respect to total length, and greater development of the caudal fin is apparent, with each ray, in turn, being subdivided into segments. In addition, the outlines of translucent ventral fins appear in the middle of the stomach. The pectoral fins are still translucent and have a larger size and fan shape. Tongue, gums and teeth much more noticeable. Cannibalism is observed on smaller specimens. At the end of the stage, greater mandibular development and very sharp snout are apparent. The ventral fins begin to develop and pigment black at their distal ends. Almost completely pigmented stomach, and the embryonic fin retracts from the cephalic region at the beginning of the spine to give way to an outline of the first dorsal fin, still translucent.

D **Larval Stage 4:** Total length from 7.549 mm +/- 0.039 to 15.789 mm +/- 0.043. The morphological development is more homogeneous for the whole body, with greater development of muscle bundles, the head acquires the shape of an adult, more sharpened and harmonious with the body, complete blood irrigation of the gills occurs. Greater development of fins and their pigmentation, the pectoral fins are pigmented at the basal level. The dorsal fin migrates towards the back of the body and is pigmented, embryonic fin very retracted. The ventral fins, completely pigmented with formation of six rays. Dotted orange chromatophores appear on the belly, apparently a mixture between xanthophores and erythrophores. At 8 mm, the light blue iridescent eyes are visible to the naked eye. There are more and more rays in the 1st dorsal fin, of which between four and six are pigmented and at the height of the abdomen, still covered by the embryonic fin. Seven spines are in the first branchial arch, and two in the second. The tongue observed under the microscope is black. At 9 mm, spines form in the ventral fins, protruding from the abdominal region, with greater development of the caudal region that acquires a rounded shape.

E **Larval Stage 5:** When its larva reaches 16 mm, four spines are observed in each ventral fin, the 1st dorsal fin is completely pigmented and an outline of the 2nd dorsal fin and the anal fin are in development, both translucent. The caudal fin is completely constituted by radii, gently bifurcated.
From 16 mm to the juvenile stage. Main feature is the appearance of scales that give a silvery shine, the upper and lower jaws are symmetrical. The dorsal, ventral and pectoral fins are pigmented. A bifurcated caudal fin is observed, although pigmentation is still poor, while the anal fin still remains translucent. The head has a 1:3 ratio with respect to the total length.

F **Larval Stage 6:** In the single representative, all the fins and scales are formed in almost the entire body. Fusiform body, 4.65 cm of total length, 4.20 cm of standard length. Dark lines have formed on the sides of the body, characteristic of the species. Marked development of the snout, as well as of the first of the dorsal fins. Twelve spinules present behind the second dorsal fin and extend to the caudal peduncle, and 12 spinules present that extend from behind the anal fin to the caudal peduncle. The anal fin and pectorals poorly pigmented.

Fig. 5. Larval, pre-juvenile and juvenile development stages proposed for *Sarda chiliensis chiliensis.*

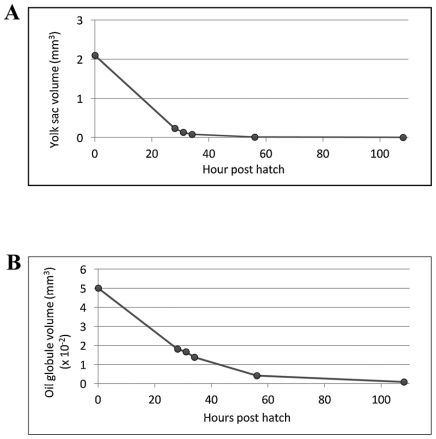


Fig. 6. Shown in A: volume of the yolk sac according to the hours post hatch and B: volume of the oil globule according to the hours post hatch.

Table 2
Component matrix for larvae of *Sarda chiliensis chiliensis.*

	Components
Height oily drop	**r = 0.998**
Oil drop volume	**r = 0.997**
Oil drop radius	**r = 0.993**
Height yolk	**r = 0.990**
Yolk length	**r = 0.988**
Yolk sac volume	**r = 0.977**
Oil drop length	**r = 0.974**

addition of enriching substances (fatty acid emulsions), or the use of marine or enriched yeasts, in their preparation are essential to feed the levels of unsaturated and long-chain fatty acids (EPA) and unsaturated (DHA) in rotifers (Yufera and Pascual, 1984; Fukusho, 1980; Watanabe et al., 1983).

Exogenous feeding began for *S. chiliensis chiliensis* (Fig. 2 and Fig. 8) when the volumes of the yolk sack and oil globule were minimal and tended to remain until approximately 108 h post-hatch. After 53 days of post-hatch, exogenous food sources seems to be the main energetic contributors. This situation would highlight the importance of ocular development to the success to capture the prey, as other authors have reported for different fish species (Peña and Dumas, 2007; Blaxter, 1986), which was directly related to the highly predatory behavior observed at that time. This scenario coincides with the Atlantic bonito *S. sarda* since larvae began to feed on rotifers at 2 days post-hatch when the larvae measured approximately 5.0 mm, like the larvae of *S. chiliensis lineolata* who started preying on rotifers and copepods in stage L2, which begins on day 4 post-hatch (counting from fertilization of the egg) when larvae were 5.5 mm, according to McFarlane et al. (2000).

It had been reported that the transition between endogenous and exogenous feeding is a critical period in which many morphological changes are happening, and generally high mortality rates might be expected (Moteki, 2002; Makrakis et al., 2005). In the case of *S. chiliensis chiliensis*, during the first days post-hatch, the ontogenetic changes were related to highly predatory behavior, which involved a rapid development of the head, eyes, and mouth, which lead to piscivorous habits. Although the 53 -h-old larvae initially preyed on rotifers (Brachionus plicatilis), as the development of the mouth became more noticeable at approximately 152 h post-hatch (6.3 dph), the larvae stopped preferring rotifers and began to exhibit cannibalism (Fig. 8), preying on smaller juvenile larvae. The same situation of cannibalism was observed for larvae of *S. chiliensis chiliensis* when offered as live food nauplii of Artemia (*Artemia franciscana*). The larvae resorted to cannibalism instead of consuming the nauplii. The cannibalistic behavior of *S. chiliensis chiliensis* begins from stage 3, and so does *S. chiliensis lineolata* and *S. sarda*, but at stage 4 (McFarlane et al., 2000). Rotifers and Artemia nauplii, while widely used in marine fish hatcheries, are not the best option for *S. chiliensis chiliensis*, and potential new live-feed sources should be a relevant variable to be addressed in future studies to avoid cannibalism, especially for larvae from stage 3 forward.

4.2. Morphological characteristic of larval developmental stages

S. chiliensis chiliensis is the only species of the genus that has shown a single oil globule during the larval stage (Fig. 5A). This globule occurred in the last stages of embryonic development, with significant resorption occurring at 81 h (~3.4 days) until total elimination of the globule at 110 h (~4.6 days) of larval life, corresponding with the extinction of the last endogenous source of food (Fig. 8). This observation was similar to that reported by Anon (2009) for S. Sarda reared at 20 °C, where resorption occurred between the 4 and 5 days post-hatch.

Fish are one of the most colorful vertebrates, where color can be determined by up to six different types of chromatophores: melanophores (black), xanthophores (yellow), erythrophores (red), iridophores (iridescent, blue, silver, or gold), leukophores (opaque, whitish) and cyanophores (blue). Together, these cells can produce almost any spectacular color (Darias et al., 2013). The small, almost imperceptible, punctate chromatophores detected in newly hatched larvae agreed with the observations of Barnhart (1927) for larvae of *Sarda chiliensis lineolata*, obtained from eggs collected from La Jolla, California, which were incubated in the laboratory. Barnhart (1927) observed that newly hatched larvae had a few chromatophores. On the other hand, Sanzo (1932) and Vodyanitsky (1936) reported that *S. sarda* larvae, besides showing xantoforos, exhibited melanophores in the eggs before hatching, which had not been reported for *S. chiliensis chiliensis*. Barnhart (1927) explained that the observed yellowish pigmentation was due to samples being conserved in Bouin's solution, which, according to Orton (1955), decolorizes melanin. Hence, the pigmentation observed by Barnhart (1927) could correspond to melanophores instead of xantoforos.

In the present investigation, all morphological analyses were from direct observation of live and fresh samples from the hatchery-reared larvae. Hence, the observed yellowish pigmentation in *S. chiliensis chiliensis* during the early periods of larval development would correspond to the presence of xantoforos and non-melanophores. Our observations are supported by Ortega (2015) for *S. sarda*, in which pigmentation was visible towards the middle of the embryonic development, and several xantoforos were observed in the newly hatched larvae. But different from the afore-mentioned observations by Sanzo (1932) and Vodyanitsky (1936) for *S. sarda* larvae.

4.3. Morphometry of the different larval developmental stages

Ortega and de la Gándara (2007) described that the newly hatched *S. sarda* larvae measured 3.87 mm +/- 0.45 mm, and showed a fast larval growth allowing then a metamorphosis being completed by day 40, whereas the larvae of freshly hatched *S. chiliensis chiliensis* measured on average 4.116 mm +/- 0.529 and were slightly longer. On the other hand, McFarlane et al. (2000) noted that the eye diameter for stage 1 *Sarda chiliensis lineolata* larvae ranges between 0.34–0.36 mm, assuming that the lower range corresponds to newly hatched larvae. For freshly hatched *S. chiliensis chiliensis* larvae, an average eye height of 0.283 mm and an average eye length of 0.346 mm was recorded, the latter being concordant with that observed by McFarlane et al. (2000). No

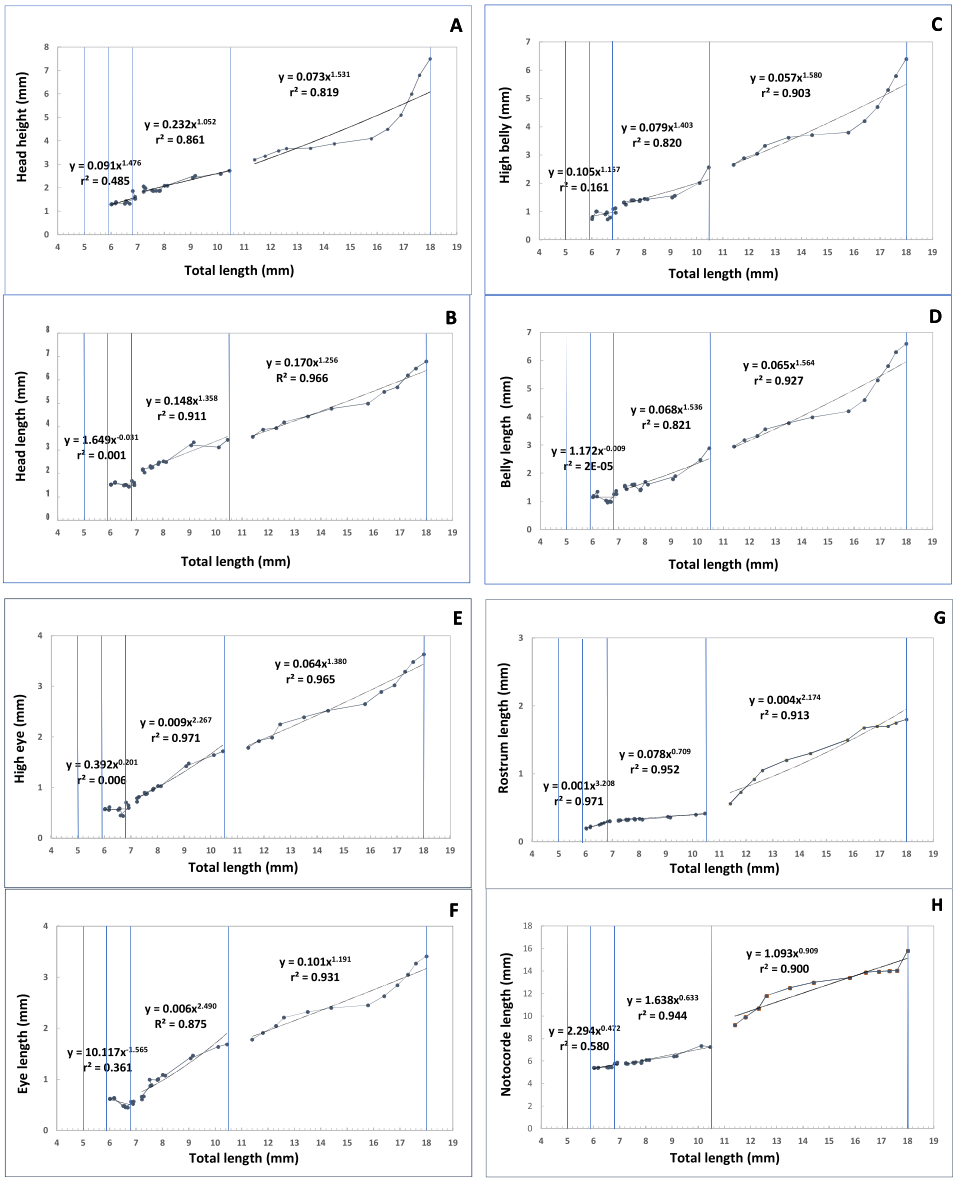


Fig. 7. Allometric growth equations and relationships for different body regions selected for total length in *Sarda chiliensis chiliensis* during development stages 3, 4 and 5.

information was found regarding the above indexes for *S. sarda*.

According to McFarlane et al. (2000), although the morphological and behavioral development occurred rapidly in the larvae of *Sarda chiliensis lineolata* during stages L1-L4, their length increased only slightly. Length increased fastest after the transition to stage L5. When larvae reached the juvenile stage, however, their length increased more slowly, and for *S. sarda*, the metamorphosis begins between 12 and 15 days post-hatch (also in L5), and continues through the juvenile stage from 20–25 days and was completed by day 40 post-hatch (Ortega and de la Gándara, 2007). In *S. chiliensis chiliensis*, this acceleration in length,

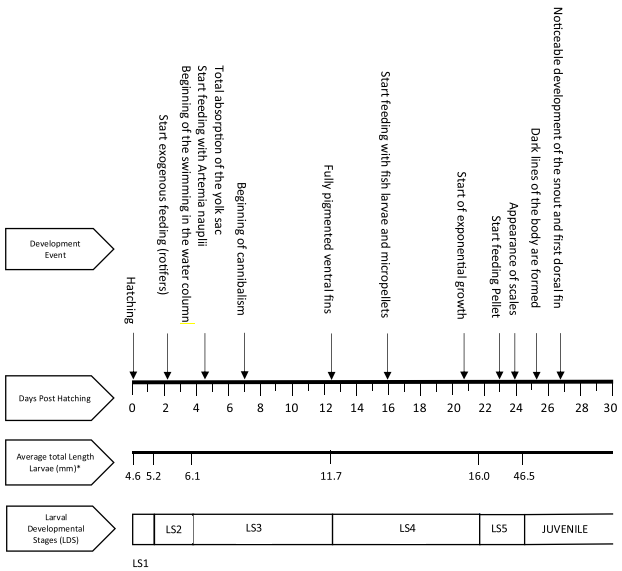


Fig. 8. Main developmental events of *Sarda chiliensis chiliensis* during larval periods.
* only larval stages are shown

which gives rise to the pre-juvenile stage, was observed after 519 h (~ 22 dph) and then slowed during the juvenile stage. This deceleration in the length of the fish was mainly due to morphological changes, which are inevitable in the larval development of all fish species. The energy available, at that time, was assigned to these metamorphosis processes, which are essential in this period of development (Miranda et al., 2014; Civero-Cerecedo et al., 2004). For *S. chiliensis chiliensis*, three growth trends can be observed (Fig. 4): one that goes from hatching to 495 h (~20.6 days); a second relatively short period, which goes from 495 to 519 h, where larval length increased up to 16 mm; and finally, a shorter period from 519 to 591 h, with a smaller growth increment, that gave rise to pre-juveniles of 18 mm. This development suggests that the metamorphosis starts from approximately 495 h (~20.6 days), which took longer than for the species mentioned. The differentiation of the dorsal and caudal fins would indicate the pre-juvenile stage, and a second metamorphosis would result in juveniles from 591 h (~24.6 days), at an average temperature of 23.75 °C. In the case of *S. sarda*, the growth was faster, reaching 25 mm by 20 days post-hatch. Studies carried out with *S. sarda* (Ortega and de la Gándara, 2009; Reglero et al., 2014) shown an advanced piscivorous feeding at 8–10 dph, which assumes an important improvement in both survival and growth. The addition of *M. cephalus* larvae (striped mullet) as a live-feed to *S. chiliensis chiliensis* did not start until day 16, which could explain the differences in growth in comparison to *S. sarda*.

The growth of *S. chiliensis chiliensis* shows three main stages after hatching (Fig. 4): larval, pre-juvenile, and juvenile. We complement the growth curve with the morphological characters and their respective statistical analyzes to better appreciate the development of the cephalic region and the internal structures. These two characters showed greater variability than that of increased length. The growth in weight of *S. chiliensis chiliensis* between egg hatching to the end of larval stage 4 (about 500 h) was approximately 6 g (0.012 g per day, slow growth). Then the growth in weight, between 500 h and almost 600 h, was greater than in the previous stage with an increase of about 8 g (0.08 g per day). There is exponential growth.

Concerning *S. chiliensis lineolata* and *S. sarda*, five larval stages, and one juvenile have been identified in their development stages (McFarlane et al., 2000; Ortega and de la Gándara (2007). For *S. chiliensis lineolata*, two discrete metamorphic events occurred during the developmental larvae stages (Youson, 1988). The metamorphic events were characterized by a rapid transition of the individuals into stage L5, which leads to the differentiation of the first dorsal and caudal fin. The criterion used to define these metamorphic events in other *Sarda* species also applies to determine the same event in *S. chiliensis chiliensis*, which was observed at approximately 519 h post-hatch (21.6 days).

In *Sarda chiliensis lineolata*, the metamorphosis was characterized by rapid larvae growth and the development of structures, particularly the trunk muscles, bony fins, and jaw. For *S. chiliensis*, an overall improvement in the locomotor capacity was as well apparent along with the transition to the first metamorphosis, which occurred in less than 12 h in larvae reared at 26 °C, while the continuity of the metamorphosis took a long time, and ends in a juvenile stage where the adult form was attained (McFarlane et al., 2000). For *S. sarda*, the metamorphosis started between 12 and 15 days post-hatch (also in L5), and it was completed by day 40 post-hatch, when the larva was already a juvenile, at an average temperature of 21 °C (Ortega and de la Gándara, 2007). The afore-mentioned authors defined the juvenile stage as a period that extends between 20–25 days after hatching. For *S. chiliensis chiliensis*, the first metamorphosis took longer to start since the differentiation of the dorsal and tail fins was recorded almost at 22 dph at an average temperature of 23.75 °C.

The formation of the first scales occurred in stage 5 for S. chiliensis chiliensis, which began in the cephalic and opercular region (Fig. 8). This change was accompanied by a differentiation of the fins and a retraction of the embryonic fin, with the rays exhibiting pigmentation, especially from the first dorsal fin and caudal fin. At this stage, the fish still did not have an adult form, yet the majority of the body was still translucent, thus corresponding to a pre-juvenile period reached between 495 (~21 days) and 591 h post-hatch (~25 days). McFarlane et al. (2000), as well as Ortega and de la Gándara (2007), reported that

the beginning of the first metamorphosis occurred in the larval stage, and the main common feature between both studies was the observed differentiation of the first dorsal fin and the caudal fin. In S. chiliensis chiliensis, the first metamorphosis occurred between 21 and 22 days of larval life and allowed the larvae to acquire an adult shape, starting with the differentiation of the first dorsal fin and therefore defining the beginning of the pre-juvenile stage (Fig. 8).

The juvenile period for *S. chiliensis chiliensis* was characterized by an almost complete scaling of the body, the adoption of a typical fusiform adult body, and the presence of the characteristic oblique dark stripes on the back of the body that extend towards the sides of the body. Other additional changes were a body becoming compressed, the spinules and the lateral line becoming defined along the body, and the formation of a pointed conical head with a large mouth, where the large conical teeth can be seen. All these morphological changes occurred after 600 h (25 days).

Most relevant characteristics for *S. chiliensis chiliensis* larval growth between 2 and 4 days post-hatching were the opening of the mouth and the exhaustion of the yolk sac and the oil globule, a period that coincides with the start of exogenous feeding and with the incipient development of the dentition. Just after 108 h post-hatch, the dentition is well developed (4.5 days). In this investigation, a relatively slow growth in length in stage 1–4 was quantified for *S. chiliensis chiliensis* compared to the rapid growth in length shown in stage 5 and 6 (Fig. 4).

4.4. Allometric growth

Considering that the genus *Sarda* is distributed in all the oceans of the world, *Sarda chiliensis chiliensis* is geographically located only on the coasts of the Southeast Pacific Ocean (Yoshida, 1980). Regarding the investigations carried out in the *Sarda* genus, there are a large number of articles published for the different problems, and however, there is no work described on allometric growth for the larvae of this species, despite having several publications on this theme for other species of fish. That said, it is of utmost importance for aquaculture to have this type of research results, which will help to have a better understanding of the biology of this resource, which can be used in different applications, mainly for the commercial cultivation of this species.

Like several species of marine fish, *Sarda chiliensis chiliensis* during its larval period shows an accelerated growth, where it experiences drastic changes both in its morphology, physiology, as well as in its functional systems (sensory, digestive, locomotor, and respiratory systems), all this to acquire the characteristics of an adult organism. In this research, we quantify that the structures of the body (i.e., development of the mandible, the pigmentation of the eye, and the development of the head in general) had faster growth in comparison with the fish growth in length. The different changes in size and body proportions observed in *S. ch. chiliensis* were related to changes in the development of the anterior and posterior regions of the body and swimming and feeding behavior. The scheme and behavior of the morphological determination described for *Sarda chiliensis chiliensis* from hatching to state six (Juvenile), passing through the sequences of morphological events, can be used to facilitate experimental research and to develop husbandry protocols for the commercial farming of this species. Civera-Cerecedo et al. (2004) reported that from the anatomical point of view, strong changes occur during specific periods in the life of the larvae, and these changes are correlated with the allometric growth observed in many fish species.

The negative allometric growth of the notochord during the development of the larval in 3 and 4 stages of *Sarda chiliensis chiliensis* gave way to slight growth, almost isometric, for larvae in stage 5. It is common, for the vast majority of fish species to develop the anterior (head) and posterior (tail) body regions before the abdominal region (Gisbert, 2003). Therefore, organs essential for primary functions (feeding, breathing, and locomotion) are developed first (Osse and van den Boogart, 1995; Papadakis et al., 2018).

The upper part of the eye showed negative allometric growth in phase three of the larval growth, and later in phase four and five, it had a rapid growth, which was allometrically positive. Gisbert et al. (2002); Gisbert and Doroshov (2006), and Khemis et al. (2012) reported similar trends for other species of marine fish. Positive allometric growth of the eye diameter is considered as an indicator of the development and differentiation of neuronal and sensory structures, allowing larvae to react to light stimuli, and to be able to detect zooplankton prey, and potential predators in the water column (Gisbert et al., 2002; Gisbert and Doroshov, 2006). In turbot (*Scophthalmus maximus*), cranium development is crucial during the larval stage primarily, and it was correlated with food ingestion and respiration, hence positive allometric growth occurred during the earlier stages and negative allometric growth during the later stages (Lv et al., 2019). Papadakis et al. (2018) reported that the eye ontogeny of meager (*Argyrosomus regius*) provides as well important information for the biology of the species, as well as for the optimization of larval rearing conditions regarding light management and prey item density under aquaculture conditions.

The head height presented, for the *S. ch. chiliensis* larval stages three to five, positive allometric growth and a rapid increase, as indicated by Gisbert et al. (2002) for this relationship. The rostrum and upper belly length showed a positive allometric growth except for the rostrum length of state four in which it shows negative allometric growth. For head, belly, and eye lengths, growth at larval stage three was allometrically negative. Later, in stages four and five of larval development, they grew very quickly with positive allometry. Khemis et al. (2012) explain that in phase three of larval development, a large number of physiological changes would be occurring, which would allow an energy flow to these functions and therefore limit the longitudinal growth of the larva (Osse and van den Boogart, 1995).

4.5. Behaviour larval

As in other marine fish, the larvae of *S. chiliensis chiliensis* with yolk sacs remained floating on the surface of the water. Once the yolk sac was reabsorbed, the larvae remained in the water column, agreeing with what was observed by Gisbert et al. (2002) for *Paralichthys californicus* and Ortega (2015) for *S. Sarda*.

5. Conclusion

Regarding the larval culture temperature, it is suggested that due to current and previous experiences with this research, as well as those carried out by McFarlane et al. (2000), a range between 22° to 26 °C, would be appropriate for the larval culture of this species.

The characteristics of larval development changes of the South Pacific Bonito *Sarda ch. chiliensis*, showed a strong relationship with characters related to the visual system (head height and eye height).

Therefore, according to the morphological and morphometric characteristics, six post-embryonic stages are proposed for this research:

- A first stage, which would run from 0 to 34 h: This stage is characterized by endogenous feeding, neutral flotation, and a single oil globule located at the rear end.
- A second stage that would run from 35 to 91 h: This stage is characterized by the end of endogenous feeding and the beginning of exogenous feeding, coincidentally with the pigmentation of the eyes (presence of iridophores)
- A third stage from 92 to 300 h: This stage is mainly characterized by the development of the cephalic region, which begins to cover almost a quarter of the total length.
- A fourth stage from 301 h to 495 h: This stage is characterized by the most homogeneous morphological development for the entire body, with a greater development of muscle bundles, which allows it greater mobility to capture its prey.

- A fifth stage (pre-juvenile) from 496 h to 591 h: This stage is mainly characterized by the appearance of scales that give the fish a silver luster, the upper and lower jaws are symmetrical.
- A juvenile stage of more than 592 h: This stage is mainly characterized by having the general characteristics of an adult, dark line on the back, typical of the *Sarda chiliensis chiliensis* species.

Regarding fish behavior, *Sarda chiliensis chiliensis* is aggressive and cannibalistic from stage three onwards, which makes it necessary to modify the standard marine fish feeding protocol.

This work is the first document for *Sarda chiliensis chiliensis* where allometric growth for larvae of this species is described. Besides, in this study are revealed for aquaculture purposes the different larval stages, their general behavior, growth, and a first tentative feeding protocol. Together they constitute a necessary knowledge that allows establishing the biological feasibility of the cultivation of *S. chiliensis chiliensis* in the north of Chile in water recirculation systems.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

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CAPÍTULO 5:

Conclusiones Generales

Conclusiones

1. Se estableció un primer plantel de reproductores de Bonitos del Pacífico Sur, *Sarda chiliensis chiliensis* capturados del ambiente salvaje. Este protocolo, podrían ser utilizadas para otras especies de peces pelágicos, principalmente túnidos de importancia comercial.
2. La metodología y resultados de la incubación de huevos de *Sarda chiliensis chiliensis* permitió actualizar y estandarizar los antecedentes hasta ahora publicados, lo que nos permitió obtener un mayor conocimiento del desarrollo embriológico de esta especie, como por ejemplo, el tiempo de eclosión de los huevos, el cual no supero las 72 horas.
3. Se establecieron 6 estado de desarrollo larvario del Bonito del Pacífico Sur, en un tiempo de poco mas de 592 horas. Durante el periodo de desarrollo larval las proporciones corporales medidas y las tasas de crecimiento cambiaron de forma variable.
4. Las tres investigaciones expuestas en esta tesis establecieron la “factibilidad biológica” de conformar un stock de reproductores a partir de una acuicultura basada en pesquerías, y que además se logró un desove espontáneo en menos de un año de cautiverio, generando huevos viables que llevo como resultado, la eclosión de larvas, también viables.
5. En la presente tesis, se entregan resultados concluyentes para considerar a esta especie, como una verdadera alternativa de cultivo comercial en el norte de Chile y así diversificar la acuicultura nacional.

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