

Frontiers Media SA

Avenue du Tribunal-Federal 34
1005 Lausanne, Switzerland
VAT Number CHE-114.168.540 TVA
www.frontiersin.org

Invoice

Invoice #: 2023-1038822-0
Date Issued: 18 Oct 2023
Date Due: 17 Nov 2023
Our Reference:

For information:
accounting@frontiersin.org
Tel +41 21 510 17 03

Bill to:
Noemi Cota
Institute of the Sea of Peru (IMARPE)
Esquina Gamarra y General Valle S/N
07021 Callao
Peru

Description of Services:

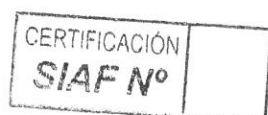
Prepayment Invoice for Ms 1287439

"Reproductive performance and egg and larvae quality from first generation peruvian grunt *Anisotremus scapularis* (Tschudi, 1846): a comparative analysis with spawns from wild broodstock", by Noemi Cota*, Lili Jannet Carrera Santos, Angélica Castro, Oneill Leon Dominguez, Leenin Flores Ramos, Anthony Ruiz Soto, Juan Pablo Lazo and Neil Duncan

	Unit Price USD	Qty	Total USD
Pre-payment	2141.75	1	2141.75
Sub Total			2141.75
VAT*			0.00
Total			USD 2141.75

* 7.7% VAT is payable on all invoices addressed to Swiss- or Liechtenstein-resident persons or organizations.

Please see next page for Payment Options and Instructions and Frequently Asked Questions



Payment Options and Instructions

- **per Credit Card online (Visa, Mastercard, Diners)**

Log on to your Frontiers account, mouse over your name, select >Invoices >View Details >Proceed to Payment.
Alternatively, please call us at +41 21 510 17 03 (during office hours Central European Time, GMT+1).

- **per direct Bank Transfer**

Beneficiary name:	Bank address:	IBAN:	Account number:
Frontiers Media SA	UBS Switzerland AG	CH800024324347968870K	243-479688.70K
Beneficiary address:	Place St Francois 16	BIC/SWIFT:	Reason for payment (required):
Avenue du Tribunal-Federal 34	CH-1003 Lausanne	UBSWCHZH80A	Invoice n. 2023-1038822-0
1005 Lausanne, Switzerland	Switzerland		

Frequently Asked Questions

- **Can I pay by Purchase Orders?**

Please email (to accounting@frontiersin.org) or fax (to +41 21 51 01 701) your purchase order to have the invoice re-issued accordingly. An email address is required for electronic delivery.

- **How can I modify the invoice?**

Log on to your Frontiers account, mouse over your name, select >Invoices >View Details >Modify Payer, then edit:

- the "Billing Address" section to modify the billing address;
- the "Your Reference" section to add other information needed by your institution (such as VAT number, Purchase Order number, etc);
- the "Email this invoice to" section to send this invoice to another person for payment.


- **I am not a Registered User, what can I do?**

If the invoice was issued directly to your attention, please consider registering at www.frontiersin.org (with same email address) to have access to full features and functionality. Registration is completely for free!
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CALCULO DE RETENCIONES E IMPUESTOS POR SERVICIO DE NO DOMICILIADO

PROVEEDOR	:	FRONTIERS MEDIA SA
PRODUCTO/SERVICIO	:	Servicio de publicación de artículo científico en revista internacional
INVOICE N°	:	2023-1038822-0 del 18.10.2023
REFERENCIA	:	Correo electrónico del Sr. Anthony Sandoval – ÁFLel del 10 y 13 de noviembre del.2023

CONCEPTOS	Dólar USD \$	T/C del 10/11/2023	Soles S/	% Tributos	Montos a Pagar
MONTO DE LA COTIZACIÓN	2,141.75	3.813	8,166.49		
RETENCIÓN DE IMPTO A LA RENTA NO DOM					-
MONTO A PAGAR AL PROVEEDOR	2,141.75		8,166.49		8,166.49
IGV NO DOMIC	385.52		1,469.97	18	1,469.97



.....
CPC. María Elena Gonzales Saba
IMARPE
AFT-Tributos

Callao, 13 de noviembre 2023


RV: Request - Invoice # 2023-1038822-0

Rene Anthony Sandoval Lizana <rsandoval@imarpe.gob.pe>

Lun 13/11/2023 8:27

Para: Maria Elena. Gonzales Saba <mgonzales@imarpe.gob.pe>

CC: Noemi Jackelith Cota Mamani <ncota@imarpe.gob.pe>; Haydee Saenz Apari <hsaenz@imarpe.gob.pe>

 1 archivos adjuntos (46 KB)

2023-1038822-0 (3).pdf;

Estimada Sra. Maria Elena,

Por medio de la presente, reitero la solicitud de poder realizar la modificación del cálculo de tributos y/o impuestos aplicables a la contratación de referencia, teniendo en cuenta que el monto neto de impuestos a recibir por el proveedor asciende a la suma de \$ 2141.75.

Asimismo, favor de incluir un margen adicional (de ser factible) en el tipo de cambio, con la finalidad de poder cubrir la variación constante del tipo de cambio.

Quedo atento a sus comentarios.

Saludos cordiales.

Atentamente,

Rene Antony Sandoval Lizana

Área de Programación e Información - Área Funcional de Logística e Infraestructura

Instituto del Mar del Perú - IMARPE

Esquina Gamarra y General Valle S/N, Chucuito - Callao

Telf. 2088650 Anexo 895

**IMARPE**
INSTITUTO DEL MAR DEL PERÚ**PERÚ**Ministerio
de la Producción**De:** Rene Anthony Sandoval Lizana <rsandoval@imarpe.gob.pe>**Enviado:** viernes, 10 de noviembre de 2023 8:32**Para:** Maria Elena. Gonzales Saba <mgonzales@imarpe.gob.pe>**Cc:** Noemi Jackelith Cota Mamani <ncota@imarpe.gob.pe>**Asunto:** RV: Request - Invoice # 2023-1038822-0

Estimada Sra. Maria Elena,

De acuerdo a lo coordinado, solicito a Ud. poder realizar la modificación del cálculo de tributos y/o impuestos aplicables a la contratación de referencia, teniendo en cuenta que el monto neto de impuestos a recibir por el proveedor asciende a la suma de \$ 2141.75.

Asimismo, favor de incluir un margen adicional (de ser factible) en el tipo de cambio, con la finalidad de poder cubrir la variación constante del tipo de cambio.

Quedo atento a sus comentarios.

Saludos cordiales.

Atentamente,

Rene Antony Sandoval Lizana

Área de Programación e Información - Área Funcional de Logística e Infraestructura

Instituto del Mar del Perú - IMARPE

Esquina Gamarra y General Valle S/N, Chucuito - Callao

Telf. 2088650 Anexo 895



IMARPE
INSTITUTO DEL MAR DEL PERÚ



PERÚ

Ministerio
de la Producción

De: Noemi Jackelith Cota Mamani <ncota@imarpe.gob.pe>

Enviado: viernes, 10 de noviembre de 2023 7:47

Para: Rene Anthony Sandoval Lizana <rsandoval@imarpe.gob.pe>

Cc: Evelyn Fiestas Querevalú <efiestas@imarpe.gob.pe>; Lili Carrera Santos <lcarrera@imarpe.gob.pe>

Asunto: Re: Request - Invoice # 2023-1038822-0

Estimado Anthony

El monto de 2141.75 es el monto correcto para su pago a FRONTIERS MEDIA SA por la publicación del artículo del Laboratorio de Cultivo de Peces.

Saludos

Noemi Cota

Obtener [Outlook para iOS](#)

De: Rene Anthony Sandoval Lizana <rsandoval@imarpe.gob.pe>

Enviado: miércoles, noviembre 8, 2023 2:57 p. m.

Para: Noemi Jackelith Cota Mamani <ncota@imarpe.gob.pe>

CC: Evelyn Fiestas Querevalú <efiestas@imarpe.gob.pe>

Asunto: RE: Request - Invoice # 2023-1038822-0

Buenas tardes estimada Noemi Cota,

Por medio de la presente se solicita a Ud. poder confirmar si el pago a la empresa FRONTIERS MEDIA SA, neto de impuestos es de 2141.75 dólares.

Favor de confirmar lo indicado para continuar con el procedimiento correspondiente.

Atentamente,

Rene Antony Sandoval Lizana

Área de Programación e Información - Área Funcional de Logística e Infraestructura

Instituto del Mar del Perú - IMARPE

Esquina Gamarra y General Valle S/N, Chucuito - Callao

Telf. 2088650 Anexo 895



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Ministerio
de la Producción

De: Noemi Jackelith Cota Mamani <ncota@imarpe.gob.pe>

Enviado: lunes, 6 de noviembre de 2023 10:26

Para: Rene Anthony Sandoval Lizana <rsandoval@imarpe.gob.pe>

Cc: Lili Carrera Santos <lcarrera@imarpe.gob.pe>; Diana Villalobos Salas <dvillalobos@imarpe.gob.pe>

Asunto: RV: Request - Invoice # 2023-1038822-0

Estimado Anthony

La semana pasada escribimos a Frontiers para la modificación del INVOICE y puedan considerar el IGV; sin embargo, no han respondido, lo más probable es que no sea posible.

Ya que el INVOICE tiene un vencimiento al 17 de noviembre, comentarte que el año pasado también realizamos una transferencia internacional con el Sr. Sovero, donde él emitió 2 órdenes de servicio, una con el monto que le debe llegar al proveedor y otro con por el pago del IGV. De esta manera no fue necesario que la empresa, en ese caso fue la WAS (World Aquaculture Society), realice una modificación de los INVOICE emitidos para la inscripción a un congreso. Adjunto las órdenes de servicios comentadas.

Por favor para tu revisión

Muchas gracias por el apoyo en la gestión

Saludos

--

M. Sc. Noemi Jackelith Cota Mamani

Laboratorio Cultivo de Peces

Área Funcional de Investigaciones en Acuicultura

Dirección General de Investigaciones en Acuicultura

Instituto del Mar del Perú

IMARPE

De: Noemi Jackelith Cota Mamani <ncota@imarpe.gob.pe>

Enviado: martes, 31 de octubre de 2023 14:50

Para: accounting@frontiersin.org <accounting@frontiersin.org>

Cc: Rene Anthony Sandoval Lizana <rsandoval@imarpe.gob.pe>; Lili Carrera Santos <lcarrera@imarpe.gob.pe>;

Diana Villalobos Salas <dvillalobos@imarpe.gob.pe>

Asunto: Request - Invoice # 2023-1038822-0

Dear Mrs Wilgus,

13/11/23, 10:31

Correo: Rene Anthony Sandoval Lizana - Outlook

We would like to request some changes to be made on the invoice # 2023-1038822-0 from Oct 18th . Due to local taxation policies we need to withhold income taxes on payments. So, in order for you to receive the due amount (2141.75), the invoice should be changed as follow:

- Unite price USD should be 2519.71, same goes the pre-payment total and the sub total.
- A line should be added below VAT : Income Tax Withheld (- 377.96)

Which should bring the total to 2141.75 (same as it is right now). We are going to pay the 377.96 in taxes and transfer to you 2141.75.

If you had any questions, please do not hesitate to contact us.

Thank you in advance.

Best regards,

Rene Anthony Sandoval Lizana
Logistics Office of IMARPE

--

M. Sc. Noemi Jackelith Cota Mamani
Laboratorio Cultivo de Peces
Área Funcional de Investigaciones en Acuicultura
Dirección General de Investigaciones en Acuicultura
Instituto del Mar del Perú
IMARPE

De: Rene Anthony Sandoval Lizana <rsandoval@imarpe.gob.pe>

Enviado: lunes, 30 de octubre de 2023 16:22

Para: Noemi Jackelith Cota Mamani <ncota@imarpe.gob.pe>

Asunto: INVOICE

Estimada Noemi,

Por medio de la presente se remite el monto a ser cotizado por el proveedor para que se le pueda pagar el total de \$ 2141.75

Costo del Servicio: 2519.71

Retención del Impuesto a la Renta: 377.96

Monto a pagar al Proveedor: 2141.75

Quedo atento al envío del nuevo Invoice, para continuar con el tramite correspondiente.

Saludos cordiales.

Atentamente,

Rene Antony Sandoval Lizana
Área de Programación e Información - Área Funcional de Logística e Infraestructura

RE: Solicitud de Cotización N° 1580-2023-IMP, en referencia al "SERVICIO DE SECUENCIAMIENTO DE ADN DE ALGAS"

Frontiers Accounting Support <accounting@frontiersin.org>

Lun 30/10/2023 7:53

Para: Rene Anthony Sandoval Lizana <rsandoval@imarpe.gob.pe>

CC: Noemi Jackelith Cota Mamani <ncota@imarpe.gob.pe>

Hello Rene Anthony Sandoval Lizana,

Thank you for your email.

We would be happy to assist with your query; please provide us with your Manuscript ID or Invoice # so we may investigate your request further, and provide you with the quotation as requested.

Best regards,

Bonnie Wilgus

Frontiers | Accounting Office
Avenue du Tribunal-Fédéral 34
CH-1005 Lausanne, Switzerland

T +41 (21) 510 17 03

----- Original Message -----

From: Rene Anthony Sandoval Lizana [rsandoval@imarpe.gob.pe]

Sent: 27.10.2023 15:58

To: accounting@frontiersin.org

Cc: ncota@imarpe.gob.pe

Subject: Solicitud de Cotización N° 1580-2023-IMP, en referencia al "SERVICIO DE SECUENCIAMIENTO DE ADN DE ALGAS"

Estimado Proveedor:

Es grato dirigirme a usted para alcanzar la **Solicitud de Cotización N° 1580-2023-IMP**, en referencia al "SERVICIO DE PUBLICACIÓN DE ARTÍCULO CIENTÍFICO EN REVISTA INTERNACIONAL", según el detalle adjunto.

Asimismo, para consultas técnicas y de visita respecto al servicio, comunicarse con:

- Noemi Cota - ncota@imarpe.gob.pe

Agradeceré remitir su respuesta a este correo electrónico en un plazo no mayor de 02 días hábiles de recepcionado el presente correo.

Quedo a la espera de su pronta respuesta.

IMPORTANTE

El IMARPE aprobó y publicó en su portal la Directiva N° 002-2018-IMARPE/SG/OGPP – “CONTRATACIÓN DE BIENES Y SERVICIOS CUYOS MONTOS SEAN IGUALES O MENORES A 8 UIT EN EL IMARPE”, aprobada mediante Resolución de Secretaría General N° 028-2018-IMARPE/SG de fecha 03 de agosto de 2018, la cual rige a partir de su aprobación - "Revisar el numeral 7.5.- APLICACION DE PLAZO Y PENALIDADES".

Atentamente,

Rene Antony Sandoval Lizana

Área de Programación e Información - Área Funcional de Logística e Infraestructura

Instituto del Mar del Perú - IMARPE

Esquina Gamarra y General Valle S/N, Chucuito - Callao

Telf. 2088650 Anexo 895



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ref:!00D580JetR.!5008e0JfDT5:ref

Fwd: Visto de TDR

Noemi Jackelith Cota Mamani <ncota@imarpe.gob.pe>

Vie 27/10/2023 8:26

Para: Rene Anthony Sandoval Lizana <rsandoval@imarpe.gob.pe>

CC: Lili Carrera Santos <lcarrera@imarpe.gob.pe>; Diana Villalobos Salas <dvillalobos@imarpe.gob.pe>

 1 archivos adjuntos (405 KB)

EE TT para publicación de artículo científico - oct. 2023.pdf;

Estimado Anthony

Envío las especificaciones técnicas coordinadas para el trámite correspondiente.

Muchas gracias por tu apoyo en la gestión

Saludos

Noemi Cota

Obtener [Outlook para iOS](#)

De: Lili Carrera Santos <lcarrera@imarpe.gob.pe>

Enviado: Friday, October 27, 2023 8:23:22 AM

Para: Noemi Jackelith Cota Mamani <ncota@imarpe.gob.pe>

Asunto: Fwd: Visto de TDR

Obtener [Outlook para Android](#)

From: Victor Yopez Pinillos <vyopez@imarpe.gob.pe>

Sent: Friday, October 27, 2023 12:07:15 AM

To: Lili Carrera Santos <lcarrera@imarpe.gob.pe>

Cc: Jorge Flores Mego <jflores@imarpe.gob.pe>; Jorge Flores <jafm3377@gmail.com>

Subject: RV: Visto de TDR

Lili:

Alcanzo el TdR visado, para el trámite correspondiente.

Saludos,

Víctor.

De: Lili Carrera Santos <lcarrera@imarpe.gob.pe>

Enviado: miércoles, 25 de octubre de 2023 9:23

13/11/23, 10:30

Correo: Rene Anthony Sandoval Lizana - Outlook

Para: Victor Yepez Pinillos <vyopez@imarpe.gob.pe>

Cc: Jorge Flores Mego <jflores@imarpe.gob.pe>

Asunto: Visto de TDR

Estimado Sr. Yépez,

Para el trámite de la publicación del proyecto, el logístico ha solicitado hacer un TDR, el cual adjunto para su visto y dar continuidad al trámite.

Gracias,

Lili

1 **Reproductive performance and egg and larvae quality from**
2 **first generation peruvian grunt *Anisotremus scapularis***
3 **(Tschudi, 1846): a comparative analysis with spawns from**
4 **wild broodstock**

5
6 Noemi Cota^{1*}, Lili Carrera¹, Angélica Castro¹, Oneill León¹, Leenin Flores², Anthony
7 Ruiz², Juan Pablo Lazo³ and Neil Duncan⁴

8
9 ¹Laboratorio de Cultivo de Peces, Instituto del Mar del Perú (IMARPE), Callao, Perú

10 ²Laboratorio de Análisis Instrumental, Instituto del Mar del Perú (IMARPE), Callao,
11 Perú.

12 ³Centro de Investigaciones Científicas y de Educación Superior de Ensenada (CICESE),
13 Ensenada, Baja California, México.

14 ⁴Instituto de Investigación y Tecnología Agroalimentaria (IRTA), Sant Carles de la
15 Ràpita, Tarragona, Spain.

16
17 *** Correspondence:**

18 Noemi Cota

19 ncota@imarpe.gob.pe

20
21 **Abstract**

22
23 *Anisotremus scapularis* is a marine fish distributed along the Peruvian coast that is
24 commercially important in local markets. IMARPE started research on this species aimed
25 to condition breeders and control reproduction in captivity and avoid dependence on wild
26 broodstock. Therefore, the present study characterized and compared the reproductive
27 performance, quality of eggs and larvae between a first generation domesticated
28 broodstock (F1B) and a wild broodstock (WB). The F1B and WB broodstocks were
29 conditioned in recirculation systems (RAS) to spawn naturally from August 2021 to
30 August 2022. The following parameters were recorded for each spawn, the total number
31 of eggs, percentages of viable eggs and fertilization, and hatching, and biochemical
32 analyses on each spawn were performed. Progeny from each spawning were assessed for
33 survival activity index (SAI), and at 20 days post-hatch (DPH), the temperature tolerance
34 index (TTI) was analyzed. Wild group, WB had significantly higher ($P<0.05$) egg
35 production, egg diameter, oil globule, and SAI than those of F1B. However, the
36 percentage of viable eggs and hatching showed significantly higher values ($P<0.05$) in
37 F1B spawns. A low correlation ($r<0.50$) was observed between the evaluated
38 reproductive parameters in *A. scapularis* broodstock; nevertheless, it was significant in
39 some cases. No significant differences were observed in protein, lipid, carbohydrate, or
40 ash content in eggs, but ash content in the 20 DPH larvae differed, with higher values in
41 F1B. A principal component analysis (PCA), indicated a strong relationship between F1B
42 broodstock eggs and fatty acids DHA, 16:0, 18:1n-9, and 18:2n-6; whereas fatty acids
43 18:0, 20:4n-3, 22:5n-3, and EPA had a high connection with WB eggs. At 20 DPH, F1B
44 larvae had a strong link with 18:1n-9, DHA, and 18:2n-6; while WB larvae were found
45 to have a high association with 16:1n-7, EPA, 22:5n-3, and ARA. Based on these results
46 we concluded that F1B broodstock has better egg quality and similar larval quality
47 parameters compared to WB, making the F1 domesticated broodstock (F1B) an
48 alternative to establish a broodstock under captive conditions and thus contributing to the
49 aquaculture development of the species.

Keywords: native marine fish, captive broodstock, egg quality, larval quality, fatty acids.

1. Introduction

The peruvian grunt *Anisotremus scapularis* is a marine fish distributed along the coasts of Ecuador, Peru, and Chile, from Manta (Ecuador) to Antofagasta (Chile), in rocky areas at depths of up to 25 m (Chirichigno and Vélez, 1998). It is a resource highly valued in Peru for its commercial and gastronomic qualities, known for the quality of its meat and high fillet yield (30% of total weight). *Anisotremus scapularis* also sustains artisanal coastal fisheries along the entire Peruvian coastline (Mendo and Wosnitza-Mendo, 2014). In relation to this, the National Aquaculture Policy to 2030 (Ministerio de la Producción, 2023) and the "Roadmap for Marine Fish Farming in Peru" ["Hoja de Ruta del Cultivo de Peces Marinos en el Perú"] (Molares, 2021) stressed the need to support and direct research towards the technological development of an aquaculture based on prioritized species, such as *A. scapularis*. Therefore, it is important to achieve a sustained production of seed and juveniles, and for this purpose having captive broodstock and controlling reproduction are key aspects to achieve this goal.

In this respect, it is necessary to consider that the development of a sustainable aquaculture industry requires closing the culture cycle in captivity. Closing the lifecycle of any potential species and initiating a breeding selection program is essential and is best achieved establishing broodstock reared in captivity from juveniles (Jeréz et al., 2018). The production of domesticated broodstock batches reduces the pressure on wild populations, moreover it serves as the underpinnings for successful farming operations in many countries, especially in Asia and the Mediterranean (Ibarra-Castro and Álvarez-Lajonchere, 2011). Additionally, the costs of feeding and maintaining broodstock are high, which is why it is important to develop methodologies to compare different stocks of broodstock in order to select individuals with high reproductive capacity (Quiñones-Arreola et al., 2015). In this regard, the quality of spawn is defined as the potential of an egg to hatch and develop into a viable larva (Kjorsvik et al., 1990; Brooks et al., 1997). In fish, the quality of eggs and larvae is highly variable and is influenced by several factors including nutritional status, stress, over-ripeness, induction of spawning, genetics and age of the broodstock, as well as water quality and other environmental factors such as photoperiod, temperature, and salinity (Ceccon et al., 2012), and the biochemical composition of eggs (Wright-Moore et al., 2019).

Therefore, developing reliable criteria to characterize egg quality and select spawns that produce highly viable offspring, is fundamental to evaluate and establish growout protocols. Extensive research on egg quality has been conducted in recent decades (Migaud et al., 2013; Bobe and Labbé, 2010; Brooks et al., 1997; Kjorsvik et al., 1990). However, the success of these efforts is still moderate in marine fish (Thorsen et al., 2003), and comparisons between studies are difficult due to the lack of standardization of methodologies and criteria used to define egg quality. Most studies include fertilization and hatching rates when evaluating egg quality, as recommended by Brooks et al. (1997).

The Instituto del Mar del Perú (IMARPE) initiated research on the conditioning and captive reproduction of *A. scapularis*, achieving the first spawns and, in recent years, improving the cultivation of larvae and juveniles. However, seed production relies on a batch of broodstock composed by specimens captured from the natural environment.

Therefore, in order to improve the production of eggs and larvae under laboratory conditions, we propose as the main objective of this study, the conditioning and characterization of the reproductive performance and spawn quality of a first-generation broodstock born in captivity (F1), and compare egg and larval quality with spawns from wild broodstock.

2. Material and methods

2.1 Broodstock conditioning and spawning

Broodstock fish were conditioned in a seawater recirculation systems (RAS) composed of 2 set of tanks with a total water capacity of 2.5 m³, a water pump (3/4 HP, IWAKI, Japan), a heat pump (9500 BTU, Aqua Logic, USA), a biofilter (4.5 ft³ floating bed filter, Aqua Logic, USA), and a seawater sterilizer with ultraviolet lamp (Emperor Aquatics, Aqua Logic, USA) (Carrera et al., 2018). Sixty first-generation (group F1) Peruvian grunts broodstock were housed in recirculation system 1 (RAS 1), with females weighting on average 439.78 ± 111.72 g with 27.16 ± 1.80 cm in length and males 409.10 ± 2.54 g with 26.62 ± 144.42 cm. Broodstock had been cultured for 5 years (group F1B) prior to the experiment. The wild broodstock group (WB, 30 specimens) were placed in system 2 (RAS 2) with females averaging 727.72 ± 248.59 g and 31.05 ± 4 cm and males 599.55 ± 200.75 g and 29.54 ± 3.56 cm. The sex ratio in the tanks was 1:1 in both broodstock batches. Based on the gonadosomatic index variation reported for the species in their natural environment and data that was obtained from daily satellite monitoring of oceanographic parameters in the Peruvian sea by the Remote Sensing Functional Area of IMARPE and from the National Oceanic and Atmospheric Administration (NOAA) solar calculations, fish were subjected to a natural photoperiod and thermoperiod (ranging from 11 to 13 hours of light and 17 to 20°C).

Broodstock were fed three times a week with Peruvian anchovy (*Engraulis ringens*) chunks supplemented with gelatin capsules containing vitamins and omega-3 fatty acids (Cota et al., 2022), at a feeding ratio between 4% and 5% of the total biomass for each culture tank. Both *A. scapularis* broodstock groups spawned naturally, and eggs collected from the adjacent 100 L collection tanks attached to each culture system, which were fitted with 500 µm mesh bags. Egg production was monitored every morning from August 2021 to August 2022 for WB and from September 2021 to August 2022 for F1B. Physicochemical parameters and nitrogen compounds of the culture systems were monitored every other day. The temperature, oxygen level, and pH were maintained at averages of $18.78 \pm 0.79^\circ\text{C}$, 6.65 ± 1.21 mg L⁻¹, and 7.30 ± 0.56 , respectively. Nitrogen compounds values for total ammonia nitrogen were (0.09 ± 0.15 mg L⁻¹), for nitrite (0.34 ± 0.24 mg L⁻¹), and for nitrate (73.59 ± 52.50 mg L⁻¹).

2.2 Reproductive Performance and Egg Quality Evaluation

Eggs were collected in 10 L containers from the spawn collection tanks, immediately rinsed on 500 µm mesh sieves using abundant UV sterilized seawater. The eggs were then transferred to 1 L beakers and samples were taken for counting the total number of eggs per spawn.

To estimate the percent fertilization rates, three aliquots of 50 eggs each were taken and placed in Petri dishes, in each of which the number of eggs showing 2-64 blastomeres

were counted using an optical microscope (Leica, Germany) and the LAS 3.4 image program.

Fertilization rate was calculated with equation 1:

$$\text{Fertilization rate (\%)} = Nh \times 100 / Nt \quad \dots \quad \text{Equation 1}$$

Where:

Nh = Number of eggs with 2-64 blastomeres,

Nt = Total number of eggs.

Then, the percentage of viable (floating) and non-viable (non-floating) eggs was determined. Additionally, the diameter of the eggs and the oil droplet were measured using the LAS 3.4 image program and an optical microscope (Leica, Germany).

Hatching rates (%) were calculated using 50 fertilized eggs, placed in 500 mL beakers for incubation at 19°C, and the number of live larvae counted, approximately 40 hours after fertilization, and the following equation 2 used:

$$\text{Hatching rate (\%)} = LV \times 100 / (LV + LM + HM) \quad \dots \quad \text{Equation 2}$$

Where:

LV = Number of live larvae,

LM = Number of dead larvae,

HM = Number of remaining eggs.

2.3 Evaluation of larval quality

One spawn for every month from October 2021 to July 2022 (with fertilization rate > 80%) was selected from each broodstock group to assess larval quality. Larvae were cultured from day 0 DPH until 20 DPH, which corresponds to the rotifer feeding stage. The larval culture was performed using our well established protocol from the IMARPE's Fish Culture Laboratory (Castro et al., 2021). Briefly, larvae were transferred to 0.5-0.7 m³ fiberglass tanks, filled with 50% of the total volume and fitted with air stones to provide gentle aeration. Additionally, the tanks were inoculated with "green water", using a mixture of microalgae, *Isochrysis galbana* and *Nanochloropsis oculata* (ratio 1:4). Larvae were cultured at a temperature of 19°C, under static conditions (no water flow), and a photoperiod of 24 hours of light (HL): 0 hours of darkness (HO) during the first 10 DPH, followed by 12 HL: 12 HO until 20 DPH using a white fluorescent lights (1000 lux).

Larval quality was assessed using larval tolerance to starvation with the Survival Activity Index (SAI) following the procedure described by Shimma and Tsujigado (1981). Briefly, three replicates of 30 newly hatched larvae were placed in 1 L beakers without feeding, and the cumulative mortality was recorded daily, using equation 3:

$$SAI = 1/N \sum_{i=1}^k (N - h_i) * i \quad \dots \quad \text{Equation 3}$$

Where:

N = Total number of larvae,

h_i = Cumulative mortality on the i -th day,
 k = Number of days elapsed until all larvae die due to starvation.

The SAI also indicates the cumulative mortality on the 3rd day after hatching. This is a potential indicator of larval quality as it reflects the quality of endogenous reserves contained in the yolk sac and the potential for larval survival (Giménez et al., 2006).

Additionally, the quality of the larvae was evaluated at the end of the experiment, on the 20 DPH, using the temperature tolerance test developed by Dhert et al. (1992), which can be considered an indicator of survival capacity in their natural environment related to the resistance inherited from the parental broodstock and the quality of the feeding during the firsts DPH (Santamaria-Miranda et al., 2021; Fuentes, 2014). The test involved subjecting the larvae to acute stress at a temperature of 32°C. For this, 500 mL beakers with seawater from the culture systems were used, which were placed inside an 80 L plastic container with the same culture water (water bath) and a 1000-watt heater to maintain a constant water temperature of 32°C. When the selected temperature was reached, 5 larvae from each tank (in duplicate) were placed in each beaker. Mortality was recorded every 5 minutes for 120 minutes. Larvae were considered dead if they appeared opaque, with a bent body, and unresponsive to any contact stimulus. The temperature tolerance index (TTI) was calculated by adding the cumulative mortalities recorded in each mentioned time interval.

During the larval culture, larval growth was monitored by measuring their length in millimeters using the LAS 3.4 image program and an optical microscope coupled with a digital camera. The larvae were anesthetized with MS-222, and their measurements were taken on the 1, 15, and 20 DPH. Survival was evaluated at the beginning and at the end of the larval culture.

2.4 Biochemical analysis

The samples for biochemical analysis were obtained from 3 spawns from each group (WB and F1B) every month during the study (October 2021 to August 2022), as well as from the larvae collected at the end of the larval culture (October 2021 to July 2022). Approximately 10 g of viable eggs and 5 g of 20 DDE larvae per sample were collected using a 500 μ m sieve and rinsed with distilled water. They were then placed in 50 mL Falcon tubes and frozen at -80°C until further analysis.

The assays were conducted by the Instrumental Analysis Laboratory of IMARPE. Total soluble proteins were estimated using the Hartree method (1972), total carbohydrates were determined using the spectrophotometric method (Dubois et al., 1956), and total lipids were assessed using a modified Folch method (Folch et al., 1957) by IMARPE, which involves lipid extraction using a Chloroform-Methanol 2:1 solvent mixture with ultrasound assistance. The fatty acids were then identified using a gas chromatograph (Varian, Netherlands) according to Ichihara and Fukubayashi (2010). For moisture analysis, 0.1 g of sample was used, which was dried in a vacuum oven at a temperature of 105°C for 16 hours and a pressure below 0.1 bar. In the case of ash analysis, 0.1 g of sample was incinerated in a muffle furnace at 550°C for 16 hours. The amino acids were hydrolyzed with 6 M HCl for 22 hours at 112°C in a block heater, then derivatized according to the Waters manufacturer's kit, and analyzed by HPLC-FL (Hitachi, Japan).

2.5 Statistical Analysis

The data were expressed as mean and standard deviation. Statistical comparisons of reproductive performance and indicators of egg and larval quality were performed between different batches of broodstock (WB and F1B) using the Student t-test and correlations were assessed using the Pearson test (Schober et al., 2018). Normality was tested using the Shapiro-Wilk statistic, and homoscedasticity of variances was tested using the Bartlett test. The non-parametric Mann-Whitney test was applied to variables that did not meet the assumptions of the Student t-test. To determine whether the fatty acid or amino acid profiles of the eggs could be used to differentiate spawns from wild and cultured broodstock (F1), a principal component analysis (PCA) and discriminant analysis were conducted. A significance level of 0.05 was used for all analyses, and the data were processed using the R statistical software (R Core Team, 2022).

3. Results

3.1 Reproductive performance and egg quality evaluation

Egg production during the study period in both F1B and WB groups is shown in Figure 1. Production from wild specimens was significantly higher ($P < 0.05$) than that of the F1 generation (Figure 1). On average, wild specimens spawned a total of $90,804 \pm 77,273$ eggs per month, equivalent to $206,372 \text{ eggs kg}^{-1} \text{ female}$, while the F1 generation spawned $50,688 \pm 30,598$ eggs, equivalent to $115,200 \text{ eggs kg}^{-1} \text{ female}$. In terms of the number of spawns during the monitoring period, the F1B group produced 147 spawns, and the wild specimens produced 197 spawns (Table 1 and Figure 1).

Egg viability resulted in significant differences between groups; WB reached an average of $58.88 \pm 24.55\%$, while F1B showed $66.57 \pm 21.99\%$, with the latter being significantly higher. In terms of egg diameter and oil droplet size, a similar trend was observed in both cases; however, significant differences were found with larger values in wild specimens, with an average egg diameter of $0.82 \pm 0.02 \text{ mm}$ and oil droplet size of $0.18 \pm 0.02 \text{ mm}$, compared with F1B with $0.80 \pm 0.01 \text{ mm}$ and $0.18 \pm 0.01 \text{ mm}$, respectively. Fertilization (%) was $68.17 \pm 20.48\%$ for WB and $69.06 \pm 19.80\%$ for F1B, with no significant differences between the two groups. F1B had significantly higher hatching rates with an average of $90.52 \pm 11.60\%$, compared with WB with an average of $86.35 \pm 14.23\%$.

3.2 Larval quality evaluation

There were no significant differences in the total length of hatched larvae between the two broodstock groups. With respect to the survival activity index, the larvae survived without feed up to 8 DPH, and significant differences were observed between the two broodstock groups: the SAI for WB was $12.18 \pm 3.39\%$ and $11.34 \pm 2.90\%$ for F1B (Table 1). The cumulative mortality on the 3rd day after hatching had an average of $20.07 \pm 10.23\%$ for F1B and $19.39 \pm 10.91\%$ for WB. Spawns with accumulated mortality below 10% were considered good quality spawns, while those with mortality above 35% were considered poor quality and between 10 and 35 % mortality were considered intermediate quality. All spawns had intermediate quality (Figure 2).

The temperature tolerance index (TTI) of larvae from the WB batch began to show signs of low tolerance from $7.08 \pm 4.69 \text{ min}$, and F1B from $9.29 \pm 7.32 \text{ min}$ for most of the

months evaluated. The average values of the TTI from August to July for WB larvae were 102.23 ± 15.46 , while for F1B larvae from August to May, it was 100.62 ± 5.90 . However, no significant differences were observed between the two broodstock groups. It is also worth mentioning that the best results for F1B spawns were observed in November 2021, and March and May 2022, while for WB spawns in January, March, and July 2022 (Figure 3).

Specific growth rate in $\% \text{ cm day}^{-1}$ was also determined in larvae; the average for F1B was $4.19 \pm 0.72\% \text{ cm day}^{-1}$, and $3.86 \pm 1.20\% \text{ cm day}^{-1}$ for WB, with no significant differences between the two groups. On the other hand, the average larval survival during the study period was $32.14 \pm 25.51\%$ for WB larvae and $37.29 \pm 30.99\%$ for F1B larvae, with higher values observed for the latter group in most months evaluated. However, no significant differences were observed between groups.

3.3 Correlation

In general, a low correlation ($r < 0.50$) was observed between the evaluated reproductive parameters in *A. scapularis* broodstock. However, in some parameters significant correlations were observed (Figure 4). For example, a moderate and positive correlation was observed between percentage viable eggs and fertilization rate ($r = 0.331$), egg diameter and oil droplet diameter ($r = 0.304$), and egg diameter and larval survival ($r = 0.561$). Moreover, a moderate and negative correlation was observed between hatching rate and larval survival ($r = -0.627$), and SAI and cumulative mortality at 3 DPH ($r = -0.537$).

3.4 Biochemical analysis of eggs and larvae

The proximal composition of eggs and 20 DPH larvae from both groups of *A. scapularis* broodstock is shown in Tables 2, 3, and 4. No significant differences between groups were observed in protein, lipids, carbohydrates, or ash content in eggs, but significant differences were found in ash content in the 20 DPH larvae, with higher values for the F1B broodstock. Fatty acid profiles of eggs and larvae are reported in Table 3. Polyunsaturated fatty acids, in particular n-3 PUFA dominated in both groups of eggs (F1B and WB). More specifically docosahexaenoic acid (C22:6n-3) [DHA] was the most abundant fatty acid in eggs, followed by palmitic acid (16:0) and oleic acid (18:1n-9). In larvae, palmitic acid (16:0) was the highest fatty acids present in both groups (F1B and WB), followed by DHA and stearic acid (18:0). Additionally, significant differences were found in stearic acid (C18:0), eicosatetraenoic acid (20:4n-3), and docosapentaenoic acid (22:5n-3), with higher values in the WB eggs. Palmitic acid (16:0) and DHA had the highest values in the F1B eggs. In 20 DPH larvae, significant differences were observed in palmitoleic acid (16:1n-7), eicosapentaenoic acid (20:5n-3) [EPA], and arachidonic acid (20:4n-6) [ARA], with higher values in WB.

Results from the principal component analysis (PCA) of the fatty acids in eggs (Fig. 5a), revealed that the first principal component explained 27.76% of the observed variability, and the second component explained 21.51%. The discriminant analysis of two components explained 47.46% of the variability and confirmed that there is no separation between the two groups of broodstock (Fig. 5b). However, from the vector projections, a greater relationship between the eggs of F1B broodstock and DHA, palmitic acid (16:0), oleic acid (18:1n-9), and linoleic acid (18:2n-6); while stearic acid (18:0),

eicosatetraenoic acid (20:4n-3), docosapentaenoic acid (22:5n-3), and EPA have a higher relationship with WB eggs.

Results from the principal component analysis (PCA) of the larvae fatty acids (Fig. 6a), showed that the first principal component explained 38.77% of the observed variability, and the second component explained 31.85%. The discriminant analysis of two components explained 74.44% of the variability, and it was observed that there is no separation between the two batches of broodstock (Fig. 6b). Based on the vector projections, results show that the larvae of F1B broodstock have a higher relationship with oleic acid (18:1n-9), DHA, and linoleic acid (18:2n-6); while the larvae of WB broodstock have a higher relationship with palmitoleic acid (16:1n-7), EPA, docosapentaenoic acid (22:5n-3), and ARA.

The total amino acid composition (Table 4) was not significantly different between the eggs of the two groups of broodstock. The amino acids found in higher proportion were glutamic acid, leucine, alanine, aspartic acid, and lysine. In 20 DPH larvae, significant differences were observed in the amino acid leucine, which was found in higher proportion in the WB batch larvae. The amino acids found in higher proportion in the larvae were glutamic acid, lysine, and arginine.

4. Discussion

Comparisons of egg quality and composition between wild and first-generation domesticated broodstock have been conducted in several marine fish species (Lund et al., 2008; Ceccon et al., 2012; Quiñones-Arreola et al., 2015; Soares et al., 2015; Santamaría-Miranda et al., 2021). We expected eggs from wild broodstocks would outperform F1 eggs in most quality traits evaluated, as eggs obtained from wild broodstocks are generally considered to be of higher quality (Salze et al., 2005; Ceccon et al., 2012; Santamaría-Miranda et al., 2021). However, in the F1B spawns, higher percentage of viable eggs fertilization rate, and hatching rate were observed. Reproductive performance in fish depends on various factors such as fish size and condition, the use of hormonal treatments, nutrition, and stress management (Bobe and Labbé, 2010; Schaefer et al., 2018). In this regard, the continuous production of large quantities of high-quality gametes is essential for aquaculture (Schaefer et al., 2018). Therefore, achieving a reliable supply of high-quality eggs is a major challenge that needs to be overcome when developing aquaculture for new marine species (Mylonas et al., 2011; Bardon-Albaret and Saillant, 2017), such as *Anisotremus scapularis*, which is considered a prioritized new species for aquaculture in Peru (Ministerio de la Producción, 2012).

However, the total number of eggs, egg diameter, and oil droplets were lower in the F1B batch, which may be related to the fact that broodstock individuals were smaller (430.28 g and 26.99 cm) compared with their wild counterparts (655.27 g and 30.20 cm). For many fish species, the age or size of the female can determine the size of the eggs (Bromage et al., 1990; Kamler, 2005). On the other hand, fish that spawns for the first time often show low fecundity and low-quality spawn (Bardon-Albaret and Saillant, 2017). Therefore, the occurrence of first-time spawning females in the F1B group could explain the lower production of total eggs in this group. Similar conditions were observed in other marine fish species such as *Seriola rivoliana*, where wild broodstocks showed a higher spawning frequency and produced more eggs compared with the F1 generation (Quiñones-Arreola et al., 2015), which is also similar for *Cyclopterus lumpus* (Pountney

et al., 2022). However, a different situation was observed in *Lutjanus campechanus*, in which wild females produced fewer eggs than captive females (Bardon-Albaret and Saillant, 2017). This result coincides with the findings reported for *Gadus morhua*, where the higher number of eggs produced were from the F1 group, indicating that the results vary according to the species and origin of the broodstock (Kjørsvik, 1994).

The morphological characteristics of *A. scapularis* eggs were described as pelagic, spherical, completely smooth, with a transparent chorion, uniform yolk, and an oil droplet (Montes et al., 2019); similar to most cultured marine fish species (e.g., *Sparus aurata*, *Dicentrarchus labrax*, *Dentex dentex*, *Gadus morhua*, *Scophthalmus maximus*, *Solea senegales*, *Hippoglossus hippoglossus*). These characteristics can also be used as parameters for determining egg quality (Lahnsteiner et al., 2008; Aristizabal, 2009; Mandal et al., 2021). Egg quality can also be defined based on the proportion of spawned and floating eggs (i.e., which are considered viable) (Giménez et al., 2006). The percentage of floating eggs is commonly used as an indicator of spawning quality in marine fish culture (Aristizabal, 2009), and this parameter is related to egg viability in some species (Martín et al., 2019). Flotation is achieved through the hydration process, which is a key step in successful reproduction (Carnevalli et al., 2001). In the present study the quantity of viable or floating eggs, were significantly higher for F1B specimens of *A. scapularis* than for WB in most of the months during the bioassay, which differs from the findings reported for *Lutjanus campechanus* and *L. guttatus*, where wild females produced a greater number of viable eggs compared with captive females (Bardon-Albaret and Saillant, 2017; Chacón-Guzmán et al., 2020).

The size of fish eggs can be influenced by various factors such as the nutritional status of the broodstock, size/age of the female, spawning type (natural, induced, etc.), or the timing of spawning (beginning, middle, and end of the spawning season). In a fish species with asynchronous spawning like *A. scapularis*, which exhibits continuous and multiple spawns (Carrera et al., 2022), the average egg size may decrease due to the successive spawns of each individual (Lahnsteiner et al., 2008). The diameter of *A. scapularis* eggs has been reported to range between 0.75 to 0.77 mm for eggs from wild specimens (Montes et al., 2019), which is relatively smaller than the values observed in the present study for fish of the same origin, with an average diameter of 0.81 ± 0.07 mm. Similarly, for *L. guttatus*, the average egg diameter was 0.75 mm, with similar sizes for both wild and captive specimens (Chacón-Guzmán et al., 2020). In another study with the same species, Boza-Abarca et al. (2011) reported an average egg diameter of 0.72 ± 0.027 mm. In *L. argentimaculatus*, reported egg diameter ranged from 0.74 to 0.81 mm (Leu et al., 2003). For *Sparus aurata*, the egg diameter was higher than the aforementioned species, ranging from 0.97 to 0.99 mm under captive conditions (Jerez et al., 2012). In other species such as *Argyrosomus regius*, F1 specimens had significantly larger egg diameters (1.04 ± 0.10 mm) compared to wild specimens (0.97 ± 0.13 mm) (Soares et al., 2015), which is different from the findings in the *A. scapularis* broodstock group, where F1B exhibited a smaller average diameter than that of the wild specimens (0.80 ± 0.01 mm).

Other important parameters for determining fish egg quality are the fertilization percentage and the hatching percentage (Lahnsteiner et al., 2008; Ciji et al., 2022). Percent fertilization rates for F1B specimens of *A. scapularis* exhibited a slightly higher average compared to those of wild origin. Similar differences were observed in *L. campechanus* (Bardon-Albaret and Saillant, 2017), but the opposite was found in *Tor putitora*, where wild specimens had a higher fertilization rates than captive ones (Ciji et

al., 2022). Generally, it can be said that depending on the origin of the broodstock and the rearing conditions, percent fertilization can vary. For instance, in captive specimens of *Sparus aurata*, the values range between 83% and 92% (Jerez et al., 2012), while in wild specimens of *L. argentimaculatus*, an average percent fertilization of 74.3% was obtained (Leu et al., 2003), and in wild *Diplodus puntazzo*, over 3 years of monitoring, values ranged from 76%, 78%, to 81% (Papadaki et al., 2008). On the other hand, in *Latris lineata*, the percent fertilization did not show significant differences between wild and captive specimens (Morehead et al., 2001), similar to values observed in the present study for *A. scapularis*. Percent hatching rates for *A. scapularis*, were higher for F1B group compared to the WB group. These values coincide with *Argyrosomus regius*, where F1 broodstock had significantly higher hatching percentages than that of wild broodstock (Soares et al., 2015). However, variations can be found depending on the origin of the broodstock. For example, in *L. campechanus*, the percent hatching for eggs of captive females was significantly lower than that of wild females (Bardon-Albaret and Saillant, 2017). In *Sparus aurata*, captive specimen percent hatching ranged from 72% to 77% (Jerez et al., 2012), and for *L. argentimaculatus*, percent hatching was 81.9% for eggs from wild specimens (Leu et al., 2003). In *Tor putitora*, wild specimens had a higher hatching rates than that of captive specimens (Ciji et al., 2022), and in a study of wild *Diplodus puntazzo* broodstock, the hatching rates did not vary significantly, remaining at around 86% through all the years of study (Papadaki et al., 2008).

In a study with *Lutjanus peru* under laboratory conditions, the authors did not find a correlation between egg diameter and hatching or survival rate, while the egg oil droplet showed a positive correlation with larval survival (Moguel-Hernández, 2015). Santamaria-Miranda et al. (2021) found a positive correlation between the weight of *L. peru* females and the size of the oil droplet, but not with egg size. In *L. campechanus* (Bardon-Albaret and Saillant, 2017), found a low and non-significant correlation among the spawning quality parameters. In the present study, a moderate but significant positive correlation was found with larval survival and egg diameter and a negative correlation with percent hatching. This suggests that the hatching percentage in *A. scapularis* could be used as a predictor of subsequent larval survival. On the other hand, SAI had a negative and significant moderate correlation with cumulative mortality at 3 DPH. Other significant relationships were the number of total eggs with fertilization percentage and the egg diameter with the oil droplet diameter, however only a weak correlation was found.

The evaluation of the “hardiness” of the larvae using the TTI, results obtained in the present study with WB broodstock were higher than those reported by Kanazawa (1997) with 77% thermal tolerance for *Pagrus major* larvae and the 73.9% for *Lutjanus guttatus* larvae from wild broodstock (Burbano et al., 2019). In larvae of the California halibut, *Paralichthys californicus*, an index of 1.88% was obtained at the end of the larval rearing period (Fuentes and Lazo 2018). Ccecon et al. (2012) compared spawns from both origins in *Gadus morhua*, and found that larvae from wild broodstocks were more resistant to these tolerance tests. This is contrary to what was observed in the present research, where no significant differences were found between both groups. It is worth noting that TTI values are inversely proportional to the hardiness of the larvae, where higher values represent reduced tolerance (Fuentes and Lazo, 2018).

Values from the biochemical analysis, for protein, lipid, carbohydrate, and dry matter content were similar between the eggs and larvae from both broodstock groups. In

contrast, Santamaría-Miranda et al. (2021) working with *Lutjanus peru*, found lower values for total proteins and carbohydrates in F1 eggs, and lipid content was lower in eggs from wild broodstock. However, Ceccon et al. (2012) in *Gadus morhua*, reports similar findings where no temporal effects were observed for the total lipid content in both groups of eggs, and to those of Quiñones-Arreola et al. (2015) in *Seriola rivoliana*, where there was no difference between the two experimental groups in terms of total protein, lipid, carbohydrate, or triacylglyceride content.

Total fatty acid profiles (TFA) were similar between eggs of the two broodstock groups, which can be related to the similar diet feed to both experimental groups. Similar, correlation between diets and eggs were observed in gilthead seabream (Harel et al., 1992). These results were contrary to the findings of de Lund et al. (2008) in *Solea solea*, where the TFA content was lower in F1 broodstock eggs, although similar to the results of Ceccon et al. (2012) for *Gadus morhua*, where the relative total fatty acid content was similar for both cultured and wild broodstock eggs. In general, the quality of spawns can be influenced by diet and environmental conditions during oogenesis (Brooks et al., 1997; Migaud et al., 2013), which is why many aquaculturists try to manipulate fish diets with various concentrations and proportions of essential fatty acids to reach maximum egg quality (Izquierdo et al., 2005). However, for this study, the diet was similar for both groups, fed fish chunks supplemented with vitamin and fatty acid capsules (Cota et al., 2022).

In general, marine fish eggs with a high amount of DHA are considered to be of high quality, as DHA is essential nutrient for many important biological functions including the neural development of the larvae (Sargent et al., 1999). In F1B eggs and larvae, a higher proportion of DHA was found, which could be correlated to the better growth and survival rates observed in the experimental group. Sargent et al. (1999) discussed how both the concentration and proportions of DHA, EPA, and ARA are important for the nutrition of fish larvae and suggested that appropriate proportions are in the range of 2:1 DHA:EPA and 5:1 EPA:ARA (Papanikos et al., 2008). Therefore, in addition to a DHA:EPA ratio a EPA:ARA ratio or even better a DHA:EPA:ARA ratio has been proposed as an indicator of adequate broodstock and larval diets at each stage, species, and habitat (Sargent et al., 1999; Bell and Sargent, 2003). Here, we found that the DHA:EPA ratios for the eggs were 2.57:1 and 2.27:1 for F1B and WB, respectively, and 2.07:1 and 0.99:1 in the larvae. The EPA:ARA ratio was 8.11:1 and 8.30:1 in the eggs of F1B and WB broodstock, and 1.67:1 and 2.25:1 in the larvae of each of these experimental groups. It is worth noting that the eggs of the two broodstock groups did not show differences in EPA and ARA content, but the 20 DPH larvae from wild broodstock showed a higher content of these fatty acids.

In a study conducted by Lund et al. (2008) with *Solea solea*, using a multivariate analysis of the fatty acid profiles, it was possible to discriminate between spawns from the natural environment and cultured groups. The eggs of cultured broodstock had high levels of eicosenoic acid (20:1n-9), linoleic acid (18:2n-6), and alpha-linolenic acid (18:3n-3), while the eggs of wild fish had high levels of palmitoleic acid (16:1n-7), EPA, and ARA, indicating dietary differences between groups. Ceccon et al. (2012), reported that the total n-3 PUFA, DHA, and eicosatetraenoic acid (20:4n-3) were positively correlated and were characteristic of the wild broodstock groups. On the other hand, n-6 fatty acids, such as linoleic acid (18:2n-6) and gamma-linolenic acid (18:3n-6), were negatively correlated and were characteristic of the cultured broodstock group. In the present study, a similarity

in FA profiles was observed between the two broodstock groups as it was not possible to discriminate between the two of them. However, there was a high association of F1B eggs and 20 DPH larvae with palmitic acid (16:0), oleic acid (18:1n-9), DHA, and linoleic acid (18:2n-6). In the case of wild broodstock eggs and larvae, a stronger association to stearic acid (18:0), palmitoleic acid (16:1n-7), eicosatetraenoic acid (20:4n-3), docosapentaenoic acid (22:5n-3), EPA, and ARA was found.

The most prevalent amino acids measured in *A. scapularis* eggs from both experimental groups were glutamic acid, leucine, alanine, aspartic acid, and lysine. Essential amino acids, with the exception of tryptophan, which was not quantified, constituted 50.95% (F1B) and 50.99% (WB) of the total amino acids, with leucine, lysine, and arginine being the most abundant. In the case of 20 DPH larvae from both batches, the amino acids found in higher proportions were glutamic acid, lysine, and arginine. Essential amino acids constituted 48.94% (F1B) and 49.20% (WB) of the total, with lysine, leucine, and arginine being the most prominent. Faulk and Holt (2008) observed that glutamate, leucine, alanine, proline, lysine, and aspartate are the most abundant amino acids in captive-spawned coho (*Oncorhynchus kisutch*) eggs. Ceccon et al. (2012) showed that the total amino acid content was significantly higher in eggs from wild broodstock compared to cultured *Gadus morhua*. In both groups, the most abundant amino acid was glutamic acid. Additionally, the authors report that leucine and threonine, were significantly higher in eggs from wild broodstock, while arginine, phenylalanine, and tryptophan were significantly higher in eggs from cultured broodstock. However, the PCA did not provide a good model to separate wild and cultured broodstock eggs. In this study, the amino acid composition was not significantly different between eggs and 20 DPH larvae from the two broodstock groups, except for leucine, which was found in higher proportions in the WB larvae. In the PCA of amino acids in eggs and 20 DPH larvae, no difference was observed in the projection of vectors by broodstock group, and there was no discrimination of amino acids by group.

5. Conclusions

Based on the results from the present study, we can conclude that the *A. scapularis* F1 broodstock group had better egg quality in terms of egg viability ($66.57 \pm 21.99\%$) and hatching rate ($90.52 \pm 11.60\%$). However, a lower egg production was found ($115,200$ eggs kg^{-1}) and egg size (0.80 ± 0.01 mm) was smaller compared to that of wild specimens (WB). Nonetheless, F1 broodstock larvae exhibited good temperature tolerance index (100.62 ± 5.90) and similar growth rates ($4.19 \pm 0.72\%$ cm day^{-1}) and larval survival ($37.29 \pm 30.99\%$) up to 20 DPH, compared to those of the larvae from the wild spawns. Spawns showed similar total fatty acid (TFA) content between experimental groups (F1B Eggs: 97.90 ± 16.35 mg g^{-1} , F1B 20 DPH larvae: 62.46 ± 8.08 mg g^{-1}) and total amino acid (TAA) content (F1B Eggs: 696.79 ± 82.73 mg g^{-1} , F1B 20 DPH larvae: 598.42 ± 37.65 mg g^{-1}). However, in terms of fatty acid composition, the F1 batch exhibited a higher proportion of 16:0 and DHA in eggs and a higher DHA/EPA ratio in 20 DPH larvae. Therefore, we can recommend the use of F1 broodstock as a good alternative for the recruitment of new broodstock to produce high quality eggs and larvae under captive conditions, thereby contributing to the aquaculture development of this promising species.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the Ethics Committee of the Research, Innovation, and Venture Unit of the National University Federico Villareal (Letter N°001-2023-CEI-UIIE-FOPCA-UNFV).

Author contributions

Noemi Cota, Lili Carrera and Angélica Castro conceived and designed the study, performed data collection and wrote the manuscript. Noemi Cota carried out the analysis of the results and statistical analysis. Chris León conducted the animal management and sample collections. Leenin Flores and Anthony Ruíz performed the biochemical analyzes and analysis of these results. Neil Duncan and Juan Pablo Lazo contributed to writing - reviewing & editing, and with supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.1033718/full#supplementary-material>

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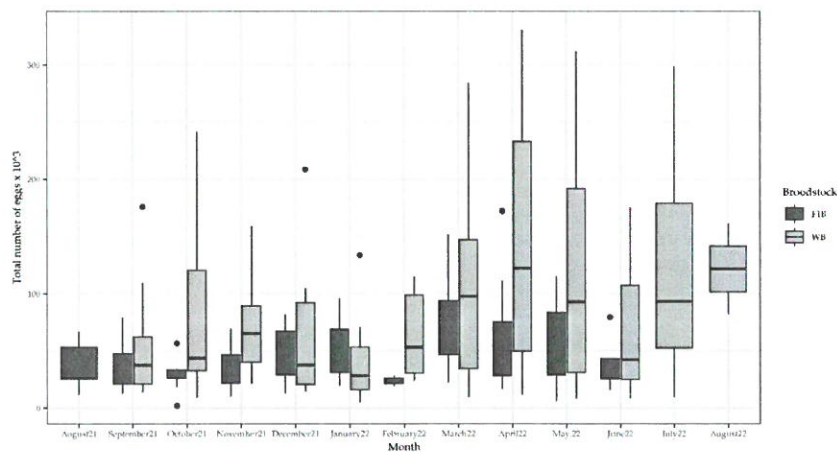


Figure 1. Total egg production ($\times 10^3$) from first-generation domesticated (F1B) and wild caught (WB) *Anisotremus scapularis* broodstock.

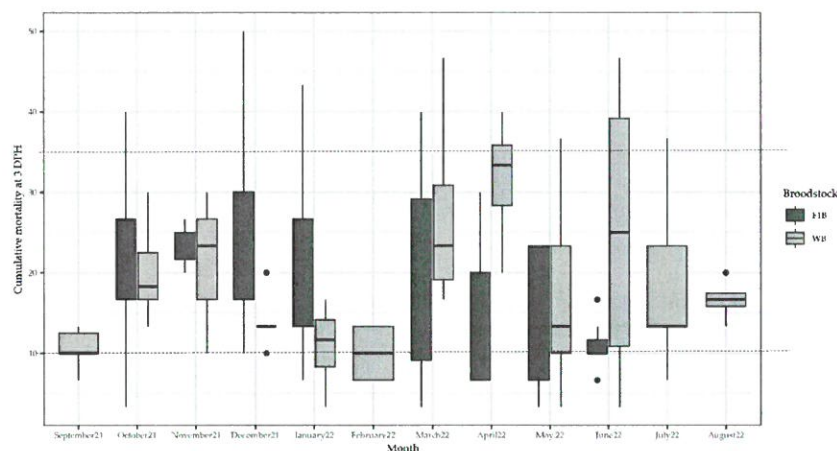


Figure 2. Box plot of accumulated mortality by day 3 (MA3) of *Anisotremus scapularis* first-generation domesticated (F1B) and wild caught (WB) broodstocks.

Comentado [NJCM1]: Las figuras se incluirán automáticamente al final del manuscrito enviado. Para cargar más de una figura a la vez, guarde las figuras (etiquetadas en orden de aparición en el manuscrito) en un archivo zip y cárguelas como 'Presentación de material complementario'. Todas las imágenes deben tener una resolución de 300 ppp en tamaño final. Tenga en cuenta que guardar una figura directamente como un archivo de imagen (JPEG, TIF) puede afectar en gran medida la resolución de su imagen. Para evitar esto, una opción es exportar el archivo como PDF y luego convertirlo a TIFF o EPS usando un software de gráficos.

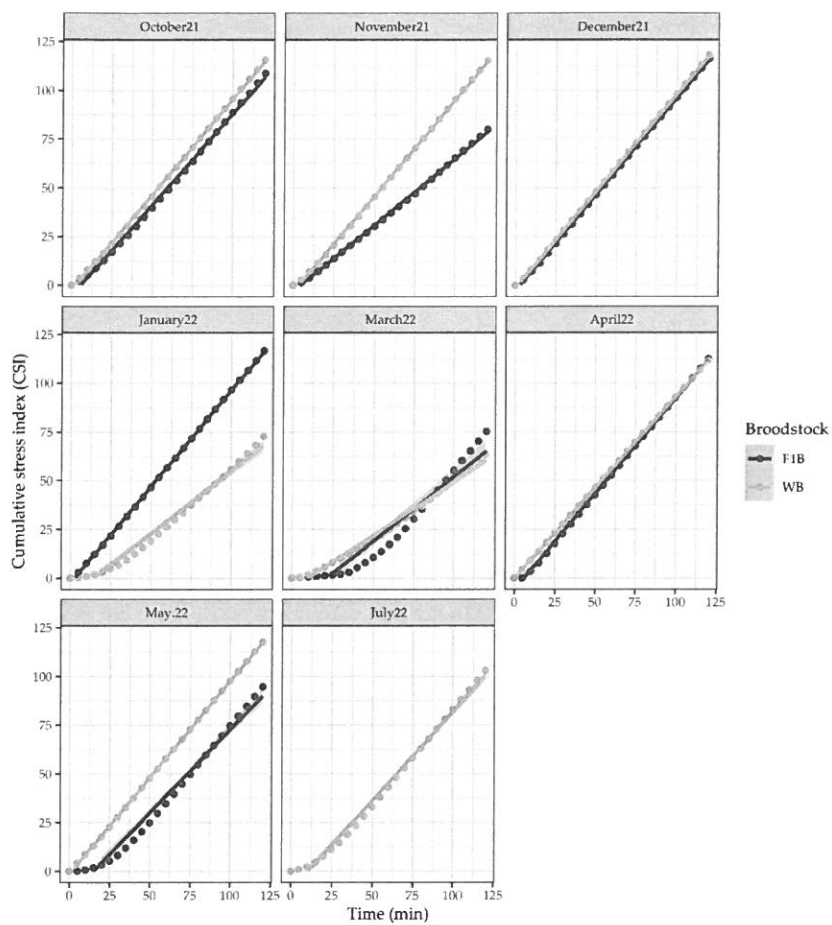


Figure 3. Temperature tolerance index (TTI) of larvae of *Anisotremus scapularis* first-generation domesticated (F1B) and wild caught (WB) broodstocks.

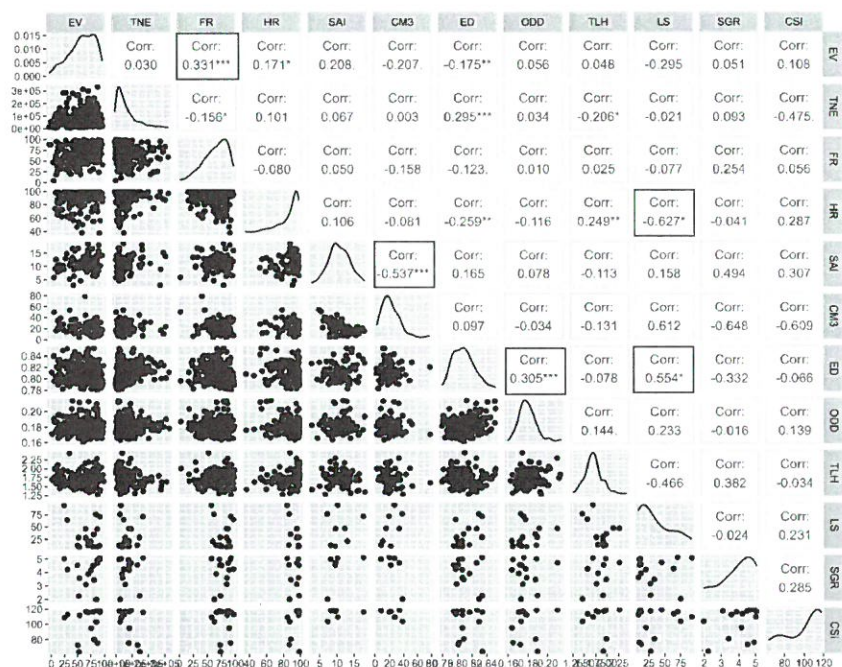


Figure 4. Pearson's correlation coefficients between parameters measured on wild caught (WB) and first generation domesticated (F1B) spawns. Moderate and weak correlations with significant values are marked with red boxes. Symbols (*) denote the level of significance of the correlations: * $p < 0.05$, ** $p < 0.01$, or *** $p < 0.001$. EV = eggs viability, TNE = total number of eggs, FR = fertilization rate, HR = hatching rate, SAI = survival activity index, CM3 = cumulative mortality at 3 DPH, ED = eggs diameter, ODD = oil droplet diameter, TLH = total length at hatching, LS = larval survival, SGR = specific growth rate, CSI = cumulative stress index.

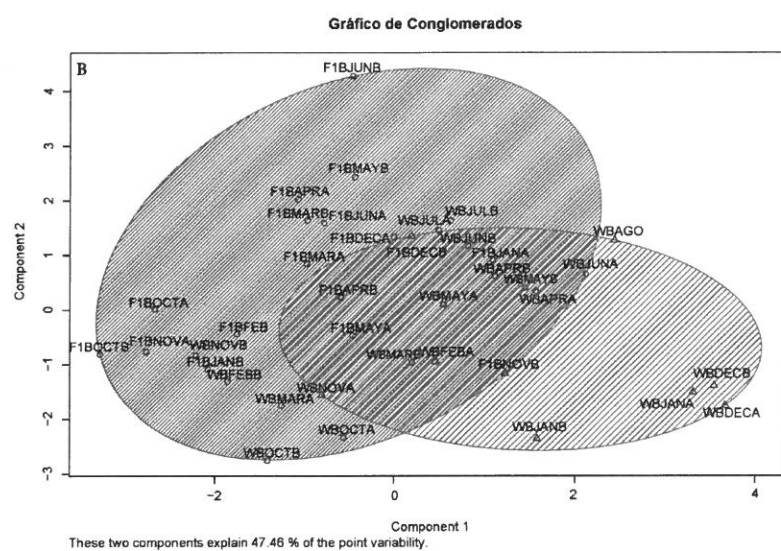
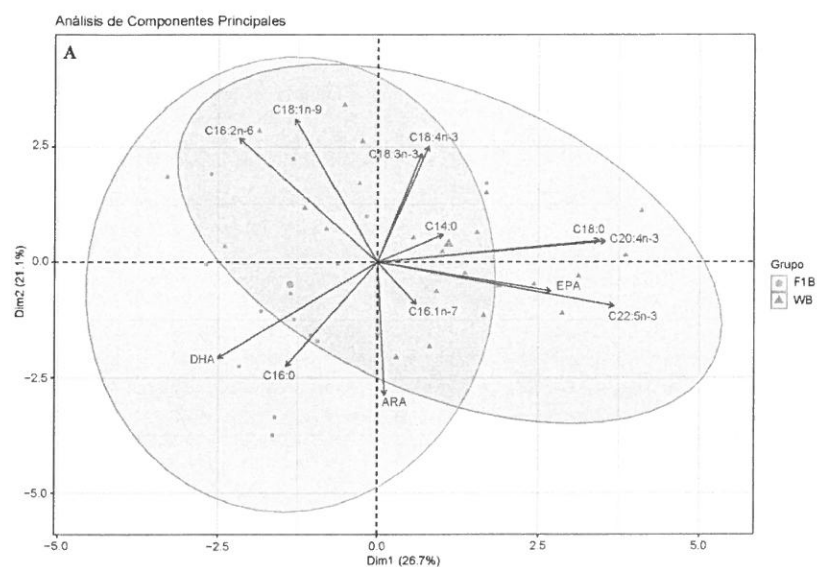


Figure 5. Principal component analysis (A) and discriminant analysis (B) of the fatty acids in eggs from *Anisotremus scapularis* first-generation domesticated (F1B) and wild caught (WB) broodstocks.

Table 1.

Reproductive and larval performance of *Anisotremus scapularis* first-generation domesticated (F1B) and wild caught (WB) broodstock evaluated from August 2021 to August 2022.

	Broodstock	
	F1 (n = 147)	Wild (n = 197)
Total body weight (kg)	439.77 ± 111.72	655.27 ± 226.90
Total number of eggs	50 688 ± 30 598^a	90 804 ± 77 273^b
Relative fecundity (egg kg ⁻¹)	115 200	206 372
Eggs viability (%)	66.57 ± 21.99^a	58.88 ± 24.55^b
Eggs diameter (mm)	0.80 ± 0.01^a	0.82 ± 0.02^b
Oil droplet diameter (mm)	0.18 ± 0.01^a	0.18 ± 0.02^b
Fertilization rate (%)	69.06 ± 19.80	68.17 ± 20.48
Hatching rate (%)	90.52 ± 11.60^a	86.35 ± 14.23^b
Larvae total length at hatching (mm)	1.70 ± 0.17	1.72 ± 0.20
Survival activity index (SAI)	11.34 ± 2.90^a	12.18 ± 3.39^b
Cumulative mortality at 3 DPH	20.07 ± 10.23	19.39 ± 10.91
Temperature tolerance index (TTI)	100.62 ± 5.90	102.23 ± 15.46
Specific growth rate (% cm day ⁻¹)	4.19 ± 0.72	3.86 ± 1.20
Larval survival (%)	37.29 ± 30.99	32.14 ± 25.51

*Significant differences ($p < 0.05$) between treatments are represented with letters.

Table 2

Proximal composition of eggs from *Anisotremus scapularis* first-generation domesticated (F1B) and wild caught (WB) broodstocks.

	Eggs		Larvae	
	F1 (n=17)	Wild (n=21)	F1 (n=7)	Wild (n=6)
Crude protein (%)	72.82 ± 8.08	73.52 ± 7.11	59.84 ± 3.76	60.37 ± 7.61
Crude lipid (%)	16.76 ± 1.85	16.91 ± 1.74	13.55 ± 1.65	14.39 ± 2.24
Carbohydrates (%)	3.28 ± 0.38	3.32 ± 0.71	4.38 ± 1.13	5.56 ± 1.57
Ash (%)	10.44 ± 4.07	9.28 ± 3.18	14.80 ± 4.44^a	10.55 ± 0.77^b
Dry matter (%)	8.05 ± 0.87	8.04 ± 0.75	14.60 ± 1.31	14.38 ± 1.98

*The statistical analyses were performed on eggs and larvae.

Table 3

Mean total fatty acid content (TFA, mg g⁻¹ dry weight), and fatty acid composition (in %TFA) of eggs from *Anisotremus scapularis* first-generation domesticated (F1B) and wild caught (WB) broodstocks. Data are expressed as mean ± S.E.

Fatty acid composition	Eggs		Larvae	
	F1 (n=17)	Wild (n=21)	F1 (n=7)	Wild (n=6)
TFA (mg g ⁻¹)	97.90 ± 16.35	98.28 ± 16.89	62.46 ± 8.08	73.79 ± 17.77
Saturated (%)				
Myristic (14:0)	3.14 ± 0.25	3.28 ± 0.30	2.44 ± 0.68	2.68 ± 0.74
Palmitic (16:0)	22.90 ± 1.12^a	21.82 ± 0.63^b	19.18 ± 0.92	18.40 ± 0.88
Stearic (18:0)	6.12 ± 0.42^a	7.12 ± 0.80^b	11.91 ± 2.15	10.39 ± 1.56

Monounsaturated (%)				
Palmitoleic (16:1n-7)	6.48 ± 0.45	6.63 ± 0.61	5.25 ± 0.96^a	7.44 ± 1.47^b
Oleic (18:1n-9)	12.82 ± 1.57	13.14 ± 1.17	9.47 ± 2.06	8.61 ± 1.72
Polyunsaturated (n-3) (%)				
Alpha Linolenic (18:3n-3)	0.36 ± 0.06	0.39 ± 0.08	0.78 ± 0.27	0.66 ± 0.21
Stearidonic (18:4n-3)	0.77 ± 0.08	0.82 ± 0.1	0.79 ± 0.27	0.68 ± 0.24
Eicosatetraenoic (20:4n-3)	0.66 ± 0.05^a	0.79 ± 0.06^b	0.93 ± 0.22	0.85 ± 0.31
Eicosapentaenoic (20:5n-3)	10.32 ± 1.08	10.62 ± 1.42	9.11 ± 1.86^a	14.09 ± 3.55^b
Docosapentaenoic (22:5n-3)	2.65 ± 0.24^a	2.92 ± 0.29^b	5.01 ± 1.17	6.46 ± 1.72
Docosahexaenoic (22:6n-3)	26.20 ± 1.13^a	25.10 ± 1.20^b	16.63 ± 4.05	12.29 ± 4.48
Polyunsaturated (n-6) (%)				
Linoleic (18:2n-6)	2.45 ± 0.42	2.25 ± 0.68	5.55 ± 1.74	5.03 ± 0.87
Arachidonic (20:4n6)	1.29 ± 0.13	1.29 ± 0.11	5.45 ± 0.38^a	6.27 ± 0.71^b
Total saturated fats	32.16 ± 1.22	31.19 ± 5.07	28.74 ± 12.89	31.47 ± 1.45
Total monounsaturated fats	19.29 ± 1.64	19.79 ± 1.09	13.98 ± 3.95	16.05 ± 1.71
Total polyunsaturated fats	44.50 ± 1.56	42.99 ± 6.47	41.1 ± 12.73	45.49 ± 3.63
Total n-3 fatty acids	40.91 ± 1.32	39.36 ± 6.09	30.88 ± 9.5	35.02 ± 2.02
Total n-6 fatty acids	3.59 ± 0.58	3.49 ± 0.61	10.22 ± 3.33	10.47 ± 2.38
DHA:EPA	2.57 ± 0.30	2.39 ± 0.34	2.07 ± 0.52^a	0.99 ± 0.63^b
EPA:ARA	8.11 ± 0.97	8.30 ± 1.24	1.67 ± 0.32^a	2.25 ± 0.51^b

*The statistical analyses were performed on eggs and larvae.

Table 4

Mean total amino acid content (TAA, mg g⁻¹ dry weight), and amino acid composition (in %TAA) of eggs from *Anisotremus scapularis* first-generation (F1B) and wild caught (WB) broodstocks. Data are expressed as mean ± S.E.

	Eggs		Larvae	
	F1 (n=17)	Wild (n=21)	F1 (n=7)	Wild (n=6)
TAA (mg g ⁻¹ fry wt)	696.79 ± 82.73	741.56 ± 101.29	598.42 ± 37.65	603.67 ± 76.09
Non-essential amino acids (%)				
Arginine	6.81 ± 0.61	6.5 ± 0.67	7.47 ± 0.11	7.50 ± 0.65
Histidine	2.75 ± 0.11	2.74 ± 0.09	2.67 ± 0.06	2.65 ± 0.09
Isoleucine	6.07 ± 0.24	6.13 ± 0.23	4.53 ± 0.08	4.64 ± 0.10
Leucine	9.51 ± 0.33	9.65 ± 0.36	8.00 ± 0.11^a	8.19 ± 0.10^b
Lysine	8.09 ± 0.26	7.96 ± 0.53	8.56 ± 0.11	8.56 ± 0.09
Phenylalanine	4.36 ± 0.18	4.31 ± 0.12	4.44 ± 0.03	4.5 ± 0.07
Threonine	4.91 ± 0.13	4.96 ± 0.15	5.01 ± 0.05	5.04 ± 0.04
Valine	6.40 ± 0.2	6.49 ± 0.18	5.28 ± 0.07	5.37 ± 0.09
Essential amino acids (%)				
Alanine	7.93 ± 0.29	7.90 ± 0.35	6.36 ± 0.06	6.4 ± 0.07
Aspartic acid	7.63 ± 0.44	7.47 ± 0.44	10 ± 0.03	9.71 ± 0.57
Glutamic acid	13.82 ± 0.41	13.87 ± 0.42	14.8 ± 0.1	14.74 ± 0.13
Glycine	3.52 ± 0.14	3.46 ± 0.11	6.31 ± 0.28	6.12 ± 0.18
Proline	6.10 ± 0.29	6.21 ± 0.39	4.70 ± 0.1	4.69 ± 0.09
Serine	5.47 ± 0.62	5.78 ± 0.22	5.28 ± 0.03	5.16 ± 0.2
Tyrosine	4.35 ± 0.12	4.33 ± 0.15	3.6 ± 0.08	3.57 ± 0.09

*The statistical analyses were performed on eggs and larvae.



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Invoice

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Date Due: 17 Nov 2023
Our Reference:

Bill to:
Noemi Cota
Institute of the Sea of Peru (IMARPE)
Esquina Gamarra y General Valle S/N
07021 Callao
Peru

Description of Services:

Prepayment Invoice for Ms 1287439

"Reproductive performance and egg and larvae quality from first generation peruvian grunt *Anisotremus scapularis* (Tschudi, 1846): a comparative analysis with spawns from wild broodstock", by Noemi Cota*, Lili Jannet Carrera Santos, Angélica Castro, Oneill Leon Dominguez, Leenin Flores Ramos, Anthony Ruiz Soto, Juan Pablo Lazo and Neil Duncan

	Unit Price USD	Qty	Total USD
Pre-payment	2141.75	1	2141.75
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OFICINA REGISTRANTE	DIRECCION GENERAL DE INVESTIGACIONES EN ACUICULTURA	USUARIO	dvillalobos@imarpe.gob.pe (mailto:dvillalobos@imarpe.gob.pe)
REFERENCIA		FECHA REGISTRO	02/10/2023
OBSERVACIONES			
DOCUMENTO DIGITAL	Ver Documento		
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PROVEIDO 07915-2023-IMARPE/OGA DOCUMENTO INTERNO MEMORANDUM 00203-2023-IMARPE/DGIA TOMAR ACCIÓN 03/10/2023 POR ACERTAR

Información del documento en curso

DOCUMENTO	PROVEIDO 07915-2023-IMARPE/OGA	REGISTRO	03/10/2023
ASUNTO	MEMORANDUM 00203-2023-IMARPE/DGIA Autorización para publicación de artículo científico		
OFICINA REGISTRANTE	OFICINA GENERAL DE ADMINISTRACION	USUARIO	jgalarza@imarpe.gob.pe (mailto:dvillalobos@imarpe.gob.pe)
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OBSERVACIONES	Por encargo de la Jefa de la Oficina General de Administración, se remite el MEMORANDUM 00203-2023-IMARPE/DGIA - Autorización para publicación de artículo científico, para su atención y trámite correspondiente.		

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FECHA : Callao; 02 de octubre de 2023

Es grato dirigirme a usted, a fin de expresarle que, a efectos de cumplir con sus objetivos en el presente año, el Área Funcional de Investigaciones en Acuicultura (AFIA) requiere efectivizar la publicación de los resultados de sus experimentos, desarrollados en el marco de ejecución de sus líneas de investigación.

Al respecto, alcanzo para su consideración el informe técnico acerca de “Reproductive performance and egg and larvae quality from first generation peruvian grunt *Anisotremus scapularis* (Tschudi, 1846): a comparative analysis with spawns from wild broodstock”, elaborado por especialistas del Laboratorio de Cultivo de Peces de AFIA; del cual planteamos autorice la procedencia de su publicación, para su difusión entre la comunidad científica y público interesado.

En tal sentido, le solicito tenga a bien considerar la aprobación de la publicación del indicado documento científico; para cuyo efecto se adjunta el pedido de servicio correspondiente, requiriéndose el visto bueno de su Despacho.

Atentamente,


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.....
Rgo. Ms. Víctor Yáñez Pinillo
Director General de Investigaciones
en Acuicultura

Cc: Arch.



