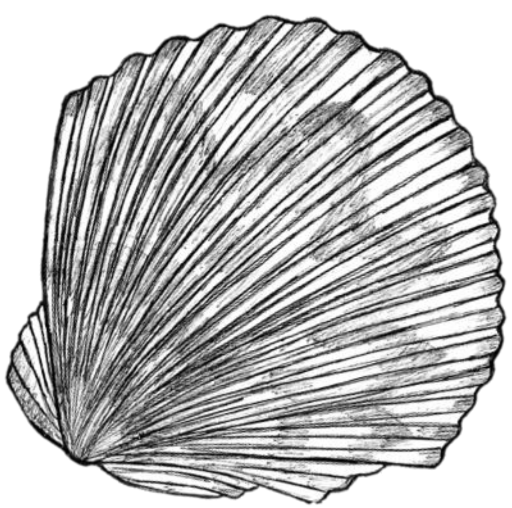

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Marine species associated to Peruvian scallop *Argopecten purpuratus* culture: trophic interactions and contaminant exposure

Iván Loaiza Alamo

2020

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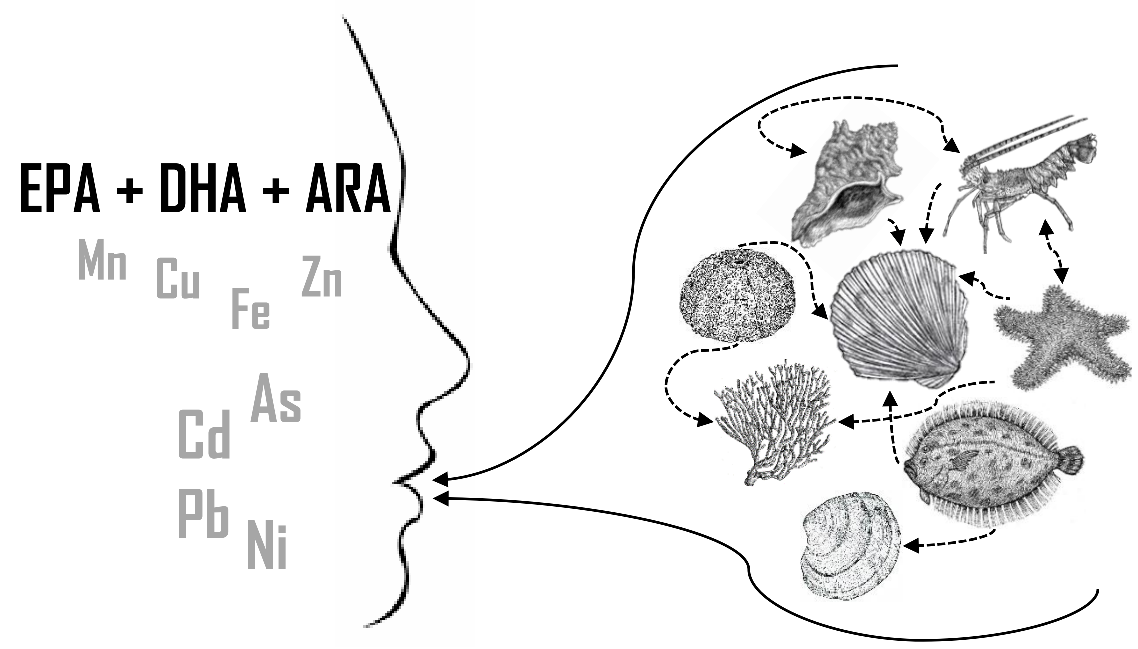
by

***Iván Loaiza Alamo***



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Universiteit Antwerpen

# Marine species associated to Peruvian scallop *Argopecten purpuratus* culture: trophic interactions and contaminant exposure

**Iván Loaiza Alamo**

A dissertation submitted to Ghent University and University of Antwerp in fulfillment of the requirements for the degree of Doctor in Science (Marine Sciences)

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# LIST OF ABREVIATIONS

**AN** Abrupt Niño 2017

**ARA** Arachidonic acid (20:4n6)

**BDL** Below the detection limit

**BDO** Bottom dissolved oxygen

**BMI** Body mass index

**CVD** Cardiovascular disease

**DHA** Docosahexaenoic acid (22:6n-3)

**EDI** Estimated daily intake

**EDTA** Ethylenediaminetetraacetic acid

**EFA** Essential fatty acids

**EHS** Environmental health status

**ENSO** Niño Southern Oscillation

**EPA** Eicosapentaenoic acid (20:5n-3)

**ERL** Effects Range-Low

**ERM** Effects Range-Median

**FAs** Fatty acids

**FBS** Food Balance Sheets

**FFQ** Frequency feeding questionnaire

**GDP** Gross Domestic Product

**GHG** Greenhouse gas

**HIs** Total hazard indices

**HR-ICP-MS** High resolution inductively coupled plasma mass spectrometer

**ICP-MS** Inductively coupled plasma mass spectrometry

**IMARPE** Instituto del Mar del Peru

**IR** Ingestion rate

**IRZ** Illescas Reserved Zone

**ISQG** Interim Sediment Quality Guideline

**ITCZ** Inter tropical Convergence Zone

**JPQ** Juan Paolo Quay port

**LC-PUFA** Long-chain polyunsaturated fatty acid

**MPAs** Marine Protected Areas

**MRLs** Maximum residual levels

**MT** Metric tonnes

**NHCS** Northern Humboldt Current System

**NL** Northern location

**NN** No El Niño event

**NSSs** Nutritional species-specific-score

**OMZ** Oxygen Minimum Zone

**OSPA** Organizaciones Pesqueras Artesanales

**PB** Paracas Bay

**PC** Peru Current

**PCA** Principal component analysis

**PCBM** Monitoring program of mollusk bivalves in Peru

**PEL** Probable effect level

**PL1** Paracas location 1

**PL2** Paracas location 2

**POM** Particulate organic matter

**PRODUCE** Ministerio de la Producción

**PSJ** Location in front Punta San Juan Reserved Zone

**PTDI** Provisional Tolerance Daily Intake

**PTWI** Provisional Tolerance Weekly Intake

**PUFA** Polyunsaturated fatty acid

**RD** River discharge

**RNSIIPG** Peruvian Guano Islands, Isles and Capes National Reserve

**RSM** Response surface methodology

**SANIPES** Organismo Nacional de Sanidad Pesquera

**SB** Sechura Bay

**SBT** Sea bottom temperature

**SDGs** Sustainable Development Goals

**SDO** Surface dissolved oxygen

**SERNANP** Servicio Nacional de Áreas Naturales Protegidas

**SFA** Saturated fatty acid

**SHO** Location in front of the iron company Shougang Hierro Peru S.A.A

**SIAR** Stable isotope analysis in R

**SL** Southern location

**SRM** Standard reference material

**SS** Surface salinity

**SST** Sea surface temperature

**Tb1/2** Estimated biological half-lives

**THQs** Target hazard quotients

**TMFs** trophic magnification factors

**TP** Trophic positions

**TRs** Target cancer risks

# SUMMARY

The present study addressed current concerns related to the seafood safety in Peru. The potential strategies to determine the current environmental health status (EHS), through bio-monitoring approaches were investigated. The levels of chemicals (i.e. metals, fatty acids, stable isotopes) in environmental compartments (from POM to top-predators) were assessed and their consequences at different levels of organization were discussed, e.g. contaminant transfer through the food web, potential value as nutritive seafood and/or risk for seafood consumers. As result, an integrated approach for the assessment of risks and benefits for consuming marine species from Peru was addressed.

In order to test whether the Peruvian seafood consumption could pose a human health risk due to the accumulated metal levels of these products, metal bioaccumulation was studied throughout the food chain (chapter 2). From this study it became apparent that in 6 of the studied marine edible species, around 10-20% of the molluscs (e.g. *Argopecten purpuratus*, *Bursa ventricosa*…) and 30-40% of the crustaceans (e.g. *Romaleon setosum*, *Hepatus chilensis*…) exceeded the maximum residual levels (MRLs) for human consumption in inorganic As and Cd. Integrated risk indices, e.g. target hazard quotient (THQs), total hazard indices (HIs), provisional tolerable weekly intake (PTWI) did not exceed their respective limits, however the target cancer risks (TRs) for inorganic As were always higher than the threshold ($1x10^{-6}$), therefore an actual cancer risk is present. The results confirmed that Peruvian marine species are loaded with As and Cd and that MRLs and TRs were

again higher than the thresholds for some species (from the 54 studied species), such as the crab *H. chilensis*, the mantis shrimp *Squilla* sp., the mussel *Semimytilus algosus, and the* snail *B. ventricosa*, among others (chapter 4).

Nevertheless, some marine species are also high in beneficial compounds: long-chain polyunsaturated fatty acids (LC-PUFAs): EPA, DHA, ARA; and micro-nutrients: Cu, Fe, Mn and Zn. The north of Peru was characterized by marine species with the highest LC-PUFAs (up to 180 mg EPA/100g), followed by those from the center and southern regions. Moreover, the species considered as potentially edible or non-edible species (e.g. *C. sexdecimdentatus*, *C. filiformis*, *C. plebejus*) are promising future foods due to their high nutritional values (high LC-PUFAs and micro-nutrients). Based on risk indices, it was concluded that the consumption of the estimated safe amounts of seafood could lead to a high contribution (up to 80% of the recommended values) of beneficial LC-PUFAs and micro-nutrients in Peruvian populations (chapter 4). Based on nutritional indices, the gastropods (e.g. *B. ventricosa*) were scored as the least beneficial species for human consumption.

The quality of marine species as seafood was further monitored in the frame of the complexity of the Peruvian marine ecosystems, which are driven by the northern Humboldt Current system (NHCS), and simultaneously impacted by oceanographic drastic variations (i.e. El Niño event). The intensities of El Niño phenomenon effect on fatty acids and metal contamination were monitored and analyzed using the Peruvian scallop *A. purpuratus* in different regions of Peru (chapter 3).

*A. purpuratus* was identified as potential bioindicator species to be used along the coast of Peru. Gills, digestive gland and intestine were the tissues where metal accumulation was the highest in *A. purpuratus*. Fatty acids were good biomarkers when annual (El Niño effect) comparisons were performed, while metals allowed to discriminate amongst locations (degree of pollution). The application of a series of biomarkers (i.e. metals, fatty acids) is key to understand the response of organisms to natural and/or anthropogenic stressors. However, cost-benefit relationships should be considered for a long-term and sustainable scallop monitoring program in Peruvian water domains.

Food web interactions and metal transfer in Peruvian marine ecosystems were integrated to understand the flow of energy or mass (incl. contaminants) in these ecosystems. Overall, $\delta^{15}N$ values increased southward, from the northern to center and southern regions. A logical trend of increasing $\delta^{15}N$ and $\delta^{13}C$ from food sources to top consumers, passing through the scallop *A. purpuratus*, could be observed. This points at *A. purpuratus'* crucial role as intermediate consumer and main food item for predators in the marine food webs under study, which is confirmed with the SIAR-mixing models and trophic regression lines. The trophic magnification factors (TMFs) in Peruvian marine food webs were highest for Cu (1.58), As (1.45) and Cd (1.08), but also TMFs < 1 were observed for Ni, Pb, Fe, Mn and Zn as non-biomagnifying metals. Therefore, it is concluded that As contamination is of serious concern in Peru, while some ecosystems are rich in natural Cu or are Cu-contaminated.

In conclusion, this PhD research emphasizes the importance of (1) determining the EHS of the different regions of Peru; (2) designing the right strategies for EHS monitoring; 3) the necessity to complement the field studies (this study) with laboratory experiments; (4) the possibility to enhance nutrition of Peruvian population through marine food consumption; and (5) improving national bio-monitoring programs in Peru.

(1) The regional analysis showed that the southernmost locations were more degraded than the northernmost ones, based on the metal contamination and/or enrichment. This was also reflected when biomarkers (e.g. fatty acids, stable isotopes) were used. When environmental compartments, such as sediment and seston were compared with different international limits for ecosystem protection, this pattern of low EHS in the southern marine ecosystems was also present.

(2) A research-based approach prior to analysis is ideal for the right strategy to design an EHS monitoring. The single species use is the most optimal approach (1 740 € · area · yr), while the multi-species (n=6) approach based on ecologically and economically important species requires a medium budget (~ 5 700 € · area · yr). Far more expensive than the previous approaches, the ecosystem-based approach which requires a large budget of ~ 11 940 € · area · yr, due to the high number of samples and sampling campaigns. For the single species approach, a substantial ecological knowledge of the species under study (e.g. *A. purpuratus*) is required, and such an approach should contain tissue-specific-metal analysis. The advantage of the multi-species approach is the dual use of metals and fatty acids analysis as tracer and biomarker,

on the one hand, and as proxy to determine (e.g. scoring) potential nutritive marine foods on the other hand. The ecosystem-based approach can give the "big picture" to understand the marine ecosystems, even under the effect of climate and oceanographic variations (i.e. ongoing Climate Change, El Niño, ...).

(3) Field studies could reflect what is actually going on in certain ecosystems, and they are a priority in developing countries (i.e. Peru), where baseline information is limited. These *in-situ* studies are necessary to understand the ecosystem functionality first; subsequently *ex-situ* experiments could be conducted. The *ex-situ* studies then should be performed to address specific research questions that are complementary to the *in-situ* collected data, e.g. metal uptake and kinetics using highly sensitive radiotracer or stable isotope techniques in lab conditions to understand the bioaccumulation and/or biomagnification processes in marine key species (e.g. *A. purpuratus*).

(4) Risk and beneficial estimations for seafood consumption are valuable information to improve healthy diets for Peruvians, but interventions on feeding practices and nutrition are the most important actions to tackle current health concerns (e.g. anemia, malnutrition, obesity,…) in Peru. The combined work of public health experts and marine biologists could lead to actions (e.g. human health evaluations) at local and national level.

(5) The development of an ecosystem-based (pelagic-benthic coupling) monitoring program as one entity is the key to understand these NHCS and El Niño driven marine ecosystems. Other (sub)monitoring programs can be also implemented for specific-research questions in certain locations targeting bioindicator and/or multi-species, e.g. from ~1 700 € · location · yr; considered as 'optimal budget' in this study.

As a result, an integrated national plan (incl. Peruvian institutions, e.g. IMARPE, ministries, universities…) with the use of a multi-disciplinary approach (biology, ecotoxicology, human health, social science, food production, etc…) should be considered in order to understand and prevent the scenario where the resources would be 1) over-exploited due to their high demand; or 2) un-exploited and un-utilized due to their degree of contamination at some locations.

# SAMENVATTING

De hier voorgestelde studie richtte zich op een bezorgdheid met betrekking tot de veiligheid van zeevruchten en andere mariene voeding in Peru. Mogelijke strategieën om de huidige gezondheidstoestand van het mariene milieu (Environmental Health Status, EHS) te bepalen door middel van biomonitoring werden onderzocht. De concentraties van verschillende elementen (bv. metalen, vetzuren, stabiele isotopen) werden beoordeeld in verschillende milieucompartimenten (van zwevende deeltjes organisch materiaal tot top-predatoren) en de consequenties van de aanwezigheid van deze stoffen op verschillende organisatieniveaus werden besproken, bv. overdracht van verontreinigende stoffen via de voedselketen, de potentiële waarde van vetzuren en nutriënten in zeevruchten als voedsel en / of hun risico voor consumenten. Dit leverde een geïntegreerde aanpak op voor de beoordeling van de risico's en de voordelen van het consumeren van mariene organismen uit Peru.

Om te testen of de Peruviaanse consumptie van zeevruchten een gezondheidsrisico voor de mens zou kunnen vormen vanwege de geaccumuleerde metaalconcentraties in deze producten, werd bioaccumulatie van metalen in de hele voedselketen bestudeerd (hoofdstuk 2). Uit deze studie bleek dat bij 6 van de bestudeerde mariene eetbare soorten, ongeveer 10-20% van de weekdieren (bijv. *Argopecten purpuratus, Bursa ventricosa, ...*) en 30-40% van de schaaldieren (bijv. *Romaleon setosum*, *Hepatus chilensis, ...*) de maximale residugehalten (MRL's) voor menselijke consumptie in anorganisch As en Cd overschreden werden. Geïntegreerde risico-indexen, bv. doel gevaar quotiënt (THQ's), totale gevarenindexen (Hazard index, HI's), en de aanvaardbare wekelijkse inname (PTWI) overschreden over het algemeen hun respectieve limieten niet, maar de beoogde concentraties om kankerrisico's te vermijden (TR's) voor anorganisch Arseen waren altijd hoger dan de drempelwaarde ($1x10^{-6}$); daarom is er een daadwerkelijk risico op kanker aanwezig. De resultaten bevestigden dat een aantal Peruviaanse mariene soorten As en Cd bevatten en dat MRL's en TR's wel hoger waren dan de drempelwaarden voor sommige soorten (van de 54 bestudeerde soorten), zoals onder andere voor de krab *H. chilensis*, de mantis of bidsprinkhaankreeft *Squilla* sp., de mossel *Semimytilus algosus* en de slak *B. Ventricosa* (hoofdstuk 4).

Niettemin bevatten sommige mariene soorten ook veel nuttige componenten zoals meervoudig onverzadigde lange-keten vetzuren (LC-PUFA's): EPA, DHA, ARA; en micronutriënten zoals Cu, Fe, Mn en Zn. Het noorden van Peru werd gekenmerkt door mariene soorten met de hoogste LC-PUFA's (tot 180 mg EPA / 100 g), gevolgd door de ongewervelde soorten uit het centrum en de zuidelijke regio's. Bovendien kunnen de soorten die als potentieel eetbare of niet-eetbare soorten worden beschouwd (bijvoorbeeld *C. sexdecimdentatus*, *C. filiformis*, *C. plebejus*) veelbelovend zijn in de toekomst vanwege hun hoge voedingswaarden (hoge LC-PUFA's en micronutriënten). Op basis van risico-indexen werd geconcludeerd dat de consumptie van de door ons geschatte veilige hoeveelheden zeevruchten zou kunnen leiden tot een hoge bijdrage aan nuttige LC-PUFA's en micronutriënten in Peruviaanse bevolking (tot 80% van de aanbevolen waarden) (hoofdstuk 4). Op basis van voedingsindexen werden de gastropoden (bv. *Bursa ventricosa*) gescoord als de minst voordelige soort voor menselijke consumptie.

De nutritionele kwaliteit van mariene soorten zoals zeevruchten werd verder gemonitord in het kader van de complexiteit van de Peruviaanse mariene ecosystemen, die worden aangedreven door het noordelijke Humboldt Current-systeem (NHCS) en tegelijkertijd worden beïnvloed door ingrijpende oceanografische variaties (d.w.z. El Niño-evenement). Het effect van El Niño op vetzuursamenstelling en metaalverontreiniging in verschillende regio's van Peru werd geanalyseerd met behulp van de Peruviaanse Sint Jacobs of kamschelp *A. purpuratus* (hoofdstuk 3). *A. purpuratus* werd geïdentificeerd als potentiële bio-indicator soort voor de kustzone van Peru. Kieuwen, de spijsverteringsklier en de darm waren de weefsels waar metaalaccumulatie het hoogst was in *A. purpuratus*. Vetzuren waren goede biomarkers wanneer de invloed van het El Niño-effect op jaarlijkse vergelijkingen werden bepaald, terwijl metalen goede indicatoren waren om onderscheid te maken tussen locaties (vervuilingsgradiënt). Het gebruik van een reeks biomarkers (d.w.z. metalen, vetzuren) is cruciaal om de respons van organismen op natuurlijke fluctuaties en / of antropogene stressoren te begrijpen. Er moet echter steeds een kosten-baten analyse gemaakt worden om een langdurig en duurzaam monitoringsprogramma in Peruviaanse mariene ecosystemen mogelijk te maken.

Interacties en metaaloverdracht binnen voedselketen in Peruviaanse mariene ecosystemen werden geïntegreerd om de doorstroming van energetische inhoud en massa (incl. verontreinigingen) in deze ecosystemen te begrijpen. Over het algemeen namen de $\delta^{15}N$-waarden zuidwaarts toe, van de noordelijke tot centrale en zuidelijke regio's. Een logische trend van een stijging van $\delta^{15}N$ en $\delta^{13}C$ waarden vanuit voedselbronnen tot topconsumenten kon worden waargenomen, met tussenwaarden in het mantelweefsel van *A. purpuratus*. Dit wijst op de cruciale rol van *A. purpuratus* als intermediaire consument en als de belangrijkste prooi voor predatoren die in de mariene voedselketens werden bestudeerd, wat wordt bevestigd met de SIAR-modellen en trofische regressielijnen. De trofische biomagnificatie factoren (TMF's) in Peruviaanse mariene voedselketens waren het hoogst voor Cu (1,58), As (1,45) en Cd (1,08). Maar er werden ook TMF's <1 waargenomen voor Ni, Pb, Fe, Mn en Zn als niet- biomagnificerende metalen. Daarom wordt geconcludeerd dat As-contaminatie een ernstig probleem is in Peru, terwijl sommige ecosystemen rijk zijn aan natuurlijk Cu of gecontamineerd zijn met Cu.

Dit doctoraatsonderzoek onderstreept het belang van (1) het bepalen van de EHS van de verschillende regio's van Peru; (2) het ontwerpen van de juiste strategieën voor EHS-monitoring; 3) de noodzaak om de veldstudies (zoals deze) aan te vullen met laboratoriumexperimenten; (4) de mogelijkheid om de voeding van de Peruviaanse bevolking te verbeteren door consumptie van zeevruchten; en (5) de noodzaak van een verbetering van nationale biomonitor programma's in Peru.

(1) De regionale analyse toonde aan dat in de meest zuidelijke locaties slechter was dan in de meest noordelijke, op basis van de metaalverontreiniging en / of -verrijking in de organismen. Dit werd ook weerspiegeld wanneer biomarkers (bijv. vetzuren, stabiele

isotopen) werden gebruikt. Als verschillende milieucompartimenten zoals sediment en seston werden vergeleken met verschillende internationale limieten voor de bescherming van ecosystemen, was dit patroon met lage EHS in de zuidelijke mariene ecosystemen ook aanwezig.

(2) Een op onderzoek gebaseerde aanpak voorafgaand aan de staalname en analyse is ideaal om de juiste strategie voor een EHS-monitoring te ontwerpen. Het gebruik van één soort is de meest optimale aanpak (1 740 € · $gebied^{-1}$ · $jr^{-1}$), terwijl een multisoorten-benadering (n = 6) op basis van ecologisch en economisch belangrijke soorten een gemiddeld budget vereist (~ 5 700 € · $gebied^{-1}$ · $jr^{-1}$ ). Veel duurder dan de vorige benaderingen is de ecosysteembenadering die een budget van ~ 11 940 € · $oppervlakte^{-1}$ · $jr^{-1}$ vereist vanwege het grote aantal stalen en de staalnamecampagnes. Voor de strategie waarbij een enkele soort wordt gebruikt is een substantiële ecologische kennis van de te bestuderen soort (bijvoorbeeld *A. purpuratus*) vereist, en een dergelijke aanpak moet weefsel-specifieke metaalanalyses bevatten. Het voordeel van de multisoortenbenadering is het dubbele gebruik van metalen en vetzurenanalyse als tracer en biomarker, enerzijds, en als proxy om potentiële voedzame mariene voedingsmiddelen te bepalen anderzijds. De ecosysteem-gebaseerde benadering kan een vollediger beeld geven van de mariene ecosystemen, zelfs onder invloed van klimaat- en oceanografische variaties (d.w.z. voortdurende klimaatverandering, El Niño, ...).

(3) Veldstudies kunnen een afspiegeling zijn van wat er feitelijk gaande is in bepaalde ecosystemen, en ze zijn een prioriteit in ontwikkelingslanden zoals Peru waar de basisinformatie beperkt is. Deze in-situ studies zijn nodig om eerst de functionaliteit van het

ecosysteem te begrijpen. Vervolgens kunnen ex-situ experimenten worden uitgevoerd om specifieke onderzoeksvragen te beantwoorden die complementair zijn aan de in-situ verzamelde gegevens, b.v. in laboratoriumomstandigheden metaalopname en kinetiek bepalen met behulp van zeer gevoelige radiotracer of stabiele isotooptechnieken om de bioaccumulatie- en / of biomagnificatieprocessen in zeer belangrijke soorten (bijvoorbeeld *A. purpuratus*) beter te begrijpen.

(4) Schattingen van de risico's en de voordelen van de consumptie van zeevruchten zijn waardevolle informatie om het diëet van Peruvianen te verbeteren, maar interventies naar voedingsmethoden en voeding toe zijn de belangrijkste te nemen acties om de huidige gezondheidsproblemen (bijvoorbeeld bloedarmoede, ondervoeding, obesitas, ...) in Peru aan te pakken. Het samenwerken van volksgezondheidsexperts en mariene biologen kan leiden tot positieve acties (bv. evaluaties en verbetering van de menselijke gezondheid) op lokaal en nationaal niveau.

(5) De ontwikkeling van een ecosysteem-gebaseerd monitoringprogramma (pelagisch-benthische koppeling) als één entiteit is de sleutel tot het begrijpen van deze door NHCS en El Niño aangedreven mariene ecosystemen. Andere (sub) monitoringprogramma's kunnen ook worden geïmplementeerd voor specifieke onderzoeksvragen op bepaalde locaties, waarbij deze gericht op specifieke bio-indicatoren en / of op multisoorten, b.v. vanaf ~ 1 700 € · $locatie^{-1} \cdot jr^{-1}$; beschouwd worden als beste waarde voor optimaal budget in deze studie. Daarom moet een geïntegreerd nationaal plan (incl. Peruviaanse instellingen, bijv. IMARPE, ministeries, universiteiten, ...) met een multidisciplinaire aanpak (biologie, ecotoxicologie, menselijke gezondheid, sociale wetenschappen,

voedselproductie, enz, ...) overwogen worden om het scenario te voorkomen waarbij de mariene bronnen 1) overgeëxploiteerd zouden worden vanwege de grote vraag; of 2) helemaal niet-geëxploiteerd zouden worden vanwege de mate van verontreiniging op sommige locaties.

# RESUMEN

El presente estudio es sobre las preocupaciones actuales relacionadas con la seguridad alimentaria de los productos pesqueros en el Perú. Se investigaron las posibles estrategias para determinar el estado de salud ambiental (EHS) actual, a través de diferentes enfoques de bio-monitoreos. Se evaluaron los niveles de químicos (metales, ácidos grasos e isótopos estables) en compartimentos ambientales (desde material orgánico particulado (POM) hasta predadores) y se discutieron sus consecuencias en diferentes niveles de organización, ej. transferencia de contaminantes a través de la red alimentaria, valor potencial como productos marinos nutritivos y/o riesgo por el consumo de estos productos. Como resultado, se implementó un enfoque integrado para la evaluación de los riesgos y beneficios por el consumo de especies marinas del Perú.

Para determinar si el consumo de productos pesqueros peruanos podría representar un riesgo para la salud humana debido a los niveles de metales acumulados, se estudió la bioacumulación de metales en parte en diferentes especies de la cadena trófica (capítulo 2). De este estudio, se encontró que de las seis especies comestibles marinas estudiadas, alrededor del 10-20% de los moluscos (ej. *Argopecten purpuratus*, *Bursa ventricosa*, ...) y del 30-40% de los crustáceos (ej. *Romaleon setosum*, *Hepatus chilensis*, ...) excedieron los niveles residuales máximos (MRL) para consumo humano en As inorgánico y Cd. Los índices de riesgo integrados, ej. el cociente de peligro (THQ), el índice de peligro total (HI) y la ingesta semanal tolerable (PTWI) no excedieron sus límites respectivos, sin embargo los riesgos de cáncer

(TR) para As inorgánicos siempre fueron superiores al umbral ($1x10^{-6}$), por lo que un riesgo de cáncer es posible por el consumo de estas especies. Estos resultados se confirman cuando se demuestra nuevamente que las especies marinas de Perú están cargadas de As y Cd, y que los MRL y TR fueron más altos que los umbrales para algunas especies (de las 54 especies estudiadas) posteriormente evaluadas, ej. el cangrejo *H. chilensis*, el camarón mantis *Squilla* sp. el chorito *Semimytilus algosus* y el caracol *B. ventricosa*, entre otros (capítulo 4). Sin embargo, algunas especies marinas también son ricas en compuestos beneficiosos: ácidos grasos poliinsaturados de cadena larga (LC-PUFA): EPA, DHA, ARA; y micronutrientes: Cu, Fe, Mn y Zn. El norte de Perú se caracterizó por tener especies marinas con los LC-PUFA más altos (hasta 180 mg EPA / 100g), seguidos por los del centro y la región del sur. Además, las especies consideradas como especies potencialmente comestibles o no comestibles (ej. *C. sexdecimdentatus*, *C. filiformis*, *C. plebejus*) son alimentos prometedores para el futuro, esto debido a sus altos valores nutricionales (altos en LC-PUFA y micronutrientes). En base a los índices de riesgo, se concluyó que el consumo seguro de las cantidades estimadas de especies marinas podría conducir a una alta (beneficiosa) contribución (hasta el 80% de los valores recomendados) de LC-PUFA y micronutrientes en las poblaciones peruanas (capítulo 4). Según los índices nutricionales, los gasterópodos (ej. *B. ventricosa*) fueron calificados como las especies menos beneficiosas para el consumo humano.

La calidad de las especies marinas como productos pesqueros se monitorearon en el marco de la complejidad de los ecosistemas marinos peruanos, que son influenciados por el sistema de corriente Humboldt del Norte (NHCS), y además impactados por drásticas variaciones oceanográficas (ej. El Niño). Las intensidades del efecto del fenómeno de El Niño sobre los ácidos grasos y la contaminación por metales se monitorearon y analizaron utilizando la concha de abanico *A. purpuratus* en diferentes regiones del Perú (capítulo 3). *A. purpuratus* se identificó como una especie bioindicadora potencial para ser utilizada a lo largo de la costa del Perú. Las branquias, la glándula digestiva y el intestino fueron los tejidos donde la acumulación de metales fue la más alta en *A. purpuratus*. Los ácidos grasos fueron buenos biomarcadores cuando se realizaron comparaciones anuales (efecto El Niño), mientras que los metales permitió discriminar entre locaciones (grado de contaminación). La aplicación de una serie de biomarcadores (ej. metales, ácidos grasos) es clave para comprender la respuesta de los organismos a los estresores naturales y/o antropogénicos. Sin embargo, las relaciones costo-beneficio deben considerarse para un programa de monitoreo (con uso de *A. purpuratus*) sostenible a largo plazo en ecosistemas marinos del Perú.

Se integró las interacciones de la red trófica y la transferencia de metales en ecosistemas marinos del Perú para comprender el flujo de energía o masa (incl. contaminantes) en estos ecosistemas. En general, los valores de $\delta^{15}N$ aumentaron hacia el sur, desde la región norte hacia el centro y finalmente hacia la región sur. Se pudo observar una tendencia lógica de aumentar $\delta^{15}N$ y $\delta^{13}C$, de las fuentes de alimentos a los principales consumidores, pasando por la concha de abanico *A. purpuratus*. Esto indica al papel crucial de *A. purpuratus* como consumidor intermedio y principal alimento para los predadores en estas redes tróficas, esto también se confirma con los mixing modelos de SIAR y las líneas de regresión trófica. Los factores de magnificación trófica (TMF) de las redes alimentarias marinas estudiadas fueron más altos para Cu (1.58), As (1.45) y Cd (1.08), pero también se observaron TMF <1 para Ni, Pb, Fe, Mn y Zn como metales que no se biomagnifican. Por lo tanto, se concluye que la contaminación por As es una preocupación y problema serio en Perú, mientras que algunos ecosistemas son ricos en Cu natural o están contaminados con Cu.

En conclusión, esta investigación de doctorado enfatiza la importancia de (1) determinar el EHS de las diferentes regiones del Perú; (2) diseñar las estrategias correctas para el monitoreo de EHS; 3) la necesidad de complementar los estudios de campo (este estudio) con experimentos de laboratorio; (4) la posibilidad de mejorar la nutrición de la población peruana a través del consumo de alimentos de origen marino; y (5) mejorar los programas nacionales de bio-monitoreo en Perú.

(1) El análisis regional mostró que los lugares más al sur estaban más degradados que los más al norte, debido a la contaminación y/o enriquecimiento de metales. Esto también se reflejó cuando se usaron biomarcadores (ej. ácidos grasos, isótopos estables). Cuando se compararon los compartimentos ambientales, como sedimentos y seston con diferentes límites internacionales para la protección del ecosistema, también estuvo presente este patrón de bajo EHS en los ecosistemas marinos del sur.

(2) Un enfoque basado en investigación antes del análisis es ideal para la estrategia correcta para diseñar un monitoreo de EHS. El uso de una sola especie es el enfoque más óptimo (1 740 € · área · año), mientras que el enfoque de múltiples especies (n = 6) basado en especies ecológicas y económicamente importantes requiere un presupuesto

medio (~ 5 700 € · área · año ). Mucho más costoso que los enfoques anteriores, es el enfoque ecosistémico que requiere un gran presupuesto de ~ 11 940 € · área · año, debido a la gran cantidad de muestras y campañas de muestreo. Para el enfoque de una sola especie, se requiere un conocimiento exhaustivo de la ecología de la especie en estudio (ej. *A. purpuratus*), y dicho enfoque debe contener análisis de metales en tejido específicos. La ventaja del enfoque de múltiples especies es el uso dual del análisis de metales y ácidos grasos como trazador y biomarcador, y como proxy para determinar (ej. scoring) la potencialidad nutritiva de los alimentos marinos. El enfoque basado en el ecosistema puede dar la "imagen completa" para comprender los ecosistemas marinos, incluso bajo el efecto de variaciones climatológica y oceanográficas (ej. cambio climático, El Niño, ...).

(3) Los estudios de campo pueden reflejar lo que realmente está sucediendo en ciertos ecosistemas, y son una prioridad en los países en desarrollo (ej. Perú), donde la información de referencia (ej. estudios de línea base) es limitada. Estos estudios *in-situ* son necesarios para comprender primero la funcionalidad del ecosistema; posteriormente se puede realizar experimentos *ex-situ*. Los estudios *ex-situ* deben realizarse para abordar preguntas de investigación específicas que son complementarias de los datos recopilados *in-situ*, ej. captación y cinética de metales utilizando radiotrazadores altamente sensibles o técnicas de isótopos estables en condiciones de laboratorio para comprender los procesos de bioacumulación y/o biomagnificación en especies marinas clave (ej. *A. purpuratus*).

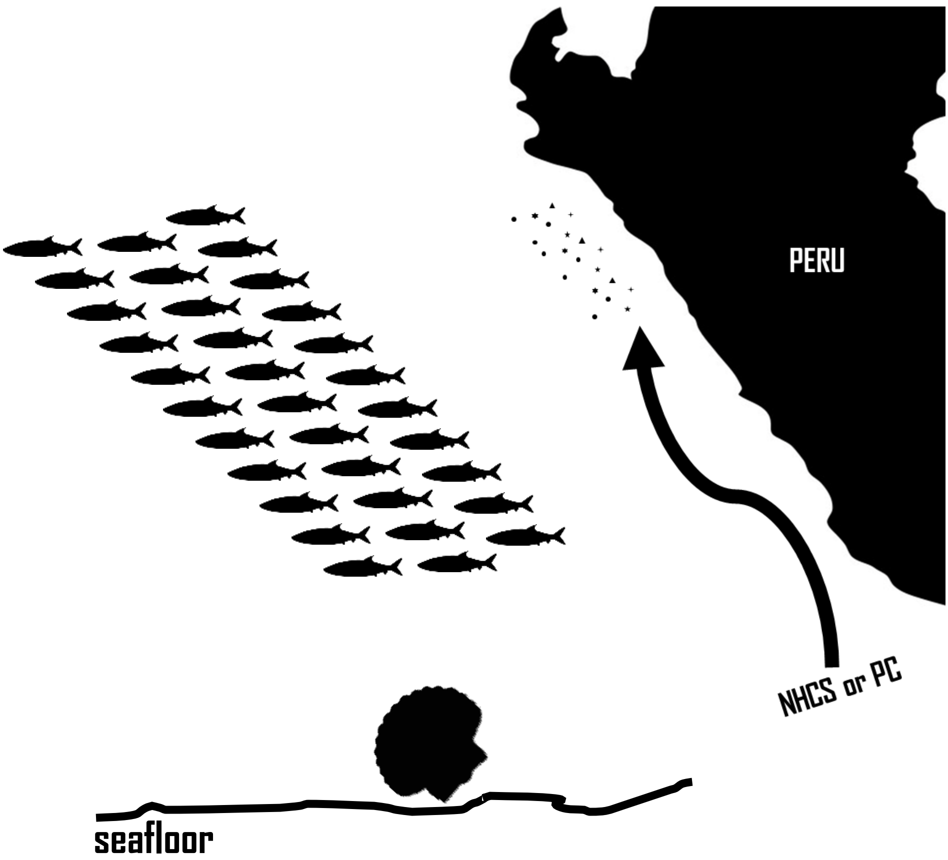
(4) Las estimaciones de riesgo y beneficios para el consumo de mariscos son información valiosa para mejorar las dietas para los peruanos, pero las intervenciones sobre las prácticas de alimentación y

nutrición son las acciones más importantes para abordar los problemas de salud actuales (ej. anemia, desnutrición, obesidad, ...) en Perú. El trabajo conjunto de expertos en salud pública y biólogos marinos podría conducir a acciones (ej. evaluaciones de salud humana) a nivel local y nacional.

(5) El desarrollo de un programa de monitoreo basado en el ecosistema (ej. acoplamiento pelágico-bentónico) como una entidad es la clave para comprender estos ecosistemas marinos influenciados por el NHCS y El Niño. También se pueden implementar otros (sub) programas de monitoreo para preguntas de investigación específica en ciertas locaciones con especies bioindicadores y/o especies múltiples, ej. desde ~ 1 700 € · ubicación · año; considerado como "presupuesto óptimo" en este estudio. Como resultado, un plan nacional integrado (incluidas las instituciones peruanas, ej. IMARPE, ministerios, universidades, ...) con el uso de un enfoque multidisciplinario (biología, eco-toxicología, salud humana, ciencias sociales, producción de alimentos, etc.) debe ser considerado para comprender y prevenir un escenario en el que los recursos estarían 1) sobreexplotados debido a su alta demanda; o 2) no explotados y no utilizados debido a su grado de contaminación en ciertas locaciones.

# CHAPTER 1

# GENERAL INTRODUCTION



# 1. Peruvian waters as home of the world's largest productivity: an ecosystem under El Niño-Southern Oscillation (ENSO)

Peruvian marine ecosystems are driven by the northern Humboldt Current system (NHCS) or Peru Current (PC) (see Fig 1), which is one of the most productive Eastern boundary upwelling systems. Upwelling of cool waters brings nutrients to the surface that substantially increase biological productivity in this area (Chavez et al. 2008). The NHCS of Peru produces more fish per unit area than any other region in the oceans worldwide (Chavez et al. 2008). The small pelagic fish Peruvian anchovy *Engraulis ringens* is the reason that Peru is the home to one of the biggest fishery of the world (Majluf et al. 2017). Nevertheless, the sinking and decay of surface-derived primary production and poor mixing causes also an intense and extremely shallow Oxygen Minimum Zone (OMZ) (Chavez et al. 2008; Espinoza et al. 2017). This permanent OMZ is in fact part of the explanation of the unique high productivity of the Peruvian anchovy *Engraulis ringens*. The OMZ concentrates their prey and at the same time reduces the predation on zooplankton and small pelagic fish (Chavez 1987; Chavez et al. 2008).

Besides the oceanic NHCS or PC, one coastal branch (Peru Coastal Current) is present in this complex current system of the Eastern Equatorial Pacific Ocean, which is clearly influenced by the Peru-Chile Current System (Cabarcos et al. 2014) (see Fig 1). Between the PC and Peru Coastal Current, the Peru–Chile Countercurrent flows in the opposite direction with moderate surface temperatures (Huyer et al. 1991). The PC waters feed the South Equatorial Current, flowing westward, while the North-Equatorial Countercurrent represents the most important equatorial surface current with a net eastward flow (Fig 1) (Tomczak and Godfrey, 2001). This North-Equatorial Countercurrent is deflected to the north, feeding the Costa Rica Coastal Current and the North-Equatorial Current. Below the South Equatorial Current, and in the opposite direction, is the Equatorial Undercurrent that provides the equatorial upwelling with nutrient-rich waters, and its role as a fertilizer is crucial in controlling primary productivity in the region (Murray et al. 1994). The Equatorial Undercurrent continues eastward, feeding the Peruvian coastal upwelling (Toggweiler et al. 1991) and the Peru Countercurrent, characterized by cool and high-salinity waters (Fig 1) (Wyrtki, 1981; Cabarcos et al. 2014).

The Coastal pre-Inca and Inca communities depended considerably on ocean resources for their survival, as described through their pottery left behind by the native South Americans. The NHCS or PC biological richness (incl. current systems) was also studied and artistically embodied in the Inca's antique art pieces (Coker, 1910; Rostworowski, 2005; cited by Chavez et al. 2008; Lavallée & Michèle, 2012; Reitz et al. 2008). In the modern world, the NHCS' productivity was also confirmed with the discovery and use of the tremendous deposits of seabird dropping or guano (Cushman, 2003), which was related to the high consumption of their main prey, the Peruvian anchovy *E. ringens*. However, the strong El Niño 1957-1958 led to a dramatic decrease in seabird population due to the decline of the already heavily fished small fish, *E. ringens* (Chavez et al. 2008). El Niño events are thought to have been occurring for thousands of years. For example, it is thought that El Niño affected the Moche in modern-day Peru, who sacrificed humans in order to try to prevent the rains (Bourget 2016; BBC News, 2019).

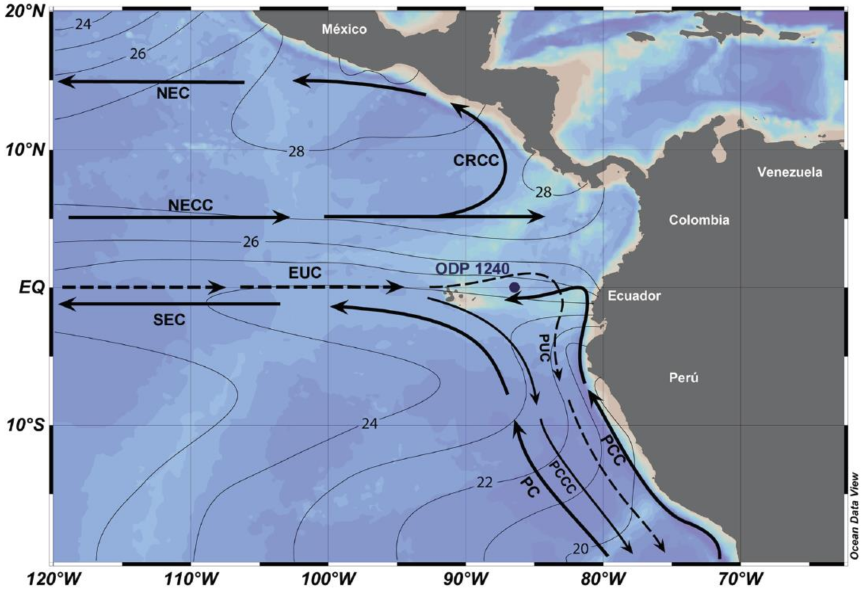



**Fig 1.** Surface and subsurface currents in the Eastern Equatorial Pacific Ocean (EEP). Surface currents: PC: Peru Current or NHCS; PCC: Peru Coastal Current; PCCC: Peru–Chile Countercurrent; SEC: South Equatorial Current; NEC: North-Equatorial Current; NECC: North-Equatorial Countercurrent and CRCC: Costa Rica Coastal Current. Subsurface currents: EUC: Equatorial Undercurrent and PUC: Peru Undercurrent. Black isotherms represent annual average temperature (Cabarcos et al. 2014).

El Niño Southern Oscillation (ENSO) is the cycle of warm and cold sea surface temperature of the tropical and eastern Pacific Ocean. El Niño is the warm phase of the ENSO, when a band of warm ocean water is developed in the central and east-central equatorial Pacific, including the area off the Pacific coast of South America. The opposite "La Niña" phase consists of a basin wide cooling of the tropical Pacific and thus the cold phase of ENSO (Trenberth, 1997; Takahashi, 2017). The term

El Niño is associated to "the boy Christ-child" since the current warm water in the Pacific near South America is often the warmest at Christmas (Trenberth, 1997). In developing countries (e.g. Peru), El Niño is associated to low air pressure, above-average (+ 0.4°C; for at least three months) sea surface temperatures and abnormal rains and winds. As consequence, the most important economic activities, e.g. agriculture and fisheries of these countries are impacted. The El Niño phenomenon is irregular, intervals of occurrence can have fluctuations from one to seven years. There is no consensus on whether climate change has an influence on the occurrence, strength or duration of El Niño events (Collins et al. 2010; Takahashi, 2017).

The NHCS is the region where ENSO, and climate variability in general, is most notable. Sea surface temperature (SST) anomalies greater than 10 ºC have been observed during strong El Niño events (Chavez et al. 2008). The modern strong El Niño events include those taking place in 1925, 1941, 1957, 1982 and 1997 (Barber and Chavez, 1983). Recently, two El Niño events occurred with different intensities in 2016 and 2017 in Peru, characterized as "El Niño Global" and as "El Niño Costero", respectively (Fig 2). ENSO events during 1982-1983 and 1997-1998 were also characterized as "El Niño Global or El Niño extraordinario" because the magnitude of these events caused world-wide impacts (Fig 2) (Takahashi, 2016; 2017). In general, El Niño event names are given in relation to the magnitude of their influence and/or the size of the impacted area, which could be more coastal or oceanic-coastal related.

On the other hand, El Niño events from 1925 and 2017 are termed "Niños Costeros" as their impact was mainly locally, e.g. Peruvian locations (see Fig 2). It is noteworthy to mention that the ENSO 2016 was part of a world-wide ENSO event, however non-substantial impacts (i.e. high rains, flooding,…) were observed in Peru (Fig 2) (Takahashi, 2017; Loaiza et al. 2018; 2020). In contrast, El Niño 2017 was drastically intense for many regions of Peru, the north was the most affected by the El Niño-driven conditions. The coast exhibited the weakening of the winds with the development of the rain band called Inter tropical Convergence Zone (ITCZ) and the sea surface warming (Takahashi, 2017). The coastal waters of northern Peru were also impacted by the intense rain periods that led to abrupt change of the salinity, and abnormal discharges from lotic ecosystems (incl. rivers, estuaries) (Takahashi, 2017; Loaiza et al. 2020).

Rivers transport eroded particulates, including contaminants and in this specific case: agriculture and mining derived-wastes from the Northern Andean areas of Peru (Forrest et al. 2007; Loaiza et al. 2020). In general, these environmental changes and discharges alter the entire marine ecosystems by 1) increasing contaminant levels (e.g. metals) in water and particulate matter; 2) changing salinity that could play a crucial role for metal speciation and bioavailability; and 3) shifting bioaccumulation rates of metals due to the high temperatures; among others. As result, the marine species from these ecosystems might be more susceptible to accumulating contaminants (i.e. metals), which can also cause adverse effects on the health of Peruvian (consumers) populations.

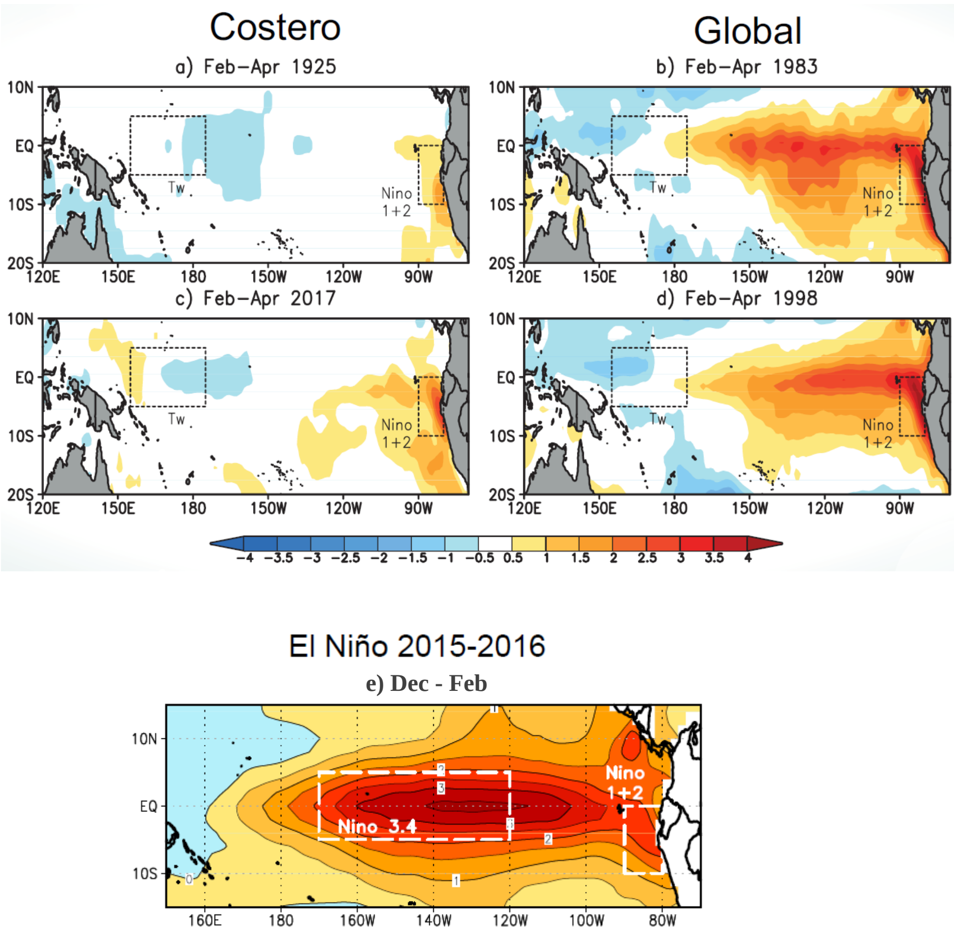



**Fig 2.** Sea surface temperature anomalies from February-April during the last hundred years. "El Niño costero" events of a) 1925 and c) 2017, and the "El Niño Global" events of b) 1983 and d) 1998. e) Patterns of sea surface anomaly in the "mature" phase (Dec-Feb) for El Niño 2015-2016 ("El Niño Global"). Adapted from Takahashi 2016; 2017.

## 2. Peruvian scallop *Argopecten purpuratus*

Besides the Peruvian anchovy *E. ringens*, there are other important Peruvian marine species affected by the diverse ENSO' occurrences in the Pacific Ocean, such as the Peruvian scallop *Argopecten purpuratus* and its culture (incl. the associated macrobenthic communities) (Kluger et al. 2018; Loaiza et al. 2020). The Peruvian scallop *A. purpuratus* is distributed in the Southern Pacific Ocean from Paita (5.1°S), Peru to Valparaio (33.1°S), Chile. This scallop is mainly found in shallow water of 5-40 m in semi-protected bays on sandy, stony or sandy-muddy substrate. The most important *A. purpuratus* natural banks and aquaculture areas are in Sechura Bay, Paracas Bay, Independencia Bay, Samanco Bay, Tortugas Bay and Lobos de Tierra Island (Wolff et al. 2007; PRODUCE, 2019).

The Peruvian scallop *A. purpuratus* is a short-lived free-living functional hermaphrodite with broad cast spawning, fertilization occurs in the water column, where eggs and sperm are released. Maximum shell height of *A. purpuratus* is from 90 to 140 mm, and maturity is reached at ~25 mm (see Fig 3) (Mendo et al. 2016). The spawning of this species occurs from around September to May, but during El Niño years, spawning is prolonged throughout the year (Wolff et al. 2007; Mendo et al. 2016). Seston quality and horizontal fluxes of food (i.e. currents and particulate organic carbon) are the most important factors in gonad growth (incl. spawning variations) (Aguirre-Velarde, 2009; Mendo et al. 2016). Pectinidae species, such as *A. purpuratus* are filter-feeding organisms, they create a water current over their gills which also serve as filter structures: particles are trapped in the mucous covering the gills and cilia move the food toward the mouth after which it is digested in the digestive gland. Food sources are zoo- and phytoplankton, seston, bacteria, organic particulates, and resuspended sediment, which, in accordance to previous studies, are probably rich in contaminants (e.g. metals) (Loaiza et al. 2015; Mendo et al. 2016).

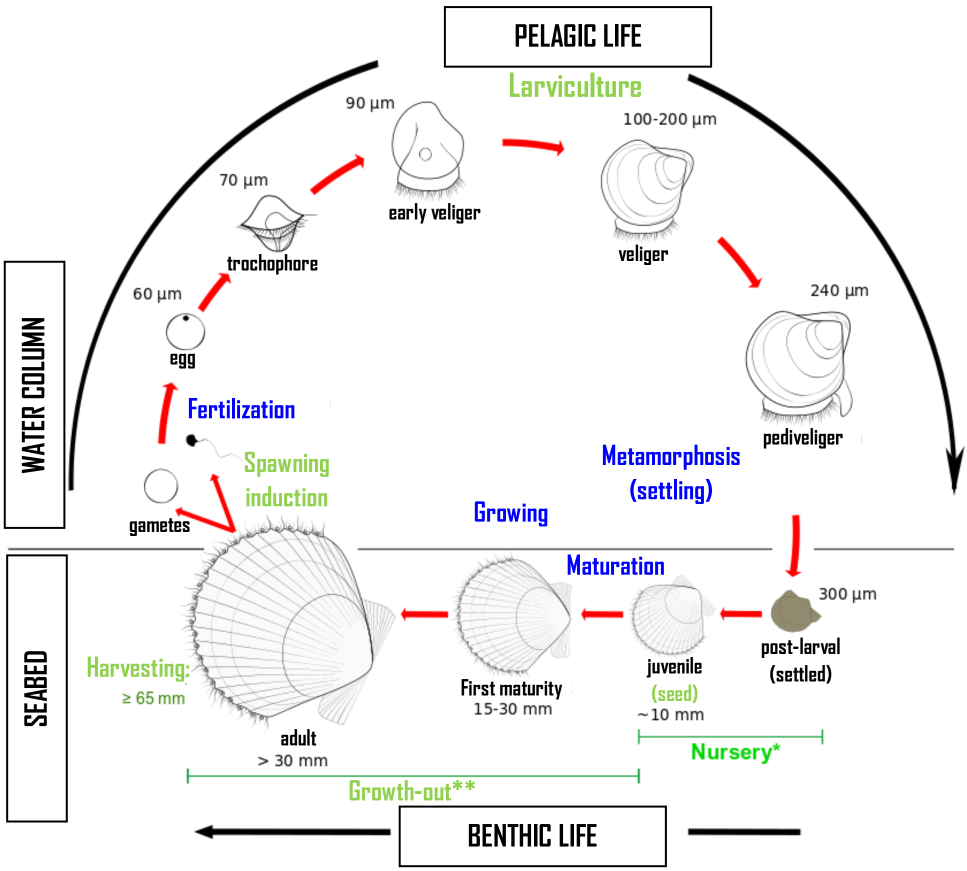



**Fig 3.** Life cycle of the Peruvian scallop *Argopecten purpuratus* and aquaculture/re-stocking interventions (printed in green) in the production process.

**Modified from Aguirre-Velarde & Flye-Sainte-Maire (2019).**

***Nursery: Cultivation in controlled environment after the metamorphosis that is performed before transfer to the natural environment.**

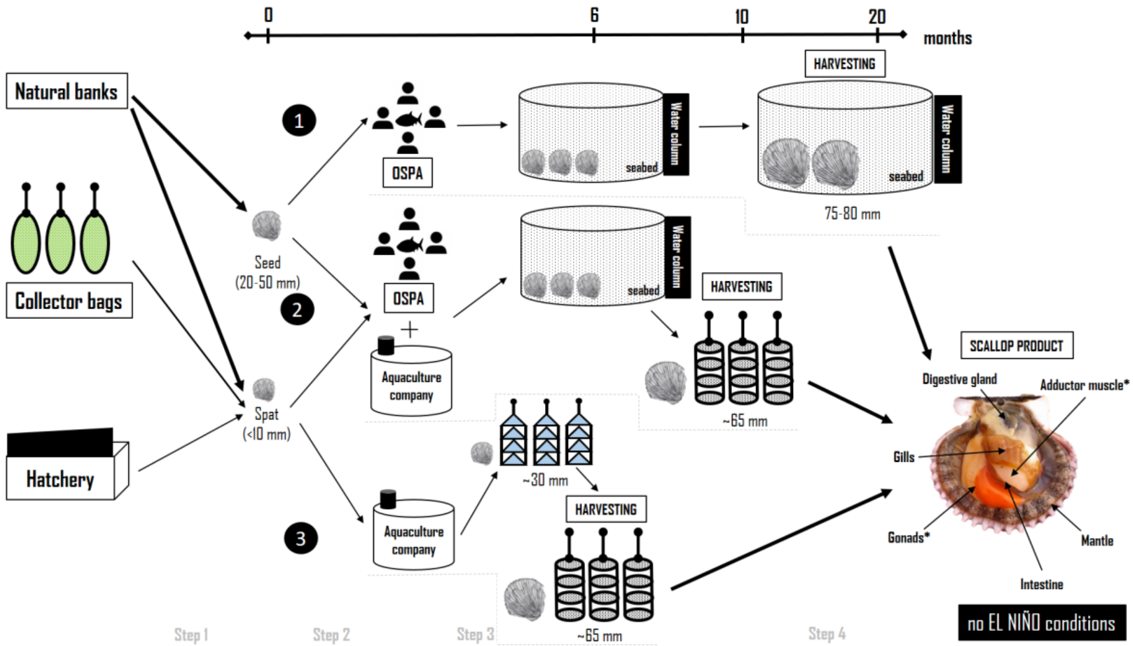
****Grow-out: Cultivation on the seabed in corrals and/or suspended system (lanterns).**

The aquaculture and/or 're-stocking' of *A. purpuratus* has been widely distributed in many parts of the coast of Peru, and therefore their production intensively increased to up to 67 700 metric tonnes MT/yr. in the last decade (PRODUCE, 2019). Peruvian scallop re-stocking is a practice mainly performed in Sechura Bay, where scallop seeds and/or spats coming from hatcheries or natural stocks (i.e. collected from natural banks or with collector bags) are cultivated in corrals or by sea ranching in natural bank areas (Mendo et al. 2016). Spats and seeds of *A. purparatus* are individuals of <10 mm and 20-50 mm, respectively. The collection of seeds from natural banks is prohibited since 2006, however fishermen associations are still conducting this practice because the implementation of collector bags and/or hatcheries involves a high economic investment (Mendo et al. 2016; PRODUCE, 2019). After the seed or spat is obtained (either through collection or production), the culture process starts with the sieving of individuals for the selection of 10, 15 and 20 mm diameter individuals. Then the following steps are according whether the producers are private aquaculture companies or fishermen associations, or a mixture (i.e. company & fishermen association) of both (Acquapisco SA, pers. comm.) (Fig 4).

In private companies, spats are suspended in pearl nets until they reach about 30 mm in shell height, then they are transferred to lantern nets until reaching a commercial size of ~65 mm as a minimum. It takes about 6 months or 180 days for the scallops to reach 60 mm in Sechura Bay, however it varies for each culture location. In Casma (e.g. Tortugas Bay), the scallops need 14 months to reach 75-85 mm in suspended culture (Acuapesca, 2019; Acquapisco SA, pers. comm.)

(Fig 4). On the other hand, fishermen from the associations normally throw the seeds from overboard in the concession areas, where corrals are installed for bottom culture (ranching). About 20 months are required to reach 80 mm of scallops in bottom culture, this is also affected by the location conditions (PRODUCE, 2019). In case there is a cooperation between private company and fishermen, a combination of methodologies is applied, the scallop culture can start in bottom corrals and then suspended for the last period of fattening to the commercial size, however this process might change in relation to the oceanographic conditions (e.g. El Niño event) (Fig 4).

Maintenance of suspended and bottom cultures is time (cost) demanding. Bottom culture requires more arduous activities than the suspended culture, the divers harvest scallop' predators (e.g. *Bursa ventricosa*, *Romaleon setosum*) while they clean and repair the corrals. In suspended culture, a diver also supports in the maintenance process, but the cleaning and control of predators are mainly performed on board by other fishermen. A hydraulic arm on board is used to lift the lantern nets of the suspended culture for maintenance (Mendo et al. 2016; pers. obs.). For harvesting, a similar process as for maintenance is performed but more intensive. The suspended cultures and seabed ranches are harvested until they are completely empty, after which the next seeding or production period can start again (Acuapesca, 2019; Acquapisco SA, pers. comm.).




**Note.- to our knowledge, the most likely pathway is related to the thickness line for Step 1.**
**(*) Adductor muscle and gonads are the most common tissues for human consumption (also exportation).**

**Fig 4.** Peruvian Scallop *A. purpuratus* culture in Peru. 1) Scallop culture technique mainly used by OSPA; 2) Scallop culture technique mainly used by the private company associated with the OSPA; and 3) Scallop culture technique mainly used by the private aquaculture company. OSPA is called to the Organizaciones Pesqueras Artesanales (in english; Artisanal Fishing Organizations).

The northern region has around 80% of that total Peruvian scallop production and re-stocking. However, this increasing pattern has been drastically affected by the last ENSO events. The north of Peru is characterized for being the most vulnerable and heavily-impacted due to ENSO-driven conditions (Takahashi 2017; Kluger et al. 2018; Loaiza et al. 2020), which led to a substantial decline of production and re-stocking of the Peruvian scallop. During the “El Niño Costero”, or also called “Abrupt El Niño” of 2017, a massive mortality of Peruvian scallops (incl. other benthic species) was observed along the entire Sechura Bay area (~1120 km$^2$) and nearby the Illescas Reserved Zone (Kluger et al. 2018; Loaiza et al. 2020; pers. obs.). All production disappeared in hours to days due to this ENSO event. These new and dramatic recent scenarios stressed the urgent need to see the Peruvian marine ecosystems as a whole and stresses the importance of studying bentho-pelagic interactions in trophic food webs (Docmac et al. 2017; Espinoza et al. 2017).

## 3. Peruvian coastal waters: a regional approach of using marine resources

In the North, Peruvian scallop aquaculture in Sechura Bay provides work for about 5 000 fishermen and 25 000 persons involved in related activities, e.g. divers, motorists, and scallop’ processing and transport, among others (Mendo et al. 2008; 2016; Kluger et al. 2019). Nevertheless, other living marine resources (also part of the Peruvian scallop associated communities) are also relevant in that region, e.g. octopus (e.g. *Octopus mimus*), sea snails (e.g. *Bursa ventricosa*), and crabs (e.g. *Romaleon setosum*) amongst others. The Piura region hosts the largest number of artisanal fishing vessels when compared to other regions of the country (31.7% of all national artisanal vessels, Castillo et al. 2018). These fishermen have a multi-species and multi-gear focus, which has a long tradition in the northern region (Kluger et al. 2019). The highest fishing landings (per year) are also in the northern locations of Peru, in Chimbote with 657 000 metric tonnes (MT), and in Chicama with 680 000 metric tonnes (MT) (PRODUCE, 2019). The north (e.g. Sechura Bay) of Peru is also characterized by the presence of numerous industrial activities, such as high-capacity harbours, phosphate factories, oil platforms, fishery factories, artisanal and industrial ports (IMARPE, 2007; 2019).

From the 53 rivers that are part of the Pacific watershed off Peru, the northern region is influenced by major fresh and brackish water input along the coast of Peru, namely through the mouth of the Virrila Estuary which is occasionally connected with the Piura river (INRENA, 2005; MINAGRI, 2019). The Virrila Estuary heavily increases in caudal discharge when the Piura river is a contributor (up to 3000 $m^3/s$ in ENSO 2017; IMARPE, 2017) during El Niño event conditions, but only when abnormal precipitations occur (Takahashi, 2017). These discharges alter the entire marine ecosystems, and as previously mentioned most of the biodiversity and high species biomasses (e.g. Peruvian scallop) just die off in the north (Kluger et al. 2018; Loaiza et al. 2020). The high precipitation causes inundation and strong erosion around rivers which commonly comes with the release of high concentrations of pollutants (e.g. metals) as common components of these fresh-and-brackish water inputs (Diop et al. 2016; Forrest et al. 2007; Kehrig et al. 2013; Loaiza et al. 2020).

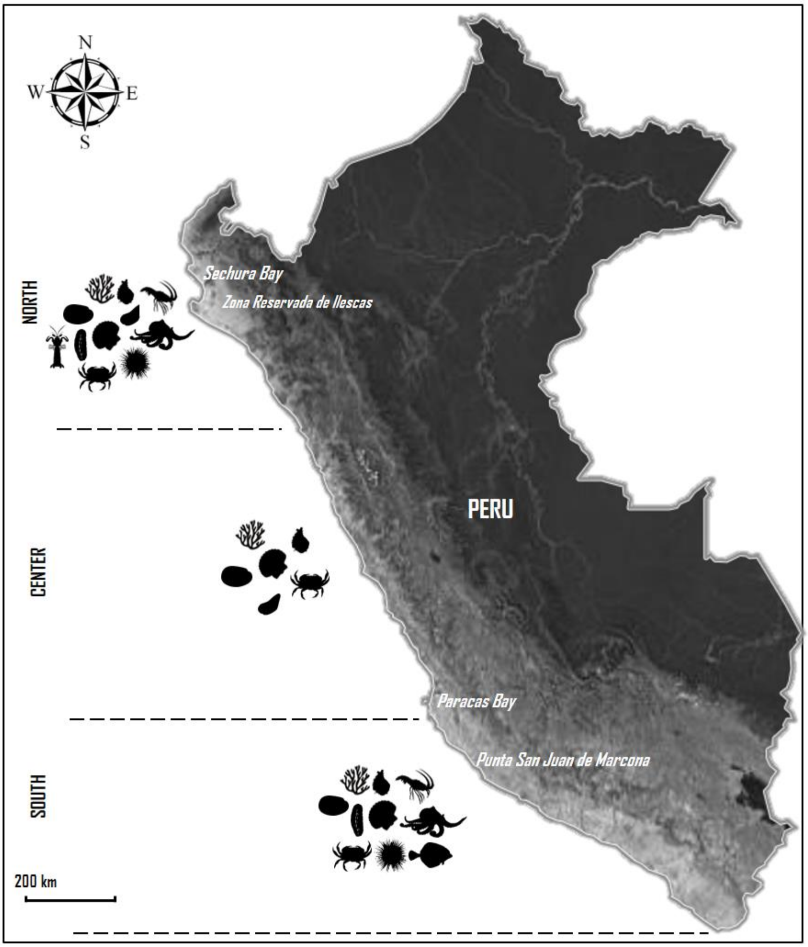



**Fig 5.** Map of Peru with indication of the regions: North, Center and South, and important considered locations (Sechura Bay, Zona Reservada de Illescas, Paracas Bay, Punta San Juan de Marcona) where samplings for this study were conducted.

The center-south of Peru is also characterized by the production of the cultivated Peruvian scallop in Pisco (Ica Region), but to a lesser extent than the northern region (see Fig 6.) (PRODUCE, 2019). Production of scallops decreased strongly in Ica since 2001 after the El Niño 82/83 and 97/98, however recoveries were observed in the recent years, about

800 metric tonnes (MT) have been harvested in 2016 and 2017 (Mendo et al. 2016; PRODUCE, 2017). Many fishermen have migrated from Pisco to the north of Peru after the El Niño 97/98, where they started sea ranching operations in Peruvian scallop natural banks that contributes to the current high production of the north (Mendo et al. 2008; 2016; PRODUCE, 2019).

Besides the Peruvian scallop, other commercial invertebrates are also abundant in the center of Peru: mussel (*Aulacomya atra*), razor clam (*Ensis macha*), snail (*Thaisella chocolata*), octopus (*Octopus mimus*), crab (*Romaleon setosum*), sea urchin (*Lexochinus albus*), among others (Mendo et al. 2008; PRODUCE, 2017). The Peru center-south region has also high landings (per year) in fishing products (incl. aquaculture), e.g. Callao and Pisco had about 376 000 and 485 000 metric tonnes (MT) of landings in 2017, respectively (PRODUCE, 2019). Center-south artisanal fishermen are about 9000 and 5000 in Lima-Callao and Ica, respectively, which is as high as in Arequipa (~7000 fishermen) and Ancash (~6000 fishermen). El Niño has impacted the center-south of Peru in different ways, rains and high river discharges also occurred during strong El Niño periods, however El Niño is favorable for scallop culture in this region (Mendo et al. 2016).

Water temperature during strong El Niño conditions rises around 10°C and the dissolved oxygen levels increase in deeper areas in the center-south of Peru (Tarazona et al. 1988; Arntz and Fahrbach, 1991; Mendo and Wolff, 2003). These two factors greatly influence the population dynamics of the Peruvian scallop as well as the carrying capacity of the bays (Wolff and Mendo, 2000; Mendo and Wolff, 2003). Mendo and

Wolff (2003) suggested that the significant increase in stocks of Peruvian scallop from the center-south is due to the combined effect of (i) an increase in the reproductive activity through the acceleration of maturation and an increase in the frequency of spawning, (ii) the shortening of the larval stage and greater larval survival, (iii) greater individual growth rates, (iv) greater survival of juveniles and adults due to the reduction of predators, and (v) an increase in the carrying capacity of the bays due to greater levels of oxygen.

The center-south, specifically Paracas Bay (Pisco) has other environmental stressors, such as the impacts of the most important fishery industry, the Peruvian anchovy fishmeal industry, as well as the emission and pollution of the Camisea Gas Fractionation Plant – PLUSPETROL (SNP, 2003). In 2004 a 14 km long submarine emitter APROPISCO was built to discharge effluents and contaminants from the fishery factories outside the buffer zone of the National Paracas Reserve, however, impacts had already occurred for several years (SNP, 2003; DIGESA, 2008; SERNANP, 2019).

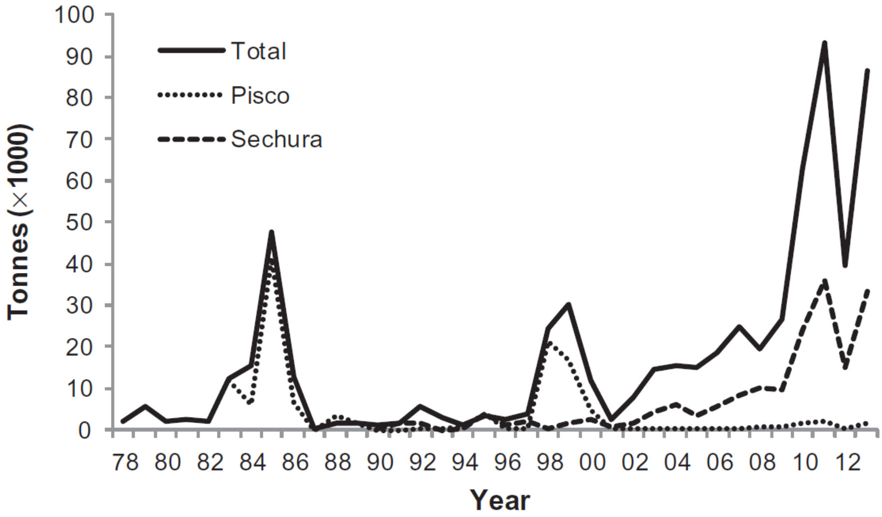



**Fig 6.** Scallop total production (thousands of tonnes) in Pisco and Sechura Bay (Piura) (Mendo et al. 2016).

Locations more southward along the Peruvian coast are also highly abundant in marine edible species, the most important are fish but also some marine invertebrates, e.g. the 'erizo rojo' *Loxechinus albus* and other shellfish, e.g. *Octopus mimus*, *Aulacomya atra*, *Concholepas concholepas*, as well as the 'sargazo' algae *Macrocystis pyrifera*. These species occur on the seafloor (benthos) and are important sources of proteins for the southern Peruvian population. The artisanal fishermen are about 13000 in the southern region, from Arequipa to Tacna (Castillo et al. 2018). The southernmost location in Ica; Marcona district (also considered as southern region in this study), contributes to the southern fishermen populations, with a group of 600 fishermen, who work from shore (i.e. sargazo harvesting) and from boats (IW:LEARN, 2019; PPSJ, 2019). Fishing landings from San Nicolas – San Juan Marcona to Ite Vila Vila – Tacna were around 120 000 metric tonnes (MT) in 2017, being Marcona the 3rd highest landing port with 10 810 metric tonnes (MT) (PRODUCE, 2017).

Non-scallop culture and/or re-stocking activities are performed in the south of Peru, and Peruvian scallop individuals are present in Marcona, but in low densities (PRODUCE, 2019; Loaiza et al. in prep.; pers. obs.). El Niño phenomenon have been poorly described in the southern Peru, and specifically less in bentho-pelagic ecosystems. More importance has been given to the El Niño effect on the Peruvian scallop aquaculture in the north (i.e. Sechura Bay) (ENFEN, 2017; IGP, 2018). However, it is well-known that El Niño' adverse effects are lower in magnitude for the center-southern locations, e.g. Paracas Bay, in comparison to those in the north (Mendo et al. 2016). In the last Abrupt El Niño or Niño Costero 2017, the water temperature showed positive anomalies in the southern region, but only up to + 1.5°C. The rains were intense in the Andes regions (from the center and south of Peru), but not on the coasts. The river flow rates were also higher than the average historic flow rates in El Niño 2017, and the reservoirs were up to their 100% hydraulic carrying capacity along the Peruvian territory (ENFEN, 2017, IGP, 2018). Nevertheless, fresh-water plumes were not observed in marine areas such as, scallop aquaculture concessions in Paracas Bay (pers. obs. and pers. comm.).

The southern region is also characterized by industrial activities (e.g. mining, anchovy meals factories,…) and artisanal fisheries and ports along the entire coast (MINEM, 2019; PRODUCE, 2019). The Iron mining from Shougang, located in the proximity of the Punta San Juan Reserve (Marcona) causes one of the main stressors in the southern marine ecosystems (Núñez-Barriga et al. 1999; Adkesson et al. 2019;

PPSJ, 2019; SERNANP, 2019; pers. comm.).

## 4. Chemical profiling for tropho-dynamic studies and environmental health status

As previously described, benthic species, e.g. octopus (e.g. *Octopus mimus*), scallops (e.g. *A. purpuratus*), snails (e.g. *Thaisella chocolata*, *Bursa ventricosa*), crabs (e.g. *Romaleon setosum*), sea urchin (*Lexochinus albus)*, algae (e.g. *Chondracanthus chamissoi, Macrocystis pyrifera*) and other taxonomic groups are part of the national fishing landings, and are the main target of the artisanal fishermen (PRODUCE, 2017; 2019). These species are relevant for the artisanal fishermen's economic subsistence (incl. eventual income), as well as for their own local consumption. In the last decade, the internal national trade of living marine resources or fishing products has increased from 600 000 to about 700 000 metric tonnes (MT). The following order is seen for different species groups (in landing per year): squid > shrimp > crab > algae > scallop > others (PRODUCE, 2017). Nevertheless, it is noteworthy to mention that part of the artisanal fishing is not declared and/or supervised by the national authorities in Peru (PRODUCE, 2019; SANIPES, 2019; pers. comm.).

Some of these more artisanal-target species have rarely been studied or registered in NHCS' management studies, therefore the effect of environmental stressors (i.e. ENSO, contaminants) are unknown for the Peruvian marine food webs (IMARPE, 2019). Moreover, the species that are considered as non-edible have not yet received substantial attention of Peruvian scientists and authorities (IMARPE, 2019; PRODUCE; 2019). A clear example is the high number of studies on specific species based on their economical contribution or Gross Domestic Product (GDP), e.g. Peruvian anchovy, Peruvian hake, Peruvian scallop,… (IMARPE, 2019; PRODUCE; 2019). Recent studies (i.e. Espinoza et al. 2017) have investigated the trophic web in Peruvian marine waters including commercial and non-commercial species to understand the functionality of the NHCS ecosystem as a whole. Docmac et al. (2017) also studied different marine species from the Pacific Ocean (off Chile), by coupling bentho-pelagic communities in an upwelling system. This study in the Northern Chile region highlighted the importance of bentho-pelagic interactions in marine ecosystems.

In accordance to the described non-steady environment conditions in the NCHS, with the addition of the El Niño-driven impacts and anthropogenic stressors, it is urgent to document *in-situ* the environmental health conditions of the Peruvian marine ecosystems. The use of a multi-parameter approach of chemical composition is an up-to-date technique to evaluate marine ecosystems functionality (Viarengo and Canesi, 1991; Sardenne et al. 2017). We used stable isotopes to uncover food web relationships, fatty acid composition to further improve our understanding of the food web as well as to discover seafood that could have beneficial health effects for humans, and metal bioaccumulation to estimate both beneficial effects of essential metals (micro-nutrients) as possible risks due to metal toxicity.

Metals can be classified as essential (or micro-nutrient: Cu, Fe, Mn, Zn) and non-essential (e.g. potentially harmful: As, Cd, Pb) elements depending on their concentration and specific functions at cellular, enzymatic and protein levels. Elements carrying out fundamental biological functions that are necessary for growth and development in all organisms (from bacteria to humans) are considered as essential, while elements that do not have any known functionality for living organisms, are considered as non-essential (Grosell et al. 2007; Peña et al. 1999). Nevertheless, elements famously considered as toxic, harmful and/or non-essential, e.g. Ni, As and Cd in some cases are essential for some living organisms (Hunter, 2008).

Some studies showed that these elements are required in minimum (or trace) concentrations, such as 12.5 µg per day of As for humans, while ~45 pM of Cd could play a role for marine diatom growth (Lane & Morel, 2000; Hunter, 2008). Arsenic exposure is associated to various diseases (cancer, cardiovascular disease (CVD),…), as well as is the cause of numerous fatal intoxications (Benramdane et al. 1999; US EPA, 2012). Nevertheless, inorganic As seems to be the only toxic form or species of this metal. Therefore, it is crucial to determine individual concentrations of every arsenical species (e.g. arsenite (As[III]), arseniate (As[V]), arsenobetaïne (Asbet)), to assess the real toxic effect of this metal (Benramdane et al. 1999). There is also evidence that Ni is an essential trace element in several animal species, plants and prokaryotic organisms, and it seems to be essential for humans (tolerable upper intake level: ≤ 1 mg/day for adults). However, no data are available concerning nickel deficiency for humans (WHO, 2019; Cempel & Nikel, 2006). In conclusion, almost all metals can have beneficial and/or harmful effects in living organisms in relation to their concentration, and when the metal-specific-threshold of any metal is exceeded, it will cause adverse effects.

Metals can also play a role as tracers of nutrient (or contaminant) flow since they are transferred and bioaccumulate within organisms, specially the non-essential metals (As, Cd, Hg,…), which are poorly regulated and might be biomagnified (i.e. increase with each trophic level) along a food web (Sardenne et al. 2017; Loaiza et al. in prep.). Contaminants (e.g. metals) can biomagnify or non-biomagnify (i.e. bio-dilute) in marine ecosystems. Normally the increase of metal transfer and accumulation (i.e. biomagnification effect) along the food web suggests that diet is the major exposure route for the metals. When the metal successively decreases in species with increasing trophic levels, it suggests that diet is not the major exposure route of that metal, or that there is a bio-dilution of metal during the transfer (Watanabe et al. 2008; Bisi et al. 2012; Vizzini et al. 2013; Signa et al. 2017). Metals are stable and persistent elements that are accumulated in the tissues of marine organisms, and their concentrations are regulated by exposure to these chemical compounds, including dietary exposure and/or other environmental exposure (Metian et al. 2009a; Sardenne et al. 2017), which means they can be used as a bioindicator for the quality of this environment.

Stable isotope techniques can provide a measure of the trophic position that integrates the assimilation of energy or mass flow through all the different trophic pathways leading to an organism. Stable isotopes have the potential to simultaneously capture complex interactions, including trophic omnivory, and to track energy or mass flow through ecological communities (Peterson and Fry 1987, Kling et al. 1992, Cabana and Rasmussen 1996). Nitrogen and carbon are two elements commonly used as stable isotope tracers for trophic interaction analysis in food webs (Sardenne et al. 2017). The ratio of nitrogen ($\delta^{15}N$) can be used to estimate the trophic level because the $\delta^{15}N$ of a consumer generally increases by 3-4 ‰ relative to its diet (Vander Zanden et al. 1999; Cabana and Rasmussen, 1996; Post et al. 2002). In contrast, the ratio of carbon isotopes ($\delta^{13}C$) changes little as carbon moves through food webs (Peterson and Fry 1987; Post et al. 2012), and therefore typically can be used to evaluate the feeding ecology of organisms when the isotopic signatures of the food sources are different, e.g. benthic vs. pelagic and/or marine vs. freshwater (incl. terrestrial) inputs (France, 1996; Post et al. 2012; Signa et al. 2017).

Fatty acids are long carbon chains constituting lipids that are necessary for a variety of physiological functions (Sardenne et al. 2017). A subset of these fatty acids, known as 'essential fatty acids' (EFA), play key functions such as growth, development and immune response in organisms (Arts et al. 2001). EFA and their precursors are mainly found in marine plants and algae and cannot be readily synthesized by all consumers (Sardenne et al. 2017). These EFA are incorporated into consumers and can therefore be used to track different primary production sources and predators-prey relations in food webs (Dalsgaard et al. 2003). Three EFA are recognized as being essential for consumers: the omega (ω3) fatty acids docosahexaenoic acid (DHA) (22:6n-3) and eicosapentaenoic acid (EPA) (20:5n-3), and the omega (ω6) arachidonic acid (ARA) (20:4n6); also well-known as long-chain polyunsaturated fatty acids (LC-PUFAs) (Arts et al. 2001; Domingo et al. 2007; Prato et al. 2019). In marine ecosystems, these LC-PUFAs are commonly used as biomarkers to understand the trophic structure and called 'fatty acids trophic markers', as well as biomarkers for environmental stressors (Müller-Navarra et al. 2000; Budge et al. 2006; Milinkovitch et al. 2015; Filimonova et al. 2016; Sardenne et al. 2017).

The advantages to use a multi-parameter approach of chemicals such as metals, stable isotopes and fatty acids is their ability to reflect the integration of consumers' diets over a relatively long timeframe (Sardenne et al. 2017). All these chemicals together could be combined to understand the flow energy, elements/compounds (incl. contaminants) transmission and connection among the organisms in food webs. Also to provide complementary insights as the estimation of the health status of different ecosystems, this in terms of contaminants and their uptake availability along the food web, from the primary producers up to top predators (incl. humans) (Hebert et al. 2009; Le Croizier et al. 2016; Sardenne et al. 2017).

## 5. Health risk assessment of marine food and new trends of consuming marine products

Metals and fatty acids have a dual use in adjacent fields of eco-toxicology and trophic ecology, such as: 1) a food safety approach since the metal (mostly non-essentials or potentially harmful) levels in the edible species from the food web could be used to estimate a possible human health risk from their consumption; and for 2) alternative diets or 'future foods' approach, which imply the use of micro-nutrients (e.g. Cu, Fe, Zn) and fatty acids (e.g. LC-PUFAs) in order to rank new species as highly beneficial for human consumption.

Seafood is an important source of persistent chemical contaminants such as Hg, As, Cd among others. These contaminants can cause adverse effects in human health, more related to long-term effects (EFSA, 2010, EFSA, 2011; Sioen et al. 2008). For example, Cd can cause toxic effects in kidney function and in bones, while Pb has been identified as a toxic element that leads to developmental neurotoxicity in young children and to cardiovascular effects and nephrotoxicity in adults. Cardiovascular diseases are the number 1 cause of death globally, taking an estimated 17.9 million lives each year (WHO, 2019). The exposure of humans to inorganic As by inhalation and ingestion causes lung, skin, bladder, and liver cancer, as previously mentioned (see section 3.). On the other hand, essential metals or micro-nutrients, such as Cu is also linked to physical and psychiatric disorders when exposure exceeds certain limits (Blanchard & Grosell 2005).

For this reason, estimation of possible risks for seafood consumption has been heavily reported in numerous locations around the world. To our knowledge only three human health risk studies have been performed on the consumption of contaminated seafood in Peru, two by the author of this study (Loaiza et al. 2015; 2018) and one by Barriga-Sanchez et al. (2018). The overall conclusion of these studies is that Peruvian seafood is Cd and As contaminated, and that their high consumption could pose a human health risk for the consumers.

Health risk assessments are based on indices as estimators of possible adverse effects for humans. From simple indices such as the comparison between the metal seafood levels with the maximum residue levels (MRLs) per taxon group (mollusk, cephalopod, etc.); to determining target cancer risks (TRs) (e.g. Loaiza et al. 2018). For almost all indices, the level of seafood contamination, seafood ingestion rates and human body measurements are used for the estimations. Therefore, it is relevant and more accurate to work with local data for index determinations. Social and nutritional survey studies in the sampling locations should be conducted, when the anthropometric measurements and nutritional information are not available (Loaiza et al. 2018).

Seafood is also one of the best providers of LC-PUFAs and other nutrients (Fe, Zn, ...) in human diets (Sioen et al. 2008; Loaiza et al. under review). In general, LC-PUFAs play a crucial role in brain function, normal growth and development, immune system regulation, and prevent several diseases (e.g. cancer, CVD, neural disorders) (Kris-etherton et al. 2002; Sioen et al. 2008; Tacon & Metian, 2013; Abedi and Sahari, 2014). EPA and DHA as omega (ω3) fatty acids have beneficial effects on diseases related to the heart or blood vessels, e.g. coronary artery disease, heart attack, among others, which are caused due to unhealthy diet, lack of exercise, overweight and smoking. LC-PUFA ω3 also prevent diseases such as skin disease, asthma, arthritis and lupus (Abedi and Sahari, 2014; Pennstate Hershey, 2019).

LC-PUFA ω6 (e.g. ARA) helps to stimulate skin and hair growth, maintain bone health, and regulate metabolism and reproductive system. These ω6 fatty acids can also prevent and/or attenuate diseases related to diabetes, allergies, and arthritis, among others (Abedi and Sahari, 2014; Pennstate Hershey, 2019). The ratio between ω6/ω3 is an efficient indicator for human health protection. For optimal infant nutrition, the ratio of ω6/ω3 must be no higher than 10 (Gerster, 1998).

The ideal ratio to protect human health is 1:5-10 ω6/ω3, however nutritional scientists suggest ratios from 2-4:1, which indicated a high consumption of seafood (Abedi and Sahari, 2014).

Micro-nutrients or minerals such as Cu, Fe, Mn, Zn, are normally present in high concentrations in marine species, this could play a crucial role to attenuate malnourishment and anameia in less developed countries (i.e. high poverty levels), such as Peru (PRODUCE, 2019; WFP, 2019). Fe and Zn are the most commonly used micro-nutrients to treat anaemic patients, i.e. children, women in pregnancy, among others (Black 2001; Zlotkin et al. 2005).

Beneficial elements and compounds such as LC-PUFAs and micro-nutrients are the key to find alternative nutritional diets for worldwide populations. Parodi et al. (2018) described that among many species, marine species were the most promising future food, this related to be sustainable in terms of their high nutritional properties, low or non-land use and low $CO_2$ emissions per production. Nevertheless, a precautionary approach must be taken into account prior to the exploitation of new nutritive marine species (i.e. non-edible species), which are mostly not substantially studied yet, as previously described (see section 3.). It is crucial to study the stock populations of these species, to establish a sustainable management plan prior to their exploitation for human consumption (Loaiza et al. under review).

## 6. Objectives

In this context, there is an urgent need to address the current status of seafood safety in Peru. This study assessed the chemical levels (i.e. metals, fatty acids, stable isotopes) in different environmental compartments (from POM to top-predators) of Peruvian marine ecosystems, in order to 1) determine the risk and benefit for consuming marine species from Peru; 2) find the potential value as nutritive food and/or new marine food of different Peruvian marine species; and 3) assess the current environmental health status (EHS), through different bio-monitoring (incl. food web contaminant transfer) approaches. For that, the following research questions were addressed in six chapters:

*Is there a human health risk for consumption of the Peruvian scallop and its most important predators?* **Chapter 2: Potential health risks via consumption of six edible shellfish species collected from Piura – Peru** addresses this research question.

*Is the Peruvian scallop a potential bioindicator species to determine environmental health status?* **Chapter 3: Peruvian scallop *Argopecten purpuratus*: From a key aquaculture species to a promising bioindicator species** addresses this research question.

*Are there more promising marine nutritional species as edible species in Peruvian marine ecosystems?* **Chapter 4: Marine species as safe source of LC-PUFA and micronutrients: insights in new promising marine food in Peru** addresses this research question.

*How is the environmental health status in terms of trophic metal transfer in food webs of Peruvian aquatic ecosystems?* **Chapter 5: Peruvian marine ecosystems: trophic interactions and metal transfer in aquatic food webs** addresses this research question.

*Which approach (incl. cost-benefit and research-question) to use to determine the environmental health status? What are the future actions and/or perspectives (based on this study results) for marine related fields in Peru?* **Chapter 6: General Discussion** addresses these research question.

Each chapter is intended to be an autonomous part, which can be read on its own. Inevitably, there may be some overlap between material and methods of the different chapters. All cited literature is listed at the end of the thesis.

# CHAPTER 2

# Potential health risks via consumption of six edible shellfish species collected from Piura - Peru

Slightly modified from the published article:

Loaiza, I., De Troch, M., & De Boeck, G. (2018). Potential health risks via consumption of six edible shellfish species collected from Piura–Peru. *Ecotoxicology and environmental safety*, *159*, 249-260. https://doi.org/10.1016/j.ecoenv.2018.05.005

## Abstract

Scallops and their potential predators were collected in Sechura Bay and in front of the Illescas Reserved Zone (north Peru), during *El Niño*-Southern *Oscillation* (ENSO) 2016, and analyzed for the metals chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd) and lead (Pb). This study showed that ~20% of the molluscs exceeded the maximum residual levels (MRLs) for human consumption in inorganic As, while ~30% of the crustaceans did. For Cd, around 10% and 40% of the molluscs and the crustaceans were above the MRLs, respectively. The cephalopod *Octopus mimus* exhibited As concentrations, but not Cd concentrations, that exceeded the MRLs. Cr, Ni, Cu, Zn and Pb in muscle exhibited generally concentrations below the MRLs. Integrated risk indices were estimated to determine if there is a health risk for consumption. Target hazard quotients (THQs) and total hazard indices (HIs) were mostly < 1, implying no human health risk. Provisional tolerable weekly intake (PTWI) for Cd was exceeded in the snail *Bursa ventricosa* at Illescas Reserved Zone. Target cancer risks (TRs) for inorganic As were always higher than the threshold ($1x10^{-6}$), therefore an actual cancer risk is present.



**Keywords:** *Argopecten purpuratus*, trace metal, predator, health risk, Piura

## 1. Introduction

The north of Peru is considered as the centre of intensive scallop *Argopecten purpuratus* aquaculture. Since 2003**,** productions have considerably increased up to 168 million US$ export per year (Kluger et al. 2016; Mendo et al. 2016). Sechura Bay is the main ecosystem for *A. purpuratus* intensive cultures (~80% of the total production), while "La Ensenada de Nonura" in front of Illescas Reserved Zone (IRZ) has an important contribution in the production in the North. Lately, this activity has been affected by numerous natural (i.e. ENSO) and anthropogenic factors (i.e. industrial activities). Production has decreased, and sanitary problems are currently in evaluation by institutions such as Organismo Nacional de Sanidad Pesquera (SANIPES) and EU, among others (SANIPES, 2017). It is noteworthy to mention that the "Health Risk Assessment in Production Areas of Bivalve Mollusks" had to be conducted by SANIPES in the recent years due to high concentrations of pollutants found in Sechura Bay. Moreover, as mentioned by Loaiza et al. (2015), only a semi-annual evaluation of metal concentrations is considered for scallop aquaculture by the Peruvian government, and measurements are only performed on *A. purpuratus.* In some cases water is also included in the evaluations. Therefore there is a lack of information on metal concentrations in water, sediment, *A. purpuratus*' predators and other filter-feeding species associated to the scallop aquaculture and in nearby areas.

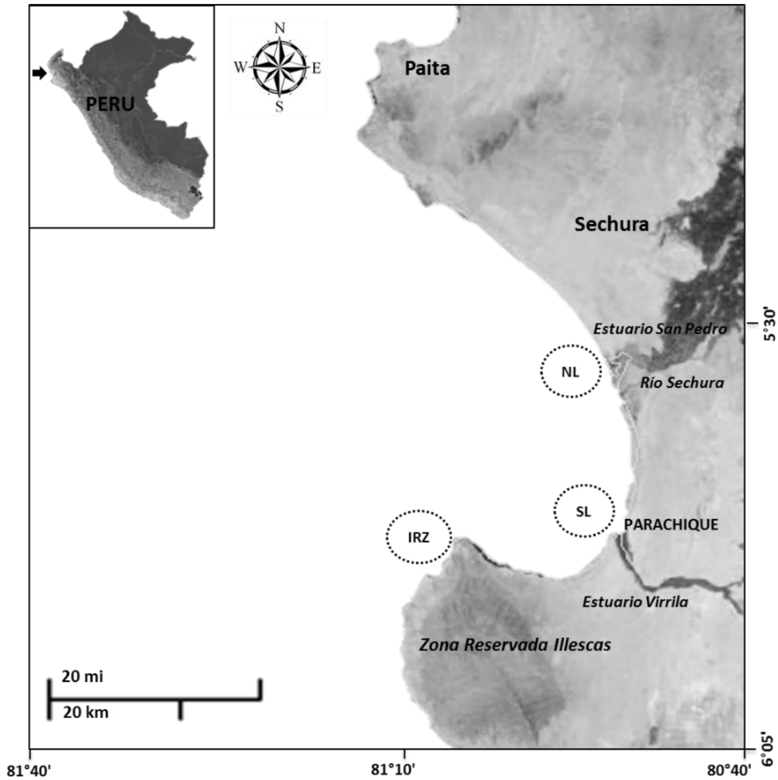



**Fig 1.** Location of the sampling areas at Sechura Bay and front Illescas Reserved Zone. Southern (SL) and Northern (NL) location in Sechura Bay; and one location in front of the Illescas Reserved Zone (IRZ) are showed as dashed black circles.

Samples of adductor muscle and gonad from *A. purpuratus* - Sechura Bay were analyzed for Cd and Pb in 2009, and surprisingly Cd concentrations (1.04 – 1.33 µg/g) exceeded the MRLs, all samples were higher than 1 ppm (weight wet (wwt.)) (Loaiza et al. 2009, unpublished results). One of the few studies about metal contamination in Peru was performed by Marín & García (2015). They determined Cd in Peruvian commercial species such as *Litopenaeus vannamei*, *A. purpuratus*, *Semele* sp, *Aulacomya atra*, among others, concluding that half of the species exhibited higher concentrations than the MRLs, and therefore their consumption can pose a potential health risk. No more information is available but there is an actual concern about sanitary problems in public and private authorities, as well as in certain populations of Peru.

Shellfish species such as octopus (*Octopus mimus*), crabs (*Romaleon setosum* and *Hepatus chilensis*), snails (*Bursa ventricosa* and *Cymatium* sp.) are potential predators of *A. purpuratus*, and are thus associated to the *A. purpuratus* culture (Mendo et al. 2016). Shellfish organisms are considered as exoskeleton-bearing aquatic invertebrate which are edible, this term is more frequently used in the field of fisheries and aquaculture (Adams, 1993; EC, 2018). These species are also highly commercialized for local consumption, either by local fishermen and/or coastal people from Sechura Bay and nearby locations. Therefore, it is urgently necessary to know the potential health risks due to their consumption.

In the proximity of the southern Sechura Bay, including areas to IRZ harbour, anthropogenic and industrial activities such as phosphate factories, the Norperuano oil pipeline, oil platforms, fishery factories, artisanal ports and the industrial Juan Paolo Quay (JPQ) port are present, while in the northern part of the bay, fishery factories, artisanal ports and fishing activities are conducted. Additionally, intensive scallop mariculture and artisanal fishing activities are performed along the Sechura Bay and in front of IRZ (IMARPE, 2007; Loaiza et al. 2015). All these activities are stressors and might cause impacts on the marine ecosystems (Loaiza et al. 2015). It is worth noting that the Reserved Zone of Illescas is restricted to the land and the intertidal area, therefore maritime and human activities (incl. fisheries) are normally performed at sea (MINAM, 2010). The Servicio Nacional de Áreas Naturales Protegidas (SERNANP) is in continuous control on land, but their vigilance of the sea part is limited to the observation and monitoring from land (Acquapisco SA, SERNANP, pers. comm.).

Fresh and brackish-water discharged from rivers/estuaries also impact the actual health conditions of Sechura Bay. The southern part of Sechura Bay is influenced by major fresh and brackish water input along the coast of Peru, from the mouth of Virrila Estuary (INRENA, 2005). On the other hand, Sechura River and San Pedro Mangrove located at northern Bay are minor contributors, although they become more important during prolonged and intense raining seasons and during “El Niño” (Mendo et al. 2016). These discharges normally come with higher concentrations of organic and inorganic pollutants, with metals as common components in these riverine inputs (Diop et al. 2016; Forrest et al. 2007; Kehrig et al. 2013).

Metals are characterised as essential (Mn, Fe, Cu, Zn) and non-essential (Cr, Ni, As, Cd and Pb) depending on their specific functions at cellular, enzymatic and protein levels. Elements carrying out fundamental biological functions that are necessary for growth and development in all organisms (from bacteria to humans) are considered as essential, while elements such as As and Cd that do not have any known functionality for living organisms, are considered as non-essential. Essential metals are also termed micronutrients when they are present in traces, but they can be toxic when their concentration increases above certain threshold, while non-essential metals are toxic in minimum concentrations (Grosell et al. 2007; Peña et al. 1999)

Food can be the major source of Cd and Pb for humans. As non-essential element, Cd can cause toxic effects in kidney function and in bones, while Pb has been identified as a toxic element that leads to developmental neurotoxicity in young children and to cardiovascular effects and nephrotoxicity in adults (EFSA, 2010, EFSA, 2011; Loaiza et al. 2015). As is a metalloid that occurs naturally or by anthropogenic impact in the environment. The exposure of humans to inorganic As by inhalation and ingestion causes lung, skin, bladder, and liver cancer. Food is also an important route for As uptake in humans (JECFA, 2010; US EPA, 2012). On the other hand, an essential metal such as Cu is linked to physical and psychiatric disorders when exposure exceeds certain limits (Blanchard & Grosell 2005).

Therefore, the aims of this study were: 1) to determine trace metal concentrations in six edible shellfish species from Sechura Bay and front IRZ; 2) to compare metal concentrations and estimated risk indices with reference values from different agencies; and 3) to identify spatial and seasonal variations in metal accumulation and risk indices from the six tested seafood products.

## 2. Materials and methods

### *2.1 Sampling procedure*

A total of 215 shellfish specimens of 6 different species i.e. octopus (*Octopus mimus*), crabs (*Romaleon setosum* and *Hepatus chilensis*), snails (*Bursa ventricosa* and *Cymatium* sp.) and Peruvian scallop (*Argopecten purpuratus*) were collected in Sechura Bay and in front of the Illescas Reserved Zone (IRZ) in January-March 2016. These species are considered as edible species in Peru, with the exception of the crab

*H. chilensis* that is consumed and commercialized only in Chile (WWF Chile, 2003). Sechura Bay and IRZ are located along the northern coast of Peru, Piura Region. Samples were collected at three locations: southern (SL) and northern (NL) location in Sechura Bay; and in one location in front of IRZ. The locations were sampled during a low-raining (1) period (January): SL1, NL1, IRZ1 and a high-raining (2) period (March): SL2, NL2, IRZ2 during ENSO, with the exception of the NL that was sampled for *A. purpuratus* during the transplanted culture in February (see Fig 1 and Table 1).

Shellfish were collected by hand while semi-autonomous diving (except for octopus which was caught with a hook) in each location. The average depth of the sampling sites was 14 ± 6 m. In case of *A. purpuratus*, they were collected with the permission of Acquapisco SA (SL and NL) and Nemo Corporation SA (IRZ). A rapid measurement and weighting were performed in order to have the same size class and weight (octopus) for each species. Organisms were kept alive by changing the seawater each 10-20 min, then dissected and preserved frozen at -20°C. For each specimen, muscle or edible tissue was carefully separated from the other tissues, subsequently cleaned and weighted for later metal analysis (Table 1).

## *2.2 Metal analysis*

After thawing the tissues at room temperature, they were dried for at least 72 h at 60°C. Thereafter, the dried tissues of ~0.2 gr were weighed and digested overnight with 2-2.5 ml of highly purified concentrated 69% $HNO_3$. The next day, they were heated to 110°C during 30 min in a Hot-block (SC154-54-Well HotBlock$^{TM}$) digester, and after cooling 0.25 ml of $H_2O_2$ was added to remove any debris from the digests, after which the samples were heated again during 30 min to complete the total digestion at 110°C. The digested samples were diluted 10 times with Milli-Q grade for the metal analysis (Cr, Mn, Fe, Ni, Cu, Zn, As, Cd and Pb) by using inductively coupled plasma mass spectrometry (ICP-MS; 7700×, Agilent Technologies) and high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS; Element XR, Thermo Scientific, Finnigan element 2, Bremen, Germany) (Hargitai et al. 2016).

The quality control was performed by the analysis of standard reference material (SRM) for mussel tissues (2976, National Institute of Standards and Technology, NIST), which was treated and analysed in the same way as the samples to test the applied analytical procedure accuracy. The obtained results indicated a stable measurement process and accurate data. The recovery ranges for the mussel tissue were 94.0 to 127 % (n = 70) for all metals. The limit of quantification (µg/L) was 0.1 for Cr, Mn, Ni, Cu, As, Cd and Pb, and 5 for Fe and Zn in ICP-MS, and 0.001 for all metals in HR-ICP-MS. All metal concentrations were calculated on a wet weight basis (µg $g^{-1}$ wwt.). Only for *A. purpuratus*, the edible tissue was considered as adductor muscle + gonad, which is the usual seafood product for consumption and trade in Peru.

**Table 1.** Sampling periods, locations, geographical coordinates (S, W), and sex, sample number (n), total length and total weight (mean ± S.E) of shellfish species from Sechura Bay and front Illescas Reserved Zone. Total length (Molluscans – shell length; Crustaceans - cephalothorax width; Cephalopods - mantle length)

| Sampling period | Location | Longitud (S) | Latitude (W) | Species | Sex | n | Size | Weight |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Total length (cm) | Total weight (g) |
| Low-raining season (January) | Illescas Reserved Zone (IRZ1) | 05°49'06.7" | 81°05'24.6" | *Octopus mimus* | ♂ | 6 | 30.3 ± 1.56 | 264 ± 29.3 |
| | | | | | ♀ | 4 | 35.5 ± 1.66 | 393 ± 41.5 |
| | | | | *Romaleon setosum* | ♂ | 7 | 8.24 ± 0.23 | 329 ± 20.7 |
| | | | | *Bursa ventricosa* | .. | 6 | 4.88 ± 0.16 | 30.0 ± 2.00 |
| | | | | *Argopecten purpuratus* | .. | 6 | 4.95 ± 0.12 | 18.9 ± 1.54 |
| | Southern location (SL1) | 05°43'58.2" | 80°54'17.8" | *Romaleon setosum* | ♂ | 5 | 8.99 ± 0.29 | 411 ± 61.4 |
| | | | | | ♀ | 5 | 7.75 ± 0.18 | 220 ± 19.4 |
| | | | | | ♀ (ovi) | 9 | 7.18 ± 0.16 | 228 ± 18.1 |
| | | | | *Hepatus chilensis* | ♀ | 3 | 5.83 ± 0.45 | 41.4 ± 7.25 |
| | | | | | ♀ (ovi) | 6 | 6.16 ± 0.20 | 48.4 ± 4.31 |
| | | | | *Bursa ventricosa* | .. | 6 | 5.72 ± 0.07 | 29.6 ± 1.96 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | *Argopecten purpuratus* | .. | 6 | 5.19 ± 0.07 | 23.2 ± 0.97 |
| | Northern location (NL1) | 05°31'27.6" | 80°56'04.2" | *Bursa ventricosa* | .. | 6 | 5.18 ± 0.15 | 20.5 ± 1.69 |
| | | | | Cymatium sp. | .. | 6 | 7.44 ± 0.22 | 37.7 ± 3.24 |
| | | | | *Argopecten purpuratus* | .. | 6 | 5.99 ± 0.11 | 38.0 ± 4.35 |
| Transplanted culture Sampling (February) | Northern location (NL1) | 05°31'27.6" | 080°56'04.2" | *Argopecten purpuratus** | .. | 6 | 4.75 ± 0.06 | 21.1 ± 0.99 |
| High-raining season (March) | Illescas Reserved Zone (IRZ2) | 05°49'06.7" | 81°05'24.6" | *Octopus mimus* | ♂ | 7 | 48.6 ± 3.97 | 451. ± 92.9 |
| | | | | | ♀ | 6 | 48.7 ± 2.29 | 492 ± 75.7 |
| | | | | *Hepatus chilensis* | ♂ | 6 | 6.08 ± 0.53 | 49.3 ± 10.9 |

### *2.3 Risk assessment*

The minimum (MIL), average (AVL) and maximum (MAL) muscle (incl. edible tissue for *A. purpuratus*) metal levels (μg $g^{-1}$ wwt) were considered for direct comparison with seafood safety guidelines based on maximum residual levels (MRLs) for human consumption set by the Organismo Nacional de Sanidad Pesquera (SANIPES), European Union (EU), US Food and Drug Administration (FDA), Codex Alimentarius from World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO). Another agency such as CFS-HONG-KONG was also used for comparison (WHO/FAO, 1995; EU, 2006; U.S. FDA, 2007; CFS-HONG KONG, 2018). It is worth to mention that Peruvian national regulations dictated by SANIPES are entirely based on European Union (EU) guidelines (SANIPES, 2017). These MIL, AVL, MAL were also considered to determine six different health risk assessments in the present study.

1) The estimated daily intake (EDI) was calculated in order to evaluate a possible alert for adverse health effects that may be caused by an individual metal. It depends on both the muscle or edible tissue levels and the amounts consumed (US EPA, 2000). The frequency of seafood consumption for Peruvians was estimated from the Food Balance Sheets of the Food and Agriculture Organization of the United Nations (FAO) (FAO, 2016).

$$\mathrm{EDI} = \frac{\mathrm{C\ x\ IR}}{\mathrm{bw}}$$

where

C: represents the element level in shellfish (μg $g^{-1}$ wwt.);

IR: the daily ingestion rate (g/day) of shellfish; and bw: the body weight (60 kg)

The ingestion rate (IR) for Peruvian people was estimated to be approximately 13.9 g/person/day for mollusks, 2.8 g/person/day for crustaceans and 0.8 g/person/day for cephalopods (FAO, 2016). It was assumed that the ingested dose is identical to the absorbed contaminant dose and that cooking has no effect on the metal (Cooper et al. 1991). The metal sources supplied by other meals or by drinking water on the same day were not taken into account, only the metal intake coming from different seafood meals (e.g. scallop, snail, crab and/or octopus) were considered (Metian et al. 2008a).

2) Weekly intakes (µg/week) of MIL, AVL, MAL were calculated for Peruvian consumers in order to compare these intakes with prescribed Provisional Tolerance Weekly Intakes (PTWIs) for Cr, Mn, Fe, Ni, Cu, Zn, As, Cd and Pb, established by Joint FAO/WHO Expert Committee on Food Additives (JECFA).

The risk for human health as a result of shellfish consumption was evaluated by calculating the percentage of the obtained weekly metal intake (µg/week) in comparison with the prescribed PTWI for each metal. The PTWI is defined as the estimated amount of a substance in food or drinking water, expressed on a body weight (bw) basis (mg/kg bw), that can be ingested weekly over a lifetime without appreciable health risk (Yap et al. 2016). JECFA (2011) established PTWI limits (in terms of µg/week for 60 kg person) for Fe, Ni, Cu, Zn, As (only inorganic) and Cd as 33 600, 2 100, 210 000, 126 000, 840 and 150 µg/week, respectively. It is noteworthy to mention that 10% of the total As was used to calculate inorganic As, and the lowest limit of 2 µg/kg bw per day was considered for inorganic As, because the 15 µg/kg bw limit has been withdrawn in 2011 (Bogdanović et al. 2014; Zhao et al. 2016). Studies conducted by the FDA indicated that foods of marine origin represent significant source of dietary arsenic exposure, however less than 10 percent of the total arsenic in finfish and approx. 30 percent of the total arsenic in shellfish. Worldwide studies shows similar results with 7.3 percent of total arsenic for finfish and 25 percent of total arsenic for shellfish present in the inorganic form. In the present study, as previously mentioned and based on the Guidance document for Arsenic in shellfish (U.S. FDA, 2003) and literature, the 10 % of the total As was considered for the inorganic form (Jara & Winter, 2014; Bogdanović et al. 2014; Zhao et al. 2016; U.S. FDA, 2019)

For Pb, the Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority (EFSA) concluded that the previously used PTWI of 25 μg/week/kg bw for Pb is no longer appropriate since there is no evidence for a threshold for critical Pb-induced effects and it was not sufficiently protective (JECFA, 2011) Therefore, three $PTWI_{1,2,3}$ values for Pb derived from blood Pb levels (BMDL) in μg/L (corresponding dietary intake values in μg/kg b.w. per day) were considered: a) $BMDL_{10}$, 15 μg/day (0.63 μg/kg b.w. per day), which is equivalent to a $PTWI_1$ of 265 μg/week and expected to have effects on the prevalence of chronic renal/kidney diseases (nephrotoxic effects) in adults; b) $BMDL_{01}$, 36 μg/day (1.50 μg/kg b.w. per day), which is equivalent to a $PTWI_2$ of 630 μg/week and induces effects on systolic blood pressure (cardiovascular effects) in adults; and c) $BMDL_{01}$, 12 μg/day (0.50 μg/kg b.w. per day), which is equivalent to a $PTWI_3$ of 210 μg/week and induces developmental neurotoxicity (neuro-developmental effects) on the central nervous system in children (EFSA, 2010). PTWI values for Cr and Mn have not been established by the JECFA (JECFA, 2016).

3) The amount of shellfish muscle (weight) that needs to be consumed per week by a 50 (women) and 70 (men) kg person in order to reach the prescribed

PTWI for each metal. It was estimated based on person weight (kg), metal concentration (µg/g) and PTWI (µg/week) per metal.

4) Target cancer risk (TR) and 5) target hazard quotients (THQ) were determined by using the US EPA Regional screening level (RSL) table and Integrated Risk Information System (IRIS) (Cooper et al. 1991; US EPA, 2016a; US EPA, 2016b). For carcinogenic effects (Cr, Ni, inorganic As and Pb), the risk is expressed as TR, and for noncarcinogenic effects (Cr, Mn, Fe, Ni, Cu, Zn, Cd and Pb) as THQ. As precautionary approach, carcinogenic effects were also estimated for Cr, Ni and Pb as CPSo values are known and available in IRIS (Ahmed et al. 2015; Nkpaa et al. 2017):

$$\mathrm{TR} = \left[\left(\frac{\mathrm{Efr\ x\ ED_{tot}\ x\ IR\ x\ C\ x\ CPSo}}{\mathrm{BWa\ x\ ATc}}\right)\right] \mathrm{x}\ 10^{-3}$$

$$\mathrm{THQ} = \left[\left(\frac{\mathrm{Efr\ x\ ED_{tot}\ x\ IR\ x\ C}}{\mathrm{RfDo\ x\ BWa\ x\ ATn}}\right)\right] \mathrm{x}\ 10^{-3}$$

where

Efr: exposure frequency (365 d/year);

$ED_{tot}$: exposure duration, total (70 years);

IR: daily ingestion rate (g/day);

C: metal concentration in the edible portion of shellfish (µg/g);

CPSo: oral carcinogenic potency slope (risk per mg/kg/day) of Cr (0.5), Ni (1.5), inorganic As (1.5) and Pb ($8.5x10^{-3}$);

BWa: body weight, adult (60 kg);

ATc: average time carcinogens (70 years x 365 d/year).

RfDo: oral reference dose (µg/g/d), RfDo for Cr (0.003), Mn (0.14), Fe (0.7), Ni (0.02), Cu (0.04), Zn (0.3), inorganic As (0.0003) and Cd (0.001);

ATn: average time noncarcinogens ($ED_{tot}$ x 365 d/year)

The European Protection Agency (EPA) has declined to set a RfDo for Pb because it found no evidence of a threshold below which a non-harmful intake could be allowed (US EPA, 2004). Therefore, the THQ for Pb was calculated using the following equation (Liu et al. 2009):

$$\mathrm{THQ} = \frac{\mathrm{C}}{\mathrm{MRL}}$$

C: metal concentration in the edible portion of shellfish (µg/g); MRL (Maximum Residue Level): the limit set by the Official Journal of the European Union (EU), Commission Regulation (EC) no 1881/2006, FDA and Codex Alimentarius WHO/FAO.

Total Cr was not available in the IRIS and RSL table; however, the US EPA assumed that the ratio of Cr (VI) to total Cr was 1:7, and gave a RfD for Cr (VI) of 0.003 µg/g/d. Thus, Cr data were divided by 7 to estimate the THQ for Cr (Tu et al. 2010).

6) A total hazard index (HI) was calculated by summing all THQs for a particular location and time:

$$\mathrm{HI} = \sum_{\mathrm{i}=0}^{\mathrm{n}} \mathrm{THQi}$$

where

THQi: targeted hazard quotient of an individual metal; HI: total hazard index for all metals (Cr, Mn, Fe, Ni, Cu, Zn, Cd and Pb) analyzed in this study or the total metal load and n was 8, except for *A. purpuratus* with n = 6 (Mn, Fe, Cu, Zn, Cd and Pb).

If the THQ or HI is < 1, there is no health risk for consumption. On the other hand, an exposed population of concern will experience health risks if the THQ or HI is > 1. For TR, a cancer risk could be posed if the TR levels are > $1 \times 10^{-6}$ (Bogdanović et al. 2014; Tu et al. 2010).

## 3. Results and discussion

For As, concentrations (µg $g^{-1}$ wwt) in muscle or edible tissue were compared to MRLs from CFS-HONG KONG and FDA and divided into two groups of species, molluscan shellfish and crustacean. In this study, *O. mimus*, *B. ventricosa*, *Cymatium* sp. and *A. purpuratus* are considered in the molluscan shellfish group, while *R. setosum* and *H. chilensis* belong to the crustacean group. For molluscan shellfish, $MRL_1$ of 10 (CFS-HONG KONG) and $MRL_2$ of 86 (FDA) µg $g^{-1}$ wwt were considered, and for crustacean a $MRL_1$ of 10 (CFS-HONG KONG) and $MRL_2$ of 76 (FDA) µg $g^{-1}$ wwt (Fig 2A and 2B). By using both MRLs, around the 20% of the molluscs exceeded the $MRL_1$ for As, and when considering the 86 µg $g^{-1}$ wwt of FDA, only 4 *B. ventricosa* individuals are above the mentioned permitted level. This species is also the most predominant represented species exceeding the 10 µg $g^{-1}$ wwt limit of CFS-HONG KONG, followed by *O. mimus* with 6 individuals exceeding the limit (Fig 2A). For crustaceans, 30% of the individuals exceeded $MRL_1$ and only 1 individual of *R. setosum* (89.4 µg $g^{-1}$ wwt) when the 76 µg $g^{-1}$ wwt of FDA is used for comparison. The same numbers (~10 ind.) of *R. setosum* and *H. chilensis* were found above the $MRL_1$ of CFS-HONG KONG (Fig 2B).

Cd concentrations (µg $g^{-1}$ wwt) in muscle or edible tissue were compared with MRLs for human consumption from values reported by EU/SANIPES, FDA and WHO/FAO. In this case, groups were divided into molluscan shellfish, crustaceans and cephalopods with the following MRLs: $MRL_1$: 1, $MRL_2$: 4 for molluscan shellfish; $MRL_1$: 0.5, $MRL_2$: 3 for crustaceans, and $MRL_1$: 1, $MRL_2$: 2 for cephalopods. *B. ventricosa*, *Cymatium sp* and *A. purpuratus* are classified in the molluscan shellfish group, while *R. setosum* and *H. chilensis* belong to the crustacean group, and *O. mimus* being the only cephalopod, in the cephalopod group (Fig 2C, 2D, 2E). Around 10% of the molluscs were above the EU/SANIPES $MRL_1$, the majority were *B. ventricosa* individuals but two of the edible tissues (adductor muscle + gonad) of *A. purpuratus* also exceeded $MRL_1$. When $MRL_2$ of the FDA (4 µg $g^{-1}$ wwt) was considered, only one individual of *B. ventricosa* was higher (5.43 µg $g^{-1}$ wwt) than that limit (Fig 2C). For crustaceans, a higher percentage exceeding the Cd MRLs was found, up to 40% of individuals exhibited concentrations above the EU/SANIPES $MRL_1$, dominated by the species *H. chilensis*. Considering a MRL of 3 µg $g^{-1}$ wwt. (FDA), only two individuals of *H. chilensis* showed higher concentrations (3.45 and 4.79 µg $g^{-1}$ wwt.) than the permitted levels (Fig 2D). The cephalopod *O. mimus* did not exhibit Cd concentrations that exceeded the MRLs, all concentrations were about 60 and 30-fold less (by average) than the MRLs: 1 (EU/SANIPES) and 2 (WHO/FAO) µg $g^{-1}$ wwt, respectively (Fig 2E).

Overall, Cr, Ni, Cu, Zn and Pb in muscle exhibited concentrations (µg $g^{-1}$ wwt) below the MRLs considered in this study. They remained 2 to 9000-fold lower than the respective MRLs, with the exception of Cr concentrations (µg $g^{-1}$ wwt) in 13 individuals of *R. setosum* and *H. chilensis* which exceeded the $MRL_1$ of 1 µg $g^{-1}$ wwt (CFS-HONG KONG), and one *R. setosum* individual that exceeded the $MRL_2$ of 12 µg $g^{-1}$ wwt (FDA). Mn and Fe levels in muscle were not compared to permitted levels due to the lack of MRLs (see Table 3 and 4; supplementary material).

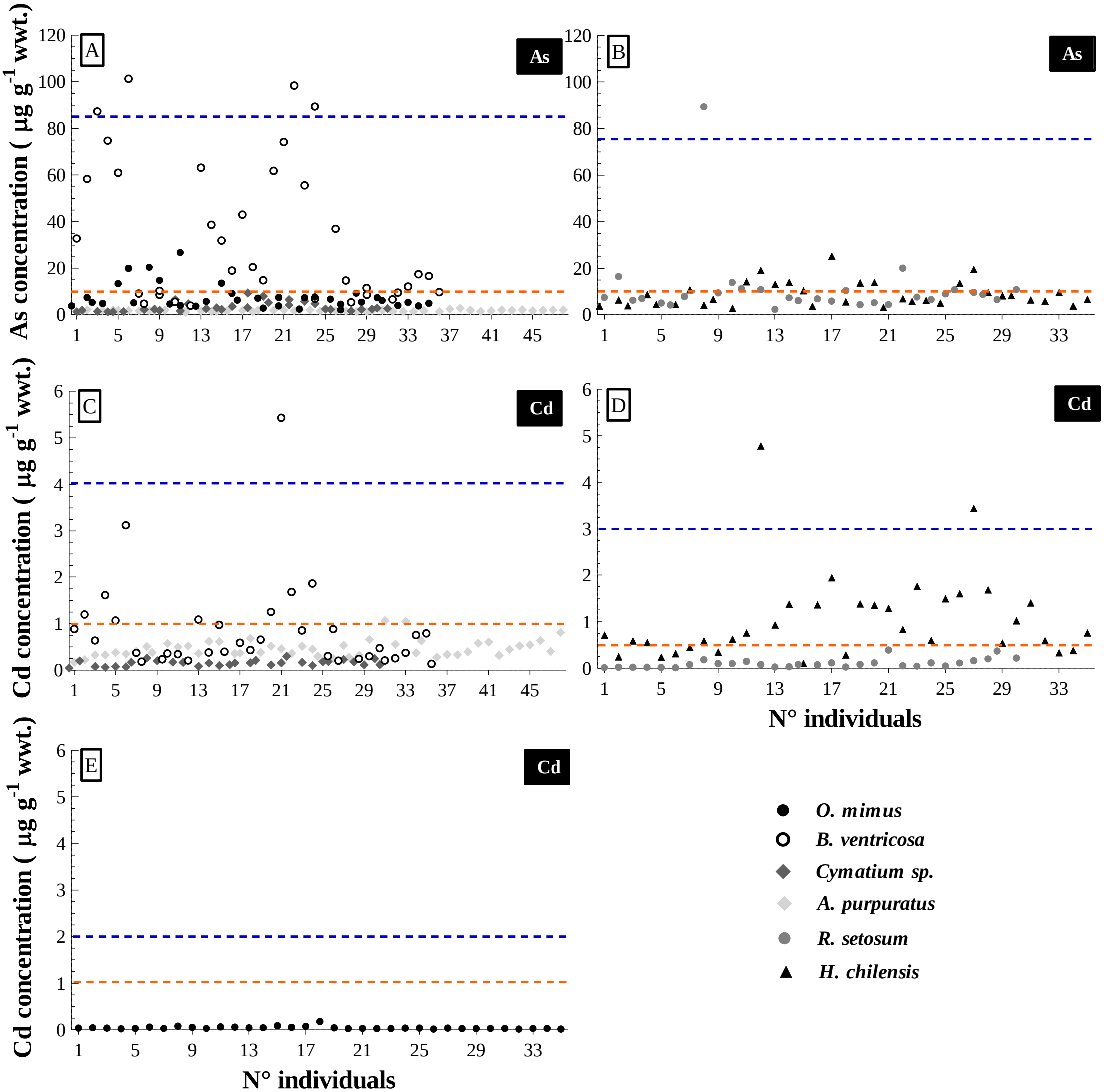



**Fig 2.** Arsenic and cadmium concentrations (µg $g^{-1}$wwt) in muscle of six shellfish species compared to the maximum residue levels (MRLs) for human consumption for (A) molluscan shellfish and (B) crustaceans for As ($MRL_1$ (---):CFS-HONG KONG and $MRL_2$ (---): FDA), and for (C) molluscan shellfish and (D) crustaceans ($MRL_1$ (---): EU/SANIPES and $MRL_2$ (---):FDA), and (E) cephalopod for Cd ($MRL_1$ (---): EU/SANIPES and $MRL_2$ (---): WHO/FAO). Edible tissue (adductor muscle + gonad) was only considered for *A. purpuratus*.

Edible molluscan shellfish were also evaluated for As, Cd and Pb at the Eastern Adriatic coast, and apparently no health risk was determined

(Bogdanović et al. 2014). A similar range of concentrations was found for Cd and Pb compared to this study. For As, our edible shellfish exhibited concentrations up to 101 µg $g^{-1}$ wwt compared to tissue levels from 1.42 to 9.58 µg $g^{-1}$ wwt. in the Eastern Adriatic coast, but this is mainly caused by the high concentrations in snails and crabs. In case only pectinidae species such as *A. purpuratus* and *Chlamys varia* are compared, a similar trend was found for all metals. A preliminary study on *A. purpuratus* in different cultures from Sechura Bay in 2010 (Loaiza et al. 2015) also demonstrated similar concentrations (µg $g^{-1}$ wwt.), with 0.28 - 0.55 for Cd, and 0.06 and 0.42 for Pb in edible tissue (adductor muscle + gonad). In that study, all concentrations were below the MRLs in contrast to our previous results that showed two samples of *A. purpuratus* that exceeded the MRLs. The scallop *Pecten jacobaeus* and other five shellfish from the Adriatic sea (Bilandžić et al. 2015), showed concentrations of trace metals (As, Cd, Pb, Cu and Zn) that were also similar to our study.

As for other pectinidae species, *Nodipecten nodosus* farmed in tropical bays was studied for the trace metals Zn, Fe, Cu, Mn, Cd and Pb in Brazil, and the Pectinidae species seemed unsafe for human consumption due to some metal levels (e.g. Cr) exceeding the permitted levels (Lino et al. 2016). Our *A. purpuratus* exhibited up to ~12-fold higher Fe concentrations than *N. nodosus*, but the opposite was true for Mn, with up to 60-fold lower concentrations. Nevertheless, similar concentrations was observed for Zn, Cu, Cd and Pb in both species. European Pecten species such as *Comptopallium radula, A. opercularis, C. varia, Pecten maximus and Chlamys islandica* have been studied in recent years, and their edible tissue (adductor muscle + gonad) concentrations (dwt. and wwt.) were on average about 8.5 (Mn), 3.0 (Cu), 3.7 (Zn), 3.5 (As) and 4.8 (Pb)-fold higher than our *A. purpuratus* (Bach, et al. 2014; Bustamante and Miramand, 2004; Bustamante et al. 2005a, 2005b;

Julshamn et al. 2008; Metian, et al. 2008b; Saavedra et al. 2017). Loaiza et al. (2015) concluded that the Peruvian scallop *A. purpuratus* is a safe product for consumption. Nevertheless, Cd exhibited a similar pattern for all Pectenidae species mentioned, Fe was two times higher in *A. purpuratus*, and Pb showed high variations among species.

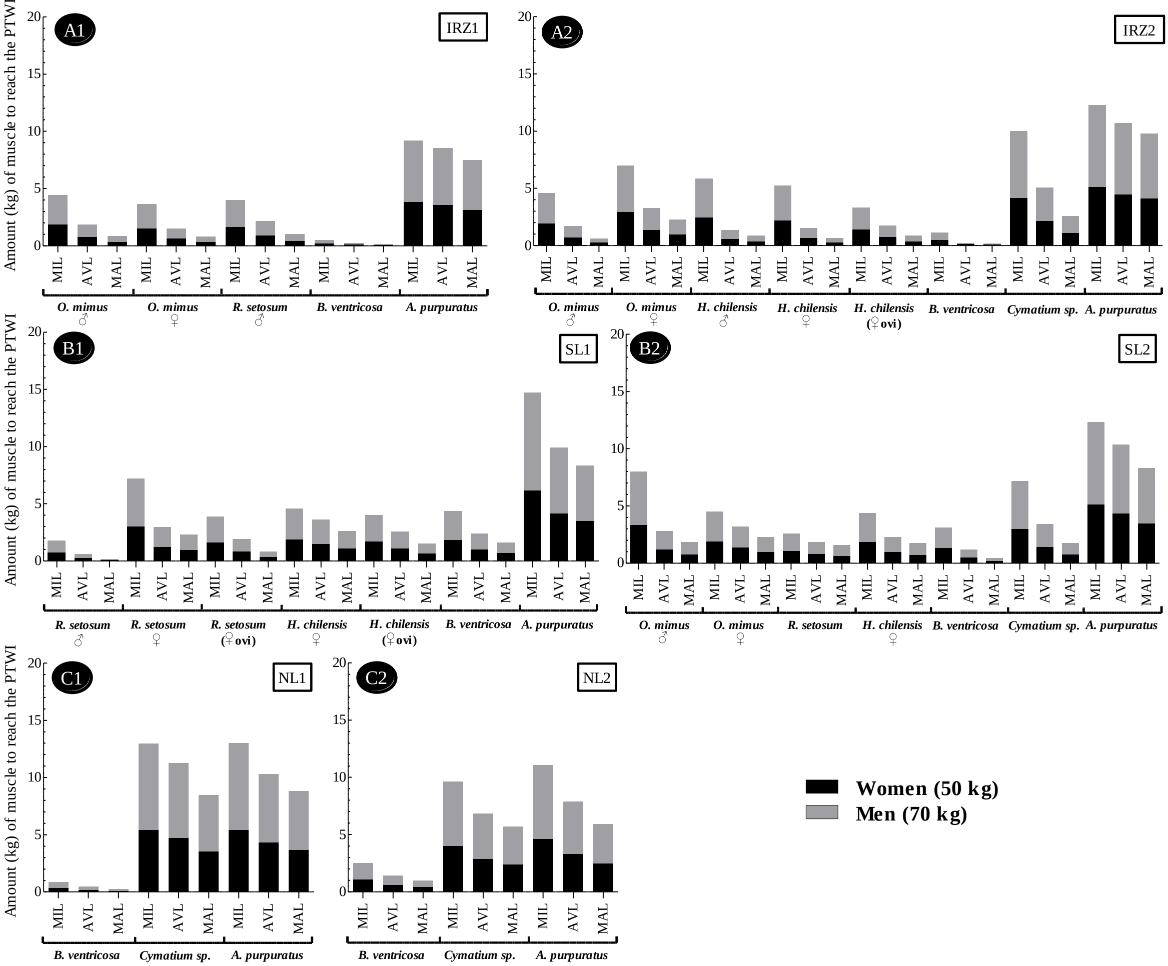



**Fig 3.** Amount of shellfish muscle allowed to be consumed by women ♀ (50 kg) and men ♂ (70 kg) in order to reach the PTWI by using minimum (MIL), average (AVL) and maximum (MAL) levels of inorganic As in muscle (incl. edible tissue: adductor muscle + gonad for *A. purpuratus*) from the southern (SL) and northern (NL) location at Sechura Bay and front Illescas Reserved Zone (IRZ), during the low (IRZ1, SL1, NL1) and high-raining (IRZ2, SL2, NL2) period.

Metal concentrations in potential predators of *A. purpuratus* such as *B. ventricosa, Cymatium sp., H. chilensis, R. setosum* and *O. mimus* were also compared to different literature. Chinese snail species exhibited considerably higher concentrations (µg/g dry weight (dwt)) of all evaluated metals (e.g. up to 116-fold more Mn) than our two snail species, with the only exception being Cr in *B. ventricosa* which exhibited similar concentration of ~3.5 µg/g dwt. (average). Sea snails such as *Patella sp.* and *Tympanotonus fuscatus* also showed similar values, for Cu, Cd and Zn (Jakimska et al. 2011).

Crabs showed more variability, *Charybdis* sp. had more elevated concentrations for Mn, Cu, and Pb, while for Ni and Zn, and Cr and Cd they showed lower concentrations than *H. chilensis* and *R. setosum.* In most cases, the consumption of Chinese mollusks (incl. the snail *L. brevicula*) could pose a health risk for the consumers, but not for the crab *Charybdis* sp. (Gao et al. 2016). The crabs *H. chilensis* and *R. setosum* in our study exhibited considerably higher As concentrations (6-116 times higher) than those in *Penaeus kerathurus, Penaeus mondon, Metapenaeus monoceros and Metapenaeus ensis*, and in some cases (30-40%) the crabs were not safe for consumption (Diop et al. 2016; Han et al. 1998; Zhao et al. 2016).

In the same study, the octopus species *Benthoctopus thielei* and *Graneledone sp.* exhibited burdens (µg/g dwt) of 3.00 and 15.0 for Cu, 0.21 and 0.37 for Cd and 138 and 113 for Zn, respectively (Jakimska et al. 2011). For Cd, these levels were similar to the average concentration (µg/g dwt) in the present study namely 0.29 for Cd in *O. mimus*, but for Cu and Zn, that study showed discrepancy with our *O. mimus* concentrations of 24.0 µg/g dwt and 78.6 µg/g dwt, respectively. Cd, Cu and Zn concentrations of *Octopus vulgaris* from Tunisian coastal regions (Rjeibi et al. 2015) were in accordance to concentrations (µg $g^{-1}$ wwt.) in *O. mimus* from this study. Concentrations exhibited the following means: 0.03 (Cd), 3.29 (Cu), 14.2 (Zn) µg/g wwt. in arms, and in according to *O. mimus* concentrations (µg/g wwt.) of 0.04 (Cd), 3.60 (Cu), 11.6 (Zn). *O. vulgaris* and *O. mimus* were both analyzed in arms as edible tissue, therefore a most suitable comparison could be applied in this case. It is worth noting that both species did not exhibited concentrations (average) that exceeded the MRLs for human consumption.

### *3.1 Risk assessment*

EDI (µg/kg/day) and weekly intakes (µg/week) were calculated to be compared to the prescribed Provisional Tolerance Weekly Intakes (PTWIs) in order to determine a possible adverse health effect caused by metal. For inorganic As, the highest weekly intake was about 987 µg/week from *B. ventricosa*, and this value was 78 % from the PTWI inorganic-As limit. The lowest % was found for *O. mimus* with a weekly intake of less than 0.1 % of the prescribed limit. On the other hand, Cd weekly intakes (µg/week) exhibited values higher than the PTWI limits of 150 µg/week, two values (MAL) of *B. ventricosa* from IRZ1 and IRZ2 were about 304 and 529 µg/week, respectively, while others varied in relative (%) range of 0.05 to 92.2 % PTWI (see Table 1 and 2; supplementary material).

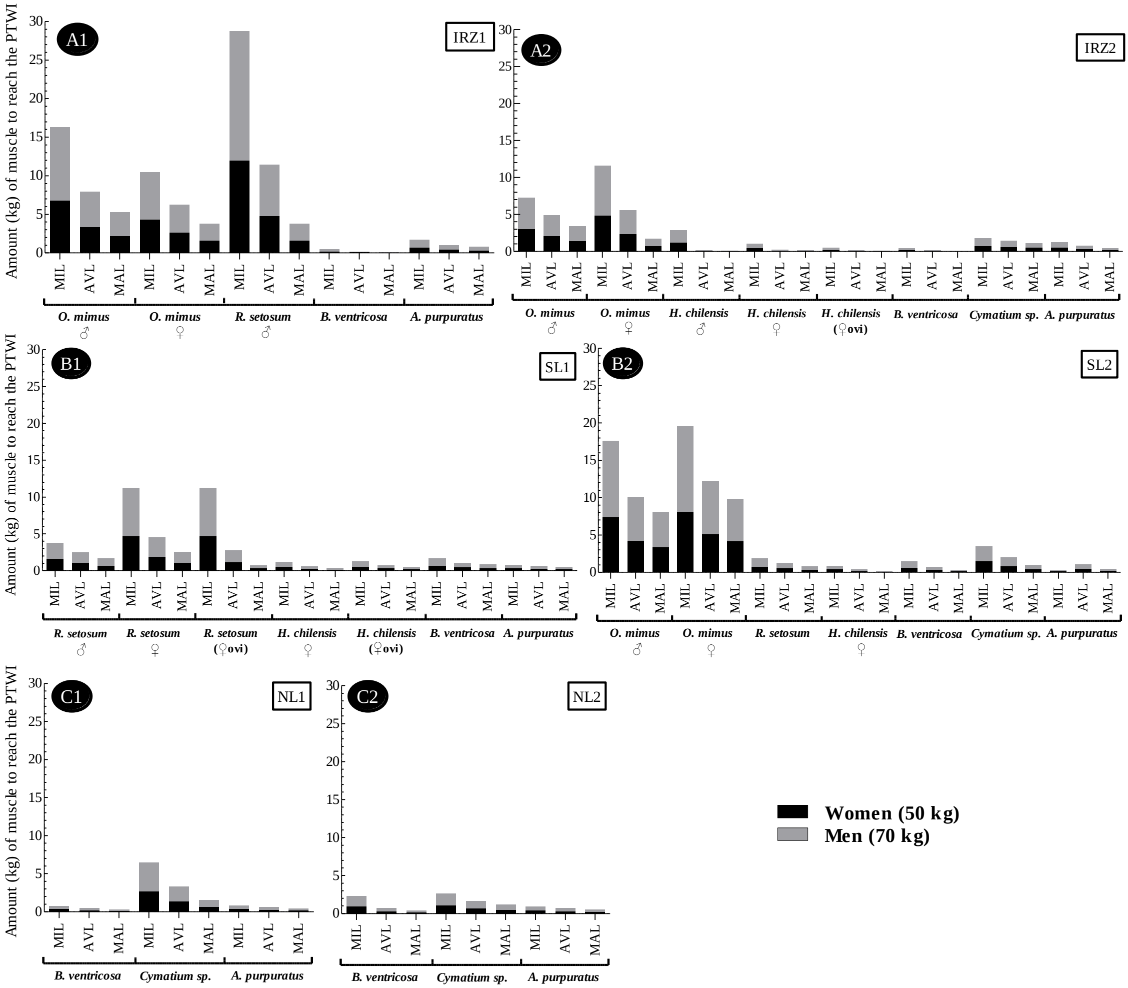



**Fig 4.** Amount of shellfish muscle allowed to be consumed by women ♀ (50 kg) and men ♂ (70 kg) in order to reach the PTWI by using minimum (MIL), average (AVL) and maximum (MAL) levels of Cd in muscle (incl. edible tissue: adductor muscle + gonad for *A. purpuratus*) from the southern (SL) and northern (NL) location at Sechura Bay and front Illescas Reserved Zone (IRZ), during the low (IRZ1, SL1, NL1) and high-raining (IRZ2, SL2, NL2) period.

Bogdanović et al. (2014) found EDI of 0.047 and 0.097 µg/kg/day for Cd, for average and maximally exposed consumers, respectively. This is in accordance with our 0.067 µg/kg/day Cd EDI (average) of the six studied shellfish. For inorganic As, our estimations are the same when is compared to maximally exposed consumers, ~0.14 µg/kg/day. However, the EDI is lower (2-fold less) when average consumption is considered in their calculations. Bogdanović et al. (2014) did not considered BWa in EDI inorganic-As calculations, but it was considered for a proper comparison with this study. In a Brazilian study, *N. nodosus* showed a similar pattern for EDIs as *A. purpuratus* with the only exception of Mn, which was about ~20 times higher in *N. nodosus*, and could be explained by the 60-fold lower concentration in Mn when *A. purpuratus* is compared to *N. nodosus* (Lino et al. 2016). *A. purpuratus* was also studied by using risk indices in 2010 (Loaiza et al. 2015), EDIs were in a range of 0.073-0.101 for Cd and 0.043-0.063 for Pb from all cultures, while this study found 0.041-0.248 for Cd and 0.004-0.148 for Pb. In fact, those values are comparable but with more variability in the latter study. It is noteworthy to mention that the previous study in 2010 only evaluated one culture area (southern Bay), while this study involves SL and NL in Sechura Bay, and the location front of IRZ. Bilandžić et al. (2015) also estimated EDI in scallops and other shellfish species, EDI of As, Cd, Pb, Cu and Zn for *P. jacobaeus* were very similar than *A. purpuratus* EDIs, with the exception of inorganic As and Pb, which were around 14 and 6-fold higher in *P. jacobaeus*, respectively.

Crustacean *Charybdis* sp. and *Oratosquilla oratoria* from Huladao City, China exhibited EDIs that were much higher than our values for *R. setosum and H. chilensis* (Gao et al. 2016). It is noteworthy to mention that these authors considered ingestion rates of 55 g/d for *Charybdis sp.* and *O. oratoria* while we considered 2.8 g/d for our study. BW was considered in the same way (60 kg) in both studies (Hulodao City and Sechura City). EDIs for Cd, Cu and Zn were also calculated in *O. vulgaris*, which were 4, 3 and 116 times higher than in *O. mimus* in this study. The applied ingestion rates were 33 and 0.8 g/d for *O. vulgaris* and *O. mimus*, respectively, it partially explains the high variation between studies (Rjeibi et al. 2015).

The % PTWI was calculated by using the weekly intakes (EDI x 7). Bilandžić et al. (2015) found contributions (%) of 26.3 (As), 54.4 (Cd), till 7.03 (Cu) and 1.53 (Zn) for *P. jacobaeus*, while in this study % PTWI were lower and around 2.07 (As), 31.0 (Cd), 0.07 (Cu) and 1.18 (Zn) for *A. purpuratus*. Concentrations (µg/g wwt) between these scallops were similar (e.g. ~1.90 for As, 0.50 for Cd, 1.40 for Cu and 17.0 For Zn) as mentioned before, however the meal size or daily ingestion (20 g/d) used for the calculations by Bilandžić et al. (2015) is much higher than the Peruvian ingestion rates of 13.9 g/d for mollusks. It explains the differences between the %PTWIs. Pb could not be compared due to the discrepancy by using PTWI prescribed values between studies.

Percent of PTWI were also estimated for Cd in *O. vulgaris*, values of 1.37, 1.44 and 6.23 % for Bizerre, Monastir and Sfax - along the Tunisian coast were found, which were ~8.90, 9.50, 40.9-fold higher than our estimated 0.15 % in *O. mimus* (Rjeibi et al. 2015). Yap et al. (2016) could estimate %PTWI for Pb in marine bivalve *Perna viridis* by using the prescribed PTWIs (0.63, 1.50 and 0.50 µg/kg b.w. per day) as considered in this study. *P. viridis* was evaluated on the east coast of Peninsular Malaysa, which exhibited contributions of 86.1, 36.2 and 108 %, which differ considerably from our values (1-5% PTWI) in *A. purpuratus*. Loaiza et al. (2015) found Cd and Pb contributions (%PTWI) in concordance with the present study.

By using the PTWI as a risk index, calculations were performed in order to determine the amount of muscle or edible tissue to reach the PTWI limit. This precautionary approach was calculated by using MIL, AVL and MAL in all species per location and period. Among locations and by using As and Cd in PTWI calculations, *A. purpuratus* did not exhibit considerably variations among locations in terms of amount of edible tissue allowed to be consumed, as well as the species *Cymatium* sp. and *H. chilensis*. On the other hand, *B. ventricosa* from IRZ can be always consumed in lower amounts than those from SL and NL, and even in up to 10-fold lower quantities compared to *B. ventricosa* from SL in the low-raining period. *O. mimus (*♂) exhibited the same trend with around 2-fold lower allowable consumption rates at IRZ compared to SL. An opposite trend was found for *R. setosum (*♂) from IRZ, which can be consumed in 2 to 8 times larger quantities than those belonging to SL. (Fig 3 and 4). It seems that *R. setosum* is less polluted in IRZ, and that SL exhibited more individuals that exceeded (As concentrations up to ~90 µg $g^{-1}$ wwt) the MRLs (Fig 2).

By using both As and Cd in the analysis, the species that can be consumed in the lowest amounts is *B. ventricosa* from IRZ. The maximum amount of muscle or edible tissue that can be consumed is no more than 0.02 and 0.03 kg for women and men per week, respectively, in the most precautionary scenario. *B. ventricosa* from IRZ did not exhibit considerably variations between seasons (Fig 3 and 4). Based on Cd PTWI, *H. chilensis* (♂) from IRZ also exhibited the same trend with 0.03 and 0.04 kg for women and men, respectively (Fig 4A2). For inorganic As, animals from SL exhibited the highest difference in amount of muscle that can be consumed after the high-raining period in *B. ventricosa* using the MAL, which was 3.6-fold lower during the high-raining period compared to the low-raining period (see Fig 3B1 and 3B2). A same trend was found for *B. ventricosa* and *H. chilensis* (♀)

when Cd is used in calculations. *O. mimus* (♂) (IRZ), *A. purpuratus* (SL) and *Cymatium sp.* (NL) also showed high decreases in amount of edible tissue that can be consumed after the high-raining period (by using MIL of Cd) (Fig 4). Overall, since both metals are present at the same time at all locations, the risk analysis should consider both metals. Therefore, *B. ventricosa* and *H. chilensis* from the IRZ are the species that can be consumed in the lowest quantities, and *B. ventricosa*, *H. chilensis* (♀), *A purpuratus* (SL), *O mimus* ♂ (IRZ) and *Cymatium sp.* (NL) decrease in amount of edible tissue that can be consumed after the high-raining period (March).

A study in France concluded that no more than 300 (Château d'Oléron) and 800 (Ré Island) g of scallop *C. varia* can be consumed by a person of 65 kg per week (Miramand et al. 2002), which is comparable to our average results of 300 (for women) and 420 (for men) g per week. However, as precautionary approach (by using MAL of Cd), we would advise a maximum consumption of 117 (women) and 164 (men) g/week for *A. purpuratus*. Bach et al. (2014) estimated the number of scallops *C. islandica* that can be ingested without Cd risk to be 45 (women) and 72 (men) per week. When using relationship between size and edible tissue weight (n=509), and the maximum amount allowed to consume of *A. purpuratus* (g/week average) to reach the PTWI for Cd, we advise similar numbers of 49 (women) and 68 (men). However, a lower PTWI of 2.5 μg/kg/bw was considered for *A. purpuratus,* while 7.0 μg/kg/bw was used for *C. islandica*. *C. radula* was also evaluated for Cd, As, Cu, Fe and Zn in Maa Bay and Sainte Marie Bay – France (Metian et al. 2008b). As seemed to be the limiting element posing a risk for *C. radula* consumption, and the numbers of scallops that can be eaten before reaching the PTWI were 27 (women) and 44 (men). For our *A. purpuratus* this is clearly not the case*:* by using the maximum amount allowed to reach PTWI inorganic As (4000 (women) and 5600 (men) g per week), 655 (women) and

919 (men) *A. purpuratus* could be consumed, which are 24 and 20-fold much higher than those for *C. radula*.

**Table 2.** Target cancer risk (TR) and target hazard quotient (THQ) by consuming shellfish with minimum (MIL), average (AVL) and maximum (MAL) levels of As-inorganic and Cd in muscle (incl. edible tissue for *A. purpuratus*) from the southern and northern location at Sechura Bay and front Illescas Reserved Zone.

| | | | | **As-inorganic Target cancer risk (TR)** | | **Cd Target hazard quotient (THQ)** | |
|---|---|---|---|---|---|---|---|
| Area | Especie | Tissue | | January (low-raining period) | March (high-raining period) | January (low-raining period) | March (high-raining period) |
| IRZ | *O.mimus* | muscle | MIL | **0.000007** | **0.000005** | 0.000235 | 0.000314 |
| | | | AVL | **0.000019** | **0.000015** | 0.000533 | 0.000733 |
| | | | MAL | **0.000039** | **0.000051** | 0.001012 | 0.002261 |
| | *R. setosum* | muscle | MIL | **0.000030** | | 0.000490 | |
| | | | AVL | **0.000055** | | 0.001235 | |
| | | | MAL | **0.000116** | | 0.003711 | |
| | *H. chilensis* | muscle | MIL | | **0.000020** | | 0.004903 |
| | | | AVL | | **0.000077** | | 0.067799 |
| | | | MAL | | **0.000178** | | 0.225119 |
| | *B. ventricosa* | muscle | MIL | **0.001138** | **0.000515** | 0.147646 | 0.151617 |
| | | | AVL | **0.002407** | **0.002284** | 0.329187 | 0.453393 |
| | | | MAL | **0.003523** | **0.003421** | 0.724536 | **1.260033** |
| | *Cymatium sp.* | muscle | MIL | | **0.000059** | | 0.038893 |
| | | | AVL | | **0.000115** | | 0.046711 |
| | | | MAL | | **0.000227** | | 0.060475 |
| | *A. purpuratus* | edible tissue* | MIL | **0.000064** | **0.000048** | 0.040855 | 0.018841 |
| | | | AVL | **0.000069** | **0.000055** | 0.069294 | 0.090483 |
| | | | MAL | **0.000078** | **0.000060** | 0.088934 | 0.152399 |
| SL | *O.mimus* | muscle | MIL | | **0.000004** | | 0.000196 |
| | | | AVL | | **0.000011** | | 0.000348 |
| | | | MAL | | **0.000018** | | 0.000475 |
| | *R. setosum* | muscle | MIL | **0.000016** | **0.000046** | 0.001251 | 0.007667 |
| | | | AVL | **0.000090** | **0.000063** | 0.004744 | 0.011105 |
| | | | MAL | **0.000630** | **0.000076** | 0.018233 | 0.017215 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *H. chilensis* | muscle | MIL | **0.000026** | **0.000027** | 0.011249 | 0.015681 |
| | | | AVL | **0.000042** | **0.000052** | 0.021098 | 0.039545 |
| | | | MAL | **0.000076** | **0.000068** | 0.033579 | 0.079427 |
| | *B. ventricosa* | muscle | MIL | **0.000134** | **0.000186** | 0.042027 | 0.046448 |
| | | | AVL | **0.000244** | **0.000486** | 0.064986 | 0.092440 |
| | | | MAL | **0.000357** | **0.001284** | 0.086247 | 0.204380 |
| | *Cymatium sp.* | muscle | MIL | | **0.000081** | | 0.019963 |
| | | | AVL | | **0.000171** | | 0.035159 |
| | | | MAL | | **0.000328** | | 0.069484 |
| | *A. purpuratus* | edible tissue* | MIL | **0.000040** | **0.000047** | 0.088054 | 0.065499 |
| | | | AVL | **0.000059** | **0.000056** | 0.111902 | 0.153109 |
| | | | MAL | **0.000070** | **0.000070** | 0.133638 | 0.247622 |
| NL | *B. ventricosa* | muscle | MIL | **0.000660** | **0.000231** | 0.087692 | 0.030425 |
| | | | AVL | **0.001252** | **0.000417** | 0.148605 | 0.096822 |
| | | | MAL | **0.002195** | **0.000603** | 0.251327 | 0.182841 |
| | *Cymatium sp.* | muscle | MIL | **0.000045** | **0.000061** | 0.010804 | 0.026742 |
| | | | AVL | **0.000052** | **0.000085** | 0.020858 | 0.041926 |
| | | | MAL | **0.000069** | **0.000103** | 0.046182 | 0.057456 |
| | *A. purpuratus* | edible tissue* | MIL | **0.000045** | **0.000030** | 0.083347 | 0.044982 |
| | | | AVL | **0.000057** | **0.000072** | 0.116402 | 0.114704 |
| | | | MAL | **0.000066** | **0.000076** | 0.159342 | 0.188431 |

**(*) Edible tissue is considered as adductor muscle + gonad in *A. purpuratus*. TR and THQ that exceed the target cancer risk (0.000001) and the target hazard quotient (1) threshold are printed in bold.**

Based on TR and THQ and by using MIN, AVL and MAL, a high possibility of risk was found for inorganic As. TR in all species per location and period exceeded the threshold of 0.000001 as shown in Table 2. The maximum TR by using MAL was ~3500-fold higher than the TR threshold for *B. ventricosa* from IRZ, either during the low or high-raining period. *B. ventricosa* also exhibited the highest TR per location, and was around 10 times higher when IRZ is compared to SL during the low-raining period. Over time, the *B. ventricosa* (MAL) and *R. setosum* (MIL) from SL high-raining period had a 3.6 and 2.8-fold higher TR than those from low-raining period.

During the high-raining period, *B. ventricosa, O. mimus and Cymatium sp.* showed the highest TR differences between locations. *B. ventricosa* and *O. mimus* from IRZ showed a 4.7 and 2.9 times higher TR than the individuals from SL, based on AVL and MAL, respectively. Based on MAL for *Cymatium* sp., we found that SL individual exhibited a 3.2-fold higher TR than those at NL. TRs were also used as risk index by Bogdanović et al. (2014), and shellfish from the Adriatic Sea exhibited up to $5.1x10^{-7}$ TR of inorganic As which is below the reference threshold ($1x10^{-6}$), unlike our As TRs (average) of $2.1x10^{-4}$. Crustaceans such as *P. monodon* and *M. monoceros* were grouped as shrimps for TR estimations, which were up to 61.8 and 49.4-fold lower than our TR-limit exceeding values for the crustaceans *R. setosum* and *H. chilensis*, respectively (Han et al. 1998).

THQs calculated by using Cd levels showed the opposite trend than the TRs for inorganic As, and when using MAL the THQ exceeded the threshold of 1 in *B. ventricosa* from IRZ during the high-raining period (see Table 2). Despite that exception, a range of about 0.02 to 72.5 % from the Cd THQ-threshold of 1 was found in all species from all locations and periods. Between locations, individuals of *B. ventricosa* and *O. mimus* from IRZ exhibited about 8.4 (low-raining period) and 4.8 times (high-raining period) higher THQ for Cd than individuals from the SL, by using MAL. Per period, SL from the high-raining period exhibited the highest THQs which were about 6.1-fold higher for *R. setosum*, and 2.4-fold higher for *H. chilensis* and *B. ventricosa* in comparison to those at the low-raining period.

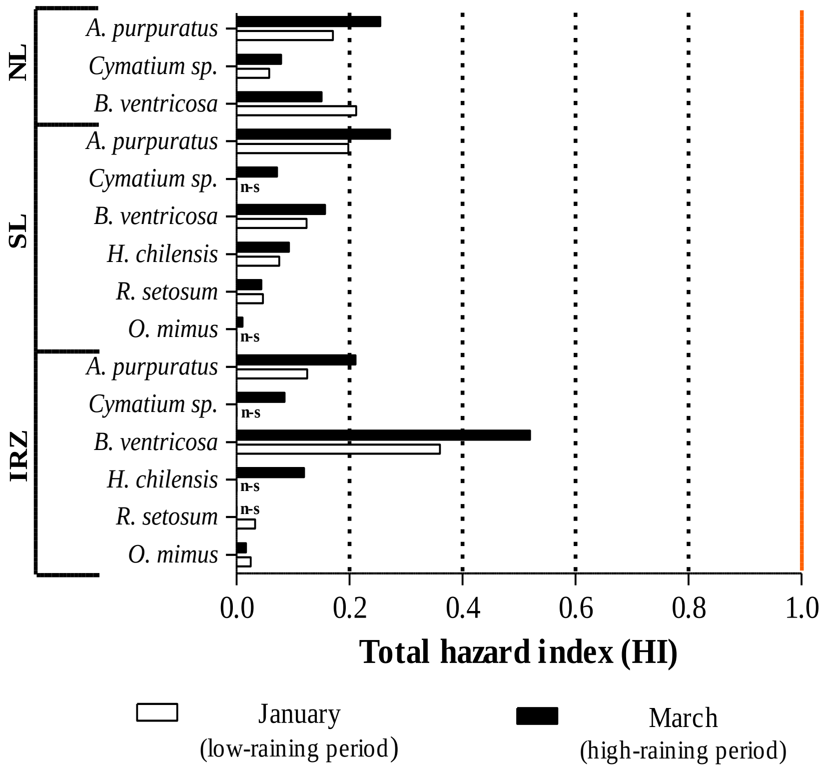
Croatian shellfish species showed up to 2.9 and 12.8-fold much higher THQ than our averages for Cd and Pb (Bogdanović et al. 2014; Gao et al. 2016; Rjeibi et al. 2015), respectively, while the Chinese crustaceans *Charybdis* sp. and *O. oratoria* showed up to 1098-fold higher THQs when were compared to our THQs in crabs *R. setosum* and *H. chilensis*. *O. vulgaris* showed a similar pattern, Cd THQ estimated values were about 18-110 times higher than those of *O. mimus* (~0.001) from Sechura Bay and front IRZ. In all cases, the ingestion rates (IRs) considered in these studies are considerably higher compared to the Peruvian daily ingestions (as mentioned before). It seems that Peruvian seafood consumption is extremely lower than consumption in countries such as Croatia, China and Tunisia. Gao et al. (2016) considered up to 55 g/d for seafood consumption for adults, while Rjeibi et al. (2015) reported 33 g/d for cephalopod consumption, this is substantially different than the FAO estimations of 13.9 g/d for mollusks, 2.8 g/d for crustaceans and 0.8 g/d for cephalopods for the Peruvian population (FAO. 2016). These differences in IRs explain part of the dissimilarities and higher THQs in Croatia, China and Tunisia compared to the Peruvian locations.

Loaiza et al. (2015) calculated THQs for *A. purpuratus* in a previous study, and results are in disagreement with our values, about 600-fold lower and 2-fold higher for Cd and Pb, respectively when both studies are compared. This can be explained by differences in the considered ingestion rates, 11.7 g/d in the previous study, while in this study it was 13.9 g/d, as well as the differences between EDtot (30 yrs vs. 70 yrs). However, THQ values of Cd and Pb were always below the threshold of 1 for this species. *P. mondon* and *M. monoceros* in Taipei City, China were also studied for THQs of Cu, Zn and Cd, and values were in the same range as our results for crabs (Han et al. 1998).

According to the PTWIs of the other evaluated metals such as Fe, Cu, Ni, Zn and Pb, the % of the weekly intake (µg/week) did not exhibited a risk, with values in a range of 0.002 to 29.587% of the PTWI limits established by JEFCA (2011). Therefore, the amount of edible tissue to be consumed per week in order to reach the PTWI was high, with an average of ~81.3 kg/week. However, there was an exception for Ni and Pb which limits the consumption of *B. ventricosa* (IRZ) *and A. purpuratus* (SL and NL) to 0.36 and 0.27 kg/week, respectively in the most precautionary scenario (by using MAL). It is worth noting that PTWI was not calculated for Cr(VI) and Mn due to the absence of PTWI' information in literature and JEFCA.

TRs was also estimated by using average concentrations of Cr (VI), Ni and Pb showing that many species (*O. mimus, R. setosum, H. chilensis, B. ventricosa*, Table 3; see supplementary material) exceeded the threshold of $1x10^{-6}$. For Cr (VI) and Ni all species exceeded TR-threshold during the low-raining period, while around half during the high-raining period. Therefore a cancer could occur by consumption of these species which present these metals. For Pb, no carcinogenic effects could be posed due to all TRs were below $1x10^{-6}$. Based on THQ, no health risk for consumption is expected due to values <1 and from 2.4 -122 973.8-fold lower than the threshold of 1 for Cr(VI), Mn, Fe, Cu, Ni, Zn and Pb (Table 3 and 4; supplementary material).

**Fig 5.** Total hazard index (HI) by using average metal levels in shellfish' muscle (incl. edible tissue for *A. purpuratus*) from the southern (SL) and northern (NL) location at Sechura Bay and front Illescas Reserved Zone (IRZ).




**(*) Edible tissue is considered as adductor muscle + gonad in *A. purpuratus*. HI's were calculated by using THQ's of Cr(VI), Mn, Fe, Cu, Ni, Zn, Cd and Pb, with the exception in *A. purpuratus*, which was calculated by using Mn, Fe, Cu, Zn, Cd and Pb.**
**HI threshold(—): 1. n-s: non-sampled species in that period.**

HIs of average metal levels did not exceed the threshold of 1 for any of the species during any location and at any sampling period (see Fig 5). Only when individual values were used, *B. ventricosa* from low-raining period at IRZ exhibited a higher value of 1.34 (unpublished result). For *A. purpuratus*, higher HI values were found after the high-raining period (March), about 1.4-1.7-fold more elevated than those at the January (low-raining period). No spatial differences were observed for HI between *A. purpuratus* individuals from the three locations. In *A. purpuratus'* predators, *B. ventricosa* (IRZ) exhibited the highest increased HI value comparing the low-and high-raining period, 1.4 order of magnitude higher after high-raining period. IRZ also contained the maximum HI of 0.52 in *B. ventricosa* during march (high-raining period) which was ~3.5-fold higher than at the other locations SL and NL. *O. mimus* and *H. chilensis* from IRZ showed around 1.5-fold higher HI compared to those from SL (see Fig 5). It is noteworthy to mention that is difficult to compare HIs between different studies due to the considered metals and n ($HI = \sum_{i=0}^{n} THQ^{i}$) which differ according to each study aim.

As previously described, locations SL, NL and IRZ have been affected by different anthropogenic activities which explains the elevated metal concentrations, as well as the estimated risk indices in the six shellfish species. Overall, and unexpectedly, the location front the Illescas Reserved Zone seems to be more affected by metal pollution. Metal concentrations and integrated risk indices reveal that species such as *B. ventricosa* and *O. mimus* are more polluted in IRZ, and thus risk indices are closer to or exceeded the limit or threshold for As and Cd. The southeast part of Sechura Bay, including the beginning of Illescas Reserved Zone has a lot of traffic and industrial activities. It is worth to emphasize that the Reserved Zone of Illescas is mainly on land including part of the intertidal area (MINAM, 2010), therefore anthropogenic activities at sea are currently ongoing, and industrial activities such as a phosphates factory, the Norperuano oil pipeline, oil platforms, fishery factories, artisanal ports and the industrial JPQ port are present and nearby the IRZ (IMARPE, 2007, Loaiza et al. 2015). Likewise, intensive scallop mariculture and important local fisherman activities are daily operating in front of IRZ.

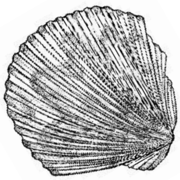
Seasonal variations such as low-and-high raining periods were considered in this study. In general, the variation among periods was different between species and dependent on the sampling location. Based on risk indices (e.g. PTWI, TR, THQ) for As and Cd, in most cases SL exhibited the most considerably variation: with a lower amount of shellfish muscle to be consumed per person, and higher TR, THQ and HI during the high-raining period, which indicates the effects of river discharges in higher metal availability and accumulation (Diop et al. 2016; Forrest et al. 2007; Kehrig et al. 2013; Mendo et al. 2016). SL is characterised by the influence of major fresh and brackish water input along the coast of Peru, i.e. at the mouth of the Virrila Estuary in the southern part of Sechura Bay (INRENA, 2005). Although ENSO 2016 did not come with abnormal and more intense rains in northern Peru, the normal high-raining period (March) influenced the organisms' accumulation and metal availability in SL. IRZ and NL have less influences from fresh or brackish water environments, this is reflected by the lack of temporal variations observed in some species, mainly in *B. ventricosa* individuals from IRZ (for PTWIs).

## 4. Conclusions

Based on metal concentrations and risk indices, this study on potential health risk for human consumption of edible seafood from the northern coast of Peru reveals that around 20 and 30% of molluscs and crustaceans exceeded at least one of the MRLs for inorganic As, while 10 and 40% for exceeded a MRL Cd. Other metals such as Cr, Ni, Cu, Zn and Pb exhibited generally concentrations below the MRLs. THQs were mainly < 1 for Cr (VI), Mn, Fe, Cu, Ni, Zn, Cd and Pb implying no human health risk. However, in *B. ventricosa*, that value was exceeded for Cd. Therefore, Cd is considered as limiting metal and only 23 g/week can be consumed before a potential health risk could occur. On the other hand, *A. purpuratus* can be consumed with a maximum of 117 g/week. TR for inorganic As were always higher than the threshold of 1x$10^{-6}$ while for Cr(VI) and Ni, TRs also exceeded the threshold in most of the studied species, therefore a cancer risk is posed. HI did not exceed the threshold of 1 for any of the species. Therefore, when excluding the TR, most of the species are safe for consumption. Only the TR integrated risk index alters that conclusion due to the high cancer risk for inorganic-As, Cr(VI) and Ni effects. Spatial and seasonal variations were different between species and more dependent on sampling location, SL seems to be more affected by the raining periods, while IRZ exhibited the high presence of metals based on organisms' accumulation.

# Chapter 3

# Peruvian scallop *Argopecten purpuratus*: from a key aquaculture species to a promising bioindicator species



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## Abstract

The present study analyzed the Peruvian scallop *Argopecten purpuratus* and its food sources for metal and fatty acid concentrations in order to determine spatial and temporal differences. Metals such as copper (Cu), manganese (Mn), and zinc (Zn) in gills and iron (Fe) and Zn in sediments were the most significant explaining factors for spatial differentiations (degree of contamination), while for fatty acids, it was C14:0, C15:0, C16:0 and C18:0 in *A. purpuratus*' muscle and in its food sources, which explained more temporal differences (El Niño-Southern Oscillation (ENSO) effect). Gills, digestive gland and intestine were the tissues where metal accumulation was the highest in *A. purpuratus*, compared to muscle, gonads and mantle. Cd in digestive gland was always high, up to ~250-fold higher than in other tissues, as previously reported in other bioindicator species for metal pollution. Fatty acids (FAs) were good biomarkers when annual comparisons were performed, while metals when locations were compared. El Niño 2017 played an important role to disentangle *A. purpuratus*' biological conditions and food sources. Metals are known as persistent contaminants that is reflected in the accumulation, over a relative long period of time. They do not reflect changes on the short term (e.g. low vs. high raining period). This could partially explain the fact that *A. purpuratus*' tissues did not substantially change in metal internalization among periods and years, while FAs did. *A. purpuratus* from Paracas locations mostly showed higher metal concentrations in gills and digestive glands, and lower fatty acid concentrations in muscle than those from Sechura and Illescas Reserved Zone.

**Keywords:** multi-biomarker approach, *Argopecten purpuratus*, bioindicator species, environmental status

## 1. Introduction

Peruvian scallop *Argopecten purpuratus* is known as bioindicator species, however little *in situ* studies have been conducted to proof this assumption (Cornejo-Ponce et al. 2011; Zapata et al. 2012; Loaiza et al. 2015; Romero-murillo et al. 2018). Other scallop species such as *Chlamys farreri, Aequipecten opercularis, Mimachlamys varia, Pecten maximus, Pecten magellanicus, Pecten alba* are used as bioindicator species or marine pollution sentinels in different studies due to their wide distribution, high bioaccumulation efficiency and important commercial value (Wu and Groves 1995; Bustamante and Miramand 2004; Metian et al. 2009b; Guo et al. 2017; Breitwieser et al. 2018a). They are mainly sedentary organisms that filter large amounts of water, allowing them to accumulate elements from the environment (Beukers-stewart et al. 2005; Mendo et al. 2016; Kanduč et al. 2018). Nevertheless, only few monitoring programs consider scallops as sentinel organism, compared to the numerous monitoring programs that use marine mussels (e.g. *Mytilus* spp.) as bioindicator species, e.g. the US Mussel Watch Program, UNEP in the Mediterranean Sea, and OSPAR at the North and Baltic, among others.

Multi-biomarker approaches and the use of ecological tracers is an up-to-date tool for monitoring environmental conditions and quality and/or for assessing pollution (Viarengo & Canesi, 1991; Sardenne et al. 2017). This approach also helps to understand the species' biological response to adverse effects from environmental pollution (Kanduč et al. 2018). Metals are considered as tracers since they are transferred and bioaccumulate within organisms, while biochemical compounds such as fatty acids are characterized as biomarkers (Milinkovitch et al. 2015; Sardenne et al. 2017). Filimonova et al. (2016)

reviewed the potentiality of fatty acids as biomarker for chemical stress such as metallic and non-metallic pollution, and concluded that fatty acid profiling is a promising  tool to understand biological responses.

The use of a biomarker battery is a more trustful approach to assess the environmental health, this minimizes the large amount of natural variables that act as confounding factors on biomarkers responses (Bouzahouane et al. 2018). Fatty acids and stable isotopes such as $\delta^{15}N$ are also commonly used to see effects of anthropogenic activities in food webs. They reflect the food quality, nitrogen enrichment and degree of eutrophication in an ecosystem (Fischer et al. 2014; Puccinelli et al. 2016). Environmental variables (e.g. temperature, salinity, etc.) can explain part of the biomarker responses and can be monitored simultaneously, to understand the role of some exogenous factors (Kanduč et al. 2018).

The most important *A. purpuratus* natural banks and aquaculture areas are available along almost the entire coast of Peru, e.g. in Sechura Bay, Paracas Bay, Independencia Bay, Samanco Bay, Tortugas Bay and Lobos de Tierra Island (Wolff et al. 2007; PRODUCE, 2019). Among the twelve international trade Pectinidae species, *A. purpuratus* is considered one of the most important commercial Pectinidae (Cisneros et al. 2008; Loaiza et al. 2015). Its high nutritional properties and great acceptation in the global market led to exports of about 168 million US$ export per year (Kluger et al. 2016; Mendo et al. 2016; Loaiza et al. 2018). Nevertheless, El Niño-Southern Oscillation (ENSO) impacted the coast of Peru during 2016 and 2017. El Niño 2016 caused flooding but was much less severe than expected, in contrast to the El Niño 2017 which drastically impacted many regions in Peru due to flooding and other ENSO-driven natural disasters (Emerton et al. 2017;

Loaiza et al. 2018). Economic activities such as *A. purpuratus* aquaculture considerably decreased during the El Niño 2017. High mortality occurred in culture areas in northern Peru (i.e. Sechura Bay), where normally the highest production is achieved (PRODUCE, 2018; pers. obs). Total *A. purpuratus* harvested was 21 000 metric tonnes (MT) and 15 600 metric tonnes (MT) in 2016 and 2017, respectively, while in previous years it reached 67 700 metric tonnes (MT) (2013). These scenarios where environmental changes produce adverse effects for marine organisms (e.g. flood-driven contaminants), are a good opportunity to evaluate the potentiality of some species as bioindicator species. Therefore we examined if *A. purpuratus* could be used as potential bioindicator species of pollution and of harsh-environmental conditions.

As previously described by Loaiza et al. (2015, 2018), anthropogenic and industrial activities are conducted in the north of Peru. Oil, metallurgic and non-metallurgic and fishery industry are performed in northern Peru, as well as artisanal fisheries and aquaculture. These activities are impacting the area of Sechura Bay and nearby Illescas Reserved Zone. In the center-south of Peru, such as Paracas Bay, historical fishery industry hardly impacted the ecosystem. In 2004 a 14 km long submarine emitter APROPISCO was built to discharge effluents and contaminants from the fishery factories outside the buffer zone of National Paracas Reserve. Other anthropogenic and industrial activities are also performed in Paracas, such as Camisea Gas Fractionation Plant – PLUSPETROL, fisheries, aquaculture and port activities (SNP, 2003; DIGESA, 2008). It is noteworthy to mention that southern Peru has been described as a natural metal-rich ecosystem, the earth crust exhibited high concentrations of metals, e.g. Cd and Pb (Barriga-Sánchez and Pariasca, 2018; DIGESA, 2018; SANIPES, 2018).

In this study, a multi-parameter approach was implemented using biomarkers and/or ecological tracers in *A. purpuratus* and its potential food sources. For this purpose, as our goal was to cover spatial and temporal variability and to determine its capability as bioindicator species, we chose to monitor spatial and temporal variations of seven metals in six different tissues and 19 fatty acids in muscle tissue of *A. purpuratus.* Metal and fatty acid concentrations in food sources were also analyzed during the El Niño 2016 and El Niño 2017.

## 2. Materials and methods

### *2.1 Sampling procedure*

In the north of Peru, samplings were conducted in Sechura Bay (SB) and Illescas Reserved Zone (IRZ) in January (1S) and March (2S) 2016, and in January (3S) and March (4S) 2017. In the center-south of Peru, samplings were conducted in Paracas Bay (PB) during January (3S) and March (4S) 2017 (Fig 1). *A. purpuratus* samples were collected by semi-autonomous diving and hand at five locations: southern (SL) and northern (NL) locations in SB; one in front of IRZ, and in two locations (PL1 and PL2) northwest of PB (Fig 1). Organisms were kept alive during transport, and then preserved frozen at -20°C (with the exception of few *A. purpuratus* individuals from 4S which were moribund due to El Niño 2017).

*A. purpuratus* individuals from different sizes (30-80 mm shell length; n=870 approx.) and origin (natural banks and aquaculture) were sampled in order to cover sufficient possible variability, and to determine their potential as bioindicator species (Bustamante et al. 2005a). Six different tissues (mantle, gonad, digestive gland, muscle, intestine and gills) were considered during the dissection. From 1 to 10 scallop individuals were used and pooled to reach the amount for one sample of intestine. All tissues were separated, cleaned and weighted for trace metal analysis, and the muscle was also analyzed for fatty acids content and stable isotope ($\delta^{15}N$). Scallops were collected with the permission of Acquapisco SA (SL, NL, PL1, PL2) and Nemo Corporation SA (IRZ).

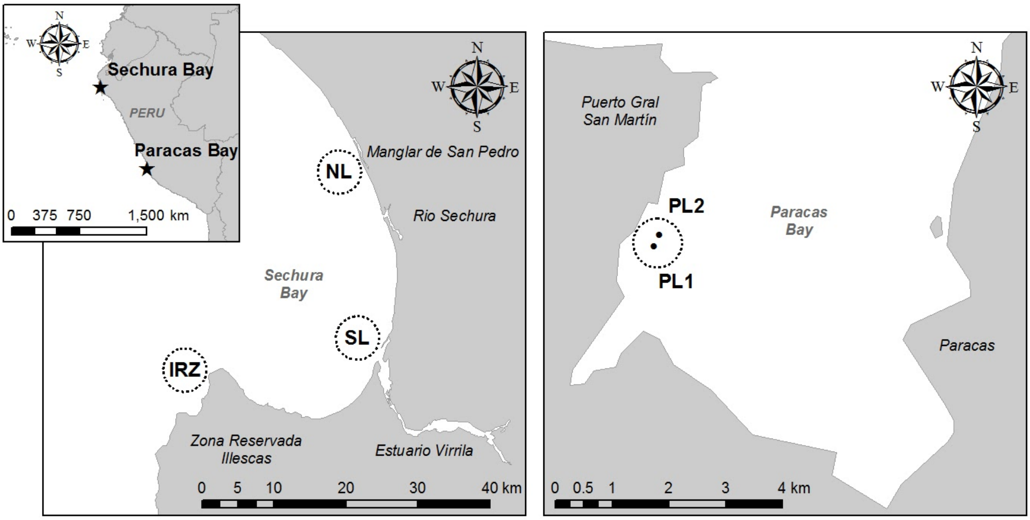



**Fig 1.** Location of the sampling areas at Sechura Bay (incl. Illescas Reserved Zone) and Paracas Bay. Southern (SL) and Northern (NL) locations in Sechura Bay; one location in front of the Illescas Reserved Zone (IRZ), and PL1 and PL2 in Paracas Bay.

*A. purpuratus*' potential food sources such as seston, particular organic matter (POM; *sensu lato*) and sediment were also collected in each location. Seston was not collected in SB northern location and in PB locations due to oceanographic conditions and/or facilities. In other locations, traps were put by semi-autonomous diving during 7-11 days, after which they were uninstalled for water collection, while water-bottom (i.e. POM; n=3-6 replicates per location)) samples were collected directly from the bottom

(Aguirre Velarde et al. 2015). Sediment was collected with a core (internal diameter: 10.16 cm) to down to a sediment depth of 10 cm. From 100 to 1000 ml of seston and water-bottom samples were filtered over a GF/F filter (0.7 μm pore size). Filters and sediments were frozen and preserved at -20°C for metal and fatty acid analysis.

### *2.2 Metal analysis*

Arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), lead (Pb) and zinc (Zn) were determined following Loaiza et al. (2018). Frozen tissues and food sources (filters and sediments) were dried for at least 72hrs at 60°C. The dried tissues were weighed and separated into small (<0.06 g) and large (>0.06 g) tissues. Small and large tissues were digested overnight with respectively 1 or 2.5 ml of highly purified concentrated 69% nitric acid ($HNO_3$). For filters, 4 ml of highly purified concentrated 69% $HNO_3$ was used. Then all samples were heated to 110°C during 30 min in the digester. After cooling (~10 min), 0.1, 0.25, 0.5 ml of hydrogen peroxide ($H_2O_2$) was added for small tissues, large tissues and filters, respectively. The samples were heated again during 30 min at 110°C to complete the total digestion. The digested samples were diluted up to 5 (small), 10 (large) and 40 (filters) ml with Milli-Q grade for the metal analysis. Dried sediment samples were put in glass tubes and digested in 2 ml of highly purified concentrated 69% $HNO_3$ in the closed system SP-Discover Microwace (CEM, USA) (15 min per sample). Digested samples were then diluted till 10 ml in 14 ml tubes with Milli-Q grade for the metal analysis (As, Cd, Cu, Fe, Mn, Pb and Zn). All metal analyses were conducted by inductively coupled plasma mass spectrometry (ICP-MS). For samples that were below the detection limit (BDL) of the ICP, an extra analysis with the high resolution inductive coupled plasma mass spectrometry (HR-ICP-MS) was performed.

For tissues, the quality control was performed by the analysis of standard reference material (SRM) of mussel tissues (2976, National Institute of Standards and Technology, NIST), which was treated and analyzed in the same way as the samples. The recovery ranges for the mussel tissue were 99.6 to 113.9% (n =66, per metal) for As, Cd, Cu, Fe, Mn, Pb and Zn. The limit of quantification (μg/L) was 0.1 for As, Cd, Cu, Mn and Pb, and 5 for Fe and Zn in ICP-MS, and 0.001 for all metals in HR-ICP-MS. For filters and sediment, the certified reference material was the Channel Sediment BCR-320R, which exhibited recoveries from 83.8 to 114.4% (n=8, per metal) for As, Cd, Cu, Fe, Mn, Pb and Zn in 2016. For 2017, the Estuarine Sediment BCR-277R (n=14) was used as reference material, and the recoveries were consistent but lower, around 70.3% for sediments and 94.4% for filters. The recoveries were used in order to calculate the final concentrations. Sediments and filters were measured in HR-ICP-MS, with a detection limit of 0.001 μg/L. All metal concentrations were calculated on a dry weight basis (μg/g dwt).

### *2.3 Fatty acid analysis*

Fatty acids (FAs) of tissues, filters and sediments were extracted and methylated to FA methyl esters (FAMEs) by a modified 1-step derivatization method following Abdulkadir and Tsuchiya (2008) as in De Troch et al. (2012). Samples were freeze-dried for at least 48hrs. Then freeze-dried samples were weighted and placed in glass tubes of 7 ml for tissues, and 10 ml for filters and sediments. Subsequently 2 or 3 ml of 2.5 % sulfuric acid ($H_2SO_4$)-methanol solution (in a proportion of 1:4) was added for respectively tissue samples and filters/sediments. Internal standard (1 mg/ml of methylnonadecanoate C19:0) was added and the solution was stirred in vortex for 10 s, this before they were placed in a water bath at 80°C for 90 min.

Samples were cooled down to room temperature (~5 min) and 1 ml of hexane and 1 ml of sodium chloride (NaCl) (0.98%) were added for tissues, and 1.5 ml of hexane and 1.5 ml of NaCL (0.98%) for filters and sediments, followed by stirring again for 10 s. Samples were placed in a centrifuge 5810 R (Eppendorf) at 160 g speed for 10 min. Then, the extracted samples in hexane (upper layer) were transferred to small vials with sodium sulfate ($Na_2SO_4$) for the last remaining water absorption for at least 1h, and subsequently placed in a micro insert spring of 1 ml for FA analysis. For sediments and some filters, which contain low organic material and FA concentration, three extractions of hexane (upper layer) after centrifugation was performed in order to obtain more concentrated FA.

The extracted FAMEs obtained were analyzed using a gas chromatograph (HP 6890N) coupled to a mass spectrometer (HP 5973). The samples were run in split10 (tissues) and splitless (filters and sediments) mode injecting 1 μl at an injector temperature of 250 °C using an HP88 column (Agilent J&W; Agilent Co., Santa Clara, CA, USA). The FAME were identified by comparing the retention times and mass spectra with authentic standards and mass spectral libraries (WILEY, NIST, FAME, own libraries) and analyzed with MSD ChemStation software (Agilent Technologies). The FAME quantification was calculated by linear regression of the chromatographic peak areas and corresponding concentrations of the external standards, ranging from 100 to 1000 µg/mL for split10, and from 50 to 200 µg/mL for spitless. All FA concentrations were calculated on a freeze-dried weight basis (ng/g fdwt).

### *2.4 Stable isotope analysis*

Muscle tissues of *A. purpuratus* were pre-treated for stable isotope ($\delta^{15}N$) analysis. Tissues were oven-dried for at least 72hrs at 60°C. After drying, the samples were ground to a fine powder using a mortar and placed in tin capsules (5-9mm) for encapsulation, and then placed in a 96-multiwell plate. The tin encapsulated samples were delivered to UC-DAVIS Stable Isotope Facility at the University of California (USA) for $\delta^{15}N$ analysis. All capsules were kept dried and pinch closed until the analysis (Pasotti et al. 2015). The $\delta^{15}N$ determination was part of the results of the dual isotopic composition (carbon and nitrogen) of the samples, by using a PDZ Europa ANCA-GSL elemental analyzer 230 interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK; UC Davis Stable Isotope Facility, http://stableisotopefacility.ucdavis.edu/).

### *2.5 Data analysis*

Statistical analysis was performed with R package 3.5.0. All data were tested for distribution normality with Shapiro-Wilk test and for homogeneity of variances with Levene's test. If these assumptions were not fulfilled, data were log-transformed and inverse transformed. When it was not possible to fulfill these assumptions, non-parametric analogues tests were used. To determine the most important parameters in our multi-parameter analysis, principal component analysis (PCA) was performed on metal and fatty acid concentrations in tissues, seston, POM and sediment grouped per location (IRZ, SL, NL, PL1, PL2) and sampling month (1S, 2S, 3S and 4S). Two-way ANOVA and post-hoc Tukey multiple comparison were performed on the important variables (determined by the PCA) using location and sampling period (i.e. January, 2016, March, 2016, January, 2017, March, 2017) as fixed factors. For non-parametric data, an extension of Kruskall-Wallis test was used with the post hoc non-parametric Kruskalmc test function. Results were statistically significant when $p < 0.05$ (see Data analysis *(addendum)* for more details; supplementary material).

A total of 14 144 environmental measurements were collected from public (i.e. SANIPES, SENAMHI) and private institutions (i.e. Acquapisco SA) from Peru, as well as by using HOBO data loggers during the sampling period (see Fig 10, supplementary material). Data analysis of these environmental variables, such as sea bottom temperature (SBT), surface salinity (SS), river discharge (RD), precipitation (ppt) and bottom (BDO) and surface (SDO) dissolved oxygen were only considered for SL in SB, as its location (northern Peru) is the most affected and vulnerable to ENSO-driven oceanographic conditions (Loaiza et al. 2018).

## 3. Results

Metal concentrations in six different tissues of *A. purpuratus* from the five locations showed that metals accumulated mainly in the digestive gland, the gills and the intestine, followed by the gonad, with high variations in metal concentration. Mantle and adductor muscle contained the lowest metal concentrations (Fig 3). For metals such as As and Cd, the digestive gland concentrated levels up to 25 and 330 µg/g dwt, respectively. Fe also showed high concentrations in digestive gland and intestine, up to 2 900 and 15 730 µg/g dwt, respectively. Intestine and gills were efficient tissues for the accumulation of As, Mn and Zn. Pb accumulated at lower levels and showed no clear pattern (see Fig 3).

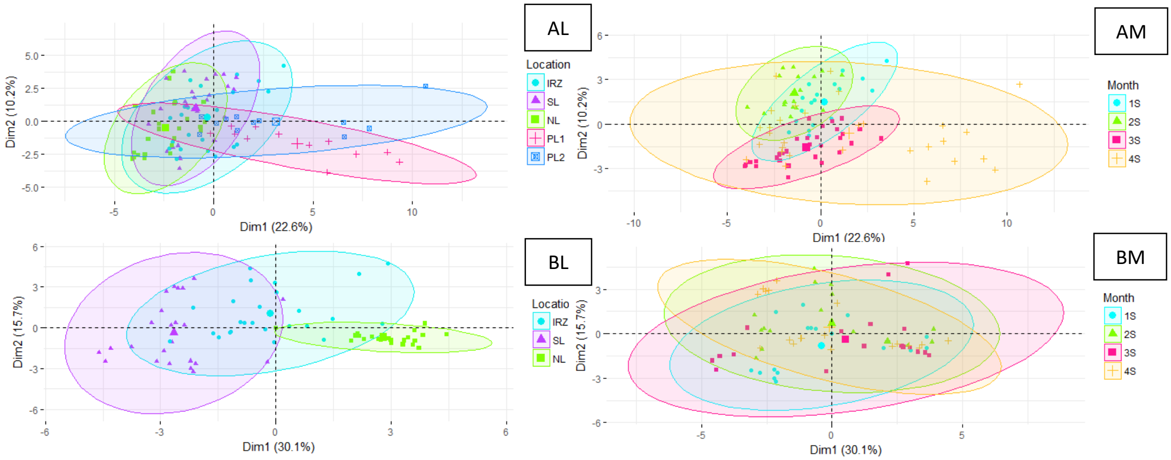



**Fig 2.** PCA results for metals in (A) *A. purpuratus* tissues and (B) food sources per location (L) and month (M). January (1S) and March (2S) 2016, and January (3S) and March (4S) 2017 as sampling months.

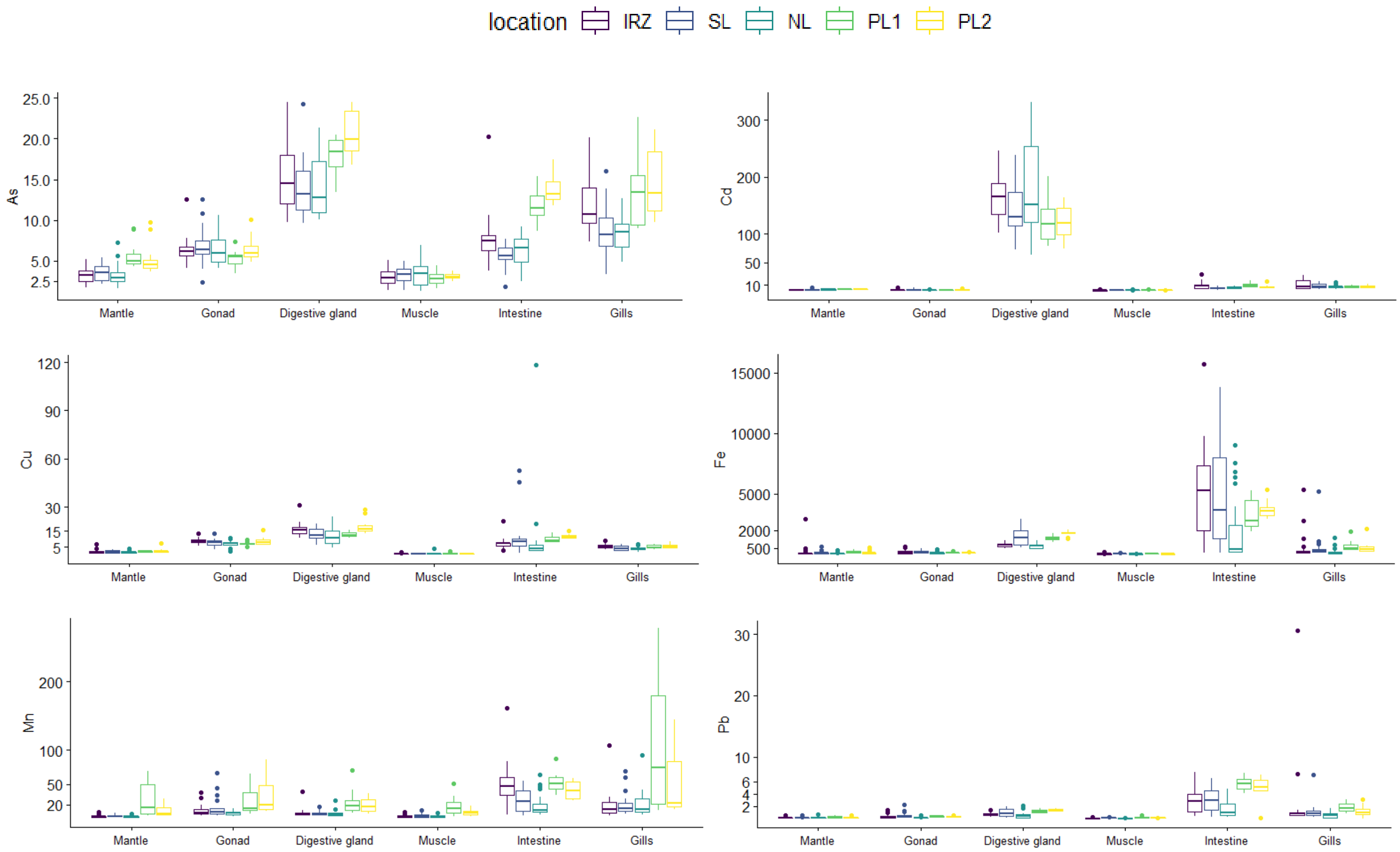
**Note.-AL and BL as metals in (A) *A. purpuratus and* (B) food sources per location (L), respectively; AM and BM as metals in (A) *A. purpuratus and* (B) food sources per month (M), respectively. For more detail in PCA results for metal concentrations per tissue *of A. purpuratus*, see Fig 7 and 8; supplementary material.**

When analyzing the results of all metals in all *A. purpuratus*' tissues together, the PCA principal component (Dim1) explained 22.6% while the second principal component (Dim2) explained 10.2% of the total variation. It was difficult to determine the most important factors for the model because of the high number of parameters (Fig 2AL, 2AM). However, metal concentrations in tissue from Paracas locations seemed to be more explained by Dim1, which were related to concentrations of Zn in gills and Mn in digestive gland, whereas metal concentrations in tissue from Sechura Bay and Illescas locations seemed to be more explained by Dim2, mainly related to Fe in gonad and As in muscle (Fig 2AL). It was difficult to find parameters to differentiate sampling month, especially because 4S showed a high variability (Fig 2AM). When analyzing metal levels in each tissue separately, results from PCA improved considerably and the two principal components explained between 54.0% and 70.8% of the total variation. Cu, Mn and Zn contributions were explaining most of the variation in these models (Fig 7; supplementary material). On the other hand, PCA on metal concentrations for each metal separately gave models where the two principal components explained between 50.9% and 78.0% of the total variation. Gill metal levels contributed most to these models (Fig 8; supplementary material).

For the variables contributing most to the separation of samples, Cu, Mn, Zn concentrations in gills, univariate analyses were performed to test the effect of location and period (Fig 4). Paracas locations (PL1 and PL2) exhibited the highest Mn and Zn concentrations during the high-rain period (March 2017). Mn and Zn concentrations at PL1 during March 2017 was significantly higher than the concentrations recorded at all the other locations, except from the concentrations recorded at PL2 during the same period. The sampling period and year had no significant effect on the Mn and Zn concentration in *A. purpuratus* gills collected in Sechura Bay and Illescas Reserved Zone (Fig 4). Location did not influence Cu concentration recorded in *A. purpuratus* gills within the same period (Fig 4). However, there was a time effect on the Cu concentration in gills for some locations, especially during 2016. IRZ and SL locations in January 2016 exhibited higher Cu concentrations in *A. purpuratus* gills than in March 2016, and only significantly higher for SL. In 2017, there were no significance differences in Cu *A. purpuratus* gills among low (January) and high (March)-rain periods and among years (Fig 4).

PCA on metal concentrations was also performed using food source data (seston, POM and sediment; Fig 2BL, 2BM) from IRZ, SL and NL. The two principal components explained 45.8% of the total variation (Dim1: 30.1% and Dim2: 15.7%) (for more detail of the food source metal concentrations, see Chapter 5: Fig 15; addendum section). The main contributors were Fe and Zn in this model, and sediment was the main contributor to differentiate locations. IRZ, SL and NL were clearly separated along the first axis (Fig 2BL). There was no clear temporal pattern (Fig 2BM).

The metals contributing most for food sources were Fe and Zn, especially in sediments. Thus the univariate analysis was performed using those variables. Sediments from SL exhibited the lowest concentrations of Fe and Zn in all months (Fig 5). NL was always significantly higher than SL, while IRZ when Zn concentrations were compared to SL during March, 2016, January, 2017 and March, 2017 (Fig 5). Only IRZ showed significance differences between the low- and high-rain periods during 2017 for both metals, and the low-rain period exhibited the highest metal sediment concentrations. Annual comparisons indicated also significant differences between IRZ when January 2016 is compared to January 2017 for both metals, with higher metal sediment concentrations in January 2017. On the other hand, SL exhibited more variability for Fe, with significantly higher concentrations in January 2016 compared to January 2017. However, the opposite trend was found for March, 2016 against March, 2017. NL did not exhibit any significantly variations in sediment metal levels among years (Fig 5).


location
IRZ
SL
NL
PL1
PL2
As
Cd
Cu
Fe
Mn
Pb
Mantle
Gonad
Digestive gland
Muscle
Intestine
Gills

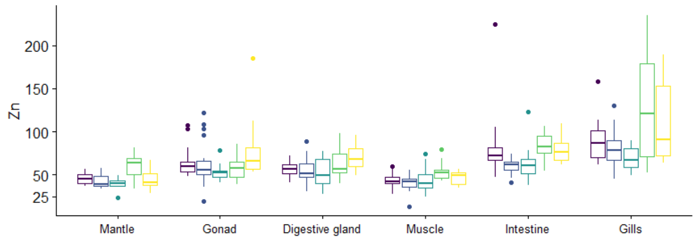



**Fig 3.** Metal concentrations (µg/g dwt) in six different tissues of *A. purpuratus* from Illescas Reserved Zone (IRZ), Sechura Bay (SL, NL) and Paracas Bay (PL1, PL2), 2016 – 2017.

Fatty acids (FAs) in muscle of *A. purpuratus* and in food sources from different locations and months were also used for PCA. Two principal components explained 65.8% and 45.9% of the total variation, respectively. The main contributors were: C14:0, C15:0, C16:0, C18:0, C20:2n-6, C20:4n-6 ARA, for muscle; and C14:0, C15:0, C16:0, C18:0, C18:1n-9 for food sources (Fig 9A, 9B; supplementary material).

Saturated fatty acid (SFA) (e.g. C14:0) in muscle of *A. purpuratus* decreased in 2017 compared to 2016. At NL, concentrations of C14:0 in *A. purpuratus*' muscle were significantly lower in the high-rain period (March) than in the low-rain period (January) in 2017 (Fig 6). Among locations, differences were only observed in 2017. *A. purpuratus* muscles were significantly higher in C14:0 in SL than IRZ and PL2 in January, and also higher than NL and PL2 in March. Annual comparisons were significantly different for IRZ among low-rain periods, and for NL among high-rain periods. In both cases C14:0 concentrations in *A. purpuratus* muscle decreased in 2017 (Fig 6).

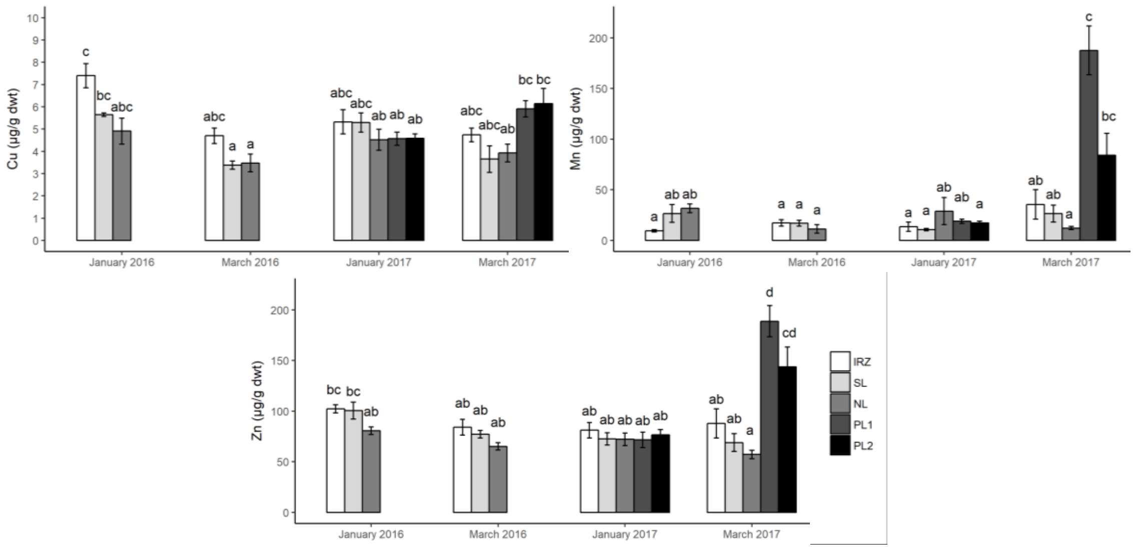



**Fig 4.** Cu, Mn and Zn concentrations (mean ± S.E; n=6) in *A. purpuratus* gills from Illescas Reserved Zone (IRZ), Sechura Bay (SL, NL) and Paracas Bay (PL1, PL2), 2016-2017. Different letters indicate significant differences between groups ($p<0.05$).

C15:0 concentration slightly decreased in muscles of *A. purpuratus* in 2017 compared to 2016. Among periods, IRZ and NL showed significantly lower concentrations of C15:0 in muscle of *A. purpuratus* from the low-rain period (January) compared to the high-rain period (March) in 2017 (Fig 6). The other locations did not exhibit significant differences for C15:0 in *A. purpuratus*' muscle in 2016 or 2017. Among locations, only PL2 was significantly higher in C15:0 concentration than IRZ during January, 2017. C15:0 concentrations were the highest in January, 2016 in NL and IRZ, and significantly higher than in January, 2017 (Fig 6). The same pattern of lower FA concentrations in 2017 in comparison with 2016 was found for C16:0 in muscles of *A. purpuratus*. IRZ exhibited significantly lower C16:0 concentrations during the low-rain period compared to the high-rain period in 2017. Among years, only the muscle of *A. purpuratus* from IRZ-low-rain period exhibited significant differences in C16:0 between years (Fig 6). For C18:0, more important variations were found during the low-rain period among years for all locations, but 2017 exhibited slightly less C18:0 concentration in *A. purpuratus*' muscle than 2016. No significant differences of C18:0 in muscle of *A. purpuratus* were observed among locations per period, and within location among periods. January, 2016 exhibited the highest C18:0 concentrations which were significantly higher than January, 2017 for Sechura and Illescas locations (Fig 6).

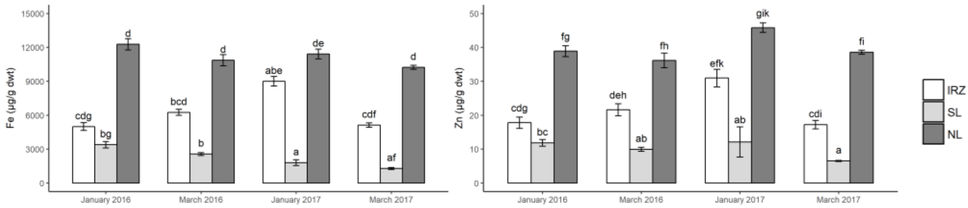



**Fig 5.** Fe and Zn concentrations (mean ± S.E; n=4-6) in sediment from Illescas Reserved Zone (IRZ) and Sechura Bay (SL, NL), 2016-2017. Different letters indicate significant differences between groups ($p<0.05$).

The polyunsaturated (PUFA) (e.g. C20:2n-6) showed a slight different pattern in terms of FA changes: only C20:2n-6 content in *A. purpuratus*' muscle in IRZ decreased significantly in 2017, compared to 2016. The highest C20:2n-6 in muscle of *A. purpuratus* were found in NL and SL in March 2017, they were significantly higher than in March 2016. In 2016, C20:2n-6 of SL in low-rain period was significantly higher than high-rain period. For 2017, the opposite trend was found where IRZ and SL exhibited significantly lower C20:2n-6 concentrations in muscle of *A. purpuratus* during low rains when compared to those at high rains. The other locations did not exhibit significant differences in C20:2n-6 concentrations among locations and periods (Fig 6). The other selected PUFA C20:4n-6 ARA also showed decreases of the FA in 2017, as found for the non-saturated FA. Sechura and Illescas locations exhibited significantly lower concentrations of C20:4n-6 ARA in muscle of *A. purpuratus* in January, 2017 compared to those in January, 2016. Among locations, SL contained the higher and significantly different C20:4n-6 in *A. purpuratus*' muscle than Paracas locations (PL1 and PL2) during March, 2017. The previous year, NL and SL exhibited higher C20:4n-6 ARA concentrations in muscle of *A. purpuratus* than IRZ (Fig 6).

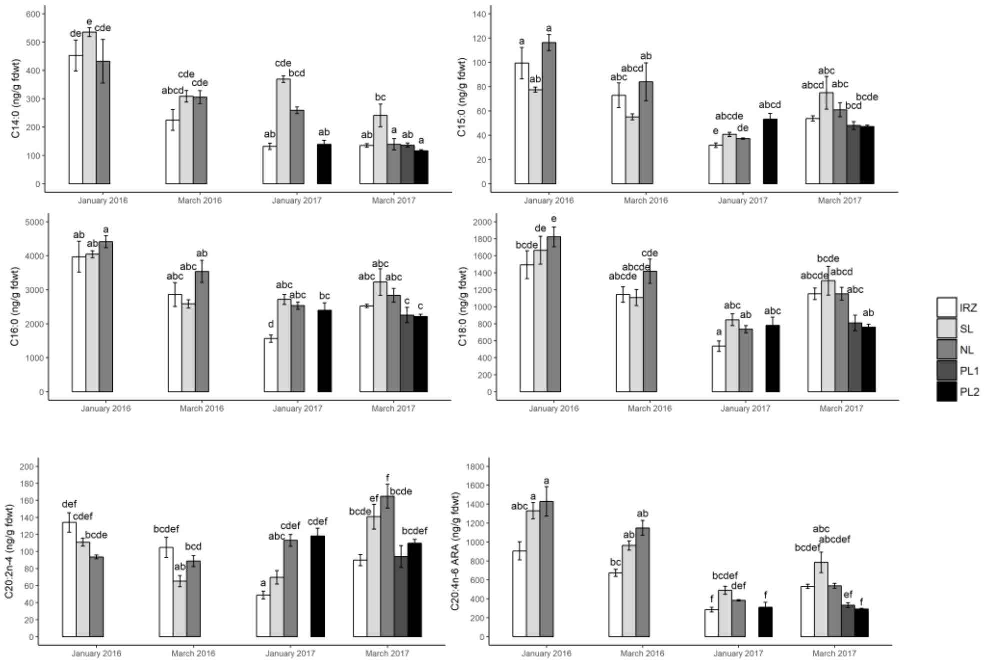



**Fig 6.** FA concentrations (mean ± S.E; n=3-6) in *A. purpuratus* muscle from Illescas Reserved Zone (IRZ), Sechura Bay (SL, NL) and Paracas Bay (PL1, PL2), 2016-2017. Different letters indicate significant differences between groups ($p<0.05$).

The FAs in potential food sources (seston, POM and sediment) exhibited a similar pattern as FAs in *A. purpuratus* muscle. The most significant and highest variations were observed in the annual comparison, almost none when periods within the years were compared. Among locations IRZ was depleted of some FAs such as C16:0 and C18:0. SL and NL exhibited significantly higher concentrations of FA in food sources than IRZ. An opposite pattern than for the FAs for *A. purpuratus* muscle was observed as food sources showed the highest increases of concentrations during 2017. Also, a high variability was observed for all FA concentrations, and hence overall no clear difference between locations and periods was found (Table 1).

The stable isotope $\delta^{15}N$ in muscle of *A. purpuratus* showed lower variations in low-rain periods than those in high-rain periods (Table 2, supplementary material). Among periods, IRZ, SL and NL showed higher $\delta^{15}N$ in muscle of *A. purpuratus* from the high-rain period (2S, 4S) compared to the low-rain period (1S, 3S) in both years. Among locations, more clear differences in $\delta^{15}N$ *A. purpuratus*' muscle were observed during the low-rain period (January), 2016 and high-rain period (March), 2017. The other periods did not exhibit clear differences in $\delta^{15}N$ *A. purpuratus*' muscle among locations. The highest $\delta^{15}N$ of 9.32 ± 0.29 ‰ was found in *A. purpuratus*' muscle from SL during (4S) high-rain period, while the lowest (6.96 ± 0.19 ‰) in IRZ from (1S) low-rain period (Table 2, supplementary material).

Environmental variables differed between 2016 and 2017: a clear increase of sea bottom temperature (SBT) was observed in 2016 (from 20 to 24 ºC) and in 2017 (from 15 to 28 ºC) along the sampling periods (ENSO-summer: Jan-March) (see Fig 10, supplementary material). Piura' river discharge (RD) and precipitations (ppt) considerably increased during 2017, up to 2 800 $m^3/s$ and 80 mm, respectively. The increases were even more intense when only the high-rain (March) period was considered. There was a lack of surface salinity (SS) registration, however the decrease of salinity (up to 20 PSU) at the end of February and beginning of March 2017 was impressive (see Fig 10, supplementary material). A plume was also observed during the Summer Sampling Campaign 2017, seawater became brackish-water with a brownish color. At the same time a massive number of *A. purpuratus* individuals and other benthic species started to die in SL when the El Niño 2017 occurred (PRODUCE, 2018; pers.obs).

## 4. Discussion

The use of a series of elements and compounds as biomarkers and/or ecological tracers in *A. purpuratus* (incl. potential food sources) could help to disentangle different spatial and temporal effects on the species and its environment. For that, a PCA analysis was performed to determine the most important factors that explain the variations; either in metal and/or fatty acid concentrations. The application of this multi-parameter approach analysis was based on previous studies in marine species (e.g. *C. farreri, Stramonita haemastoma*) that demonstrated a different environmental health status in different areas (Guo et al. 2017; Bouzahouane et al. 2018).

The PCA analysis of metals in food sources and specifically sediments showed a clear distinction between Illescas Reserved Zone (IRZ), and southern location (SL) and northern location (NL) of Sechura Bay in this study. When using the most contributor metals (Cu, Mn, Zn) in *A. purpuratus*' gills, only Paracas Bay locations were distinguished from Sechura Bay and Illescas Reserved Zone. Paracas Bay has been impacted during many decades, unlike Sechura Bay. Despite the fact that the National Paracas Reserve is in embedded in this bay, anthropogenic and industrial activities are conducted along the buffer zone. The submarine emitter built in 2004 considerably helped to decrease contaminant concentrations along the bay, however historical pollution is still present (SNP, 2003; DIGESA, 2008).

Guo et al. (2017) also performed a multi-biomarker approach using the scallop *C. farreri* in China. In this study, the most and least polluted locations could be distinguished in the Qingdao coastal area. The snail *S. haemastoma* was also used as bioindicator species at the Algerian coastline. In this study, no clear pattern was found spatially or temporally, however differences between 2 sites and a third one were found when Axis 1 was considered, and Axis 2 helped to slightly differentiate the seasons (winter, autumn, summer and spring) indicating that the potential to use this methodology is present (Bouzahouane et al. 2018). Not seasons but rain periods were considered in the present study, and sampling was done during low (January)- and high (March) rain periods during 2016 and 2017. Nevertheless, in our study no clear differences between the rain periods or the years were found for metal concentrations in *A. purpuratus* tissues and/or food sources in the PCA analysis.

In order to determine the possible effect of the analysis of a few *A. purpuratus* individuals that were moribund in March, 2017 (4S), ∑FA concentrations were compared between the alive and the moribund individuals (n=12). This allowed to estimate the possible degree of fatty acid degradation in the samples. Only individuals from SL exhibited significant differences among the two groups. The highest ∑FA concentrations were found in the moribund individuals, which could imply that degradation was not ongoing in those samples (Table 2; supplementary material).

Milinkovitch et al. (2015) evaluated biomarkers and metals in *M. varia* from the French Atlantic Coast. From 14 trace metals, only Ag exhibited significantly differences among locations and therefore no clear pattern was found when metals were used in the analysis. *M. varia* was also studied by Breitwieser et al. (2016) in the same area, and again no clear pattern was found for seasonal and spatial comparisons of metals either. This confirms our results for period and annual comparisons. Studies *in situ* with Pectinidae always showed high variability and complexity due to endogenous and exogenous variables. Endogenous factors contribute to intra-specific differences in individuals from the same Pectinidae species; e.g. differences in capacity of accumulation, depuration or elimination of metals, which are related to organism' physiological performances. These differences were also influenced by the metabolism and the size or age (incl. sexual maturity stage) of the individual (Neff, 2002; Loaiza et al. 2015).

When considering exogenous factors, food (quality and quantity) is the most important external factor that influence scallop' metal accumulation according to several authors (Metian et al. 2007; 2008a; 2009a; Hédouin et al. 2010). The dietary (i.e. phytoplankton) pathway constituted the 84-99% of the estimated global metal (i.e. Cd, Ag, Co, Zn) bioaccumulation in different scallops (Metian et al. 2007; 2008a; 2009a). Therefore, the concentration, biochemistry and bioavailability of metal-contaminated food plays an important role in accumulation. In this study, *A purpuratus*' food sources (incl. seston, POM and sediment) were affected by the environmental conditions. El Niño was present in 2016 and 2017, however the abrupt El Niño 2017, which induced increases of temperature, high-rain periods and a drop of salinity by 9 PSU (Kluger et al. 2018), might have considerably impacted and changed the food composition for *A. purpuratus*. It is clear that studies on metal uptake and kinetics (by using highly sensitive radiotracer techniques) in *A. purpuratus* are urgently needed in order to better understand the metal bioaccumulation mechanisms of this species.

*A. purpuratus*' digestive gland was the target organ for accumulation of As and Cd. Cd concentrations were relatively high (62 to 330 µg/g dwt). Other Pectinidae species such as *M. varia* exhibited similar values, ranging from 9 to 80 µg/g dwt in the French Atlantic ocean during two measurement periods in the years 1995:1996 and 2013:2014 (Bustamante and Miramand 2004, Bustamante et al. 2005a, 2005b; Milinkovitch et al. 2015; Breitwieser et al. 2016). *A. opercularis* and *P. maximus*, which were also studied during those periods and *P. maximus* which was sampled in 2000, showed Cd concentrations around 120 and 264 µg/g dwt (Bustamante and Miramand 2004; Saavedra et al. 2017), in accordance with our *A. purpuratus* digestive gland concentrations. The species *Amusium balloti*, *Adamussium colbecki* and *P. maximus* from Australia, Antarctica and the English Channel, exhibited similar values of about 111, 142 and 373 µg/g dwt respectively as well, as seen here in *A. purpuratus*' digestive gland (Bryan 1973; Mauri et al. 1990; Francesconi et al. 1993).

**Table 1.** FA concentrations (ng/g fdwt) (mean ± S.E; n=3-5) in food sources from IRZ, SL and NL, 2016-2017.

| Food source | Year | Sampling period | Location | C14:0 | C15:0 | C16:0 | C18:0 | C18:1n-9 |
|---|---|---|---|---|---|---|---|---|
| Seston | 2016 | 1S | IRZ | 79.02 ± 31.93 | $8.96 \pm 3.56^{1}$ | 0.00 ± 0.00 | 0.00 ± 0.00 | 6.93 ± 2.86 |
| | | | SL | $59.48 \pm 8.06^{1}$ | $9.51 \pm 1.83^{1}$ | $78.26 \pm 9.63^{1}$ | 19.10 ± 1.26 | 9.37 ± 0.99 |
| | | | NL | n.s | n.s | n.s | n.s | n.s |
| | | 2S | IRZ | 27.90 ± 3.16 | $7.57 \pm 0.92^{\alpha}$ | 0.00 ± 0.00 | 0.00 ± 0.00 | 4.23 ± 0.50 |
| | | | SL | $79.90 \pm 6.48^{1}$ | $19.80 \pm 1.42^{\beta 2}$ | 225.66 ± 20.09 | 32.41 ± 3.67 | 11.87 ± 0.99 |
| | | | NL | n.s | n.s | n.s | n.s | n.s |
| | 2017 | 3S | IRZ | $138.80 \pm 11.13^{\alpha}$ | $26.60 \pm 2.50^{2}$ | 0.00 ± 0.00 | 0.00 ± 0.00 | 63.36 ± 5.81 |
| | | | SL | $346.41 \pm 24.42^{\beta 2}$ | $68.97 \pm 3.67^{2}$ | $799.84 \pm 48.65^{2}$ | 125.49 ± 7.54 | 139.59 ± 8.58 |
| | | | NL | n.s | n.s | n.s | n.s | n.s |
| | | 4S | IRZ | $55.99 \pm 7.21^{\alpha}$ | $16.03 \pm 2.93^{\alpha}$ | 0.00 ± 0.00 | 0.00 ± 0.00 | 18.85 ± 2.15 |
| | | | SL | $412.59 \pm 37.70^{\beta 2}$ | $156.73 \pm 14.75^{\beta 2}$ | 739.04 ± 67.60 | 122.32 ± 15.03 | 155.56 ± 13.08 |
| | | | NL | n.s | n.s | n.s | n.s | n.s |
| POM | 2016 | 1S | IRZ | $14.76 \pm 10.57^{\alpha 1}$ | $2.69 \pm 1.39^{\alpha 1}$ | $0.00 \pm 0.00^{\alpha}$ | $0.00 \pm 0.00^{\alpha}$ | $3.55 \pm 1.97^{\alpha 1}$ |
| | | | SL | $850.37 \pm 73.08^{\beta c}$ | $63.79 \pm 3.37^{\beta c}$ | $1144.97 \pm 142.11^{\beta}$ | $294.23 \pm 45.20^{\beta}$ | $186.91 \pm 16.92^{\beta c}$ |
| | | | NL | $206.91 \pm 15.15^{\beta}$ | $47.48 \pm 7.73^{\beta}$ | $457.38 \pm 71.98^{\alpha\beta}$ | $123.14 \pm 20.80^{\alpha\beta}$ | $63.16 \pm 10.29^{\beta}$ |
| | | 2S | IRZ | $35.29 \pm 9.96^{1}$ | $5.09 \pm 2.19^{\beta 1}$ | 0.00 ± 0.00 | 0.00 ± 0.00 | $7.21 \pm 2.89^{1}$ |
| | | | SL | $58.13 \pm 30.24^{d}$ | $0.00 \pm 0.00^{\alpha d1}$ | 453.54 ± 279.14 | 137.52 ± 72.84 | $15.21 \pm 11.55^{d1}$ |
| | | | NL | 100.08 ± 27.89 | $13.75 \pm 3.29^{\beta 1}$ | 222.19 ± 62.70 | $48.19 \pm 12.72^{1}$ | $19.89 \pm 5.38^{1}$ |
| | 2017 | 3S | IRZ | $775.07 \pm 307.34^{2}$ | $91.12 \pm 34.82^{\alpha\beta 2}$ | $0.00 \pm 0.00^{\alpha}$ | $0.00 \pm 0.00^{\alpha}$ | $209.20 \pm 83.58^{2}$ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | SL | 257.28 ± 134.25 | 41.64 ± 23.00$^{\alpha}$ | 629.48 ± 303.99$^{\alpha\beta}$ | 122.34 ± 57.48$^{\alpha\beta}$ | 144.20 ± 78.98 |
| | | | NL | 1775.34 ± 98.31 | 175.19 ± 18.90$^{\beta}$ | 3642.77 ± 262.94$^{\beta}$ | 583.49 ± 69.88$^{\beta}$ | 380.56 ± 19.49 |
| | | 4S | IRZ | 509.25 ± 134.44$^{1}$ | 86.46 ± 24.64$^{2}$ | 0.00 ± 0.00 | 0 .00 ± 0.00$^{\alpha}$ | 205.17 ± 34.42$^{2}$ |
| | | | SL | 300.10 ± 97.51 | 116.19 ± 35.44$^{2}$ | 836.56 ± 295.20 | 165.21 ± 70.78$^{\alpha\beta}$ | 248.47 ± 72.77$^{2}$ |
| | | | NL | 483.78 ± 185.31 | 70.08 ± 18.46$^{2}$ | 2013.18 ± 794.48 | 718.27 ± 316.20$^{\beta 2}$ | 292.00 ± 98.76$^{2}$ |
| Sediment | 2016 | 1S | IRZ | 0.50 ± 0.04$^{1}$ | 0.41 ± 0.06$^{1}$ | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.23 ± 0.06 |
| | | | SL | 0.58 ± 0.04$^{1}$ | 0.52 ± 0.03 | 2.95 ± 0.39 | 0.44 ± 0.03$^{1}$ | 0.16 ± 0.02 |
| | | | NL | 0.69 ± 0.23$^{1}$ | 0.39 ± 0.14$^{1}$ | 2.14 ± 0.68 | 0.62 ± 0.19 | 0.11 ± 0.04 |
| | | 2S | IRZ | 0.40 ± 0.06$^{\alpha 1}$ | 0.50 ± 0.10$^{\alpha\beta}$ | 0.00 ± 0.00$^{\alpha}$ | 0.00 ± 0.00$^{\alpha}$ | 0.15 ± 0.08 |
| | | | SL | 1.15 ± 0.09$^{\beta}$ | 0.81 ± 0.02$^{\beta}$ | 5.75 ± 0.04$^{\beta}$ | 1.20 ± 0.24$^{\beta}$ | 0.16 ± 0.01 |
| | | | NL | 0.40 ± 0.13$^{\alpha 1}$ | 0.22 ± 0.07$^{\alpha 1}$ | 1.21 ± 0.34$^{\alpha\beta}$ | 0.27 ± 0.07$^{\alpha\beta}$ | 0.12 ± 0.06 |
| | 2017 | 3S | IRZ | 2.89 ± 1.23$^{2}$ | 1.22 ± 0.33$^{2}$ | 0.00 ± 0.00$^{\alpha}$ | 0.00 ± 0.00$^{\alpha}$ | 3.37 ± 1.65 |
| | | | SL | 1.75 ± 0.09$^{2}$ | 1.22 ± 0.08 | 5.97 ± 0.31$^{\alpha\beta}$ | 1.29 ± 0.12$^{\alpha\beta 2}$ | 2.87 ± 0.31 |
| | | | NL | 3.13 ± 0.37$^{2}$ | 1.94 ± 0.27$^{2}$ | 10.10 ± 0.95$^{\beta}$ | 2.23 ± 0.15$^{\beta}$ | 5.97 ± 0.74 |
| | | 4S | IRZ | 1.18 ± 0.13$^{\alpha 2}$ | 1.13 ± 0.09 | 0.00 ± 0.00$^{\alpha}$ | 0.00 ± 0.00$^{\alpha}$ | 2.78 ± 0.07 |
| | | | SL | 1.42 ± 0.27$^{\alpha}$ | 1.15 ± 0.10 | 4.46 ± 0.56$^{\alpha\beta}$ | 0.84 ± 0.06$^{\alpha\beta}$ | 2.98 ± 0.17 |
| | | | NL | 5.03 ± 1.22$^{\beta 2}$ | 2.57 ± 0.42$^{2}$ | 12.43 ± 2.30$^{\beta}$ | 3.24 ± 1.26$^{\beta}$ | 7.71 ± 1.35 |

**For each fatty acid: different symbols (α,β) indicate differences between locations per period. Different letters (c,d) indicate significant differences between periods per year, and different numbers (1,2) indicate differences between years per location.**

Other metals such as As were also found in similar (or slightly lower) concentrations as in previous studies on other Pectinidae species. Gonad, digestive gland, muscle and gills were found in a similar range of 8-30, 10-25, 8-16, and 15-40 µg/g dwt, respectively in *C. varia* (La Rochelle and Ré Island) and *P. maximus* (Ría de Arousa) as in our study (Bustamante et al. 2005a; Saavedra et al. 2017). *P. maximus* also exhibited around 10 µg As/g dwt in mantle tissue, similar to the *A. purpuratus*' mantle of this study. Nevertheless, the species *Comptopallium radula* from New Caledonia exhibited higher As values in digestive gland, muscle and gills, with values up to 340, 30 and 75 µg/g dwt respectively (Metian et al. 2008b), which could imply that Maa Bay and Sainte Maire Bay are more As-rich environments.

For Cu, a similar trend was found among the scallops, concentrations in a range of 2-15, 5-40, 2-10 and up to 5 µg/g dwt were found for the gonad, digestive gland, gills and muscle, respectively. These values were similar for *A. purpuratus* (this study), *A. opercularis* (Bay of Biscay and Faroe Island), *P. maximus* (Bay of Biscay and Ría de Arousa) and *C. radula* (New Caledonia) (Bustamante and Miramand 2004; Metian et al. 2008b; Saavedra et al. 2008). The Cu-digestive gland concentrations of *A. purpuratus* were also in line with the concentrations in *A. colbecki* (Antarctica), *A. opercularis* (English Channel) and *P. jacobeus* (Meditarranean Sea), respectively 12.6, 36.7 and 16.6 µg/g dwt (Bryan 1973; Mauri et al. 1990).

Fe concentrations in gonad, digestive gland, muscle and gills from *C. radula* (Maa Bay and Sainte Maire Bay) were similar to those from *A. purpuratus* (by average: 166, 1132, 34 and 511 µg Fe/g dwt, respectively) (Metian et al. 2008b). For Mn, *A. purpuratus* also showed average concentrations in the gonad, digestive gland, muscle and gills, with values up to 68, 58, 8 and 93 µg/g dwt, respectively, similar to those previously found in *C. varia* and *C. radula* from La Ré Island and New Caledonia (Bustamante et al. 2005a; Metian et al. 2008b).

The opposite trend was found for Pb and Zn; concentrations in scallops, e.g. *C. varia* (France), *P. maximus* (Spain) and *P. alba* (Australia) considerably exceeded Pb concentrations in *A. purpuratus* (present study, only one exception of *A. purpuratus* gills that exhibited a concentration of ~30 µg/g dwt). Concentrations up to 0.9, 19, 5, 6 and 4 µg/g dwt were found in mantle, gonad, digestive gland, muscle and gills of those species (Bryan 1973; Bustamante et al. 2005a; Saavedra et al. 2008). For Zn, concentrations up to 95, 885, 787, 175 and 569 µg/g dwt were found in mantle, gonad, digestive gland, muscle and gills in *A. opercularis*, *C. varia*, *P. maximus* and *C. radula* from different locations and studies (Bustamante and Miramand 2004; Bustamante et al. 2005a, 2005b; Metian et al. 2008b; Saavedra et al. 2008). These concentrations were about 2-10 fold higher than those found in *A. purpuratus* from this study.

The digestive gland is the most important organ for incorporation and retention of metals in scallops according to numerous authors, which is confirmed in our study. All the mentioned Pectinidae species are considered as bioindicator species due to their capacity of metal uptake, which also reflect the metal concentrations in the environment (Bustamante and Miramand 2004; Metian et al. 2009b; Milinkovitch et al. 2015; Breitwieser et al. 2016). Metal retention was estimated for the digestive gland of *P. maximus*, *C. varia*, *Chlamys nobilis* and other bivalves. The digestive gland is the most important organ for the uptake of Ag, Cd and Pb, and the second most important for Mn and Zn (after the kidney), regardless of the exposure pathway (i.e. waterborne, food or sediment) (Metian et al. 2007; 2008a; 2009a; 2009b; Pan & Wang. 2008; Hédouin et al. 2010). The uptake and depuration kinetic analysis also allowed to estimated biological half-lives ($T_{b1/2}$), based on the depuration rate per metal and species. This elucidated relatively long biological half-lives, e.g. $T_{b1/2}$ > 1.5 for Pb and $T_{b1/2}$ >4 months for Cd in *C. varia* and *P. maximus* (Metian et al. 2007; 2009b). *C. nobilis* also exhibited good metal retentions at subcellular level, the organelles and cellular debris contained ~20% more Cd and Zn, after 30 days of depuration (Pan & Wang, 2008).

In this study, the digestive gland as well as the gills of *A. purpuratus* from Paracas locations showed higher metal concentrations (with exception of Cd) compared to those from Sechura and Illescas, which was also reflected in the sediment concentrations. For example; concentrations of Mn were about 70 and 280 µg/g dwt in digestive gland and gills respectively, while the sediment reached up to 170 µg/g dwt in the same year. These concentrations were up to 55, 20 and 16-fold higher than the other locations for digestive gland, gills and sediments, respectively. Pectinidae species, e.g. *P. maximus* in general show low metal bioaccumulation efficiency from sediment-bound metals; however, once the metals are incorporated, they are strongly retained. *P. maximus* retained 50-90% of the Ag, Cd and Pb transferred from sediments after 16-31 days of depuration period (Metian et al. 2007; 2008a; 2009b).

*A. purpuratus* gills also played an important role as most explaining factor for spatial differentiation in the PCA analysis. Therefore this tissue is also a promising indicator organ for environmental pollution. Gills are in constant contact with the environment as main interface, thus this organ efficiently incorporate contaminants (Jing et al. 2006; Metian et al. 2008a). Filter-feeding organisms such as *A. purpuratus* use the gills for respiration and food consumption. Food sources are zoo- and phytoplankton, seston, bacteria, organic particulates, and resuspended sediment, which probably are rich in metals (Loaiza et al. 2015; Mendo et al. 2016).

PCA analysis on FA concentrations showed more differentiation between years, and this is confirmed when univariate analyses were performed with the most contributing fatty acids. Based on FA in *A. purpuratus*' muscle tissue and food sources, a clear pattern was observed in the annual comparison per location, and an even more clear picture was observed when only the low-rain period (January) was considered in the analysis. Environmental conditions such as temperature (SBT), river discharge (RD), precipitations (ppt) and surface salinity (SS) drastically changed in 2017, and even more during the high-rain period (March) around the southern location in Sechura Bay. Higher FA variability in tissues and food sources were observed in 2017 compared to 2016. An alternative explanation of the fact that we did not observe substantial changes between the low- and high-rain periods is the short term between the evaluations. In contrast, the severe or strong El Niño occurring in Peru seems to influence the degree of change in FA profiles and constitutions. Therefore an annual analysis is more suitable when biomarkers such as FA are used, while for spatial comparisons, metals are the most suitable as previously described.

It is noteworthy to mention the presence of opposite patterns for FAs in muscles and food sources: muscles showed the highest decreases in FA concentrations, while the food sources showed the highest increases in FA concentrations in 2017. Metals and FAs are stable and time-integrated compounds that reflect accumulation and diets, over a relative long period of time. They do not reflect changes on the short term (Dalsgaard et al. 2003;

Puccinelli et al. 2016). This could partially explain the fact that *A. purpuratus*' muscles did not internalize those FA-rich food sources in 2017.

FA as biomarker exhibited more consistency and showed differentiation in the annual comparison, when the El Niño played a more important role. Filimonova et al. (2016) indicated a decrease of FA concentration in bivalve mollusks in harsh-environment conditions. This is in accordance with our results where *A. purpuratus*, showed significant decreases of FA concentrations in muscle, when the severe El Niño was occurring in 2017. In general, bivalve mollusks decrease the FA concentrations in contaminant-exposed conditions, both in the laboratory or *in situ*. C14:0, C16:0, C18:0 and C20:4n-6 ARA decreased in the yesso scallop *Mizuhepecten yessoensis*' gills after exposure with Cd. The bivalves *Cerastoderma edule* and *Scrobicularia plana* reduced their concentrations of SFA, monounsaturated fatty acid (MUFA) and PUFA when they were exposed to herbicides. The blue mussel *M. galloprovincialis* exhibited differences FA composition (i.e. PUFA) in reference vs impacted sites (Chelomin & Belcheva, 1991; Gonçalves et al. 2016; Signa et al. 2015). In this study, FA as biomarker seems to be more effective when additional stressors (other than metal pollution) such as El Niño 2017 was present. FAs were not capable to reflect the differences of metal pollution among sites, but when ENSO-driven oceanographic conditions, (e.g. also reflected in FA-food sources concentrations) was occurring, they showed to be good biomarkers. It is noteworthy to mention that the stable isotope $\delta^{15}N$ in muscle of *A. purpuratus* partially confirmed the FA profile in this study. *A. purpuratus*' muscle $\delta^{15}N$ increases up to 9.32 ± 0.29 ‰ in SL during (4S) El Niño 2017 (high-rain period). This also reflects the degree of impact (i.e. eutrophication-nitrification) in that location, in comparison with other locations (IRZ: 8.02 ± 0.16 ‰; NL: 7.76 ± 0.21 ‰) and with 2016 (~7.80 ‰ in average for SL) (Table 2; supplementary material). In general, FA concentrations increased in food sources in 2017 compared to 2016. This is significantly more pronounced for POM than seston and sediments for C18:1n-9. The same result was also observed for estuarine fish close to wastewater discharges (Sakdullah & Tsuchiya, 2009). A high proportion of C18:1n-9 was found in the diets of these fishes, as we observed in the POM of this study in 2017, the year of the severe EL Niño. More rain and river-discharges impacted the environmental conditions, e.g. a plume was observed along the Sechura Bay (pers. obs.). Fischer et al. (2014) determined also higher concentrations of SFA at the plume (harbor exit) in Monterey Bay, compared to further locations.

What determines if a species is a good bioindicator? According to several authors the following criteria should be fulfilled: a) relatively sedentary organism; b) easy to collect (e.g. by hand); c) widely-distributed; d) high capability for contaminant-incorporation; e) species reflect the degree of environmental pollution (incl. linear relationships); and f) response-effect due to stressor. All of these parameters are fulfilled by the Peruvian scallop *A. purpuratus* in this study. Bel'cheva et al. (2002) also mentioned that Pectinidae species (e.g. *A. purpuratus*) have the potentiality as bioindicator species due to their higher degree of differentiation in organs. These organs are well developed and can easily be distinguished from each other, in comparison to other mollusk bivalves (e.g. mussels) used in monitoring programs. Bustamante et al. (2002) also found that scallops could accumulate higher concentrations of contaminants in different organs (e.g. our study: *A. purpuratus* digestive gland up to 330 µg/g Cd dwt), which seem to be higher than in the currently used bioindicator species such as mussels or oysters

(Metian et al. 2009a, 2009b). On the other hand, criteria e) and f) were only partially fulfilled as e) *A. purpuratus* exhibited only differences between locations in metal concentrations and also reflected in environment metal levels (e.g. sediments). No clear simple linear relationship (n=72, $r^2 < 0.09$) was found between the age or size (e.g. total length) and ∑metal concentrations (As, Cd, Cu, Fe, Mn, Pb, Zn) of *A. purpuratus*' tissues (unpublished results); d) then only FA were suitable biomarkers in order to see the response-effect during the severe El Niño 2017. Temporal variations were clearer in long-term (annual) analysis than in short-term (period).

## 5. Conclusions

Peruvian scallop *A. purpuratus* was identified as potential bioindicator species to be used along the coast of Peru. This species is well distributed as both wild and cultivated species. This scallop is able to accumulate high concentrations of metals (also reflected in environment compartments) and exhibits response-effects when adverse conditions are present (i.e. ENSO driven-conditions). For spatial and temporal monitoring, it is crucial to consider the scale and distances between locations, as well as the time between evaluations. When regions were compared, such as Sechura (Piura) vs Paracas (Ica) a better differentiation was observed. When the time-lapse of evaluations was too short (two-month period), comparisons were not sufficient to see patterns, whereas a yearly comparison showed clear patterns. We advise seasonal evaluations (three-monthly) as most suitable for this species. The application of a battery of biomarkers and/or tracers (i.e. molecular compounds, stable isotopes, etc.) is a key for understanding the response of organisms due to anthropogenic and/or natural stressors. However, cost-benefit should be considered for a long-term and sustainable scallop monitoring program in Peruvian water domains. Metals and FAs are promising biomarkers and tracers for *A. purpuratus* monitoring for spatial (degree of pollution) and temporal (ENSO effect), respectively.

# Chapter 4

# Marine species as safe source of LC-PUFA and micronutrients: insights in new promising marine food in Peru

Slightly modified from the submitted article (under review):

Loaiza, I., De Troch, M. & De Boeck, G., (under review). Marine species as safe source of LC-PUFA and micronutrients: insights in new promising marine food in Peru

## Abstract

Seafood could be a promising way to supplement healthy fatty acids and trace elements to the Peruvian diet. Seafood from northern Peru was characterized with the highest relative concentrations of long-chain polyunsaturated fatty acids (LC-PUFAs), while in the center region marine species had the lowest As and Pb contents. Peruvian marine species are rich in LC-PUFAs and micro-nutrients (Cu, Fe, Mn, Zn), including species considered as potentially edible (e.g. *Cycloxanthops sexdecimdentatus*), but also non-edible species (e.g. *Caulerpa filiformis)*. Nevertheless, it is crucial to consider potentially harmful metals, e.g. As and Cd, which could pose a risk for consumers. High levels of beneficial LC-PUFAs and micro-nutrients would be taken up (up to 80% of the recommended values) when the Peruvian population would consume the estimated safe amount of seafood. Scoring species for fatty acid and metal content resulted in gastropods (e.g. *Bursa ventricosa*) as being the least beneficial species.

**Keywords:** polyunsaturated fatty acids; micro-nutrients; metals; alternative diets; future foods; health risk assessment; Peruvian populations

## 1. Introduction

Peru is one of the most important fishery countries in the world with very large landings of small pelagic fishes and shellfish representing an enormous source of protein and nutrients for the local population (Avadí & Freon, 2015). Pelagic fish occurring in the water column (mainly anchovy) represent about 80% of the total national capture of 5.3 million ton per year in Peru (PRODUCE, 2019). This high productivity is supported by the Humboldt current that brings cold water and nutrients to the surface waters yielding an exceptionally high density and diversity of marine species along the Peruvian coast. Consequently, Peru is home to one of the largest fisheries of the world (Majluf et al. 2017).

Despite the large access to high protein and low fat resources such as fish and other seafood, chronic malnutrition in children (< 5 year) and chronic diseases in adults are still severe problems in Peru. In fact, 19.8% of Peruvian children suffer from chronic malnutrition, and 6.2% and 1.5% suffer from overweight and obesity, respectively (Majluf et al. 2017). In addition, anaemia rates among children aged 6–36 months have stagnated at 43-45 % in the 6 last years (WFP, 2018). For a fishery country, the consumption of fish products is relatively low in Peru, i.e. around 14.5 kg/person/yr (PRODUCE, 2019), which is about 1.5 times lower than fish consumption in Spain, and up to 3 times less than in Asian countries such as China (FAO, 2019). This is partially explained by the lack of knowledge on seafood nutritional properties of the Peruvian population, being more severe in remote and vulnerable populations (Avadí & Freon, 2015; Majluf et al. 2017). Vulnerable populations include the economically disadvantaged, racial and ethnic minorities, children in low-income families, people with chronical diseases, and coastal populations which are more susceptible to disaster' impacts (e.g. El Niño phenomenon) among others (MIMP, 2019; WHO, 2019).

Long-chain polyunsaturated fatty acids (LC-PUFAs), among other nutrients, can be found at the highest concentrations in marine organisms (Kris-etherton et al. 2002; Tacon & Metian, 2013). The omega-3 (ω3) fatty acids eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3), and the omega-6 (ω6) fatty acid arachidonic acid (ARA) (20:4n6) are important beneficial compounds classified as LC-PUFAs (Domingo et al. 2007). Several clinical and epidemiological studies demonstrated that LC-PUFAs play a positive role in improved mental development and cognition in infants and children, and in preventing several diseases e.g. cancer, cardiovascular disease (CVD) or neural disorders, among others (Kris-etherton et al. 2002; Sioen et al. 2008; Tacon & Metian, 2013). Moreover, it has been demonstrated that positive developmental effects associated to LC-PUFAs modify the negative effects expected from contaminants exposure, i.e. methylmercury (MeHg) in high fish consuming populations (Black, 2001; Ström et al. 2011).

EPA and DHA as omega (ω3) fatty acids have beneficial effects on diseases related to the heart or blood vessels, e.g. coronary artery disease, heart attack, among others, which are caused due to unhealthy diet, lack of exercise, overweight and smoking (Abedi and Sahari, 2014; Pennstate Hershey, 2019). While, LC-PUFA ω6 (e.g. ARA) helps to stimulate skin and hair growth, maintain bone health, and regulate metabolism and reproductive system. These ω3 and ω6 fatty acids can also prevent and/or attenuate diseases related to diabetes, arthritis, multiple sclerosis, among others (Abedi and Sahari, 2014; Pennstate Hershey, 2019). The ratio between ω6/ω3 is an efficient indicator for human health protection. In accordance to nutritional scientists, the ideal ratio to protect human health is 1:5-10 ω6/ω3, while ratios from 2-4:1 indicate a high consumption of seafood (Abedi and Sahari, 2014).

Anthropogenic activities such as mining, food industries, oil and gas production have increased along the coast of Peru in recent years (Loaiza et al. 2018), with a potential impact on the fish and shellfish biochemical composition (nutrients, pollutants,…). Fish and other seafood are also a source of chemical contaminants that accumulate in the marine environment. For instance, metals such as arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) are known to accumulate in seafood (Domingo et al. 2007; Sioen et al. 2008).

Most metals have dual effect on living organisms health, some are more potentially harmful (or toxic) than others, as some can give more beneficial properties. In the present study, the most potentially harmful, e.g. As, Cd, Pb and Ni are considered as harmful elements. Ni was specifically considered as harmful element and/or contaminant, since this metal exceeded some of the risk indices (i.e. TR) in previous studies (Loaiza et al. 2018). On the other hand, metals such Cu, Fe, Mn and Zn are named as micro-nutrients or beneficial elements, since in most cases they can be essential for living organisms when their concentrations do not exceed certain limits (U.S. FDA, 2020). It is known that As, Cd and Ni can be essential for certain animals, plants and/or organisms (Hunter, 2008), however in this study, they are more possible to act as harmful element in accordance to previous studies in Peru (Barriga-Sánchez and Pariasca, 2018;  Loaiza et al. 2015; 2018; Marin & Garcia, 2016).

In addition, the abrupt El Niño (2017) has also impacted many regions of Peru, and mostly the vulnerable populations. The impact is mainly seen by changes in the marine ecosystems and high mortality of important commercial species, such as the Peruvian scallop *Argopecten purpuratus* (Loaiza et al. 2020). In this non-steady and changing environment it is required to screen the nutritional properties and the contaminants of Peruvian marine species, in order to document the benefits and risks of their consumption.

Cd levels found in edible tissues from *A. purpuratus* (Sechura Bay, Peru) exceeded the maximum residue levels (MRLs) for human consumption (Loaiza et al. 2009; unpublished results; Loaiza et al. 2015). Marin & Garcia (2016) also determined that half of the studied Peruvian marine species (*Litopenaeus vannamei*, *A. purpuratus*, *Semele* sp, *Aulacomya atra,…)* exceeded the MRLs for Cd, and therefore their consumption could pose a potential health risk. The mussel *A. atra* was also analyzed for metal levels along the southern coast of Peru during 2009-2012, and Cd was up to 4-fold higher than the MRLs (Barriga-Sánchez and Pariasca, 2018). Studies on fatty acids of Peruvian marine species are lacking, but some commercial species such as the Peruvian anchovy *Engraulis ringens* have been analyzed, with levels up to 2250 and 3050 mg/100g freeze dry weight for EPA and DHA (Albrecht-Ruiz et al. 2015). The flatfish *Paralycthis adspersus* showed a concentration (mg/100g wet weight.) of 201.9 ± 7.5 for EPA + DHA in Chile (Rincón-Cevera et al. 2019).

In line with the Sustainable Development Goals (SDGs), alternative diets in order to find the most nutritional food with a low environmental-impact production are a new trend. Among many species, some marine species (e.g. primary consumers: mussels) seem to be the most promising future food in the world (Parodi et al. 2018; SDGs, 2019). In this study we classified the marine species from Peru as edible, potentially edible and non-edible species for Peruvians and rest of the world, according to the results of several studies and reports from Peru and the wider region. For example, species that are consumed in nearby countries such as Chile and Ecuador, or in Asian countries, but not in Peru are considered as potentially edible species, while species that are considered as "toxic" or "have a bad taste" by fishermen or local population (pers. comm.) were categorized as non-edible. Additionally, species with lack of meat that are not known to be consumed are also considered as non-edible, e.g. the crab *Inachoides lambriformis, Pinnaxodes chilensis* (Lavallée & Michèle, 2012; Moscoso, 2012; Carbajal et al. 2017, 2018; IFOP, 2019; IMARPE, 2019; PRODUCE, 2019; pers. comm.).

The aim of this study is to screen the biochemical composition (LC-PUFAs, trace metals) of Peruvian marine species from the northern, center and southern region of Peru. Beneficial and harmful elements and compounds have been quantified in marine species (e.g. edible, potentially edible and non-edible species), in order to determine the most promising species with the best combination of minimal risk and optimal intake of beneficial compounds (LC-PUFAs) and micro-nutrients (Cu, Fe, Mn, Zn). The nutritional profile and the contaminants of the marine species considered as potentially edible and non-edible species could also give an idea of new alternative diets ("future foods") for northern, center-southern and southern Peruvian populations.

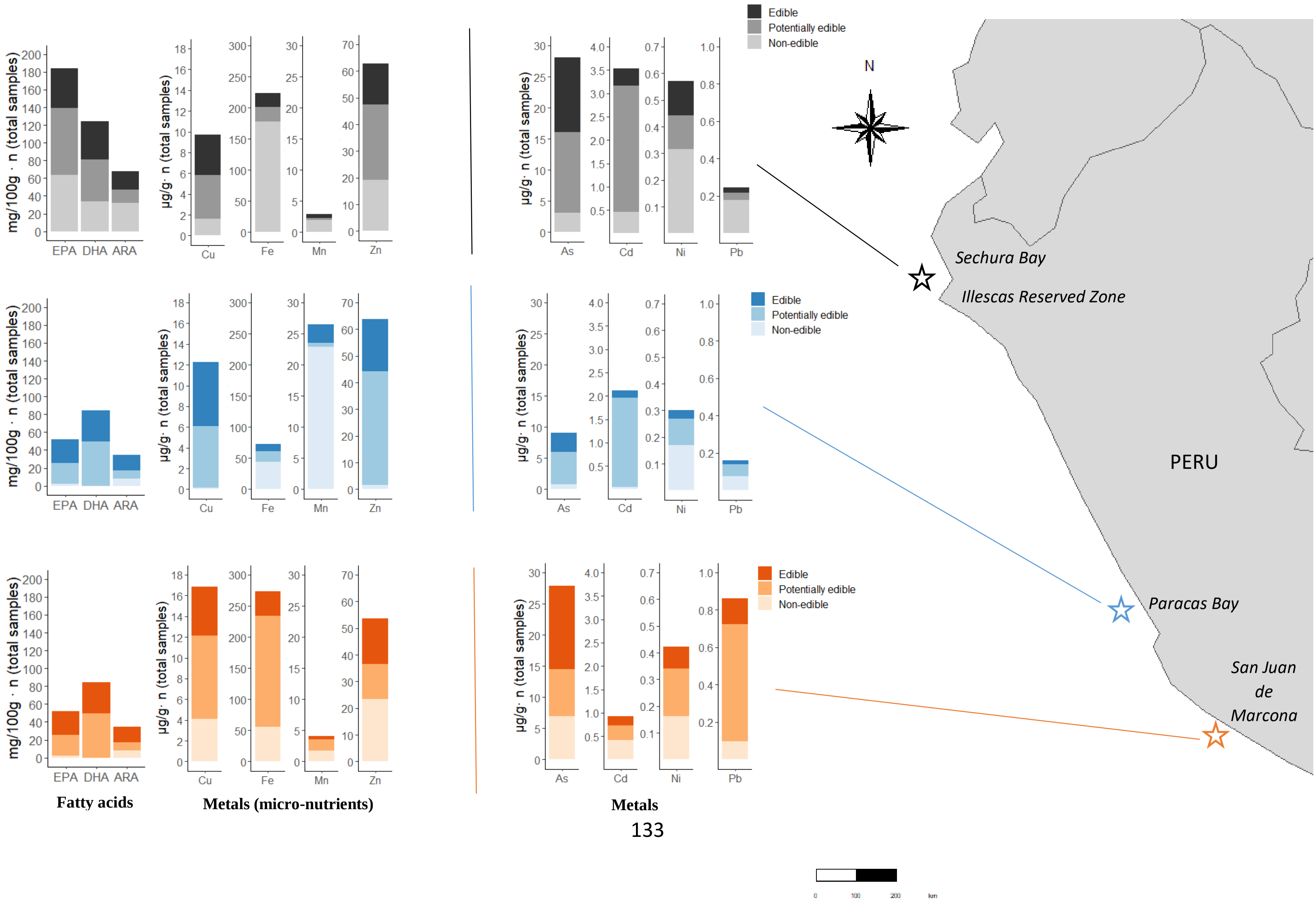

Edible
Potentially edible
Non-edible
N
Sechura Bay
Illescas Reserved Zone
Paracas Bay
San Juan
de
Marcona
PERU
mg/100g · n (total samples)
µg/g· n (total samples)
EPA DHA ARA
Cu
Fe
Mn
Zn
As
Cd
Ni
Pb
Fatty acids
Metals (micro-nutrients)
Metals

**Fig 1.** Bars representing the relative concentration (mg/100g wwt. or µg/g wwt. per total samples) of fatty acids and metals (incl. micronutrients) in edible, potentially edible and non-edible species along the Peruvian coast in the north: Sechura Bay and Illescas Reserved Zone, center-south: Paracas Bay, and south: San Juan de Marcona, as samples in the period 2016 – 2018. Edible, potentially edible and non-edible species are described in Supplementary material; Table 3. Wet weight or fresh weight as wwt. was considered for the nutritional and food safety approach.

## 2. Materials and methods

### *2.1 Sampling procedure*

Edible, potentially edible and non-edible marine species (54 species in total) were collected along the coast of Peru from 2016 to 2018 (supplementary material, Table 3). Thirty-five species were collected in the north of Peru (Sechura Bay, southern (SL) and northern (NL) locations) and front Illescas Reserved Zone (IRZ)), eleven species in the center-south (Paracas Bay, PL1 and PL2 at the northwest of the bay) and twenty-nine species in southern Peru (San Juan de Marcona, PSJ in front of the Punta San Juan Reserved Zone; and SHO in front of the iron company (Shougang Hierro Peru S.A.A). All species were collected by semi-autonomous diving as reported in Loaiza et. al. (2018, 2020), with the exception of the lobster *Pleuroncodes monodon* which was caught by fishermen at Marcona's off-shore. Scallop samples from the north and center-south were collected under the permission of the fishery and aquaculture companies, Acquapisco S.A. and Nemo Corporations.

After collection, all samples were kept cooled and transported to the laboratory to be stored at -20°C. Each individual was measured and weighed in order to register biometric data (see supplementary material, Table 3). Subsequently, the muscle and/or edible tissues of each species was dissected, cleaned and stored for metal and fatty acid analyses. In case of small species (with lack of meat) such as *I. lambriformis*, *P. chilensis*, *Pilumnoides perlatus and P. monodon* the entire specimen was processed (*in toto*). Since the marine species were pooled as edible, potentially edible and non-edible and per taxonomic group per region for the analyses, the ability to perform comparative statistical analysis for these data sets was limited. In addition, fatty acids (LC-PUFA) were compared per species without region distinction, and therefore the statistical analysis was again limited.

## *2.2 Chemical analysis*

### *2.2.1 Metal analysis*

For metal analysis, frozen tissues were dried for at least 72hrs at 60°C. The dried tissues were weighed and separated into small (<0.06 g) and large (>0.06 g) tissues. Small and large tissues were digested overnight with respectively 1 or 2.5 ml of highly purified concentrated 69% nitric acid ($HNO_3$). Then all samples were heated to 110°C during 30 min in the digester. After cooling (~10 min), 0.1 and 0.25 ml of hydrogen peroxide ($H_2O_2$) was added for small tissues and large tissues, respectively. The samples were heated again during 30 min at 110°C to complete the total digestion. The digested samples were diluted up to 5 (small) and 10 (large) ml with Milli-Q grade for the metal analysis. Digested samples were then diluted to 10 ml in 14 ml tubes with Milli-Q grade for the arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) analysis. All metal analyses were conducted by inductively coupled plasma mass spectrometry (ICP-MS; 7700×, Agilent Technologies). For samples that were below the detection limit (BDL), an extra analysis by using the high resolution inductive coupled plasma mass spectrometry (HR-ICP-MS; Element XR, Thermo Scientific, Finnigan element 2, Bremen, Germany) was performed. For more detail and information of the methodology, see Fig 8B; supplementary material.

### 2.2.2 *Fatty acid analysis*

Fatty acids (FAs) of tissues were extracted and methylated to FA methyl esters (FAMEs) by a modified 1-step derivatization method following Abdulkadir and Tsuchiya (2008) as in De Troch et al. (2012). Samples were freeze-dried for at least 48hrs and weighed and placed in glass tubes of 7 ml. Subsequently 2 ml of 2.5 % sulfuric acid ($H_2SO_4$)-methanol solution (in a proportion of 1:4) was added. An internal standard (1 mg/ml of methylnonadecanoate C19:0) was added and the solution was vortexed for 10 s, prior to placing them in a water bath at 80°C for 90 min. Samples were cooled down to room temperature (~5 min) and 1 ml of hexane and 1 ml of sodium chloride (NaCl) (0.98%) were added, followed by stirring again for 10 s. Samples were placed in a centrifuge 5810 R (Eppendorf) at 160 g speed for 10 min. Then, the extracted samples in hexane (upper layer) were transferred to small vials with sodium sulfate ($Na_2SO_4$) for absorption of the last remaining water for at least 1 h, and subsequently placed in a micro insert spring of 1 ml for FA analysis. The extracted FAMEs obtained were analyzed using a gas chromatograph (HP 6890N) coupled to a mass spectrometer (HP 5973). The samples were run in split10 mode injecting 1 µl at an injector temperature of 250 °C using an HP88 column (Agilent J&W; Agilent Co., Santa Clara, CA, USA). The FAME were identified by comparing the retention times and mass spectra with authentic standards and mass spectral libraries (WILEY, NIST, FAME, own libraries) and analyzed with MSD ChemStation software (Agilent Technologies). The FAME quantification was calculated by linear regression of the chromatographic peak areas and corresponding concentrations of the external standards, ranging from 100 to 1000 µg/mL. For more detail and information of the methodology, see Fig 8B; supplementary material.

### 2.2.3 Quality controls

The quality controls for metal analysis consisted of standard reference material (SRM) for mussel tissues (2976, National Institute of Standards and Technology, NIST). The recovery ranges were 98.9–113.5% (n=71) for each metal (see Table 5; supplementary material). The limit of quantification (µg/L) was 0.1 for Mn, Ni, Cu, As, Cd and Pb, and 5 for Fe and Zn in ICP-MS, and 0.001 for all metals in HR-ICP-MS. All metal concentrations were calculated on µg/g wet weight basis (wwt.). For fatty acid analysis, control samples (n=30) were treated and analyzed in the same way as the samples to test the applied analytical procedure accuracy. Internal standard (1 mg/ml of methylnonadecanoate C19:0) was also added in order to ensure precision control for the extraction procedure. All FA concentrations were calculated on mg/100g wet weight basis (wwt.). These units were selected in view of the relevance of the obtained data for the nutrition and food safety and industry approach (Sirot et al. 2008; Tacon & Metian, 2013).

## 2.3 Frequency feeding habits

A social and nutritional survey study was conducted among Peruvian coastal populations during the sampling periods. A frequency feeding questionnaire (FFQ incl. body weight and height measurement) was developed in order to interview 764 people from villages in the north: Sechura Bay and Illescas Reserved Zone (382 ppl.), and in the center-south: Paracas Bay (382 ppl.). A ratio of ~1:1 was considered for male/female (Table 4; supplementary material). According to our questionnaires (by average), men had a body weight of 69 kg and height of 1.62 m, while women weighted 63 kg and were 1.55 m tall in the northern coastal population. Body mass index (BMI) was 25 and 26 kg/m$^2$ for me and women, respectively. For the center-south coastal population the average body weight was 71 kg and 65 kg for men and women, respectively. Height was about 1.64 m (men) and 1.56 m (women). BMIs ($kg/m^2$) were 26 for men and 27 for women. Ingestion rates (gr/day) for scallop, snail, crab, octopus and fish were 4.1, 0.5, 0.4, 6.2 and 41.4, respectively for the northern coastal population, while ingestion rates of 5.8, 0.4, 0.4, 3.8 and 36.3 were found for center-south coastal population (see supplementary material, Table 4).

### *2.4 Risk assessment*

In agreement with our previous results (Loaiza et al. 2018), the risk assessment for consumption of the most important seafood is based on four risk indices (1-4), and one nutritional (5) species-specific-scoring for edible, potentially edible and non-edible species. The ingestion rates and anthropometric measurements of Peruvian populations were based on the FFQ results, for the northern (incl. Sechura Bay and Illescas) and southern (incl. center-south: Paracas Bay; and San Juan de Marcona) populations. We calculated the following risk indices:

1) Target cancer risk (TR) and 2) target hazard quotients (THQ) were determined using the US EPA Regional screening level (RSL) table and Integrated Risk Information System (IRIS). For carcinogenic effects (inorganic As), the risk is expressed as TR, and for noncarcinogenic effects (Cd) as THQ.

$$1)\ \mathrm{TR} = \left[\left(\frac{\mathrm{Efr\ x\ ED_{tot}\ x\ IR\ x\ C\ x\ CPSo}}{\mathrm{BWa\ x\ ATc}}\right)\right] \mathrm{x}\ 10^{-3}$$

$$2)\ \mathrm{THQ} = \left[\left(\frac{\mathrm{Efr \times ED_{tot} \times IR \times C}}{\mathrm{RfDo \times BWa \times ATn}}\right)\right] \times\ 10^{-3}$$

where

Efr: exposure frequency (365 d/year);

$ED_{tot}$: exposure duration, total (70 years);

IR: daily ingestion rate (g/day);

C: metal concentration in the edible portion of marine species (µg/g);

CPSo: oral carcinogenic potency slope (risk per mg/kg/day) of inorganic As (1.5);

BWa: body weight for women and men per region, see Table 4 (supplementary material);

ATc: average time carcinogens (70 years x 365 d/year).

RfDo: oral reference dose (µg/g/d), RfDo for Cd (0.001);

ATn: average time noncarcinogens ($ED_{tot}$ x 365 d/year)

The following indices (3 and 4) are considered as the two most straight-forward risk indices that could be applied by governmental institutions; e.g. Organismo Nacional de Sanidad Pesquera (SANIPES), Ministerio de Producción (PRODUCE) in Peru.

For 3), a) the safe amount of seafood muscle (weight) that can be consumed per week by women and men before reaching the prescribed Provisional tolerable weekly intake (PTWI) was estimated based on person weight (kg), average metal concentration (µg/g wwt.) and PTWI (µg/week) per metal. Thereafter, as precautionary approach, only the lowest amount (limited by the most harmful metal) that could be consumed of each species per region was considered as the safe amount per species; b) Nutritional and beneficial (EPA+DHA+ARA, Cu, Fe, Zn) intakes (mg/day) were then calculated with that safe amount that would be consumed per person (women or men) per region, multiplied by the average compound or element concentration. Recommended beneficial intakes for LC-PUFAs (EPA+DHA+ARA) and micro-nutrients Cu, Fe, Zn were 350, 18, 2 and 15 mg/day (Bauch et al. 2006; Ström et al. 2011; Kris-etherton et al. 2002; US FDA, 2020). Mn beneficial intakes could not be calculated due to the lack of PTWI-Mn reference values in literature.

3) a. Safe amount of seafood muscle that can be consumed per week: $\left(\frac{BWa_1}{C} \text{ x } \frac{PTWI_M}{BWa_2}\right) \text{ x } 1000$

3) b. Respective nutritional and beneficial intakes (mg/day):

$$\text{Safe amount of seafood muscle that can be consumed per week x } C_{\text{beneficial element or compound(s)}} \text{ x } 10$$

where:

$BWa_1$: body weight, women (60 kg) and men (70 kg);

C: metal concentration in the edible portion of marine species (µg/g);

$PTWI_M$: Provisional tolerable weekly intake per metal (e.g. Inorganic As, Cd);

$BWa_2$: body weight for women and men per region, see Table 4 (supplementary material);

$C_{\text{beneficial element or compound(s)}}$: concentration of EPA + DHA + ARA or Cu, Fe, Mn, Zn of the marine species (mg/100g)

For 4) intake assessment of nutrient and contaminant ingestions for the consumption of Peruvian seafood, we used the individual-species-specific seafood consumption. This assessment helps to categorize the Peruvian seafood from the most and least beneficial, in terms of a) LC-PUFA (EPA+DHA+ARA) intake/recommendation vs. b) Inorganic-As or Cd intake/respective Provisional Tolerance Daily Intake (PTDI) (Sioen et al. 2008).

Intake assessment was determined as follows:

$$4)\ a. \frac{C_{\text{beneficial compound(s)}} \text{ x IR}}{\text{recommended intake}}$$

$$4)\ b. \frac{C_{\text{contaminant}} \text{ x IR}}{\text{PTDI}}$$

where

$C_{\text{beneficial compound(s)}}$: concentration of EPA + DHA + ARA in mg/g wwt.;

$C_{\text{contaminant}}$: Inorganic-As or Cd concentration in the edible portion of seafood (µg/g wwt.);

IR: daily ingestion rate (g/day) per group of species per gender and region from Peru (based on FFQ);

recommended intake: 4.7 mg/kg bw/day, based on the average of 350 mg/day of the recommended 200-500 mg/day, and the assumption that 80% is the real contribution or internalization from seafood' fatty acids EPA + DHA + ARA intake (Bauch et al. 2006; Ström et al. 2011; Kris-etherton et al. 2002);

PTDI: 2 and 0.4 µg/kg bw/day, based on the respective PTWI for Inorganic-As and Cd divided by 7.

We used the 5) nutritional species-specific-score (NSSs), which is based on a score index from 1 to 5 to determine the degree of the nutritional properties of edible species, and also of other species as promising alternative diets or future foods.

Nutritional species-specific-score:

$$5)\ \mathrm{NSSs} = \frac{\left[\left(\Sigma\left(\frac{\text{beneficial compound(s)}^{b}\text{or element(s) intake}}{\text{recommended intake}}\right)\right)\right] - \left[\left(\Sigma\left(\frac{\text{C contaminant(s)}^{r}}{\text{MRLs}}\right) + \Sigma\left(\frac{\text{non} - \text{beneficial compound(s) or element(s) intake}}{\text{recommended intake}}\right)\right)\right]}{\Sigma\ \mathrm{NSS}_{\text{species}}} \text{ x } 100 \text{ x } \mathrm{K}_{\text{index}}$$

where

$_{\text{beneficial compound(s) or element(s) intake}}$: concentration of EPA + DHA + ARA and Cu, Fe, Mn, Zn multiply by the daily based consumption of 100g portion of the marine species.

[b]: factor of weighting (x5) was used for the polyunsaturated fatty acids (EPA + DHA + ARA) due to their high benefits in nutrition;

$C_{\text{contaminant}}$: As, Cd, Ni, Pb concentration in the edible portion of marine species (µg/g).

[r]: factor of weighting (x5) was used for As and Cd due to their high risk for human consumption (Loaiza et al. 2018);

MRLs: respective maximum residue levels (µg/g wwt.) for As, Cd, Ni, Pb in crustacean: 10, 0.5, 70, 0.5, in mollusk shellfish 10, 1, 80, 1.5, in cephalopod: 10, 1, 80, 1, and for those with no MRLs available, the minimum was considered, and as follows: 10 (As), 0.05 (Cd), 70 (Ni), 0.3 (Pb) for fish, algae and sea urchin (EU, 2006; CFS-HONG-KONG, 2018; SANIPES; 2019; US FDA, 2019).

Non-beneficial compound(s) or element(s) intake: in case the beneficial element exceeds the beneficial intake, (i.e. Fe: 18 mg/day). This metal also acts as contaminant or non-beneficial element. Therefore, the remaining intake amount divided by the recommendation is used as harmful element. As precautionary approach, Ni was used as contaminant (not as micro-nutrient or beneficial element) in this study, since this metal exceeded some of the risk indices (i.e. TR) in Loaiza et al. (2018).

$NSS_{species}$: $(((\sum$ beneficial compound(s)$^{b}$ or element(s) intake / recommended intake) – $((\sum C$ contaminant (s)$^{r}$ / MRLs) + $\sum$ (non-beneficial compound(s) or element(s) intake - recommended intake)/ recommended intake))) per species

$K_{index}$: a multiply factor to round the scoring index to a range from 0 to 5.

## 3. Results and discussion

### *3.1 Chemical levels in marine species*

The specimens from the north were characterized with the highest relative concentrations of EPA, DHA and ARA, up to around 180, 120 and 60 and mg/100g wwt., respectively. The specimens from the center and southern regions exhibited lower relative fatty acid concentrations, with similar concentrations between them (LC-PUFA concentrations all < ~80 mg/100g wwt.). The micronutrient Zn was the highest in the northern and center specimens, with about 60 µg/g wwt., while Mn was the highest only in specimens from the center (~25 µg/g wwt.). Cu and Fe were considerably elevated in specimens from the south, with 16.9 and 273.7 µg/g wwt., respectively. The center seems to have the specimens with the lowest As, Ni and Pb concentrations (e.g. As: < 10 µg/g; Ni: < 0.3 µg/g; Pb < 0.2 µg/g wwt.). As, Cd and Ni were also high in specimens from the north, relative concentrations (µg/g wwt.) of around 28.0, 3.5 and 0.6, respectively. The southern specimens also exhibited the highest relative concentrations for As and Pb, with 28 and 0.9 µg/g wwt. respectively. (see Fig 1 and Table 1).

When edible, potentially edible and non-edible species are compared, the species categorized as potentially edible are the most important contributors of the total relative concentrations of beneficial chemicals such as LC-PUFAs for all regions, and important for Cu, Fe, Mn and Zn (see Fig 1). Nevertheless, they contain also a significant part of the total relative concentrations of As and Cd in the northern and center species, and of Pb in species from the southern region. Non-edible species contained substantial total relative concentration of Fe and Ni in the south, and Mn and Pb in center and southern regions (Fig 1). These results could give the first insight that the characterized species as potentially edible and non-edible could be used as alternative diet and future foods.

Per taxonomic group, at the higher taxon level, it is clear that sea urchins have the highest ARA concentration, followed by chitons, cephalopods, shrimps and gastropods. Oysters and sea urchins were the richest group of species in DHA, in the following order: oysters> sea urchins> mussel>cephalopod>others. The lowest group of species in DHA contents were algae, limpets and sea cucumbers. The following order: sea urchin>mussel>oyster>shrimp>chiton>others was measured for the EPA concentrations (Fig 5; supplementary material). At the species level, LC-PUFA (EPA+DHA+ARA) concentrations were the highest for *Tetrapigus niger*, *Pteria sterna*, *Squilla* sp and *Semimytilus algosus*, the lowest in LC-PUFAs concentrations are the algae (e.g. *Ulva* sp., *Rhodymenia* sp., *Chondracanthus chamissoi*, *Macrocystis pyrifera*…) (see Fig 2).

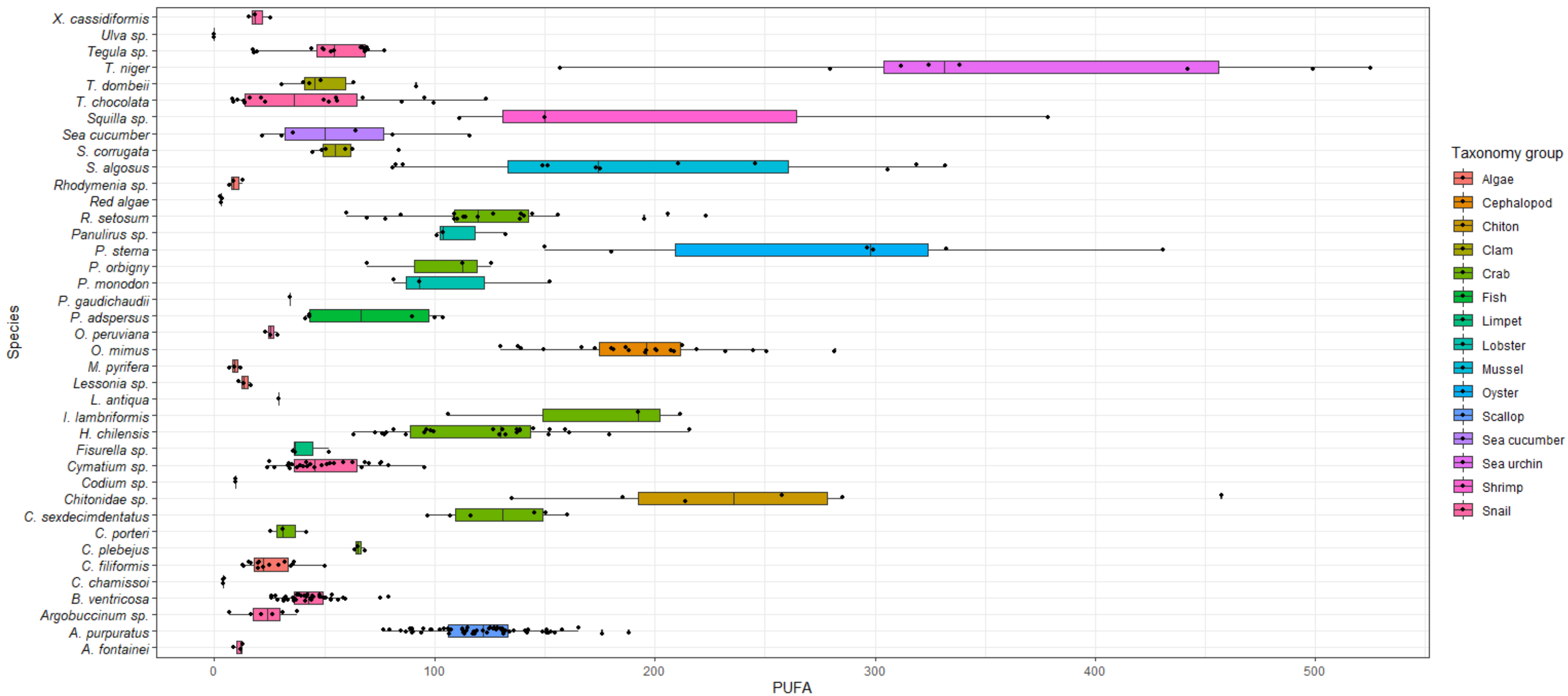



**Fig 2.** PUFA (EPA+DHA+ARA) concentrations (mg/100g wwt.) per marine species.

Nevertheless, algae were the most predominant group of species in micro-nutrients such as Fe and Mn. For Cu, chitons and gastropods showed high concentrations while the crabs had the highest Zn concentrations. Algae had the lowest Zn concentration with relative concentrations <1% (Fig 6; supplementary material). For potentially harmful elements or contaminants, it is clear that gastropods were high in As at all locations, when compared to other taxonomic groups that were analyzed, followed by crab>cephalopod>others. For Cd, mussel is the predominant species followed by shrimp and oyster. Also some crabs and snails exhibited high Cd concentrations followed by sea urchins and clams. Sea cucumber was the predominant species showing the highest Ni concentrations, in a range of 1-4 µg/g wwt., which can act as beneficial or harmful element. Some snails, crabs and scallops also showed high Ni concentrations. Pb in algae exhibited the highest concentrations, up to 6 µg/g wwt. (Fig 7; supplementary material).

As previously mentioned, potentially edible and non-edible species exhibited in some cases high concentration of beneficial compounds but also of some harmful elements (see Table 1). The most promising potentially edible species was the crab *C. sexdecimdentatus*, which exhibited high DHA, ARA and Zn concentrations, as well as substantial concentrations of EPA and Cu. For this crab, potentially harmful elements (i.e. As, Cd, Ni, Pb) were found in lower concentrations than their respective MRLs. As non-edible species, the other crab *Cancer plebejus* was also low in potentially harmful contaminants, and good in its nutritional profile: Cu and Zn were the highest micro-nutrients, while its ARA concentrations (~40 mg/100g wwt.) were also high. For iron, the algae contain the highest concentrations of 20 up to 950 µg/g wwt. as good source of this element (Table 1). Unfortunately, the species *Squilla* sp., *S.*

*algosus*, *T. niger*, *I. lambriformis* with the best nutritional profile (based on polyunsaturated fatty acids and some micro-nutrients) were also Cd-contaminated (Table 1).

**Table 1.** Potential edible and non-edible marine species from Peru as "alternative and future foods" for their chemical and nutritional composition

| Species | Taxonomic group | Categorized (use) | EPA | DHA | ARA | Cu | Fe | Mn | Zn | As | Cd | Ni | Pb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Squilla* sp. | Shrimp | potentially edible | *30.54 ± 17.72* | 62.67 ± 43.82 | *120.16 ± 82.98* | 6.07 ± 2.64 | 11.22 ± 2.64 | 0.19 ± 0.06 | 24.98 ± 3.86 | 2.22 ± 2.03 | **5.10 ± 3.26** | 0.07 ± 0.02 | 0.01 ± 0.01 |
| *S. algosus* | Mussel | | 12.68 ± 2.49 | *88.64 ± 29.90* | 91.30 ± 60.64 | 0.96 ± 0.32 | 49.71 ± 35.30 | 1.44 ± 0.85 | 11.03 ± 6.07 | 3.10 ± 1.32 | **15.55 ± 5.77** | 0.34 ± 0.09 | 0.18 ± 0.14 |
| *C. sexdecimdentatus* | Crab | | 8.13 ± 2.61 | 33.22 ± 4.92 | 88.17 ± 20.64 | 4.32 ± 3.15 | 13.79 ± 8.48 | 0.24 ± 0.14 | *46.59 ± 18.26* | 3.59 ± 2.59 | 0.06 ± 0.03 | 0.06 ± 0.03 | 0.03 ± 0.03 |
| *H. chilensis* | | | 11.23 ± 4.29 | 40.73 ± 13.66 | 69.77 ± 21.63 | 5.92 ± 3.30 | 13.23 ± 9.20 | 0.21 ± 0.12 | 43.61 ± 17.27 | **10.37 ± 7.93** | **0.93 ± 0.97** | 0.09 ± 0.07 | 0.03 ± 0.05 |
| *P. monodon* | Lobster | | 4.04 ± 2.67 | 41.86 ± 8.49 | 62.95 ± 27.37 | 7.45 ± 3.73 | 52.37 ± 14.27 | *5.13 ± 1.66* | 9.39 ± 2.43 | 0.89 ± 0.33 | **1.15 ± 0.59** | 0.27 ± 0.05 | 0.12 ± 0.06 |
| *Tegula* sp. | Snail | | 21.63 ± 7.85 | 7.86 ± 4.39 | 23.38 ± 9.55 | 8.89 ± 6.85 | 38.71 ± 66.00 | 0.61 ± 1.17 | 21.99 ± 6.58 | 4.08 ± 3.70 | 0.21 ± 0.31 | 0.11 ± 0.14 | 0.08 ± 0.16 |
| *A. scabrum* | | | 12.50 ± 6.35 | 2.24 ± 1.15 | 8.53 ± 4.27 | *10.28 ± 9.57* | 62.10 ± 41.54 | 0.71 ± 0.38 | 10.08 ± 2.63 | **17.73 ± 20.08** | 0.11 ± 0.03 | 0.08 ± 0.03 | 0.09 ± 0.04 |
| *A. fontainei* | | | 4.90 ± 1.21 | 1.46 ± 0.41 | 4.81 ± 0.70 | 8.80 ± 6.35 | 69.36 ± 34.14 | 1.66 ± 0.32 | 12.77 ± 1.17 | **20.58 ± 9.96** | 0.09 ± 0.05 | 0.06 ± 0.01 | 0.04 ± 0.02 |
| *L. antiqua* | Clam | | 2.16 | 20.24 | 7.00 | 0.31 ± 0.03 | 21.93 ± 0.14 | 0.76 ± 0.49 | 12.24 ± 5.97 | 1.56 ± 0.19 | 0.13 ± 0.06 | 0.39 ± 0.42 | 0.05 ± 0.02 |
| *Rhodymenia* sp. | Algae | | 1.63 ± 0.28 | 0.00 ± 0.00 | 7.93 ± 2.92 | 1.09 ± 0.28 | 160.77 ± 68.88 | 0.99 ± 0.11 | 4.51 ± 1.07 | 0.49 ± 0.09 | 0.06 ± 0.02 | 0.11 ± 0.03 | **1.00 ± 0.56** |
| Red algae | | | 1.64 ± 0.30 | 0.00 ± 0.00 | 1.46 ± 0.36 | 7.47 ± 7.52 | *955.41 ± 917.45* | 3.38 ± 3.14 | 10.01 ± 3.12 | 1.16 ± 0.48 | 0.16 ± 0.03 | 0.47 ± 0.46 | **3.46 ± 1.98** |
| *Ulva* sp. | | | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.37 ± 0.19 | 49.09 ± 40.44 | 1.02 ± 0.42 | 4.59 ± 5.32 | 0.22 ± 0.10 | 0.11 ± 0.05 | 0.13 ± 0.06 | 0.22 ± 0.22 |
| *T. niger* | Sea urchin | non-edible | *119.95 ± 39.29* | 66.95 ± 62.85 | *172.84 ± 68.4* | 1.13 ± 0.77 | *245.49 ± 518.25* | 3.84 ± 7.80 | 22.26 ± 15.44 | 5.49 ± 4.36 | **1.05 ± 0.90** | 0.46 ± 0.50 | 0.14 ± 0.28 |
| *I. lambriformis* | Crab | | 24.04 ± 6.62 | *82.89 ± 27.69* | 63.19 ± 22.10 | 1.80 ± 0.52 | 49.00 ± 14.01 | 5.87 ± 2.10 | 13.79 ± 2.91 | 1.95 ± 0.71 | **1.54 ± 0.21** | 0.48 ± 0.09 | 0.06 ± 0.02 |
| *C. plebejus* | | | 3.27 ± 0.28 | 19.95 ± 1.16 | 42.47 ± 1.32 | *5.77 ± 2.73* | 5.91 ± 3.77 | 0.18 ± 0.12 | *31.42 ± 11.93* | 8.92 ± 6.95 | 0.13 ± 0.15 | 0.06 ± 0.07 | 0.02 ± 0.00 |
| *C. filiformis* | Algae | | 9.53 ± 3.14 | 0.26 ± 0.45 | 15.82 ± 7.99 | 0.58 ± 0.69 | 213.61 ± 265.82 | *15.21 ± 36.16* | 1.71 ± 0.78 | 0.47 ± 0.20 | 0.06 ± 0.05 | 0.33 ± 0.26 | 0.25 ± 0.20 |
| *Codium* sp. | | | 7.62 ± 0.07 | 0.00 ± 0.00 | 1.83 ± 0.08 | 0.12 ± 0.07 | 22.25 ± 6.21 | 3.07 ± 3.24 | 1.27 ± 0.53 | 1.05 ± 0.25 | 0.06 ± 0.02 | 0.12 ± 0.03 | 0.04 ± 0.02 |

**FAs are as mg/100g (wwt.) and metals as µg/g (wwt.). n= 1-86 replicates. Metal (As, Cd, Ni, Pb) that exceeded the MRLs are printed in bold. Highest average concentration of nutritional compounds and elements are in *italic* per species and use. Wet weight or fresh weight as wwt. was considered for the nutritional and food safety approach**

High nutritional fish species such as cod, hake, whiting, plaice, sole, among others, are similar or lower in EPA and DHA than some Peruvian marine invertebrates in the present study (Sirot et al. 2008). For example, the edible *A. purpuratus*, *Romaleon setosum* and *Octopus mimus* exhibited EPA concentration of about 48, 86 and 68 mg/100g wwt., which is considerably higher than the normal range of 15-46 mg/100g wwt. from those fish species. The DHA concentration was up to 90 mg/100g wwt. for *O. mimus* which is higher than the DHA in plaice, whiting, sole and cod, but lower than the 123 mg DHA/100g wwt. of hake (Sirot et al. 2008; see Fig 5 (as Taxonomic groups); Supplementary material). The promising alternative diets or future foods such as *C. sexdecimdentatus* and *C. plebejus* also exhibited comparable or higher EPA and DHA concentrations than the mentioned commercial fishes (see Table 1). The only fish (flatfish) *P. adspersus* considered in this study showed similar EPA and DHA amounts compared to Sole (*P. adspersus*: 6.7 and 60 mg/100g wwt. vs. Sole: 14 and 72 mg/100g wwt., in EPA and DHA, respectively) (Sirot et al. 2008). When Peruvian marine seafood species are compared to terrestrial meat, about 40 and 10-fold more EPA and DHA, respectively, were present in these marine organisms (Tacon & Metian, 2013).

Sirot and co-workers (2008) also reported EPA and DHA concentrations in scallops (i.e. Calico and Great scallop), of 105 and 110 mg/100g wwt., which is about 2-fold higher than the EPA and DHA concentrations in *A. purpuratus* in the present study. Micronutrients such as Cu, Fe, Mn and Zn were about, 0.2, 3.8, 0.2 and 9.1 µg/g wwt. in pooled of Pectinidae species (Tacon & Metian, 2013), while our *A. purpuratus* exhibited slightly higher concentrations (µg/g; in average) of 0.2, 7.8, 1.3 and 9.3 wwt. for those metals. In that study, the common periwinkle and whelk also exhibited higher concentrations than our studied snails, with up to more than 10-fold higher EPA concentrations in *B. ventricosa*, *Cymatium* sp. and *Thaisella chocolata*

(Sirot et al. 2008). The tissue considered as edible tissue for fatty acid and/or metal analysis is crucial to interpret results, only muscle was considered for the scallop and snail in this study, while the other studies included all edible parts, which could include other edible tissues (e.g. gonads).

More in accordance to our results; octopus, shrimp, mussel and oyster showed similar LC-PUFA concentrations with the different species (from same taxonomic groups) collected in Spain and France (Domingo et al. 2007; Sirot et al. 2008). EPA and DHA contents (mg/100g wwt.) were 37 and 56, respectively in octopus, this is in accordance with the EPA and DHA of 68 and 89 from *O. mimus* of this study. Mixed shrimps (incl. scampis) exhibited concentrations (mg/100g wwt.) in the range of 70 to 140 for EPA, and about 70 for DHA, relatively similar to our mantis shrimp *Squilla* sp. (120 and 63 mg/100g wwt. for EPA and DHA, respectively). The mussel *S. algosus* contained (mg/100g wwt.) about 90 for both LC-PUFAs, while the oyster *P. sterna* showed about 100 and 160 mg/100g wwt. in EPA and DHA in this study. These concentrations are in the same range than those of the mussels and oysters sampled in Catalonia and in French coastal areas (Domingo et al. 2007; Sirot et al. 2008).

Minerals or micro-nutrients were also measured for *Octopus vulgaris*, and showed concentrations (µg/g wwt.) of 4.4, 53.0, 0.3 and 16.8 for Cu, Fe, Mn and Zn, respectively (Tacon & Metian, 2013), while our octopus *O. mimus* exhibited similar concentrations, with the exception of Fe, of 4.4, 6.7, 0.2 and 13.5. In that study crabs (i.e. *Cancer magister*, *Callinectes sanipus*) were also analyzed for Cu, Fe, Mn and Zn, their concentrations (µg/g wwt.) were similar to those in the crabs from this study (Cu: 5.8, Fe: 14.8, Mn: 0.4; Zn: 42.2). The lobster *Homarus americanus* exhibited concentrations (µg/g wwt.) of 13.5 (Cu), 2.6 (Fe), 0.6 (Mn) and 35.3 (Zn) in their edible tissues, while the lobsters of this study, *P. monodon* and *Panulirus* sp. contained about 12 and 4-fold higher concentrations for Fe and Mn respectively and slightly lower concentrations for Cu and Zn than *H. americanus* (Tacon & Metian, 2013).

### *3.2 Risk assessment*

Peruvian commercial marine species exceeded the target cancer risk (TR) > 0.000001 for inorganic-As as previously shown by Loaiza et al. (2018), therefore cancer development could occur in the long term by the consumption of these species. In this study, the northern and southern species exhibited up to 165-fold and 92-fold higher As-levels than TR threshold in the octopus *O. mimus* and fish *P. adspersus*, respectively. Both species were also the highest in ingestion rates by northern and southern populations, 6.2 and 36.3 gr/day respectively (see Table 2). THQ values were always lower than the threshold of 1, which implies no human health risk for Cd concentrations. The highest scoring species in THQ was *A. purpuratus*, with values of a range of 0.015-0.030 for both regions (Table 2). By considering the most harmful metal, the highest safe amount of seafood that can be consumed is 3.38 kg (women) and 3.60 (men) kg per week of the snail *Malea ringens*. Whereas the lowest safe threshold (0.13 kg/week) for consumption was for *Hepatus chilensis* collected in the northern region. The flatfish *P. adspersus* had also a high allowed amount of consumption, up to 7.57 kg/week before a potential health risk could occur, however estimations were only considered by using inorganic-As (see Table 2).

Respective beneficial intakes by consumption of the safe amounts were promising for Peruvian populations. The highest LC-PUFA (EPA + DHA + ARA) intake corresponded to a consumption of 0.7-1.0 kg of *O. mimus* per week for both regions, these concentrations of 200-250 mg LC-PUFA/day contribute considerably to the recommended 350 mg daily intake of polyunsaturated fatty acids for humans (Bauch et al. 2006; Ström et al. 2011; Kris-etherton et al. 2002). Safe consumption of the crab *R. setosum* and the scallop *A. purpuratus* also provided considerably EPA + DHA + ARA amounts, contributing up to 160 mg/day of these beneficial compounds in the daily human diet. *P. adspersus* also exhibited the highest EPA + DHA + ARA intake (up to 760 mg/day), but this was related to the overestimated amount of safe consumption (only considering inorganic-As) (Table 2).

**Table 2.** Target cancer risk (TR), target hazard quotients (THQ), and safe amount (kg/week) for consumption and respective beneficial intakes (mg/day) from Peruvian seafood for women (60 kg) and men (70 kg) from the northern and southern Peru.

| | | | **TR** | | **THQ** | | **SAFEST AMOUNT FOR CONSUMPTION (Amount (kg) per week)** | | **RESPECTIVE BENEFICIAL INTAKES (mg/day)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Inorganic-As** | | **Cd** | | **Based on Inorganic-As or Cd** | | **EPA+DHA+ARA** | | **Cu** | | **Fe** | | **Zn** | | **ω6 /ω3** |
| | | | **Women** | **Men** | **Women** | **Men** | **Women** | **Men** | **Women** | **Men** | **Women** | **Men** | **Women** | **Men** | **Women** | **Men** | **ratio** |
| | | **n** | **(60)** | **(70)** | **(60)** | **(70)** | **(60)** | **(70)** | **(60)** | **(70)** | **(60)** | **(70)** | **(60)** | **(70)** | **(60)** | **(70)** | |
| North | *A. purpuratus* | 109 | **0.000007** | **0.000006** | 0.0163 | 0.0149 | 0.57 | 0.61 | 101.66 | 108.29 | 0.02 | 0.02 | 0.69 | 0.73 | 0.70 | 0.75 | 1:8 |
| | *B. ventricosa* | 84 | **0.000041** | **0.000038** | 0.0054 | 0.0050 | 0.21 | 0.22 | 12.66 | 13.49 | 0.19 | 0.20 | 0.87 | 0.92 | 0.49 | 0.52 | 1:1 |
| | *C. arcuatus* | 2 | **0.000004** | **0.000003** | 0.0023 | 0.0021 | 0.39 | 0.41 | - | - | 0.21 | 0.23 | 0.40 | 0.43 | 1.88 | 2.01 | - |
| | *Cymatium* sp. | 54 | **0.000006** | **0.000005** | 0.0011 | 0.0010 | 1.03 | 1.10 | 69.01 | 73.51 | 0.27 | 0.29 | 1.58 | 1.68 | 2.02 | 2.16 | 1:1 |
| | *H. chilensis* | 65 | **0.000011** | **0.000010** | 0.0072 | 0.0066 | 0.13 | 0.13 | 22.46 | 23.92 | 0.10 | 0.11 | 0.27 | 0.28 | 0.69 | 0.74 | 1:10 |
| | *M. ringens* | 6 | **0.000003** | **0.000003** | 0.0003 | 0.0002 | 3.38 | 3.60 | - | - | 0.24 | 0.26 | 5.46 | 5.82 | 4.07 | 4.33 | - |
| | *O. mimus* | 61 | **0.000165** | **0.000151** | 0.0053 | 0.0048 | 0.71 | 0.76 | 200.14 | 213.19 | 0.43 | 0.46 | 0.67 | 0.72 | 1.38 | 1.47 | 1:4 |
| | *P. gaudichaudii* | 4 | **0.000006** | **0.000006** | 0.0045 | 0.0041 | 0.20 | 0.22 | - | - | 0.22 | 0.24 | 0.39 | 0.41 | 1.05 | 1.12 | - |
| | *P. orbigny* | 7 | **0.000040** | **0.000036** | 0.0028 | 0.0026 | 0.19 | 0.21 | 28.24 | 30.08 | 0.28 | 0.30 | 0.22 | 0.24 | 1.27 | 1.35 | 1:17 |
| | *R. setosum* | 36 | **0.000009** | **0.000009** | 0.0008 | 0.0007 | 0.82 | 0.87 | 154.42 | 164.49 | 0.66 | 0.71 | 2.13 | 2.26 | 4.36 | 4.65 | 1:11 |
| | *T. chocolata* | 12 | **0.000035** | **0.000032** | 0.0012 | 0.0011 | 0.27 | 0.29 | 26.85 | 28.60 | 0.42 | 0.44 | 0.46 | 0.49 | 0.62 | 0.66 | 1:3 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Tegula* sp. | 18 | **0.000005** | **0.000004** | 0.0017 | 0.0015 | 0.68 | 0.73 | 60.74 | 64.70 | 0.60 | 0.64 | 2.63 | 2.80 | 2.35 | 2.51 | 1:2 |
| South (incl. center-south) | *A. purpuratus* | 29 | **0.000009** | **0.000008** | 0.0266 | 0.0243 | 0.47 | 0.50 | 74.45 | 79.52 | 0.02 | 0.02 | 0.36 | 0.38 | 0.79 | 0.84 | 1:16 |
| | *B. ventricosa* | 2 | **0.000027** | **0.000025** | 0.0027 | 0.0025 | 0.26 | 0.28 | 16.59 | 17.72 | 0.24 | 0.25 | 3.31 | 3.54 | 0.48 | 0.51 | 1:1 |
| | *C. porteri* | 6 | **0.000010** | **0.000009** | 0.0005 | 0.0004 | 0.70 | 0.75 | 33.06 | 35.31 | 1.16 | 1.24 | 1.64 | 1.75 | 4.97 | 5.31 | 1:13 |
| | *Cymatium* sp. | 6 | **0.000002** | **0.000002** | 0.0013 | 0.0012 | 0.64 | 0.68 | 72.86 | 77.82 | 0.17 | 0.18 | 0.63 | 0.68 | 1.53 | 1.64 | 1:1 |
| | *H. chilensis* | 21 | **0.000008** | **0.000007** | 0.0016 | 0.0014 | 0.54 | 0.58 | 63.36 | 67.68 | 0.50 | 0.54 | 0.63 | 0.68 | 4.57 | 4.88 | 1:10 |
| | *O. mimus* | 1 | **0.000069** | **0.000064** | 0.0018 | 0.0016 | 0.98 | 1.05 | 233.82 | 249.74 | 1.61 | 1.72 | 1.54 | 1.65 | 1.58 | 1.68 | 1:3 |
| | *P. adspersus* | 6 | **0.000092** | **0.000084** | - | - | 7.09* | 7.57* | 711.32 | 759.74 | 0.18 | 0.20 | 4.35 | 4.64 | 2.98 | 3.19 | 1:20 |
| | *P. gaudichaudii* | 1 | **0.000021** | **0.000019** | 0.0001 | 0.0001 | 0.34 | 0.36 | 16.74 | 17.88 | 0.15 | 0.16 | 0.22 | 0.23 | 2.53 | 2.71 | 1:10 |
| | *R. setosum* | 19 | **0.000010** | **0.000009** | 0.0006 | 0.0005 | 0.74 | 0.80 | 128.74 | 137.50 | 0.81 | 0.86 | 0.45 | 0.48 | 5.75 | 6.14 | 1:5 |
| | *T. chocolata* | 31 | **0.000015** | **0.000014** | 0.0006 | 0.0006 | 0.47 | 0.50 | 22.44 | 23.97 | 1.02 | 1.08 | 0.84 | 0.89 | 1.16 | 1.24 | 1:1 |
| | *Tegula* sp. | 23 | **0.000004** | **0.000003** | 0.0012 | 0.0011 | 0.71 | 0.76 | 39.22 | 41.89 | 1.11 | 1.19 | 4.85 | 5.18 | 2.05 | 2.19 | 1:1 |

**TR and THQ that exceed the target cancer risk (0.000001) and the target hazard quotient (1) threshold are printed in bold. n= number of replicates for metal analysis (from 1-45 for fatty acid analysis). (*) The safest amount for consumption was estimated by using only Inorganic-As. Cd was BDL for this species. Wet weight or fresh weight as wwt. was considered for the nutritional and food safety approach. The estimated ratios (ω6 /ω3) were based on the respective intakes of ARA as ω6 and EPA + DHA as ω3 per species and region.**

These LC-PUFA contributions (> 200 mg/day) from safe consumption of Peruvian seafood is sufficient to prevent cardiovascular diseases according to several national and international expert groups (Bauch et al. 2006; Ström et al. 2011; Kris-etherton et al. 2002). The estimated ratios (ω6/ω3) based on EPA, DHA and ARA intakes were also around the recommended ω6/ω3 ratio of about 1:5-10, which is the ideal to protect human health (Abedi and Sahari, 2014). The safe consumption of species such as the scallop *A. purpuratus* and crabs *H. chilensis*, *Platymera gaudichaudii* and *R. setosum* provided a ratio of 1:5-10, while other species can also contribute to reach the ideal 1:5-10 ratio (Table 2).

In addition, other food sources (e.g. terrestrial based) should be used to improve the healthy diet of Peruvians, in order to reach higher intake of essential fatty acids and micro-nutrients (Kris-etherton et al. 2002). Consumption of various and specific-species diets and other food sources are the key to minimize environmental pollutant intakes and, at the same time, achieve the desired effects on health (Domingo et al. 2007; Kris-etherton et al. 2002; Parodi et al. 2018). Nevertheless, other food items such as rice, which is highly demanding in Peru can also contribute negatively to human health upon As chronic exposure, e.g. concentrations up to 345 ug As/kg were found in rice from the northern Peru (Mondal et al. 2020). When the food habits of coastal (i.e. this study) and inland populations are compared, the persons from the inland of Peru have a consumption seafood up to 4-fold lower than those from the coast (incl. Amazon region) (Flores & Gómez, 2013). However, the inland (e.g Andean region) in Peru are known as high consumers of different grains, e.g. quinoa; which is highly nutritive in LC-PUFAs (i.e. over 50% of total composition). This Andean grain might compensate the lack of LC-PUFA contribution from seafood in Andean population (Abedi and Sahari, 2014; MINAGRI, 2019)

The beneficial intake of micro-nutrients by quantities of seafood considered safe for consumption was also promising as a contribution to a Peruvian healthy diet. The concentrations did not exceed the recommended daily basis for each element (Cu, Fe, Zn). *M. ringens* (north) and *Tegula* sp. (south) were the species with the highest Fe contributions with around 30% of the recommended (18 mg/day) Fe for humans resulting from safe consumption quantities. For Cu, consumption of southern species exhibited higher beneficial intakes than northern species. The highest Cu intakes were slightly lower than the recommended daily ingestion of 2 mg Cu. For example, *O. mimus* and *Tegula* sp. from the south showed the highest Cu contributions (mg/day) of about 1.6 and 1.1 respectively, while the northern *O. mimus* and *Tegula* sp. were 4- and 2-fold lower. Highest Zn intakes (mg/day) varied from 4 to 5 for the following species: *R. setosum*, *M. ringens* and *Cancer porteri*, which were good Zn contributors leading to a third part of the recommended 15 mg/day. On average, the safe consumption of species such as the snails *B. ventricosa* and *T. chocalata* and the crab *P. gaudichaudii* were the lowest to provide beneficial compounds and elements for humans (see Table 2).

Peru is one of the most important fishery countries in the world with a coastline of 2 414 km. Yet, Peru is still tackling chronic malnutrition, anaemia and other chronic health disorders in children and vulnerable populations (Avadí & Freon, 2015; Majluf et al. 2017). Micro-nutrients or minerals such as Cu, Fe, Zn, Mn are present in considerable concentrations in the studied marine species, with up to, 70, 150, 4, and 150 order of magnitude higher levels than terrestrial meats respectively (Tacon & Metian, 2013; Fig 6; supplementary material). Thus, these 30-80% of the recommended dose of micro-nutrient contributions through safe consumption of Peruvian seafood are the key to really tackle children's malnourishment and anaemia in Peru.

Fe and Zn are the most commonly used micro-nutrients to treat anaemic patients, i.e. children  and women in pregnancy, among others (Black 2001; Zlotkin et al. 2005). Prevalence of overweight and obesity in Peruvian populations also play an important role in human health. Recently, Peru has been characterized as the third top country in overweight and obesity in Latin America, about 23 % of population is obese in Peru (FAO, 2019). In the present study, BMIs were high for the northern and center-southern coastal populations, an average value of 26 was estimated (764 FFQ for 1:1; m:f), with the exception of the 27 BMI for women from the center-southern coastal population. For both populations, the BMIs were in the range of "overweight" (25-29.9), this in according to the previous findings (NHI, 2019; FAO, 2019).

Further studies, and interventions, on improving nutrition of the local population on the long term are needed in Peru. Risk estimations and nutritional properties of seafood are provided in this study but the *a posteriori* actions (i.e. specific pre and post-interventions) are the important steps to tackle to attenuate the real problem. This can be realized by 1) providing seafood quality properties and education to researchers, doctors, fishermen and teachers at the local scale, and 2) increasing awareness on food safety and food quality guidelines for seafood at the national government level. The combined work of public health experts and marine biologists could lead to positive actions (e.g. human health evaluations) at local and national level.

The intake assessment by using the individual species-specific seafood consumption helped to distinguish the groups of species that were the most and least beneficial for human consumption, as shown in figure 3A. By using inorganic-As, the northern individuals of *A. purpuratus* were grouped as the most beneficial seafood due to their higher EPA + DHA + ARA intake/recommendation combined with a low inorganic-As intake/PTDI, this in contrast to *O. mimus*, which showed high EPA + DHA + ARA intake/recommendation, but also high inorganic-As intake/PTDI. The other two groups of individuals (as grey and red ellipses) were low in EPA + DHA + ARA intake/recommendation, or the individuals grouped to the right (red ellipse) were also high in inorganic-As intake/PTDI. The most predominant individuals in the red ellipse (also least beneficial) belong to the snail species *B. ventricosa* (Fig 3A). A similar pattern was found for the southern species, *A. purpuratus*' individuals were also the most beneficial species (see blue ellipse), but the fish *P. adspersus* took the place of *O. mimus* (from the north) with high EPA + DHA + ARA intake/recommendation, but also high inorganic-As intake/PTDI. The snails *T. chocolata* and *B. ventricosa* were also grouped as the least beneficial species (see Fig 3A).

Based on Cd concentrations, the individuals were more disperse without such a clear suitable grouping as when inorganic-As was used (Fig 3B). Nevertheless, the northern species *A. purpuratus* and *O mimus* exhibited the highest EPA + DHA + ARA intake/recommendation ratio, but also high Cd intake/PTDI for *A. purpuratus*, more than for *O. mimus*. Only one intake ratio (for women and men) of *O. mimus* exhibited a lower Cd intake/PTDI ratio. Intake ratios of *B. ventricosa* and *H. chilensis* mainly governed the red ellipse, which is considered the least beneficial individual grouping. Low Cd intake/PTDI and low EPA + DHA + ARA intake/recommendation were recorded for the crabs *H. chilensis* and *R. setosum* (Fig 3B). In the south, there was no obvious most beneficial group for human consumption, with the exception of one *O. mimus* individual which is slightly lower in Cd intake/PTDI and high in the EPA + DHA + ARA intake/recommendation. *A. purpuratus*' intake ratios showed the same trend as in the north. The snails *T. chocolata* and *B. ventricosa* were also the most predominant in the least beneficial group. The rest of species was grouped in the grey ellipse, i.e. low beneficial and low harmful intake ratios (see Fig 3B).

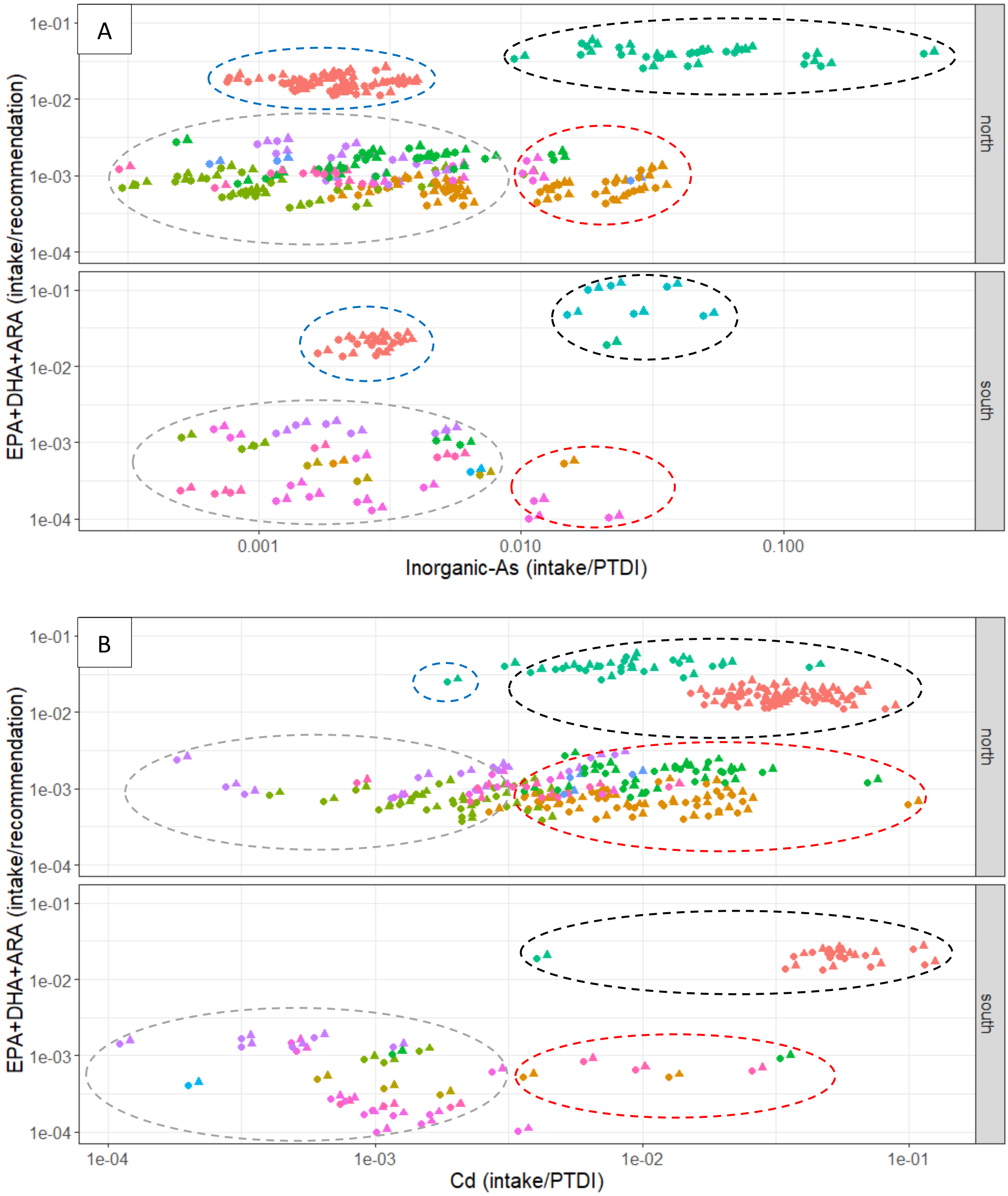
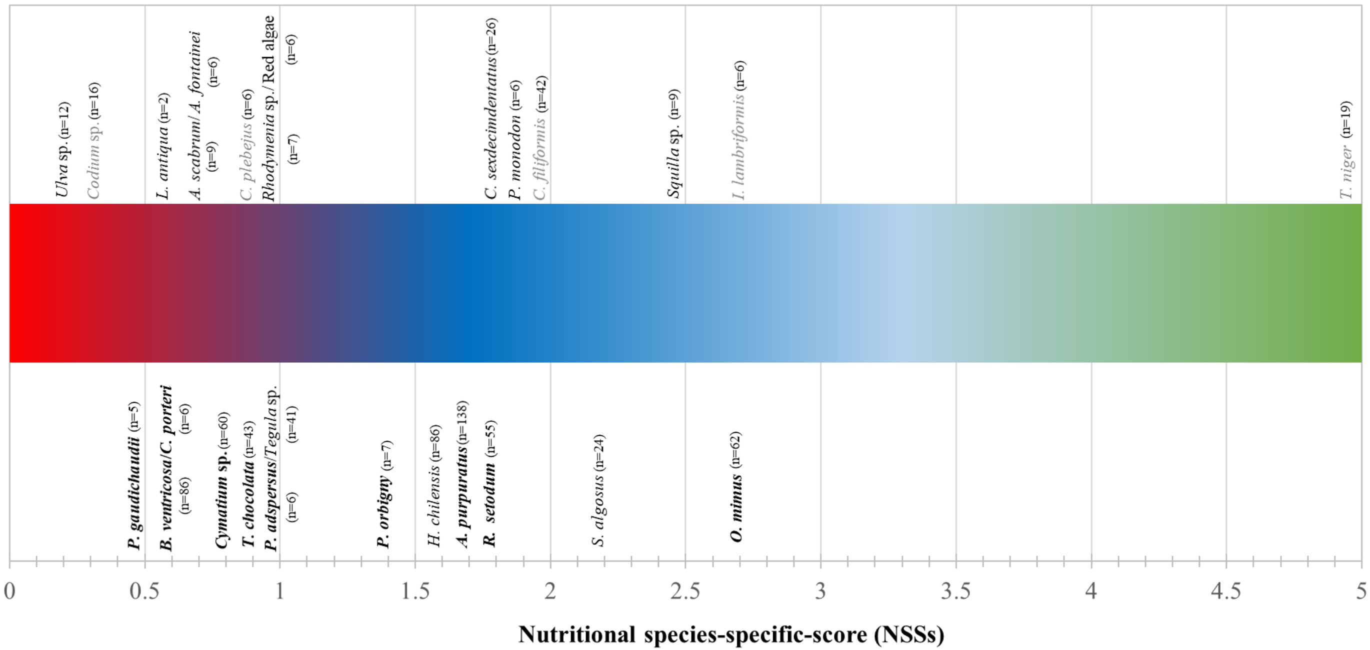



**Fig 3**. A) Inorganic-As and B) Cd intake divided by the Provisional tolerance daily intake (PTDI: 2 and 0.4 µg/kg body weight (BW) for Inorganic-As and Cd) in relation to the intake of PUFA (EPA + DHA + ARA) divided by the recommendation (4.7 mg/kg BW) for twelve Peruvian edible species consumed for women (▲) and men (●) from the north and south (incl. center-south) of Peru; note logarithmic scales

Nutritional species-specific-score (NSSs) ranked the species from 1 to 5 according to their nutritional profile. Surprisingly, potentially edible and non-edible species scored equally good as edible species, and in some cases these promising "alternative diets or future foods" were above the NSSs of edible species. For example, the sea urchin *T. niger* is considered as non-edible but had the maximum score (5) of all studied species. The following in line was the non-edible crab *I. lambriformis*, the edible *O. mimus* (2.7), and the potentially edible *Squilla* sp. (2.5). These four species were high in LC-PUFAs which can partially explain the high ranked values (Fig 4). Nevertheless, as previously described, some of them were also Cd-contaminated (see Table 1).




**Note: edible, potentially edible and non-edible species are printed in black bold, black and grey, respectively. n= number of replicates per species. For more details in the number of replicates (~variability) and the concentration (mean ± S.E) of metals and fatty acids used, see Table 6; supplementary material.**

**Fig 4.** Nutritional species-specific scoring index of edible, potentially edible and non-edible species from Peru.

The non-edible algae *C. filiformis* was also scored (2) as promising future food, in contrast to the poor nutritional (<0.3) algae, *Ulva* sp. and *Codium* sp. This NSSs confirms the low nutritional values of snails as overall, all of them (edible or not) were in the reddish zone of the NSSs (<1), and in the following order: *B. ventricosa* > *Argobuccinum (Priene) scabrum* = *Aeneator fontainei* > *Cymatium* sp.> *T. chocolata* > *Tegula* sp. Most of the crabs were ranked as good nutritional species, the (in Peru) highly consumed *R. setosum* and *P. orbigny* exhibited NSSs of 1.8 and 1.4, respectively.

The most promising crabs *C. sexdecimdentatus* and *H. chilensis*, which are clean of As and Cd were scored > 1.5 NSSs. As interesting future foods, the potential small lobster or "munida" *P. monodon* and the mussel "chorito negro" *S. algosus*, which are not yet consumed in Peru were scored as good, and sometimes better than commercial species (see Fig 4**).** It is noteworthy to mention that both species have also considerable Cd concentrations accumulated in their edible tissues (Table 1).

Recent studies are investigating new sources of food to feed the world's growing population, which currently has a more consumerist mentality (Assadourian, 2010; Parodi et al. 2018). This leads to a depletion of natural resources, especially for those used as food source for humans. Alternative diets (e.g. insects, microalgae with high nutritional profiles) could attenuate the current overexploitation and/or production of the conventional foods, which in most cases causes high greenhouse gas (GHG) emissions and requires substantial land use (Parodi et al. 2018; Van huis, 2016). Parodi et al. (2018) demonstrated that food with marine origin (mussels, algae) are promising future foods as their production does not require land use, and they are high in nutrients (e.g. EPA, DHA, Fe, Zn). This is in accordance with our findings that some species (e.g. crab *C. sexdecimdentatus and algae C.*

*filiformis*) in this study exhibited substantial concentrations of LC-PUFAs and micronutrients, while these species are actually not yet consumed in Peru. Therefore, we characterized them as promising alternative diets and future foods for Peruvian and worldwide populations.

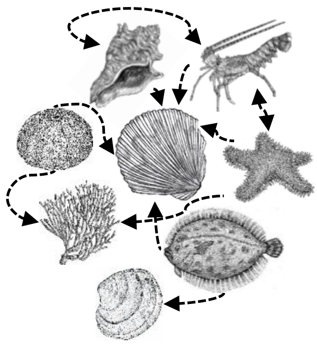
Nevertheless, some of these species (i.e. potentially edible or non-edible) seem to have "bad taste" and/or "toxicity" (pers. comm. with coastal population), which must be tested in future studies. In case there is a bad taste and/or toxicity, possibilities for appropriate food processing must be explored, to make these species pleasant and safe to consume. It is noteworthy to mention that since the majority of these species are non-exploited in Peruvian waters, they are also not substantially studied (IMARPE, 2019; PRODUCE, 2019). It is crucial to study the stock populations (incl. trophic food-web studies) of these species, to establish a sustainable management plan prior to their exploitation for human consumption.

# 4 Conclusions

Peruvian marine species are rich in LC-PUFAs but also in metals such as As and Cd, which could pose a health risk (also based on TR) if they are consumed in large amounts. The edible, potentially edible and non-edible species from the northern region exhibited the highest LC-PUFAs concentrations (up to 180 mg EPA/100g wwt.), followed by those from the center and southern regions. The edible tissues of sea urchin, oyster and mussel contained the highest LC-PUFA concentrations. Some species (e.g. *C. sexdecimdentatus, C. filiformis, C. plebejus*) considered as potentially edible or non-edible species are promising future foods due to their high nutritional values (high LC-PUFAs and micro-nutrients). The safe amounts for consumption of Peruvian seafood exceed the actual ingestion rates, only some crabs and snails should not be consumed in quantities above 130-200 g per week. The consumption of safe quantities of Peruvian seafood could have high beneficial effects and have a high contribution of LC-PUFAs and micro-nutrients for a healthy diet (up to 80% of the recommended values). Based on the intake individual-species-specific assessment and NSSs, snails are the least beneficial group of species for human consumption.

# Chapter 5

# Peruvian marine ecosystems: trophic interactions and metal transfer in aquatic food webs



Slightly modified from the article (in prep.):

Loaiza I., De Boeck G., Alcazar J., Campos D., Cárdenas-Alayza S., Ganoza M., Gómez-Sanchez M., Miglio M. and De Troch M.( in prep.). Peruvian marine ecosystems: trophic interactions and metal transfer in aquatic food webs.

## Abstract

This study investigates the trophic interactions and metals transfer (As, Cd, Cu, Fe, Mn, Ni, Pb and Zn) along seven marine ecosystems in Peru. Five of these ecosystems are driven and influenced by the Peruvian scallop aquaculture. A southward increased gradient of $\delta^{15}N$ was also observed among the three examined regions (e.g. species: *Argopecten purpuratus*, *Romaleon setosum* and food sources: POM, sediment) along the Peruvian coast. The SIAR-stable isotope mixing models helped to clarify the feeding ecology of *Argopecten purpuratus* and its important predators (e.g. *Bursa ventricosa*, *Romaleon setosum*…). The food items of *A. purpuratus* can be ranked in decreasing order of importance: seston > sediment > POM > brackish-and-fresh water POM input while *A. purpuratus* itself was found to be the main prey item for the predators. The trophic magnification factors (TMFs) in Peruvian marine food webs were highest for Cu (1.58), As (1.46) and Cd (1.07), but also TMFs < 1 for Ni, Pb, Fe and Zn confirm that these elements do not biomagnify. In both cases, *A. purpuratus* fitted the trophic metal magnification or bio-dilution regression model as intermediate consumer and/or prey, as was determined with the stable isotope signatures and SIAR-models. The TMFs and linear metal relationships implied that As contamination are of serious concern in Peru, as well as the unusual Cu biomagnification.

**Keywords:** biomagnification; metals; stable isotopes; scallop aquaculture; Peruvian ecosystems; environmental health status

## 1. Introduction

The northern Humboldt Current system (NHCS) is one of the most productive eastern boundary upwelling systems, which leads to one of the highest fish productions worldwide. The NHCS off Peru (from 3 ºS to 18 ºS) covers less than 0.1% of the World Ocean surface but sustains ~10% of the world fish catch (Chavez et al. 2008). Sechura Bay and Illescas Reseved zone (IRZ) are located in the north (5 ºS) of Peru, in the Piura Region. These ecosystems are characterized as the centre of intensive scallop (*Argopecten purpuratus*) aquaculture. Sechura Bay is a semi-enclosed bay, while Illescas is an open-shore peninsula located south of Sechura Bay.

In the proximity of the southern (SL) Sechura Bay (incl. IRZ), harbours, anthropogenic and industrial activities such as phosphate factories, the Norperuano oil pipeline, oil platforms, fishery factories, artisanal ports and the industrial JPQ port are present, while in the northern part (NL) of the bay, fishery factories, artisanal ports and fishing activities are found (IMARPE, 2007; Loaiza et al. 2015; Loaiza et al. 2020). It is worth noting that the Reserved Zone of Illescas is restricted to the land and the intertidal area, therefore maritime, industrial and human activities (incl. fisheries, aquaculture) are not regulated at sea (MINAM, 2010; SERNANP. 2019; Loaiza et al. 2020).

Fresh and brackish-water discharges from rivers/estuaries also impact the actual environmental conditions of Sechura Bay. The southern part (SL) of Sechura Bay is influenced by major fresh and brackish water input along the coast of Peru, from the mouth of Virrila Estuary (INRENA, 2005; Loaiza et al. 2020). On the other hand, Sechura River and San Pedro Mangrove located in the northern (NL) Bay are minor contributors, although they become more important during prolonged and intense raining seasons and during "El Niño" (Mendo et al. 2016; Kluger et al. 2018; Loaiza et al. 2020). These discharges normally come with higher concentrations of organic and inorganic pollutants (e.g. metals) as common components of these fresh-and-brackish water inputs (Diop et al. 2016; Forrest et al. 2007; Kehrig et al. 2013; Loaiza et al. 2020).

Paracas Bay is located in the center-south (13 ºS) of Peru, in the Ica Region, which is also considered an important area for Peruvian scallop aquaculture, although the production is considerably lower (~800 tonnes); up to 115% less than Sechura' production (PRODUCE, 2019). Paracas Bay is an enclosed bay with historical fishery industry that hardly impacted the ecosystem (Loaiza et al. 2020). However, in 2004 a 14 km long submarine emitter APROPISCO was built to discharge effluents and contaminants from the fishery factories outside the buffer zone of National Paracas Reserve, in front to Paracas Bay. Other anthropogenic and industrial activities are also present in Paracas, such as the Camisea Gas Fractionation Plant – PLUSPETROL, fisheries and port activities (SNP, 2003; DIGESA, 2008; Loaiza et al. 2020).

Punta San Juan (PSJ) is located in the south (15º S) of Peru, which is known as one of the most productive upwelling areas from NHCS (PPSJ, 2019). PSJ is an open-shore peninsula in Marcona, Ica Region. PSJ has embedded one of the 33-site network of marine protected areas in Peru known as the National Reserve System of Islands, Islets and Guano Concentration Areas, also called as Punta San Juan Reserve in this study (PPSJ, 2019). Nearby this area, the main human activities are mining (i.e. Shougang (SHO) Iron mining company) and intensive artisanal fisheries (Núñez-Barriga et al. 1999; Adkesson et al. 2019; PPSJ, 2019). The iron mining activities are currently ongoing at the north of PSJ, along the San Juan Bay which is in the proximity to the Reserve. It is worth noting that this Reserve is restricted to the land and about 3 km of area at the sea, therefore industrial (i.e. iron mining) and human activities (incl. fisheries) are still performed in nearby areas (MINAM, 2010; SERNANP. 2019; PPSJ, 2019). In addition, the southern Peru has been described as a natural metal-rich ecosystem, the earth crust exhibits high concentrations of metals, e.g. Cd and Pb (Barriga-Sánchez and Pariasca, 2018; DIGESA, 2018; SANIPES, 2018).

In order to understand the complex trophic relationships within ecosystems, the study of energy and nutrient/contaminant transfer is fundamentally important (Lindeman, 1942; Bisi et al. 2012). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope measurements have been successfully used to determine trophic levels and food sources contributions in food webs (Cabana and Rasmussen, 1996; Peterson and Fry, 1987; Vander Zanden et al. 1999). In addition, the use of contaminants (i.e. metals) as tracers is a common proxy to see possible biomagnification effects in trophic food webs to determine the environmental status and/or the contaminant' bioavailability (Borgå et al. 2011; Bisi et al. 2012; Sardenne et al. 2017; Loaiza et al. 2020).

Although there are a numerous studies on food web studies dealing with stable isotopes and contaminant transfer, these investigations usually focus on northern hemisphere areas. Moreover, these studies have been mainly conducted in freshwater systems, and in temperate or cold climates, and are scarce in the southern hemisphere (Bisi et al. 2012; Verhaert et al. 2017). In (sub)tropical areas, only a few studies have dealt with trophic relationships in estuarine and marine food webs using stable isotopes (Bisi et al. 2012; Kehrig et al. 2013) . In addition, marine benthic trophic interactions have been rarely studied in the southern hemisphere. To our knowledge this is the first investigation using stable isotopes and metal transfer on benthic communities associated to the Peruvian scallop aquaculture in Peru. Moreover, sub(tropical) regions are characterized by high species richness (Begon et al. 2006), which probably promotes more complex trophic relationships due to greater diversity of food items per species (Paine, 1966; Espinoza et al. 2017).

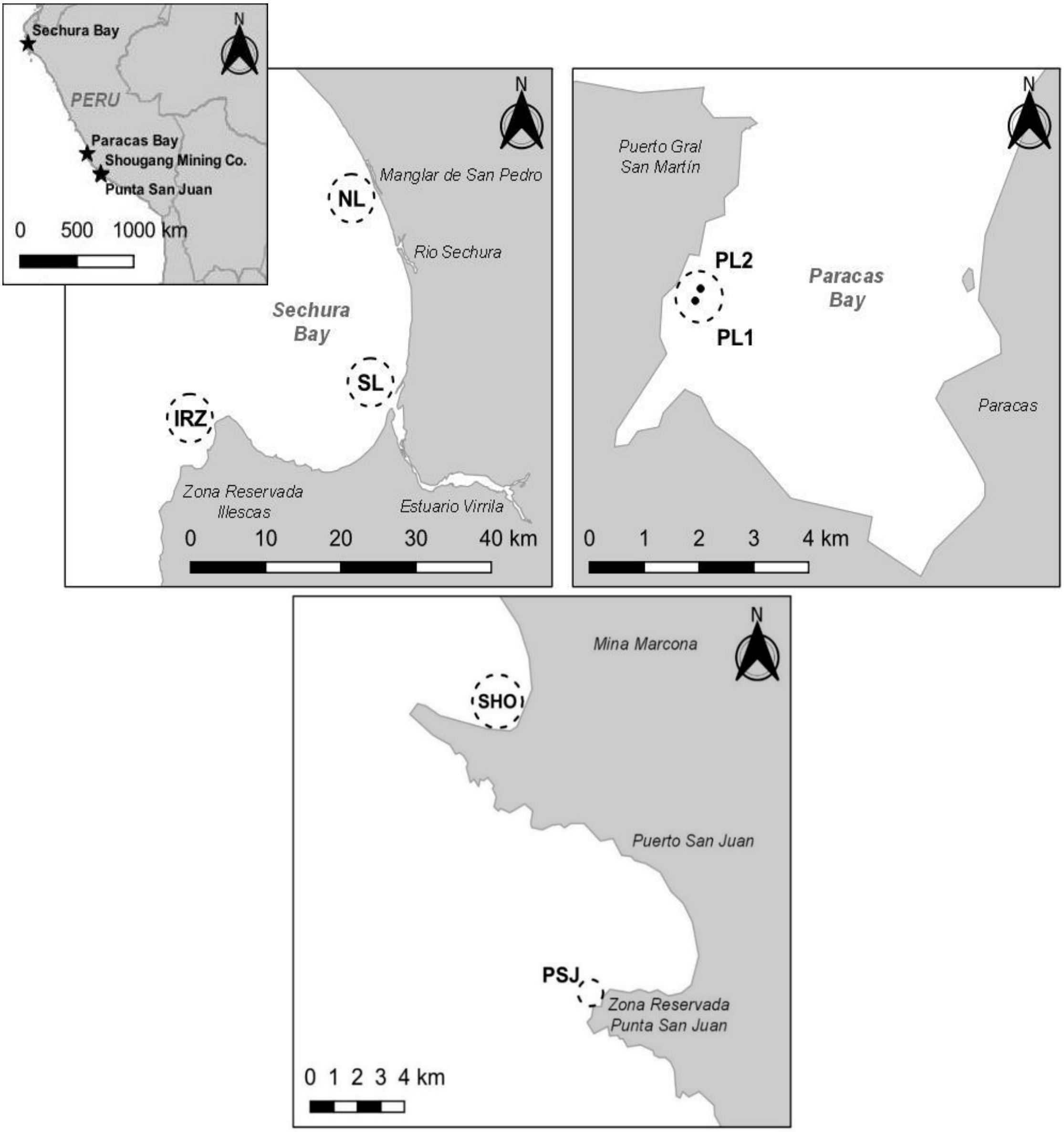



**Fig 1.** Location of the sampling areas at Sechura Bay (incl. Zona Reservada Illescas), Paracas Bay and Marcona. Southern (SL) and Northern (NL) locations in Sechura Bay; one location in front of the Illescas Reserved Zone (IRZ); PL1 and PL2 in Paracas Bay; and PSJ in front the Zona Reservada Punta San Juan, and SHO in front of the Shougang iron mining company.

The aim of this study is to understand the structure of the different marine ecosystems in Peruvian coastal waters. Trophic interactions and metal transfer (i.e. possible biogmagnification effects) was determined along the food webs. Because the presence of Peruvian scallop *A. purpuratus* aquaculture was relevant in some locations (i.e. Sechura Bay, Illescas Reserved Zone and Paracas Bay), the first insights in these ecosystems also could be used for appropriate management of this important economic activity in Peru, as well as to understand the role of *A. purpuratus* as food and as vector of metals.

## 2. Materials and methods

Marine species were collected along the coast of Peru from 2016 to 2018 (as the sampling campaigns from Chapter 4) (Fig 1). A total of 47 different species were collected along the coast of Peru. Twenty-one in the north of Peru, northern (NL) (S: 05°31'27.6"; W: 80°56'04.2") and southern (SL) (S: 05°43'58.2"; W: 80°54'17.8") locations in Sechura Bay (SB) and one (IRZ) (S: 05°49'06.7"; W: 81°05'24.6") in front Illescas Reserved Zone.

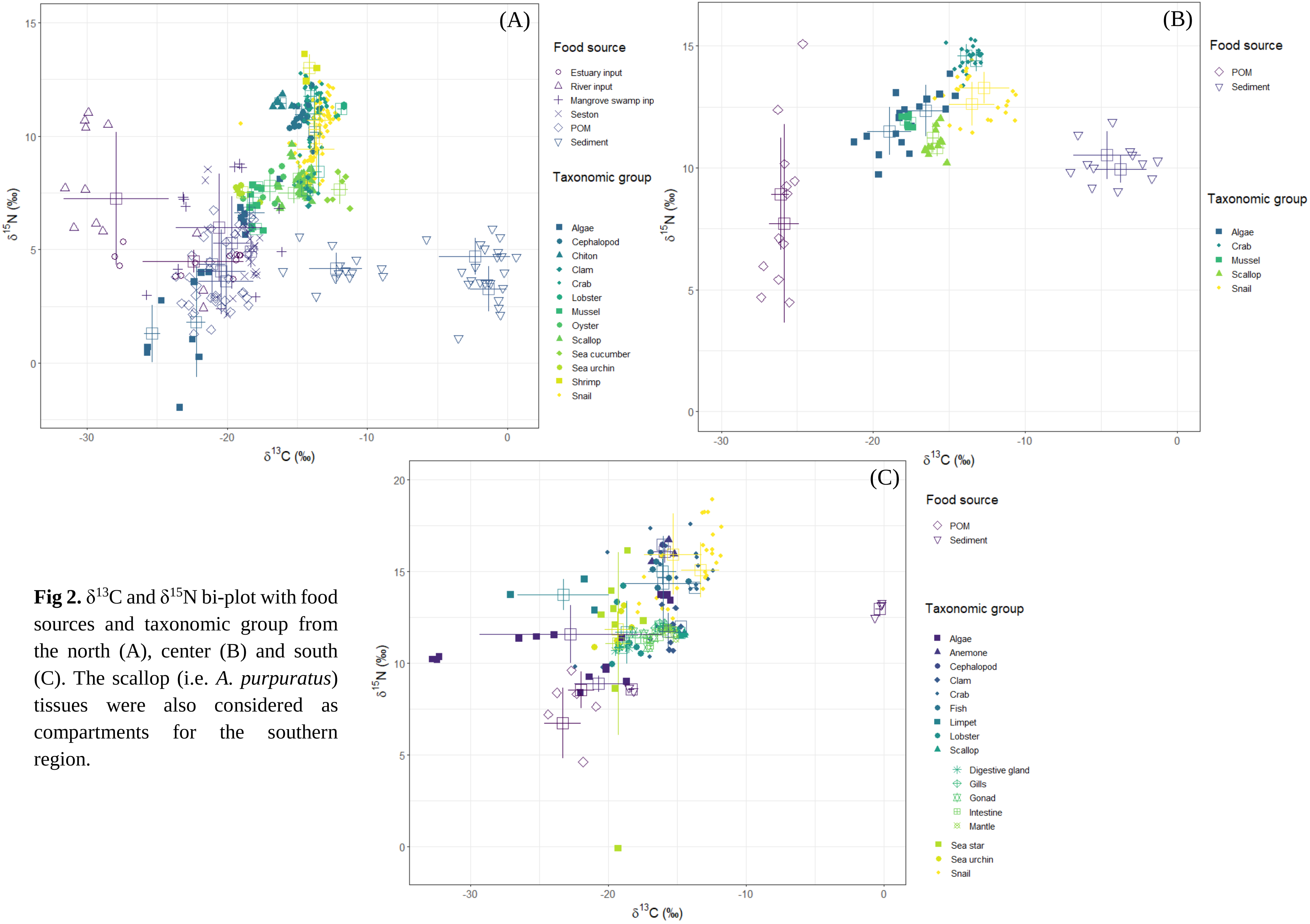



**Fig 2.** $\delta^{13}C$ and $\delta^{15}N$ bi-plot with food sources and taxonomic group from the north (A), center (B) and south (C). The scallop (i.e. *A. purpuratus*) tissues were also considered as compartments for the southern region.

Eleven species were collected in the center-south, PL1 (S: 13° 49.357'; W: 76° 17.824') and PL2 (S: 13° 49.326'; W: 76° 17.784') at the southeast of Paracas Bay (SB). Thirty-four species were sampled in southern Peru, the northwest point (PSJ) (S:15° 21′ 37.5930" ; W: 75° 11′ 19.2761″) of the Reserved zone, and one location (SHO) (S: 15° 15′ 28.8932″; W: 75° 13′ 42.5059″) in front to the iron mining company (Shougang Hierro Peru S.A.A), north from the Reserve zone in direction to San Juan Bay (San Juan de Marcona).

All species were collected by semi-autonomous diving as described in Loaiza et. al. (2018, 2020) with the exception of the lobster *Pleuroncodes monodon* which was caught by the fishermen at Marcona' off-shore (see also Chapter 4). Scallop samples from the north and center-south were collected under the permission of the fishery and aquaculture companies, Acquapisco SA and Nemo Corporations SA. After collection, all samples were transported to the laboratory to be stored at -20°C. Prior to freezing, each individual was measured and weighted in order to register biometric data. The muscle or edible tissues of each species was dissected, cleaned and stored for the chemical analysis. In case of small species (with little soft tissue), e.g. *Inachoides lambriformis*, *Patiria chilensis*, *Pinnaxodes chilensis* and *Pleuroncodes monodon* and *Pagurus* sp. samples were processed as *toto* (Table 2; supplementary material).

Ecosystem compartments such as seston, particular organic matter (POM; *sensu lato*) and sediment were also collected in each location. Seston was not collected in SB northern location, PB locations and PSJ locations due to oceanographic conditions and/or facilities. In other locations, traps were put by semi-autonomous diving during 7-11 days for water collection, while water-bottom samples were collected directly from the bottom (Aguirre-

Velarde et al. 2015). Sediment (n=3-8 replicates per location) was collected with a core (internal diameter: 10.16 cm) down to a sediment depth of 10 cm. When the location was influenced by fresh- and/or brackish water environments, fresh and brackish water POM (*sensu lato)* was also sampled. One liter of water samples (POM; *sensu lato*, n = 3 replicates) were taken at the mouth of the Sechura River (i.e. River input) and Mangrove San Pedro (i.e. Mangrove swamp input) at the northern location, and in the Virrila Estuary (i.e. Estuary input) in the southern location of Sechura Bay. Seston and water-bottom samples were filtered over a GF/F filter (0.7 µm pore size). Filters and sediments were frozen and preserved at -20°C for stable isotopes and metal. Stable isotopes and metal determination were performed as in Loaiza et al. 2018, 2020, but are briefly described below.

### *2.1 Stable isotope analysis*

Muscle or edible tissues were pre-treated for stable isotope analysis. Tissues were oven-dried for at least 72 hrs at 60°C. After drying, the samples were ground to a fine powder using a mortar and placed in tin capsules (5-9 mm) for encapsulation. In case of samples with presence of calcium carbonates such as sediment, seston, algae and some organisms that were processed in *toto* (e.g. *I. lambriformis*), the dried samples were transferred to silver cups, then acidified for 24 h under HCl vapour in a desiccator, and encapsulated again in tin capsules. The encapsulated capsules were placed and delivered in a 96-multiwell plate to UC-DAVIS Stable Isotope Facility at the University of California (USA) for carbon $\delta^{13}C$ and nitrogen $\delta^{15}N$ analysis. All capsules were kept dry and pinch closed prior to analysis (Pasotti et al. 2015). The $\delta^{13}C$ and $\delta^{15}N$ was determined with the dual (carbon and nitrogen) isotopic composition analysis by using a PDZ Europa ANCA-GSL elemental analyzer 230 interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer

(Sercon Ltd., Cheshire, UK; UC Davis Stable Isotope Facility, http://stableisotopefacility.ucdavis.edu/). A total of 630 samples (organisms, water and sediment) from the Peruvian waters were collected and measured in 2016, 2017 and 2018.

## *2.2 Metal analysis*

Frozen tissues and food sources (filters and sediments) were dried for at least 72 h at 60°C. The dried tissues were weighed and separated into small (<0.06 g) and large (>0.06 g) tissues. Small and large tissues were digested overnight with respectively 1 or 2.5 ml of highly purified concentrated 69% nitric acid ($HNO_3$). For filters, 4 ml of highly purified concentrated 69% $HNO_3$ was used. Then all samples were heated to 110°C during 30 min in the digester. After cooling (~10 min), 0.1, 0.25, 0.5 ml of hydrogen peroxide ($H_2O_2$) was added for small tissues, large tissues and filters, respectively. The samples were heated again during 30 min at 110°C to complete the total digestion. The digested samples were diluted up to 5 (small), 10 (large) and 40 (filters) ml with Milli-Q grade for the metal analysis.

Dried sediment samples were put in glass tubes and digested in 2 ml of highly purified concentrated 69% $HNO_3$ in the closed system SP-Discover Microwave (CEM, USA) (15 min per sample). Digested samples were then diluted till 10 ml in 14 ml tubes with Milli-Q grade for metal analysis (As, Cd, Cu, Fe, Mn, Ni, Pb and Zn). All metal analyses were conducted by inductively coupled plasma mass spectrometry (ICP-MS ; 7700×, Agilent Technologies). For samples that were below the detection limit (BDL) of the ICP, an extra analysis with the high resolution inductive coupled plasma mass spectrometry (HR-ICP-MS ; Element XR, Thermo Scientific, Finnigan element 2, Bremen, Germany) was performed.

The quality controls for metal analysis consisted of standard reference material (SRM) for mussel tissues (2976, National Institute of Standards and Technology, NIST). The recovery ranges were 98.9–113.5% (n=71) for each metal (see Chapter 4: Table 5; addendum section). The limit of quantification (µg/L) was 0.1 for As, Cd, Mn, Cu, Ni and Pb, and 5 for Fe and Zn in ICP-MS, and 0.001 for all metals in HR-ICP-MS. For filters and sediment, the certified reference material was the Channel Sediment BCR-320R, which exhibited recoveries from 84.6 to 121.1% (n=9, per metal) for As, Cd, Cu, Fe, Mn, Ni, Pb and Zn in 2016 and 2018. For 2017, the Estuarine Sediment BCR-277R (n=14) was also used as reference material, and the recoveries were consistent but lower, around 70.3% for sediments and 94.4% for filters. The recoveries were used in order to calculate the final concentrations (Table 2; supplementary material). Sediments and filters were measured in HR-ICP-MS, with an instrumental detection limit of 0.001 µg/L. All metal concentrations were calculated on a dry weight basis (µg/g dwt).

### *2.3 Data analysis*

Since the marine species were pooled per taxonomic group and per region for stable isotope analysis, the ability to perform comparative statistical analysis for these data sets was limited. In addition, stable isotope analysis was also performed with food sources' pooled per region, and therefore the statistical analysis was again limited. The same applied when stable isotope signatures were compared per species and food sources without region distinction. Nevertheless, main effects, e.g latitude influence (samples from North (5 ºS), center (13 ºS) and south (15º S)) on $\delta^{15}N$ values per environmental compartment (i.e. species and food sources) were tested using one-way ANOVA with Tukey multiple comparison test. Shapiro-Wilk and Levene's test were used to test were tested for normality of distribution and homogeneity of variances, respectively. Results were statistical significant when $p < 0.05$. Bayesian stable isotope mixing models could be applied to estimate the contribution (i.e. proportion) of each food source/prey to the diet of *A. purpuratus*, and for the most important predators: *Octopus mimus*, *R. setosum*, *Bursa ventricosa*, *Hepatus chilensis*, *Cymatium* sp. *Thaisella chocolata*, using SIAR (stable isotope analysis in R). As input for the models, only a limited number of the potential food sources/preys were used. This selection was based on the $\delta^{15}N$ and $\delta^{13}C$ obtained in this study, as well on literature and pers. obs. of the feeding ecology of each species (Mendo et al. 2016). The SIAR-mix models were performed as 'global' with samples from culture and wild conditions, i.e. pooled samples from northern, center and southern regions; while another SIAR mixing analysis was also performed using only Sechura Bay (incl. Illescas) samples, since this location is the most important for Peruvian scallop aquaculture. Mean (±SD) trophic enrichment factors of 0.4 ± 1.3 for $\delta^{15}N$ and 3.4 ± 1 for $\delta^{13}C$ were used (Post, 2002). Trophic positions (TP) were determined from the species and food sources δ15N values using the following equation (Post, 2002):

$$TP = [2 + \left(\frac{\delta^{15}N_{consumer} - \delta^{15}N_{base\ or\ primary\ consumer}}{\Delta\delta^{15}N}\right)]$$

where

TP is the trophic position of the environmental compartment (i.e. species or food source);

$\delta^{15}N_{consumer}$ is the $\delta^{15}N$ of the organism;

$\delta^{15}N_{base\ or\ primary\ consumer}$ is the mean $\delta^{15}N$ of the primary consumer, in this case seston was considered as baseline, 2 is the trophic position of the primary consumer; and

$\Delta\delta^{15}N$ is the trophic enrichment factor (3.4 ‰; as previously mentioned), or the shift in $\delta^{15}N$ between two consecutive trophic levels (Post, 2002). TP was estimated for the food web estructure analysis (based on SIAR-mix ('global') models) per marine species and food sources, see Fig 5.

Simple linear regression analysis was used for investigating relationships between $\delta^{15}N$ and logarithmic concentrations (ng/g) of metals in muscle (also thallus for algae) tissues (i.e. not soft tissues, gonads, whole organisms (toto), etc.), as well as for determining trophic magnification factors (TMF). All data used for the regression analysis were previously tested for distribution normality with Shapiro-Wilk test and for homogeneity of variances with Levene's test. If the assumptions were not fulfilled, data were log-transformed. When it was not possible to fulfill these assumptions, TMF values were not determined and only a simple linear relationship analysis was performed.

In total, 543 samples of species and food sources were considered for the analysis. The relationship between metal and corresponding stable isotope was as follows:

$$\mathrm{Log_{10}[metal]} \sim \delta^{15}\mathrm{N\ per\ species\ and\ location}$$

TMF was calculated as the antilog of the regression slope with base 10 and can be used for quantifying food web biomagnification (Borgå et al. 2011; Fisk et al. 2001). Therefore, this tool was used for calculating metal biomagnification in different locations or ecosystems:

$$\mathrm{Log_{10}[metal]} = \mathrm{a} + \mathrm{b}\,(\delta^{15}\mathrm{N}) \rightarrow \mathrm{TMF} = 10^{\mathrm{b}}$$

When TMFs are above 1 and $p < 0.05$, biomagnification through the food web occurs (Borgå et al. 2011).

## 3. Results and discussion

### *3.1 Trophic relationships*

Overall, the southern region exhibited the highest $\delta^{15}N$ signatures, up to ~20 ‰ compared to the ~15‰ from the northern and center regions (Fig 2). This pattern is also confirmed when marine species and food sources were latitudinally compared, significantly ($p<0.05$) higher $\delta^{15}N$ values were found in the center-southern (13-15° S) species (e.g. *R. setosum*, *Thaisella chocolata,…*) and food sources (POM, sediment) than those in the north (5° S) (see Fig 16; supplementary material). These findings are in accordance to the isotopic results from Espinoza et al. (2017), which also showed the increase in $\delta^{15}N$ values southward from 7° S to 11° S along the coast of Peru. Snails and crabs are the taxon with the highest $\delta^{15}N$ in all regions. Other groups such as shrimp, anemone and fish also exhibited high $\delta^{15}N$ concentrations, in some cases exceeding (up to 18 ‰) the $\delta^{15}N$ of snails and crabs (Fig 2). The only scallop analysed, *A. purpuratus* showed values in a range of 6-12 $\delta^{15}N$, which points at its role to be an intermediate consumer (Fig 2). *A. purpuratus*' tissues exhibited a slight $\delta^{15}N$ variation among them in the southern region, with the highest $\delta^{15}N$ values for the gills (up to 12.2 ‰) and the digestive gland being the most $\delta^{15}N$-depleted tissue (as 10.7 ‰) (Fig 2C). The *A. purpuratus* $\delta^{15}N$ signature showed also a significant ($p<0.05$) increase from the north (6.8 to 9.6 ‰), over the centre (~10 ‰) to the south (~12 ‰) of Peru (see Fig 2, and Fig 16; supplementary material).

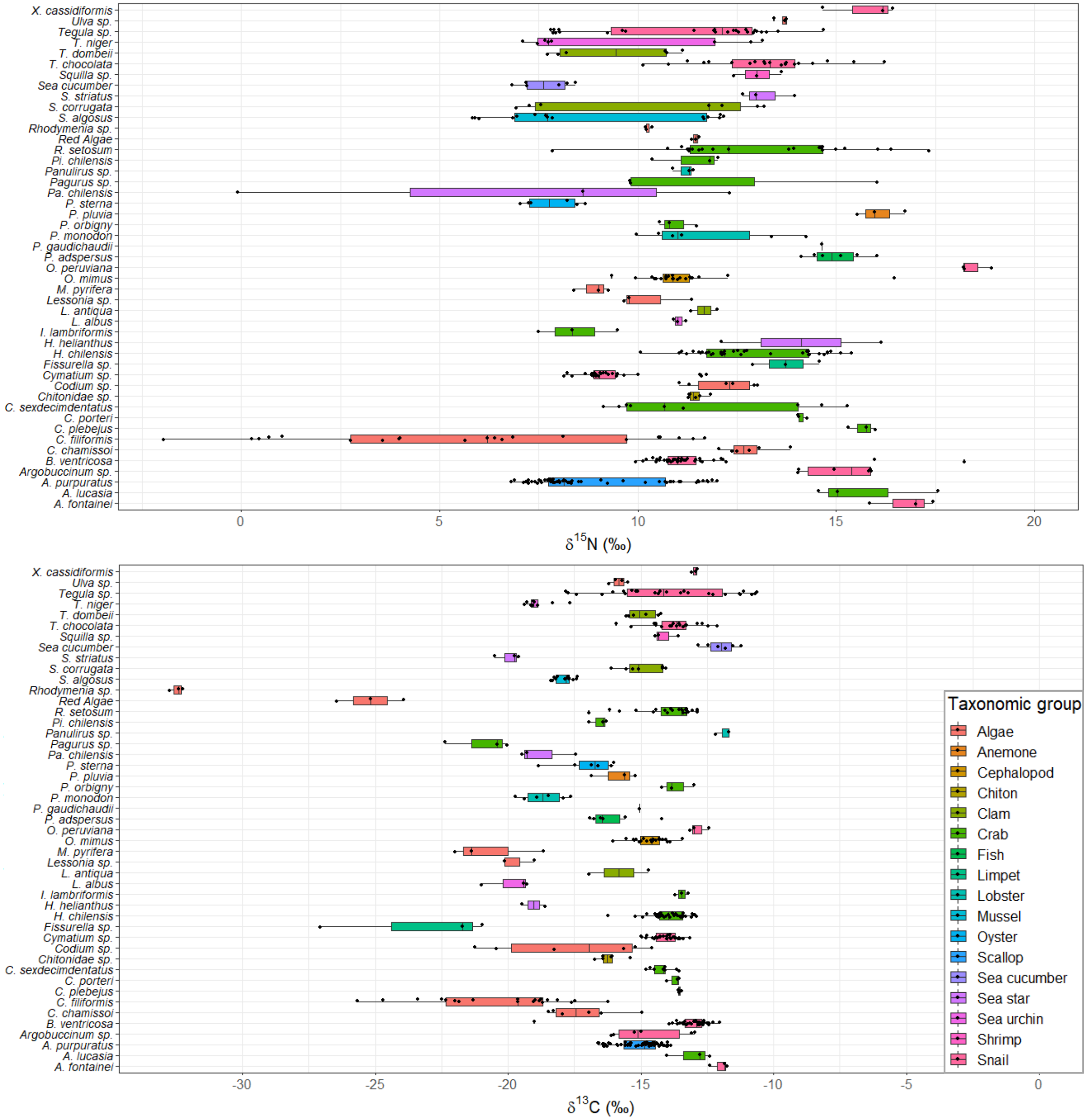



**Fig 3.** $\delta^{15}N$ (A) and $\delta^{13}C$ (B) of marine species grouped as taxonomic group. ***Note.- Pa. chilensis* as the sea star *Patiria chilensis* and *Pi. chilensis* as the crab *Pinnaxoides chilensis.***

The northern Humboldt Current system (NHCS) is the most productive eastern boundary upwelling system, the south of ~7.5° S is characterized with intense denitrification and anaerobic ammonium oxidation (anammox). This strong denitrification deals to a strong latitudinal gradient with higher baseline $\delta^{15}N$ values between Peruvian southern areas and the northern ones (Chavez et al. 2008; Lam et al. 2009; Espinoza et al. 2017). Annamox is known to occur in Peruvian marine waters (i.e. OMZ), where the denitrification in anoxic water has a large isotopic effect through the isotope fractionation, producing $NO_3$ and organic matter strongly enriched in $\delta^{15}N$ (~20‰) (Voss et al. 2001; Argüelles et al. 2012; Espinoza et al. 2017).

Our results in food sources or baseline food web components also exhibited the same pattern (see Fig 2, and Fig 16; supplementary material), the northern region (~ 5° S) exhibited lower $\delta^{15}N$-food sources up to 11 ‰, while the center and southern region showed levels up to 15 ‰. This north to south $\delta^{15}N$ gradient has also been observed in a previous study in sediments along the Peruvian and Ecuadorian margin (Mollier-Vogel et al. 2012). An increased southward gradient from north (4-5 ‰) to south (4.5-13 ‰) was found from 1ºN to 10ºS (Mollier-Vogel et al. 2012). In this study, the northern locations showed $\delta^{15}N$ sediment values relatively low and uniform, from 1 to 6 ‰, while the center and the south displayed values significantly ($p<0.05$) higher, from 9 to 12 ‰ and from 8 to 13 ‰ respectively, which is exactly as what was previously found in Peruvian domain waters. These changes in $\delta^{15}N$ baseline are then transferred through the food web, as observed in the high $\delta^{15}N$ values of consumers from the southern locations (Espinoza et al. 2017).

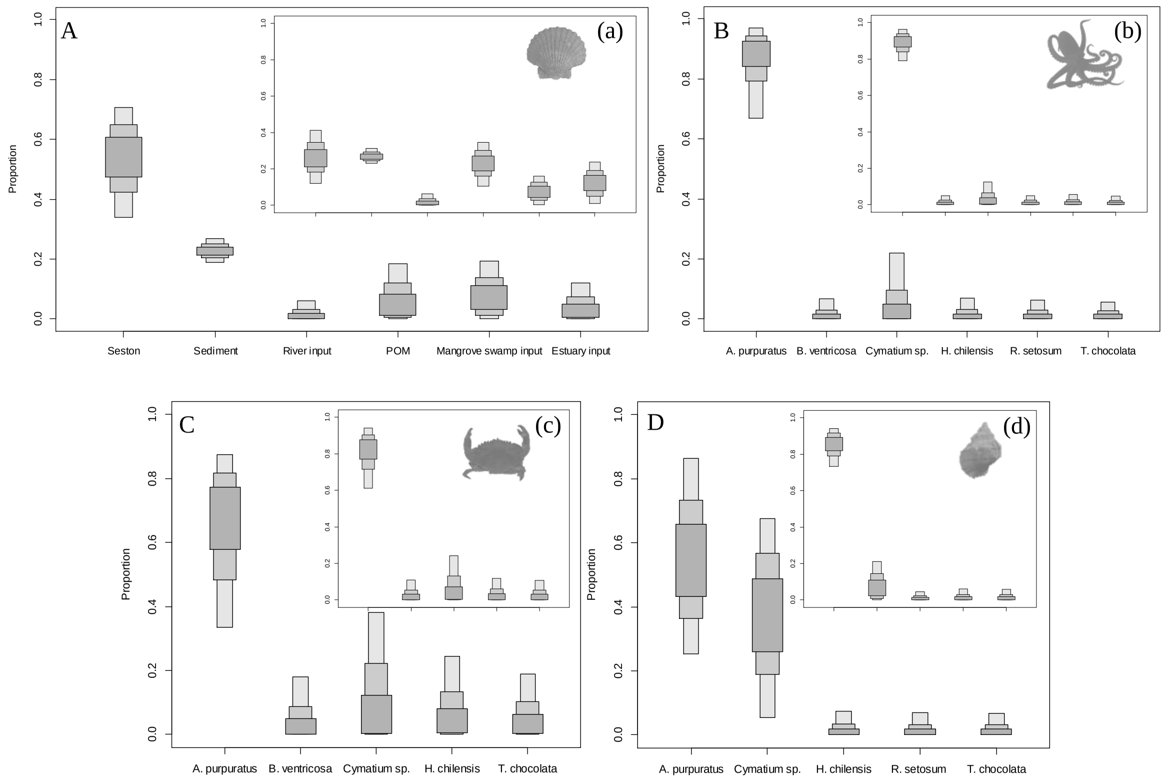
For the $\delta^{13}C$ signatures, the food sources showed the largest range in distribution, from around -32 to 0 ‰ for all regions. The consumers were in a more tight range, on average from -20 to -10 ‰ for most of the studied taxa (see Fig 2). Food sources (e.g. as seston, POM, sediment..) are samples analyzed as "bulk and/or pooled" which is reflected in the high inter-sample variability due to the inner presence of different organic material/species (McCutchan et al. 2003; Mollier-Vogel et al. 2012; Vizzini et al. 2013; Mathieu-Resuge et al. 2019). On the other hand, littoral and benthic species are more restricted in their feeding ecology, the lack of high capacity on mobility made them to have more localized and specialized diets, this in compare to pelagic and/or meso-pelagic organisms (McCutchan et al. 2003; Post, 2002; Espinoza et al. 2017; Signa et al. 2017).

The primary producer group, algae (*Rhodymenia* sp., *Caulerpa filiformis*, *Macrocystis pyrifera…*) exhibited the most $\delta^{13}C$-depleted values, up to ~-33 ‰. The scallop *A. purpuratus* showed a narrow $\delta^{13}C$ signatures range of about -16 to -14 ‰, with the individuals from the northern and center region being the most $\delta^{13}C$-depleted (~-16 ‰) the (Fig 2). The digestive gland of the scallop *A. purpuratus* showed the most $\delta^{13}C$-depleted concentrations with -19 ‰, following by gonad > intestine = gills > mantle (Fig 2C). Since *A. purpuratus* is a filter-feeding consumer, a narrow coastal foraging range is expected for this species. This scallop mainly feeds on phytoplankton (~85%), followed by zooplankton and detritus in normal conditions (e.g. NN) (Mendo et al. 2016). Under strong El Niño conditions (e.g. El Abrupt Niño (AN)), a drastic reduction of available phytoplankton has been found, implying that *A. purpuratus* uses other food sources such as bacteria, detritus, re-suspended material, among other particles (Barber and Chavez, 1983; Mendo and Wolff, 2003; Mendo et al. 2016; Loaiza et al. 2020). Noting that only particles or diets of ~5 µm diameter could be efficiently retained in filtering Pectinidae species, this considerably limits the spectrum of diet variability of this species (Aguirre-Velarde, 2009).

The potential predators of *A. purpuratus* such as snails, crabs, cephalopods and mantis shrimp were found in a high and more enriched range of $\delta^{13}C$, from around -22 to -10 ‰ than *A. purpuratus*, as expected (see Fig 2). These predators were actually having a "buffet" of *A. purpuratus* individuals because most of the samples came from scallop aquaculture areas (bottom and longline cultures) and/or are scallop' restocking areas. *A. purpuratus* densities in bottom cultures are often as high as > 60 individuals per $m^2$ (Mendo et al. 2016), which lead to an enormous supply of this species as food for these predators. Additionally, the associated macrobenthic communities of scallop aquaculture are also part of the large $\delta^{13}C$ range found for the top predators (e.g. crabs, cephalopods, shrimps,…) in the present study. The *a posteriori* analysis of metal trophic magnification will disentangle the position and relevance of each prey and/or consumer from these Peruvian marine food webs.

Per species, the snail *Oliva peruviana* showed the highest trophic level ($\delta^{15}N$ up to ~19‰), following by the snails *B. ventricosa* and *Aeneator fontainei* and the crabs *Albunea lucasia and R. setosum*, all collected from the southern region (see Fig 3A). The lowest trophic level or most $\delta^{15}N$-depleted species was the alga *C. filiformis* with concentrations up to -2 ‰, following by some individuals of the mussel *Semimytilus algosus* and scallop *A. purpuratus* that exhibited low $\delta^{15}N$ (i.e. 5-7 ‰) (Fig 3A).

For $\delta^{13}C$, the snail *Tegula* sp., followed by the sea cucumber, the lobster *Panulirus* sp. and the snail *A. fontainei* showed the highest values up to ~-10 ‰ (Fig 3B). The most $\delta^{13}C$-depleted were found in the limpet *Fissurella* sp. and the Red algae and alga *C. filiformis*, concentrations up to around -33 ‰. Most of the species exhibited a narrow range of $\delta^{13}C$ as expected in benthic species with relative low mobility, however and unexpectedly two algae species (*Codium* sp. and *C. filiformis*) exhibited the largest range of $\delta^{13}C$, followed by the limpet *Fissurella* sp. (Fig 3B). Table 1 shows that in general our $\delta^{15}N$ and $\delta^{13}C$ found in the Peruvian marine species are in accordance with previous studies from the region (i.e. Latin America) and other locations (Catenazzi & Donnelly, 2007; Bisi et al. 2012; Kehrig et al. 2013; Nerot et al. 2012; Zhao et al. 2013; Aya & Kudo, 2017; Docmac et al. 2017; Espinoza et al. 2017; Chouvelon et al. 2018). The latitude, as well as the intensity of the NHCS upwelling off Peru and Chile marine domains, are the most common factor that explain the $\delta^{15}N$ and $\delta^{13}C$ differences among species (Fig 16; supplementary material).


A
(a)
Proportion
1.0
0.8
0.6
0.4
0.2
0.0
Seston
Sediment
River input
POM
Mangrove swamp input
Estuary input
B
(b)
Proportion
A. purpuratus
B. ventricosa
Cymatium sp.
H. chilensis
R. setosum
T. chocolata
C
(c)
Proportion
A. purpuratus
B. ventricosa
Cymatium sp.
H. chilensis
T. chocolata
D
(d)
Proportion
A. purpuratus
Cymatium sp.
H. chilensis
R. setosum
T. chocolata

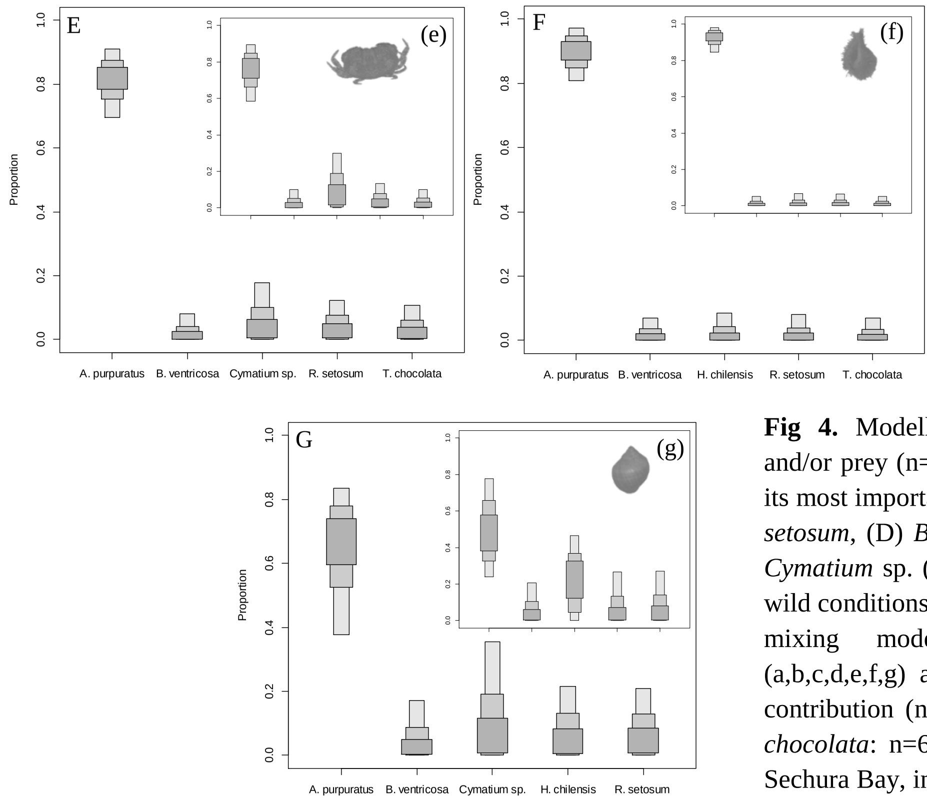



**Fig 4.** Modelled contribution of food source and/or prey (n=12-56) for (A) *A. purpuratus* and its most important predators (B) *O. mimus*, (C) *R. setosum*, (D) *B. ventricosa*, (E) *H. chilensis*, (F) *Cymatium* sp. (G) *T. chocolata* from culture and wild conditions, based on the SIAR stable isotope mixing models. The embedded figures (a,b,c,d,e,f,g) are based on the SIAR-modelled contribution (n=12-39, with the exception of *T. chocolata*: n=6) when only scallop culture (i.e. Sechura Bay, incl. Illescas) conditions were taken into account.

**Note.-Sources (food and/or prey) from x-axis are the same for big and small (or embedded) figures.**

**Table 1.** $\delta^{15}N$ and $\delta^{13}C$ in different marine species from various ecosystems.

| **Ecosystem** | **Species** | **N** | **Group** | **$\delta^{15}N$ Mean ± SD or (min-max)** | **$\delta^{13}C$ Mean ± SD or (min-max)** | **Reference** |
|---|---|---|---|---|---|---|
| Northern of Bay of Biscay | Pecten maximus† | - | Scallop | 8.0 - 10.0 | - 16.0 ~ -15.0 | Nerot et al. 2012 |
| Sea of Okhotsk | Mizuhopecten yessoensis†† | 13 | Scallop | 7.2 ± 0.2 | - 18.1 ± 0.3 | Aya & Kudo, 2017 |
| Peruvian Pacific Ocean | *Argopecten purpuratus* | 56 | Scallop | 8.9 ± 1.6 | - 15.1 ± 0.8 | *This study* |
| Northern Yellow Sea, China | *Neptunea cumingi* | 3 | Snail | 11.2 ± 0.2 | - 18.8 ± 0.3 | Zhao et al. 2013 |
| | *Rapana venosa* | 3 | Snail | 11.4 ± 0.1 | - 18.9 ± 0.2 | |
| | *Neverita didyma* | 3 | Snail | 11.7 ± 0.2 | - 18.6 ± 0.2 | |
| | *Neverita reiniana* | 3 | Snail | 12.5 ± 0.2 | - 18.7 ± 0.2 | |
| | *Asterias amurensis* | 3 | Sea star | 13.2 ± 0.2 | - 18.3 ± 0.3 | |
| | *Anthopleura xanthogrammica* | 3 | Anemone | 12.8 ± 0.2 | - 18.5 ± 0.1 | |
| Peruvian Pacific Ocean | *Bursa ventricosa* | 38 | Snail | 11.3 ± 1.5 | - 13.1 ± 1.1 | *This study* |
| | *Cymatium* sp. | 26 | Snail | 9.3 ± 0.9 | - 14.1 ± 0.5 | |
| | *Thaisella chocolata* | 21 | Snail | 13.2 ± 1.5 | - 13.7 ± 0.9 | |
| | *Patiria chilensis* | 3 | Sea star | 6.9 ± 6.4 | - 18.7 ± 1.1 | |
| | *Heliaster helianthus* | 2 | Sea star | 14.1 ± 2.9 | - 19.0 ± 0.6 | |
| | *Stichaster striatus* | 3 | Sea star | 13.2 ± 0.7 | - 20.0 ± 0.5 | |
| | *Phymanthea pluvial* | 3 | Anemone | 16.1 ± 0.6 | - 15.9 ± 0.9 | |
| Peruvian Pacific Ocean *Paracas Bay* | *Ulva* sp. | 5 | Algae | 11.0 ± 1.0 | - 11.0 ± 1.0 | Catenazzi & Donnelly, 2007 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *Chondracanthus chamissoi* | 6 | Algae | 12.8 ± 0.6 | - 17.2 ± 1.3 | *This study* |
| | *Caulerpa filiformis* | 21 | Algae | 5.7 ± 4.2 | - 20.5 ± 2.8 | |
| | *Codium* sp. | 6 | Algae | 12.2 ± 0.8 | - 17.6 ± 2.8 | |
| *San Juan Bay* | *Ulva* sp. | 4 | Algae | 13.7 ± 0.1 | - 15.8 ± 0.3 | |
| Chilean Pacific Ocean | *Perumytilus purpuratus* | 73 | Mussel | 15.3 ± 1.4 | - 16.8 ± 1.1 | Docmac et al. 2017 |
| | *Tegula atra* | 21 | Snail | 16.4 ± 1.1 | - 13.2 ± 1.1 | |
| Peruvian Pacific Ocean | *Semimytilus algosus* | 15 | Mussel | 8.9 ± 2.6 | - 17.9 ± 0.3 | *This study* |
| | *Tegula* sp. | 21 | Snail | 11.2 ± 2.2 | - 13.9 ± 2.3 | |
| North coast of Rio de Janeiro | *Xiphopenaeus kroyeri* | 23 | Shrimp | 9.8 – 11.9 | – | Kehrig et al. 2013 |
| | *Loligo sanpaulensis* | 22 | Cephalopod | 10.5 – 12.8 | – | |
| Sepetiba Bay | *Farfantepenaeus brasiliensis* | 5 | Shrimp | 11.8 ± 0.9 | - 15.1 ± 0.9 | Bisi et al. 2012 |
| | *Litopenaeus schmitt* | 5 | Shrimp | 11.3 ± 0.6 | - 14.0 ± 0.8 | |
| | *Loligo sanpaulensis* | 5 | Cephalopod | 14.2 ± 0.4 | - 15.9 ± 0.5 | |
| | *Loligo plei*††† | 3 | Cephalopod | 15.5 ± 0.1 | - 16.1 ± 0.1 | |
| | *Lolliguncula brevis* | 4 | Cephalopod | 15.2 ± 0.8 | - 14.5 ± 1.1 | |
| Guanabara Bay | *Litopenaeus schmitt* | 3 | Shrimp | 10.8 ± 1.2 | - 15.2 ± 0.2 | |
| | *Loligo plei* | 6 | Cephalopod | 12.4 ± 0.8 | - 18.9 ± 0.5 | |
| | *Lolliguncula brevis*††† | 10 | Cephalopod | 11.9 ± 1.2 | - 17.1 ± 1.1 | |
| Peruvian Pacific Ocean | *Squilla* sp. | 3 | Mantis Shrimp | 13.0 ± 0.6 | - 14.1 ± 0.5 | *This study* |
| | *Hepatus chilensis* | 37 | Crab | 12.8 ± 1.4 | - 14.0 ± 0.7 | |
| | *Romaleon setosum* | 23 | Crab | 13.0 ± 2.3 | - 13.9 ± 1.1 | |
| | *Octopus mimus* | 22 | Cephalopod | 11.1 ± 1.3 | - 14.6 ± 0.6 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Peruvian Pacific Ocean (~4º-12º S) | *Pleuroncodes monodon* | 43 | Squat lobster | 13.3 ± 1.2 | - 15.2 ± 0.4 | Espinoza et al. 2017 |
| Peruvian Pacific Ocean (~15º S) | *Pleuroncodes monodon* | 6 | Squat lobster | 11.7 ± 1.7 | - 18.7 ± 0.8 | *This study* |
| Peruvian Pacific Ocean | *Panurilus* sp | 3 | Lobster | 11.2 ± 0.3 | - 11.9 ± 0.3 | |
| NE Atlantic Ocean | *Nephrops norvegicus* | 5 | Lobster | 11.3 ± 0.2 | - 15.9 ± 0.2 | Chouvelon et al. 2018 |

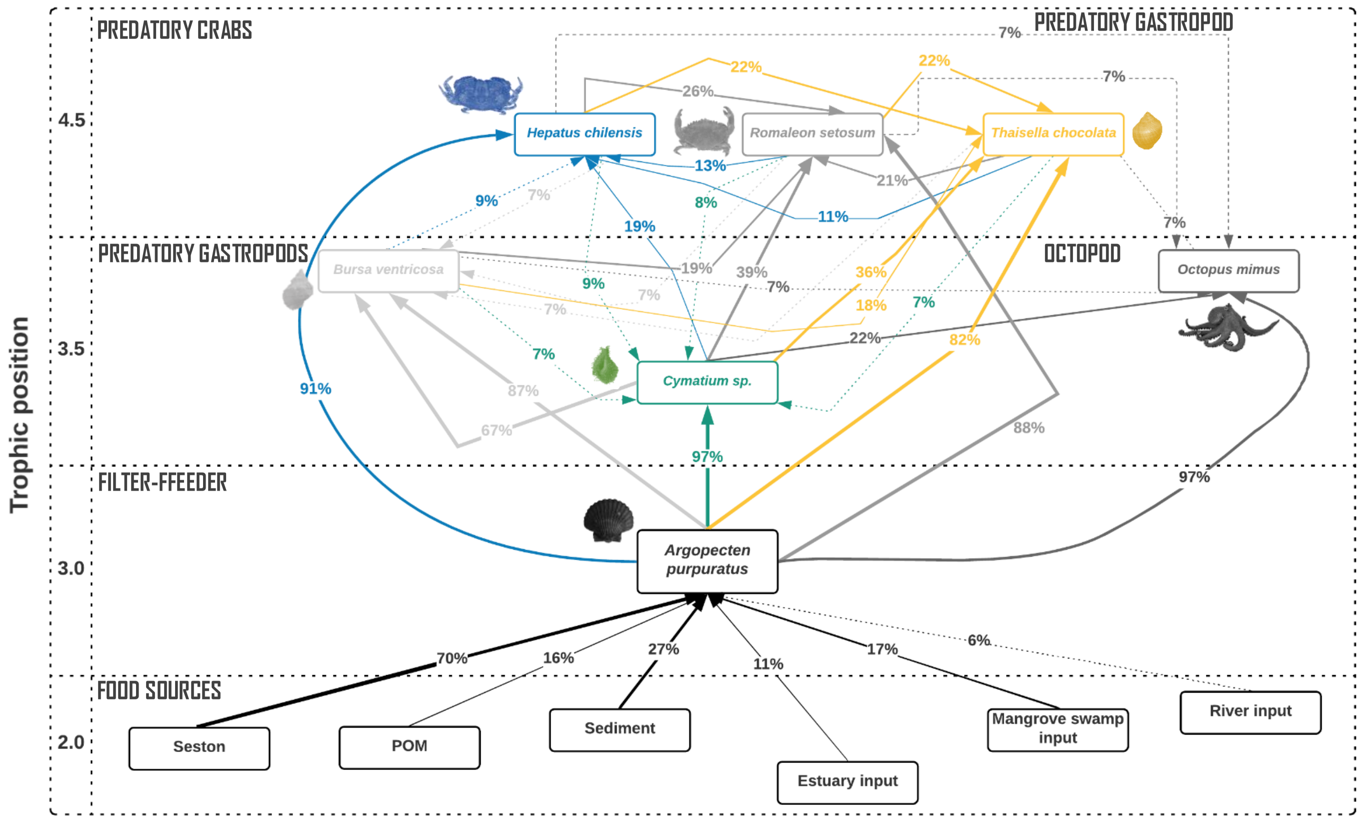
**† Only samples from the Bay and up to 50 m depth were considered.**
**†† Only individuals from 65-95 mm were considered.**
**††† Winter samples were considered due to summer samples were not taken.**

### *3.2. SIAR mixing models*

Based on SIAR-mix models (i.e. in scallop culture and wild location or 'global'), seston is the most important contributor for the Peruvian scallop *A. purpuratus* diet, representing up to 70% of the diet, followed by sediment (20-30%). River POM input seemed to be the least contributor to the *A. purpuratus* diet, it was < 10% (see Fig 4A and Fig 5). All other food sources contribute in a range between 0-20% for *A. purpuratus* (Fig 4A and Fig 5). When only northern scallop aquaculture areas were considered in the SIAR analysis, the same pattern was observed for *A. purpuratus* diet, however seston exhibited less contribution, up to 40% of the diet, while the POM increased to ~30% (Fig 4a). Some authors have studied the relevance of the seston for *A. purpuratus*, and showed that the nutritional contribution of this food source is important for *A. purpuratus*' tissues development and for physiological activities (Aguirre-Velarde, 2009; Aguirre-Velarde et al. 2015; Loaiza et al. 2020). Other studies also point at the importance of the sediment for the *A. purpuratus*' diet as re-suspended material which is rich on nutrients, but difficult to digest due to the refractive organic material (Fernández-Reiriz et al. 2004; Navarro et al. 2004).

Fresh- and brackish-water input (e.g. from rivers, estuaries,…) as food source are seasonally present in Peruvian marine ecosystems, related to the rains and caudal of these lotic aquatic systems (Mendo et al. 2016; Kluger et al. 2018; Loaiza et al. 2020). In this specific case, its role as food source for *A. purpuratus* is more pronounced when El Niño Southern Oscillation (e.g. El Abrupt Niño (AN) from 2017) occurred. During such an event, the Sechura River and the Virrila Estuary inputs are present in almost the entire Peruvian scallop aquaculture areas in the northern region (Emerton et al. 2017; Kluger et al. 2018; Loaiza et al. 2020; pers. obs.).

**Fig 5.** Food web structure of benthic community in scallop and wild conditions ('global') per trophic position and functional (feeding) group.




**Note.- Different colours were used for each species, black for food sources. The diet contributions (%) per species are related to the thickness of the line. The minimum (<10%) diet contributions are showed in dashed lines. Based on SIAR-mixing ('global') model of the main food sources and predators of the Peruvian scallop *A. purpuratus***

The predators of *A. purpuratus*; octopus *O. mimus*, crab *H. chilensis* and snail *Cymatium* sp. were characterized to have the scallop *A. purpuratus* as prey up to 90-100% (Fig 4B, 4E, 4F). The diets of crab *R. sesotum* and snail *T. chocolata* were also characterized by high contribution (incl. prey' exclusivity) of *A. purpuratus* as prey, between 35-85% for both species (Fig 4C, 4G and Fig 5). *B. ventricosa* showed similar proportions of *A. purpuratus* and *Cymatium* sp. as main prey, with a large range of 25-90% and 10-70%, respectively (Fig 4D and Fig 5). Among the *A. purpuratus*' predators, *R. setosum* and *T. chocolata* have important proportions of other predators in their diet; such as *Cymatium* sp. (~0-40%), *H. chilensis* (~0-25%) for *R. setosum* and *Cymatium* sp, *H. chilensis* and *R. setosum* (0-20%) for *T. chocolata* (see Fig 4C, 4G and Fig 5). SIAR-mix model results in scallop culture areas were very similar than the 'global' analysis for *A. purpuratus*' predators, but only the snail *B. ventricosa* exhibited considerably changes in its diet contributions, e.g. the scallop contribution is up to ~100%, while the *Cymatium* sp. was up to 10% (Fig 4d). No more similar diet contributions of these especies was observed for *B. ventricosa*, as seen with the SIAR-mix 'global' model (Fig 4D and Fig 5).

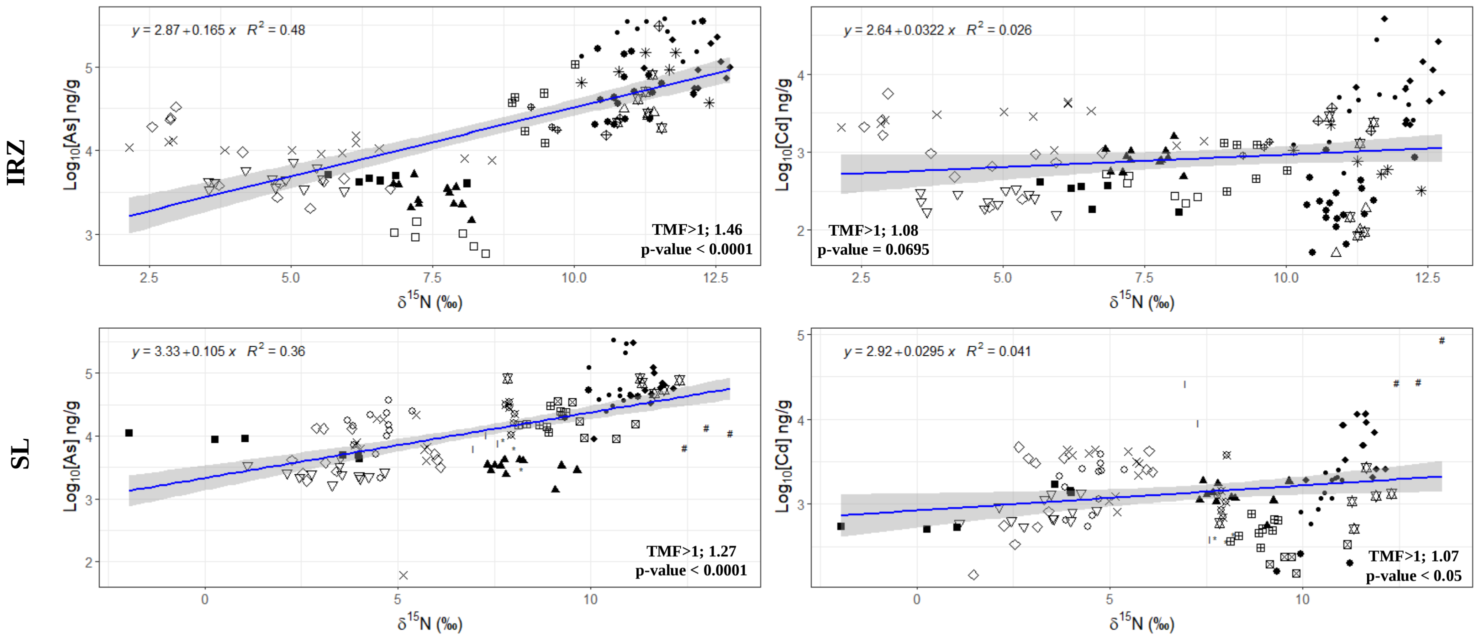
In scallop aquaculture activities, the harvest of "presumed predators" of *A. purpuratus* is an arduous activity at high economical cost which is constantly conducted in bottom corrals and longlines cultures. Our SIAR-modelled ('global') diet results elucidated the most important consumers of *A. purpuratus*, thus we suggest to prioritize and apply major fishing effort on these species, in the following order: *Cymatium* sp. > *H. chilensis* > *O. mimus* > *T. chocolata* > *R. setosum* > *B. ventricosa*; to reduce the operational costs for "cleaning corral" processes. Nevertheless, it is well-known (IMARPE, 2019; pers. comm.) that the snail *B. ventricosa* is one of the most voracious predator of *A. purpuratus* in Sechura Bay scallop culture, and it is also demonstrated in the present study when only the northern (aquaculture) region was considered (see Fig 4d). While some other studies, e.g. Mendo et al. 2016 mentioned that the crab *H. chilensis* does not consume *A. purpuratus* individuals, our study refuted that hypothesis.

In the present study the crab *R. setosum* and the snails *B. ventricosa* and *T. chocolata* are the only species that also consume other predators in considerably proportions, based on SIAR-mix ('global') models (Fig 5). We unexpectedly found that the octopus *O. mimus* did not show a large range of relevant preys as part of its diet, this is probably due to the fact that the studied ecosystems were situated in scallop culture areas (with the exception of PSJ, SHO), where the availability of scallops as prey is high. It is noteworthy to mention that more studies on predator behavior combined with biochemical proxies (e.g. fatty acids) for trophic interaction and feeding ecology should be performed in different *A. purpuratus* cultures locations.

### *3.3 Metal -$\delta^{15}$N relationships*

A positive linear relationship was found between the log transformed concentrations of As and $\delta^{15}$N for all locations (linear regression, $p < 0.0001$-0.05; with the exception of NL, $p = 0.1988$) (see Fig 6, 7 and 8). The highest R-squared was determined for the food web from PL1 ($R^2 = 0.49$), followed by those from IRZ ($R^2 = 0.48$) and SL ($R^2 = 0.36$), while the lowest of 0.018 $R^2$ was found for NL' food web (Fig 6, 7 and 8). It means that the As concentrations and $\delta^{15}$N in marine species and food sources from PL1, IRZ and SL fitted the regression lineal model better, in comparison to the other locations. This fit on the regression line reflects the possible As-biomagnification effect for those locations (Fig 6, 7). The other locations (e.g. NL, SHO) with lower R-squared (and $p > 0.05$ for NL) exhibited high variability that does not allow to conclude a proper biomagnification effect (Fig 6, 7 and 8).

For Cd, the linear relationship between log transformed metal and $\delta^{15}$N was only positive for IRZ and SL, with $p<0.05$ for SL (Fig 6) but with very low $R^2$ values. Significant and negative linear relationship was found for SHO food web from the southern region, with $R^2$ of 0.26. No regression analysis and TMF determination (incl. p-values) could be performed for NL, PL1, and PL2 for the high variability between the log transformed concentrations of Cd and $\delta^{15}$N. For biomagnification effect or positive linear relationship, Cd concentrations and $\delta^{15}$N seems to poorly fit the regression lineal model, while SL is the only location that showed a significant relationship ($p<0.05$). On the other hand, high R-squared values were more present for negative linear relationship, which means a possible Cd bio-dilution effect in these locations, such as PSJ, SHO.


IRZ
SL
y = 2.87 + 0.165 x R² = 0.48
TMF>1; 1.46
p-value < 0.0001
Log10[As] ng/g
δ15N (‰)
y = 2.64 + 0.0322 x R² = 0.026
TMF>1; 1.08
p-value = 0.0695
Log10[Cd] ng/g
δ15N (‰)
y = 3.33 + 0.105 x R² = 0.36
TMF>1; 1.27
p-value < 0.0001
Log10[As] ng/g
δ15N (‰)
y = 2.92 + 0.0295 x R² = 0.041
TMF>1; 1.07
p-value < 0.05
Log10[Cd] ng/g
δ15N (‰)

NL

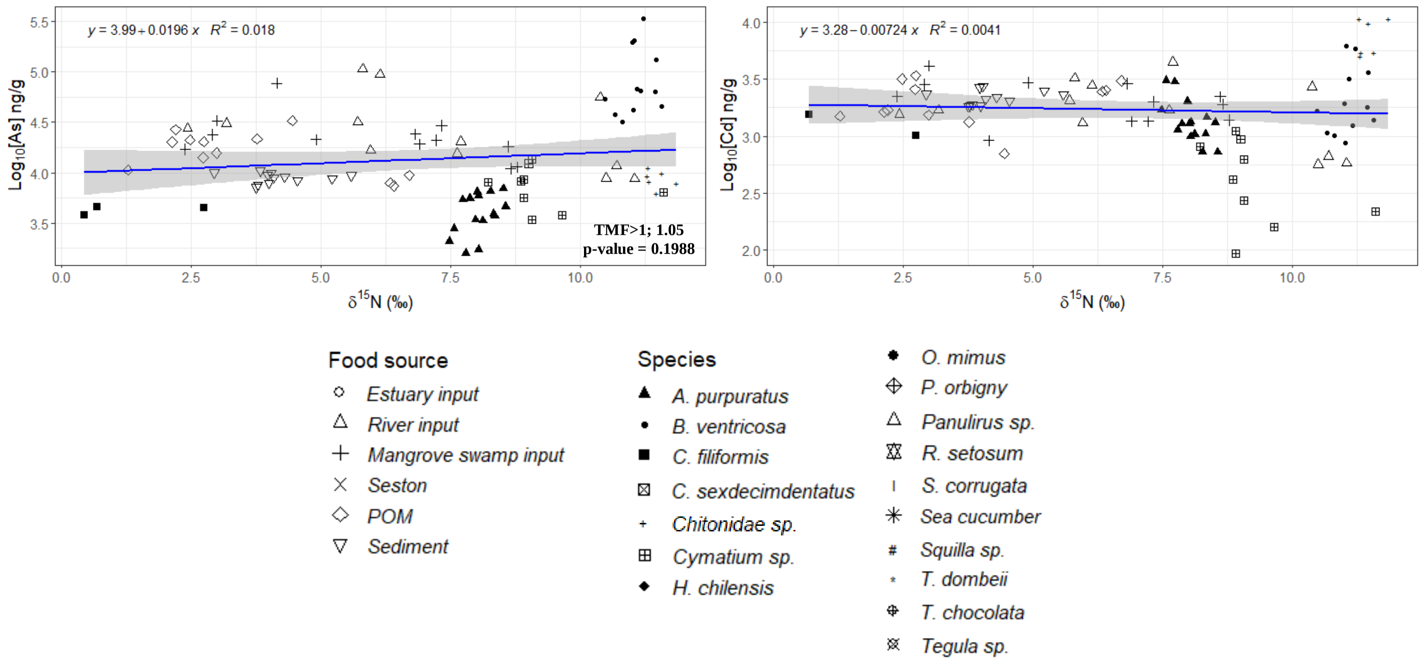



**Fig 6.** Relationship between δ $^{15}$N values and log-transformed concentrations (dwt.) of As and Cd in food sources and marine species from IRZ, SL, NL locations, Northern region of Peru.

**Note.- Metal concentrations per taxonomic group can be found in Chapter 4; addendum section. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**

For the other potentially harmful metals, e.g. Ni and Pb, only negative significant linear relationships were found between log transformed metal and $\delta^{15}N$ for all locations (see Fig 9 and 10; supplementary material). The highest R-squared was determined for the food web from SHO and SL for both metals, following by those from IRZ location (Fig 9 and 10; supplementary material). Negative linear relationships were also mainly found for the log-transformed metals (aka. micro-nutrients) Cu, Fe, Mn and Zn, with the exception of the food webs from the IRZ, PL1 and PSJ, which showed significantly ($p<0.0001$-0.0005) positive linear relationships between Cu-log-transformed and $\delta^{15}N$ (see Fig 11, 12, 13, 14; supplementary material). The highest R-squared for negative linear relationships were found for SL location, up to 0.66 for $Log_{10}[Fe]$ and 0.64 for $Log_{10}[Mn]$, while the SHO location with 0.39 for $Log_{10}[Zn]$.

TMFs were based on the relation between the $\delta^{15}N$ values and log transformed metal concentrations per location. The highest TMFs for potentially harmful metals were found when As-log-transformed concentrations were used, and above 1, e.g. TMF of 1.46 and 1.40 for IRZ and PSJ, respectively, then following by the other locations: PL1>SL>PL2>SHO>NL (Fig 6, 7 and 8). In case that Cd-log-transformed concentrations were used, the highest TMF was found for SL with 1.07, then SHO exhibited a TMF below 1; 0.84. (Fig 6, 7 and 8). TMFs for Ni and Pb were always lower than 1, from 0.51 up to 0.78 for all locations (Fig 9 and 10; supplementary material). When micronutrients (i.e.  Fe, Mn, Zn) were used, all locations (i.e. with food web linear regression analysis) exhibited TMFs < 1. In case of Cu, three locations (PL1, PSL, IRZ) exhibited TMFs >1, and considerably high TMF of 1.58, 1.28 and 1.22 for PL1, PSL and IRZ, respectively. The other locations exhibited negative linear relationships, and SHO exhibited a TMF < 1 for this metal (Fig 11, 12, 13, 14; supplementary material).

Based on estimated TMFs, a biomagnification effect (TMFs > 1; $p < 0.05$) is occurring for As in the food web from almost all locations that were analysed. Cd was only biomagnified along the food web in SL, while Cu, surprisingly showed biomagnification for the food webs from IRZ, PL1, PSJ. These increased metal transfer and accumulation along the food web suggests that diet is the major exposure route for these metals at these locations (Bisi et al. 2012). For the northern and center locations, which are scallop culture driven ecosystems, the Peruvian scallop *A. purpuratus* plays an important role as intermediate consumer and as available prey for the predators. This is reflected on their position (i.e. mostly intermediate) along the trophic metal magnification line for the biomagnified As, Cd and Cu; just after the food sources and before the most important predators, e.g. the octopus *O. mimus*, the crabs *H. chilensis* and *R. setosum* and the snails *B. ventricosa* and *T. chocolata*, among others.

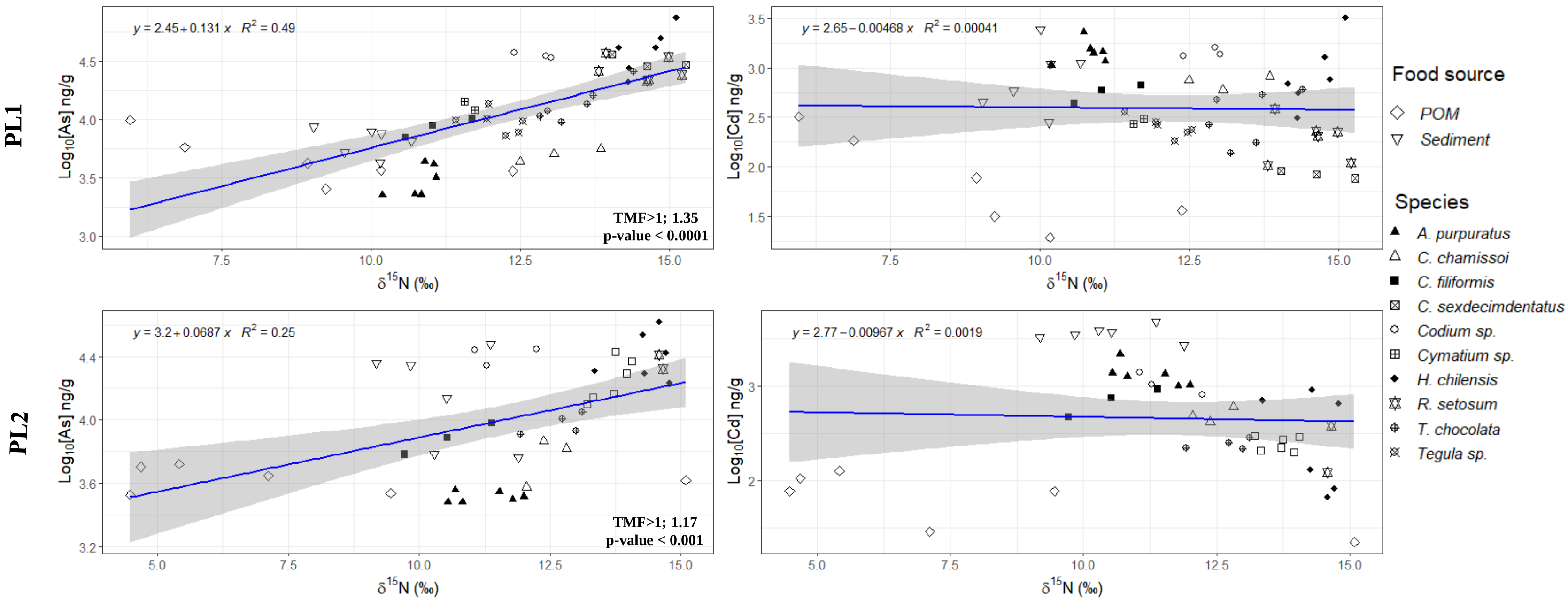



**Fig 7.** Relationship between δ $^{15}$N values and log-transformed concentrations (dwt.) of As and Cd in food sources and marine species from PL1, PL2 locations, Center region of Peru.

**Note.- Metal concentrations per taxonomic group can be found in Chapter 4; addendum section. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**

Also in non-scallop aquaculture related ecosystems such as SHO in the south of Peru, the *A. purpuratus* is highlighted as an important intermediate food item with As concentrations and $\delta^{15}N$ values nearby the middle position of the food web. These results show that this species is consumed, and therefore the metals accumulated in its muscle is probably transferred to the higher trophic levels, e.g. the snails *A. fontainei*, *Argobuccinum* sp., *T. chocolata*, *B. ventricosa*. Other benthic species that also play an important role as intermediate consumer and prey in Peruvian food webs are: the snails *Cymatium* sp. and *Tegula* sp, as shown in Fig 6, 7 and 8.

The opposite effect of biomagnification (TMFs < 1) was found for the potentially harmful elments Ni and Pb, and the micro-nutrients Fe, Mn and Zn for most locations. These metals successively decreased in species with increasing trophic levels suggesting that diet is not the major exposure route of those metals, or that there is a bio-dilution of metal during the transfer (Watanabe et al. 2008; Signa et al. 2017; Vizzini et al. 2013). The most important environmental compartment in marine ecosystems, i.e. water could be the main metal source in this case, which is reflected in the food sources and lower trophic levels (e.g. primary producers, filter-feeding consumers) (Watanabe et al. 2008; Zhao et al. 2013). The present study revealed that in fact the food sources and primary producers (e.g. *C. filiformis*) have taken up higher concentrations of those metals (Ni and Pb) and micro-nutrients (Fe, Mn and Zn) than top predators, e.g. the flat fish *Paralycthis adspersus* from SHO, and/or the previously mentioned crabs and snails from all locations.

It is noteworthy to mention that *A. purpuratus* still plays a role as intermediate consumer for food sources, and as prey for the predators on this non-metal biomagnified trophic food webs. This scallop could have accumulated these metals from food sources in first instance, but then in the case of Pb and Ni:

they were probably depurated and/or eliminated (Metian et al. 2009a; Loaiza et al. 2015; 2020), while the micro-nutrients or essential metals might be used for physiological and biological purposes, which at the end results that the transfer to higher trophic level species was minimum or little. The Peruvian scallop *A. purpuratus* has been studied *in-situ* to see metal accumulation and human health risk for consumption, and almost no Pb and Ni was found in their tissues, this in contrast to the high amounts of accumulated-Cd (Loaiza et al. 2015; 2018; 2020).

TMFs were calculated as the antilog of the slope, which implies that the higher the TMF is, the more efficient transfer of metal is occurring in that food web. For the ecosystems where biomagnification effect was found, the IRZ and SL from the north exhibited the highest TMFs for As and Cd, respectively; while the IRZ, PL1 and PSJ exhibited the highest TMFs for As and Cu. The transfer of these metals along the food webs is happening, and this could occur due to their high bioavailability, or because these ecosystems are more contaminated or metal enriched (Bisi et al. 2012).

Previous studies conducted by these authors are in according to these findings, e.g. Loaiza et al. (2018) determined that IRZ could be distinguished from the other northern region locations (i.e. SL, NL) due to the presence of more high-contaminated edible species, which could imply a human health risk for their consumption on a long term. The recent study on Peruvian scallop *A. purpuratus* as bioindicator species also determined that the center region (PL1, PL2) seems to be more affected for metal pollution, also reflected on high variations of different biomarkers (i.e. fatty acids) (Loaiza et al. 2020).

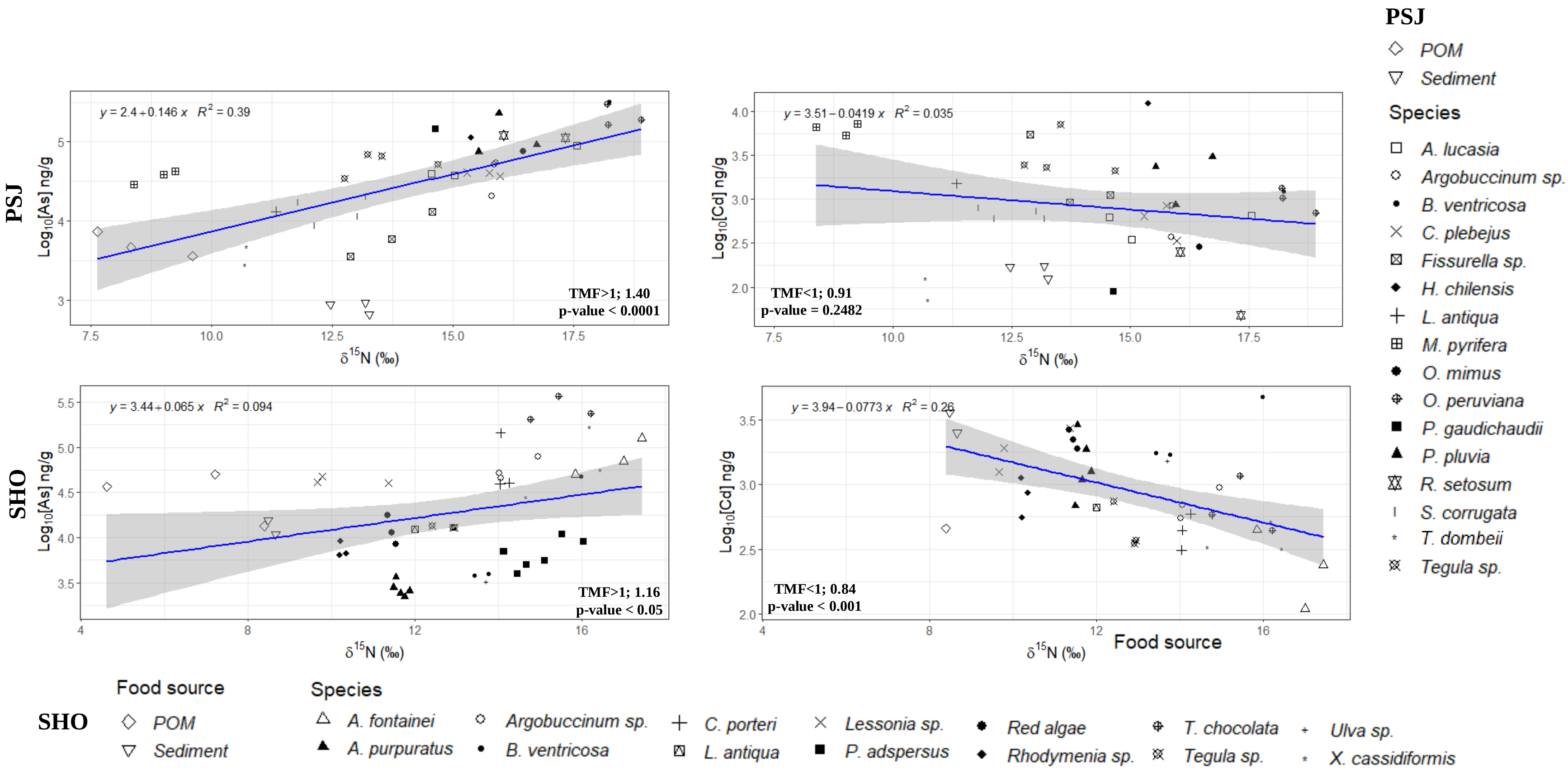



**Fig 8.** Relationship between δ $^{15}$N values and log-transformed concentrations (dwt.) of As and Cd in food sources and marine species from PSJ, SHO locations, Southern region of Peru.

**Note.- Metal concentrations per taxonomic group can be found in Chapter 4; addendum section. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**

It noteworthy to mention that marine (i.e. species and food sources) samples were collected during different El Niño effect intensities. This could have also played a role on the TMF estimations. The north (IRZ, SL, NL) was sampled during El Niño Global (NG) 2016 that did not considerably impact marine ecosystems in Peru, thus this year was considered as a 'normal condition' in this study (Loaiza et al. 2020). The northern and center (PL1, PL2) locations were also sampled during the El Abrupt Niño (AN) 2017 and this event, in comparison to the 2016' event, drastically impacted marine ecosystems in Peru e.g. rains and flood were intense (Takahashi, 2016; 2017). And as last, the southern (SHO) region was sampled in no El Niño event (NN), which is also a normal condition for this study. Therefore due to the AN, it is possible that the (mainly) northern locations, e.g. IRZ and SL were influenced by an increased presence of environmental stressors (e.g. fresh-brackish water inputs - contaminants) in 2017, which were partially reflected on the calculated TMFs.

The comparison of studies on trophic biomagnification in food webs is highly complex. The TMFs given for contaminants can vary widely among studies, depending on various factors including types of consumers incorporated in the analysis, the range of trophic levels investigated, freshwater vs. marine ecosystems, among others (Walters et al. 2016; Verhaert et al. 2017). Moreover, these studies have mainly been conducted in freshwater systems, and in temperate or cold climates, and are scarce in the southern hemisphere. This stresses the importance of this study in contributing to a significant knowledge gap on metal biomagnification in (sub)tropical aquatic ecosystems (Bisi et al. 2012; Verhaert et al. 2017). Bisi et al. (2012) determined TMFs with total mercury (THg) log-transformed concentrations and $\delta^{15}N$ for the subtropical ecosystems from Guanaraba Bay and Ilha Grande Bay in Rio de

Janeiro, and values of 1.51 and 1.67 were found, respectively, which are close to our TMFs (up to 1.58) when the food magnification effect was pronounced.

In colder or template environments such as the wetland ecosystem influenced by the Yellow River Delta in China, only TMFs > 1 were found for Cd, Zn and Hg, and non-significant p-values were found for the biomagnification of any other metal (incl. As, Cu, …) (Cui et al. 2011). A similar pattern was found in the semi-enclosed marine areas from Stagnone di Marsala, Italy, only the THg showed a TMF > 1 (1.06), the other metals (As, Cd, Pb) were below 1 (Vizzini et al. 2013). In Augusta Bay' marine food webs in Italy, TMFs in similar magnitude than the TMFs from our study were found, and were about 0.84 to 1.07 for Cd log-transformed concentrations and $\delta^{15}$N values.

In spite of differences among ecosystems, it is clear that the Peruvian marine ecosystems have a serious concern in terms of As contamination, which is reflected in all food webs from the northern, center and southern region of the country. In minor magnitude, Cd is also a concern in Peru, either in terrestrial (e.g. cacao production) or marine ecosystems (DIGESA, 2018). This study showed only a Cd trophic magnification effect in one location (i.e. SL) from the northern region, however high Cd concentrations have been found in several species along the Peruvian coast (Loaiza et al. 2018; 2020; SANIPES 2019). TMF for Cu was actually the highest from our studied marine food webs, which means that the environment is Cu-rich and/or Cu-contaminated, and therefore the availability and transfer is the most optimal for this metal in certain locations.

more studies on predator behavior combined with biochemical proxies (e.g. fatty acids) for trophic interaction and feeding ecology should be performed in different *A. purpuratus* cultures locations

## 4. Conclusions

This study revealed a trend of increasing $\delta^{15}N$ and $\delta^{13}C$ from food sources to top consumers (i.e. snails, crabs, octopus, shrimps), by passing through the scallop *A. purpuratus*. This points at *A. purpuratus'* crucial role as intermediate consumer and main food item for predators from the studied marine food webs. Overall, $\delta^{15}N$ values increase southward, from northern (Sechura Bay, incl. Illescas) to center (Paracas Bay) and the southern (Punta San Juan) regions. The cause of this gradient is the northern Humboldt Current system (NHCS), which is one of the most productive upwelling systems, with strong denitrification (higher in ≥7°S) that leads to high $\delta^{15}N$. Based on SIAR-mix models and trophic regression lines, *A. purpuratus* was again found to be a key prey species for the most important predators, as well as the main source for metal transfer to higher trophic levels. Even in food webs where a non-biomagnification (or bio-dilution) effect was observed, *A. purpuratus* played a key role in the metal transfer. The SIAR-modelled results showed that seston is the major contributor to *A. purpuratus*' diet, following by sediments and marine and brackish water POM, leaving the freshwater (river) POM input as the least contributor. Trophic magnification factors (TMF) helped to conclude that As is an actual concern in Peruvian marine ecosystems, while some ecosystems are rich in natural Cu or are Cu-contaminated.

# Chapter 6

# GENERAL DISCUSSION

## 1. Ecosystem health status (EHS)

Environmental or ecosystem health status (EHS) is an integrated approach that could help to integrate all the results obtained in this study, and translate it into useful information for the local population and policy makers from Peru. EHS is not simple to define because it integrates the complexity of human-environment interactions. Moreover, the current diverse environmental problems (e.g. pollution; Climate Change, El Niño,…) makes EHS even more difficult to address (Kruse, 2018; EEA, 2019; WHO; 2019; WISE Marine, 2019). In marine areas, humans have been operating with and within aquatic ecosystems for millennia, e.g. from the pre-Incas and Incas in the southern hemisphere to the high-technology and industrial activities nowadays, causing changes through often complex interactions (Coker, 1910; Rostworowski, 2005; cited by Chavez et al. 2008; Lavallée & Michèle, 2012; Reitz et al. 2008; EEA, 2019). The consequences of human activities on the marine ecosystem functioning around the globe have been negative in most cases (EEA, 2019). A healthy ecosystem implies that adverse or possible negative conditions are minimal or do not exist for the ecosystems' living organisms (Kruse, 2018; ODPHP, 2019). The term "health" has a positive connotation.

Healthy or good marine environment status involves the entire ecosystem, including humans. The goal is to maintain ecosystems in a healthy, clean (i.e. without pollutants), productive and resilient condition, so that they can provide services and benefits to humans (EC, 2019; EEA, 2019; WHO; 2019). In this study, EHS's were addressed using different research approaches in Chapter 2, 3, 4 and 5. To reach this aim, chemical elements and compounds were measured in several environmental compartments (i.e. from food sources to consumers) of different Peruvian marine ecosystems. Additionally, environmental variables were considered, as well as *in-situ* observations, both during the El Niño phenomenon and normal non-Niño years. These variables (metals, stable isotopes, fatty acids,…) were used to document the environmental variability between locations, regions and scenarios (i.e. El Niño intensities) along the Peruvian coast. A social component was also considered in Chapter 4 in order to precise and prioritize the human (health) component in this research.

## 2. Different approaches to estimate environmental health status (EHS)

In determined time-frame studies (i.e. 4 years), it is crucial to set priorities. Generally, research questions are determined first, and then a possible way to tackle these questions and find answers is designed. It is essential to take into account procedures that are economically, logistically and analytically feasible for each study. This is also part of the explanation that different approaches can be performed for EHS analyses (Long et al. 1995; Pereira et al. 2012; Breitwieser et al. 2018a; 2018b).

In this study (specifically in **Chapter 2: *Potential health risks via consumption of six edible shellfish species collected from Piura – Peru***) metal levels (As, Cd, Cr, Cu, Fe, Mn, Pb, Ni, Zn) of the Peruvian scallop *Argopecten purpuratus* and its potential predators were estimated as they are relevant commercial edible species in the region (IMARPE; 2019; PRODUCE, 2019). This short food web (i.e. six species) was studied to determine the actual contamination levels in their edible tissues, and in individuals from the most important scallop aquaculture area of Peru: Sechura Bay (incl. Illescas). Although the approach focussed on food safety, the fact that spatial and temporal variations were considered in the sampling methodology provided us with useful additional information, e.g. the northern area could be compared (in degree of contamination) with the southern area, and with the southernmost area (i.e. location in front of the Illescas Reserved Zone (IRZ)) of the Sechura Province. Low-raining and high-raining periods could also be compared in the frame of El Niño 2016 (aka. El Niño Global), as the samples were collected exactly in that year.

The overall conclusion of **Chapter 2** in terms of EHS is that the location in front of the only Reserved area is the most metal-contaminated (e.g. As, Cd). Metal levels and estimated health risk indexes helped to recommend that the snail *Bursa ventricosa* from that reserve should be eaten as little as possible (< 0.02 kg/week) as that IRZ is the least healthy marine ecosystem, and seafood should be consumed with care. El Niño 2016 played a small role in temporal and environmental health variations. Only some species e.g. *B. ventricosa*, *Hepatus chilensis*, *Octopus mimus*, *Cymatium sp.* were more metal-contaminated during the high-raining period than in the low-raining period, but the pattern was more individual[metal]-location dependent. It is noteworthy that due to the minimum impacts caused by El Niño 2016 to Peruvian marine ecosystems, this El Niño has been characterized as a period of almost 'normal conditions' (with the except of slightly high sea temperatures) for Peru (Takahashi, 2016; 2017; Loaiza et al. 2020).

The EHS was also addressed using a single species, the Peruvian scallop *A. purpuratus* and its food sources in **Chapter 3: *Peruvian scallop Argopecten purpuratus: From a key aquaculture species to a promising bioindicator species***. This study was conducted to test *in-situ* the capacity of *A. purpuratus* to be a bioindicator in the center-south (i.e. Paracas Bay) and north (Sechura

Bay, incl. Illescas) of Peru. A multi-biomarker approach was applied with seven metals (As, Cd, Cu, Fe, Mn, Pb, Zn) in six different tissues, 19 fatty acids (FAs) and one stable isotope ($\delta^{15}N$) of the muscle tissue of *A. purpuratus*. Metal and FA concentrations in food sources (e.g. POM, seston) of the target species were also analyzed during the sampling campaigns, in the period of El Niño 2016 and El Niño 2017.

In accordance with **Chapter 3**, the bioindicator species *A. purpuratus* also indicated that the most southward locations (i.e. PL1 and PL2 in Paracas Bay) are the most metal-contaminated systems. This insight was also reflected in the muscle FA concentrations, which were considerably lower in the center-south, compared to those from the north. EHS in time was hard to disentangle during low-raining and high-raining periods, however the FA biomarkers could explain the EHS variations between El Niño 2016 vs. El Niño 2017. The abrupt El Niño 2017 impacted the EHS in terms of high FA variations, resulting in lower levels in *A. purpuratus* and higher values in food sources. The decrease of FA in *A. purpuratus* can be related to the increase of surrounding environmental stressors (Filiminova et al. 2016). The increase of FA concentrations in food sources might be due to more particulate matter (e.g. river input) that can form agglomerations with many chemical compounds.

**Chapter 4:** ***Marine species as safe source of LC-PUFA and micronutrients: insights in new promising marine food in Peru*** discusses the beneficial elements and compounds of Peruvian marine species in three regions: north, center-south and south of Peru, this without leaving the adverse health effects of harmful contaminants. A total of 54 marine species were screened for chemical levels: micro-nutrients (Cu, Fe, Mn, Zn) and potentially harmful elements (As, Cd, Ni, Pb) metals, and the highly beneficial polyunsaturated fatty acids PUFAs (EPA, DHA, ARA). In general, the marine species from the north (IRZ, SL, NL) and south (PSJ, SHO) exhibited the highest metal contaminant (e.g. As, Ni, Pb) levels. While the PUFAs, as biomarkers, could help to confirm that the southernmost region of Punta San Juan was the most contaminated. PUFA concentrations in the southern marine species were considerably lower (e.g. up to 3.5-fold less in EPA) than in the northern marine species. Again, the southernmost locations (see Chapter 2 and 3) have the least healthy environmental conditions of all the Peruvian marine ecosystems analysed in the present study.

The use of trophic magnification factors (TMF) for biomagnification of contaminants in food webs is an up-to-date methodology for EHS determination (Bisi et al. 2012; Van et al. 2013; Verhaert et al. 2017). **Chapter 5**: ***Peruvian marine ecosystems: trophic interactions and metal transfer in aquatic food webs*** addressed the tropho-dynamics of benthic food webs by means of stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) and metal levels (As, Cd, Cu, Fe, Mn, Ni, Pb and Zn) in 53 different species and food sources. In terms of EHS, the southernmost location in the north, IRZ and the Paracas Bay' location (PL1) and Punta San Juan' location (PSJ) seem to be the most metal enriched (i.e. based on TMF levels) environments. However, when $\delta^{15}N$ was used as biomarker (Sardenne et al. 2017), the Punta San Juan' locations or the southern region is again the lowest in EHS. The $\delta^{15}N$ values from the southern region exceeded these from the center and northern regions, with up to + 5 ‰ in almost the entire food web. This nitrogen enrichment could be attributed to the anthropogenic degradation of the ecosystem (Fischer et al. 2014; Puccinelli et al. 2016), or/and the simple fact that the Humboldt Current system (NHCS) brings upwelling, which causes a strong denitrification (~

high $\delta^{15}N$ value) in Peruvian southern areas (Chavez et al. 2008; Lam et al. 2009; Espinoza et al. 2017).

We observed that different approaches led to a consistent pattern for the EHS analysis in Peruvian marine ecosystems, with the southern locations always more degraded in metal contamination or metal enriched than the northern ones. Metal contamination is currently a serious concern in Peru, that is why we focused on these chemicals (Loaiza et al. 2015; 2018; SANIPES, 2019). The southern part of Peru has been characterized as “natural metal-rich” ecosystem, which is the explanation given by the government for the elevated concentrations of harmful metals, e.g. Cd, Pb (Barriga-Sánchez and Pariasca, 2018; DIGESA, 2018; SANIPES, 2019).

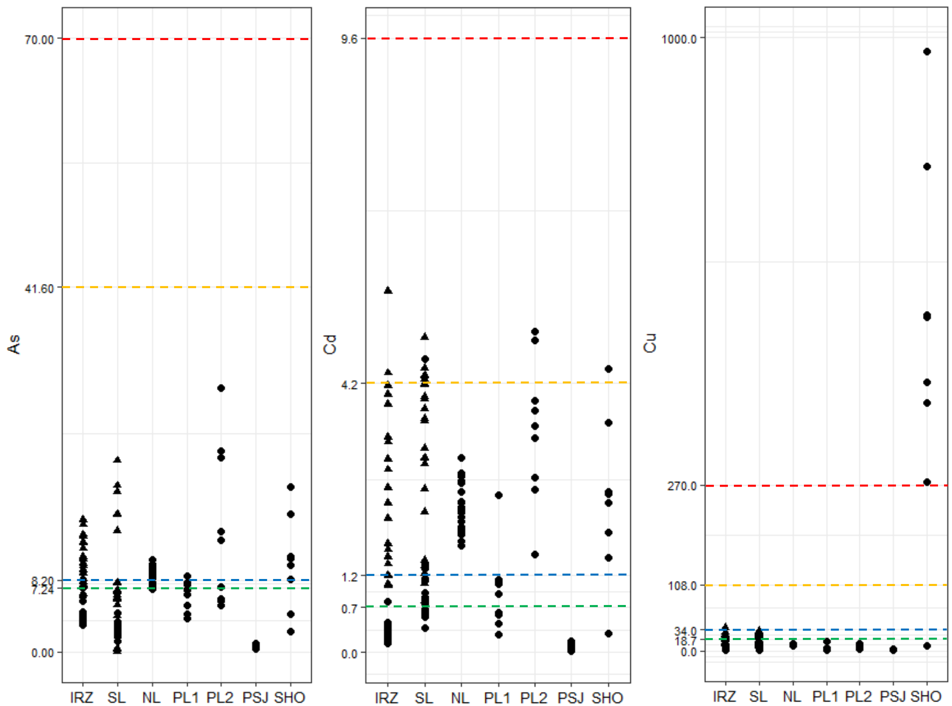
Nevertheless, and as previously mentioned, Peru is a mining country, being the first in gold and second in cadmium production in Latin America, and the second in copper, silver and zinc production worldwide in 2018 (MINEM, 2019). The southern region has a high concentration of mining activities (e.g. up to 15 concessions in one region, Arequipa), from Shougang down to Pucamarca at the border with Chile, which could substantially influence the high-metal levels and low EHS in the southern marine and coastal environments (MINEM, 2019). It is noteworthy to mention that sampling was conducted in the north during El Niño (‘weak’) 2016 and (‘abrupt’) 2017, while the center-south was sampled during El Niño 2017 and the south during no Niño 2018, which could also affect (e.g. cofounding factors) the EHS’s per region.

In order to strengthen the general conclusion about EHS, sediments and seston (also considered as re-suspended sediments) were compared with the different international limits (see Fig 1). Long et al. (1995) estimated indices to determine adverse biological effects due to chemical sediment concentrations, the Effects Range-Low (ERL) and Effects Range-Median (ERM). They defined ERL values as the lowest concentration of a metal that produced adverse effects in 10% of the data reviewed. Similarly, the ERM designates the level at which half of the studies reported harmful effects. Metal concentrations below the ERL value are not expected to cause adverse effects, while levels above the ERM value are likely to be very toxic (Long et al. 1995; U.S. EPA, 2019).

Locations are rated "good" when the concentrations of all measured metals in sediments are below the ERL limit. An "intermediate" rating applies if a metal exceeds an ERL limit, and a "poor" rating signifies exceedance of the ERM limit for a metal (Long et al. 1995; U.S. EPA, 2019). A similar approach was established by the Canadian Council of Ministers of the Environment (1999), using the Interim Sediment Quality Guideline (ISQG) and the Probable effect level (PEL). When the contaminant in sediments is ≤ ISQG, up to 9% of adverse biological effects can happen, while the probability for adverse effects is 13-27% when the concentration is between ISQG and PEL and it is up to 71% when the contaminant level is > PEL.

Based on ERM and ELM, no location in this study could be rated as "good" and only PL1, PL2 and PSJ could be rated as intermediate (Fig 1). IRZ, SL, NL and SHO could be considered the poorest in terms of environmental conditions, they exceeded the ELM-limits for Cu, Ni and Zn (Fig 1). When the ISQC and PEL are used, the most noticeably % incidence of adverse conditions were for SHO, this location exceeded the ISQC for As and Pb, and the PEL for Cd, Cu and Zn. IRZ, SL, and PL2 also exceeded PEL for Cd, so this means that there is a 71% chance that living organisms from these four locations could be affected by the found sediment and/or seston Cd concentrations (see Fig 1). Sediment pollutant levels also reflected the determined EHS, with the sediment of the southernmost Punta San Juan' location, SHO being the poorest in environmental condition. This location showed the highest % incidence of adverse biological effects and the highest metal concentrations. IRZ and SL, the southernmost locations of Sechura Bay also exhibited degraded conditions as indicated by the metal contamination in the sediment.

Oceanographic conditions (incl. El Niño effect) could also explain the EHS spatial and temporal variation in the Peruvian marine domain. The important NHCS flows northward from Chile to the north of Peru, it is oceanic but this strong current probably washed contaminants from the south to the north, and distributed them along the way (center-south of Peru) (Cabarcos et al. 2014). As strong upwelling system, the sunken nutrients, minerals and possible contaminants are also brought to the surface and along the water column, and as such will be distributed over the entire ecosystem. Peru Coastal Currents such as the PCC also play a crucial role in particulates (i.e. chemicals) distribution, also in the same direction to reinforce what is brought by the NHCS (Huyer et al. 1991; Cabarcos et al. 2014).



As
70.00
41.60
8.20
7.24
0.00
Cd
9.6
4.2
1.2
0.7
0.0
Cu
1000.0
270.0
108.0
34.0
18.7
0.0
IRZ SL NL PL1 PL2 PSJ SHO

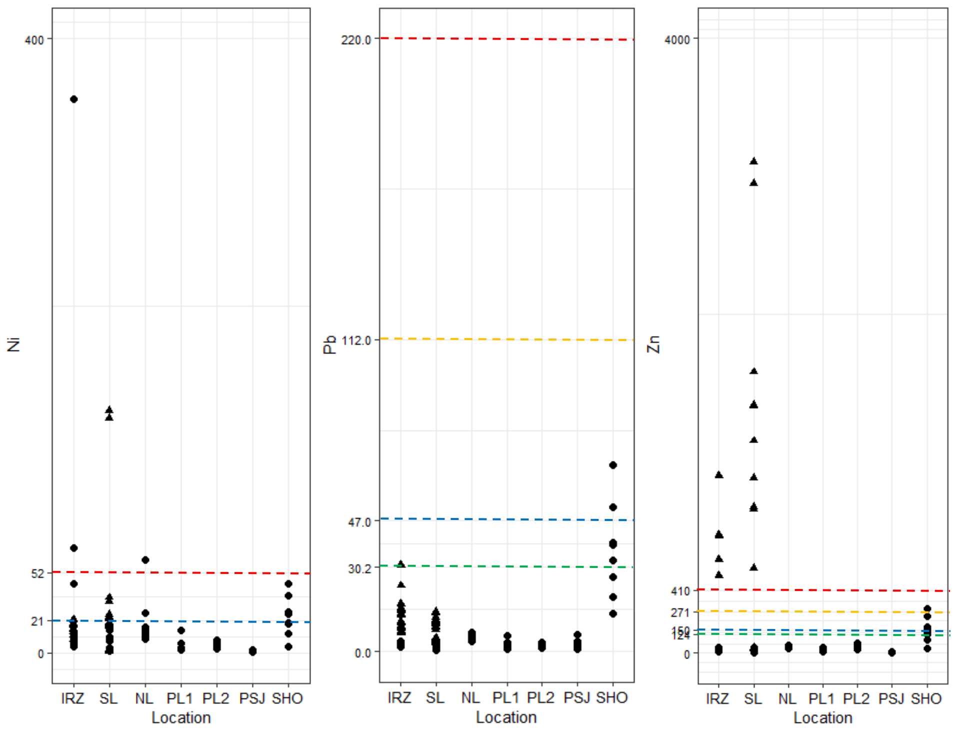



**Fig 1.** Metal concentrations (μg/g dwt.) of sediment (circle) and seston (triangle) per location compared to the Effects Range-Low (ERL) (--) and Effects Rage-Median (ERM) (--) limits from Long (1995), and Interim Sediment Quality Guideline (ISQG) (--) and the Probable effect level (PEL) (--) from the Canadian Council of Ministers of the Environment (1999

El Niño effect has different magnitude versions, e.g. El Niño Costero and El Niño Global among others, however the raise of temperature (up to 10°C) is a common El Niño phenomenon caused by the coming warm ocean water from the central and east-central equatorial Pacific (Takahashi, 2016; 2017). Temperature exerts an important effect on metal speciation, because most chemical reaction rates are highly sensitive to temperature changes (Neff, 2002). An increase of 10°C can double biochemical and biological reaction rates, therefore metal uptake by an organism could be affected (Luoma, 1983; Neff, 2002). The increase in biological process rates does not necessarily result in increased bioaccumulation of metals, because both influx and efflux rates of metals may increase. Nevertheless, as general (though not universal) rule, high temperatures act synergistically with metals to increase marine organisms' mortality (Langston, 1990).

During the strong-El Niño, river flow capacities reached their maximum in Peru due to the strong rains. Their discharges (including contaminants) were extremely high along the Peruvian coast, which substantially changed the environmental conditions (or health) of the marine ecosystems (ENFEN, 2017; Loaiza et al. 2018; 2020). Fig 2. gives an idea of the metal contribution of fresh and brackish environments to the marine environments in the present study. In some cases the metal levels (e.g. As, Ni) were higher for the POM from the Sechura River, Virrila Estuary and San Pedro Mangrove than the concentrations in the marine ecosystems, and significantly different of the concentrations of As, Ni and Pb in sediments. The river and mangrove input was also significantly higher in As and Ni concentrations than those in marine compartments (with the exception of Ni-seston). High variability was found for Cd in both environments, similar concentrations (up to ~5 µg/g) and non-significant differences were observed for the fresh-brackish and marine basal resources (Fig 2).

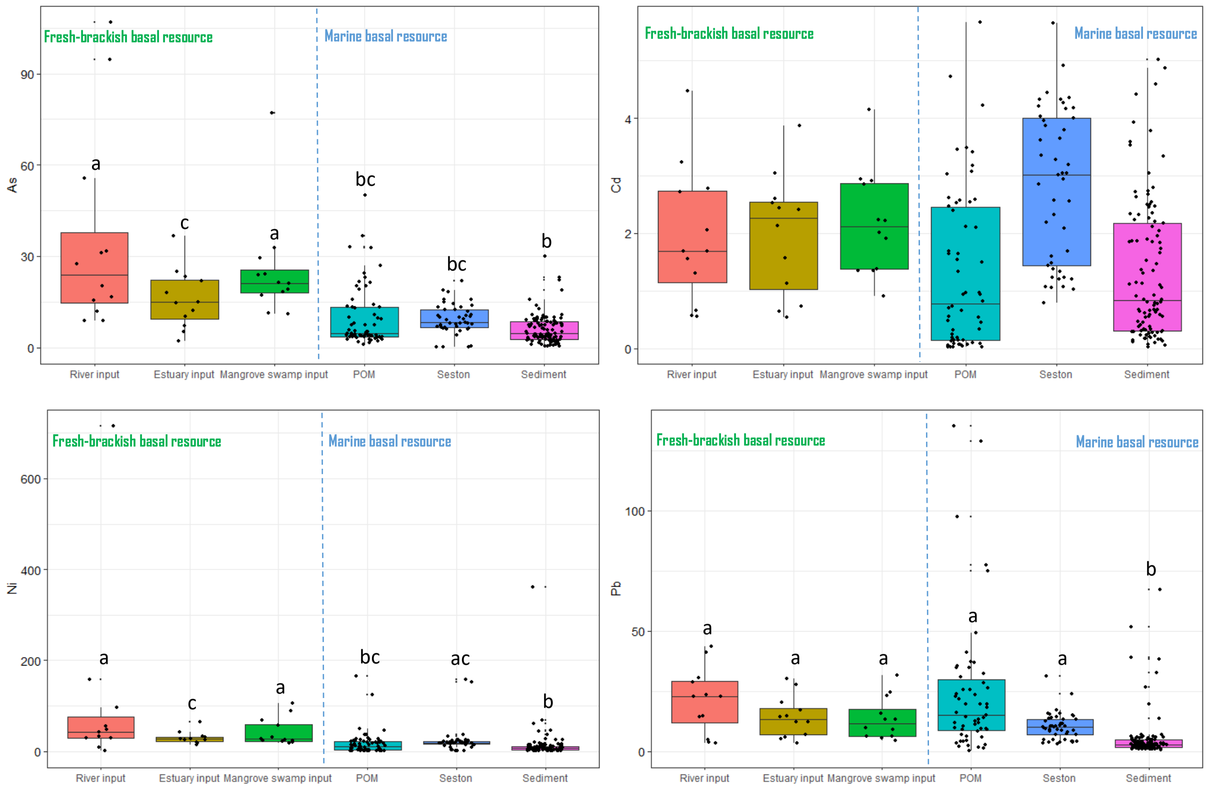
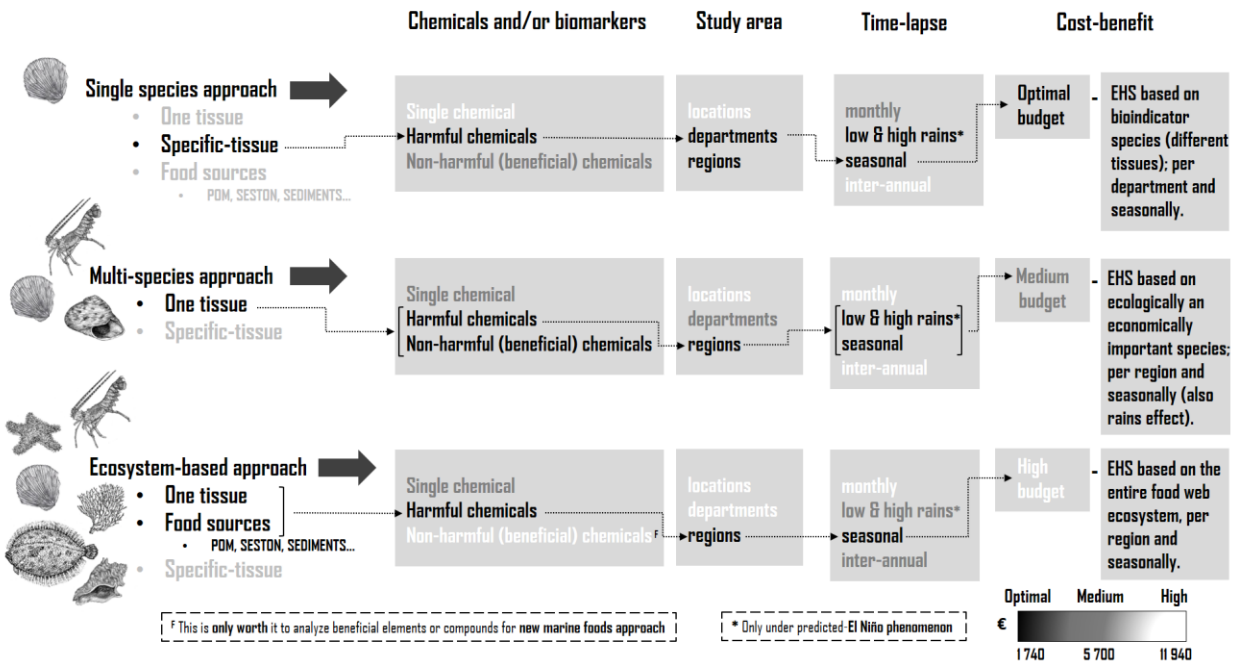



**Fig 2.** Metal concentrations (µg/g dwt.) in environmental compartments from the fresh-brackish water and marine water environments. Different letters indicate significant differences between fresh-brackish and marine basal resources.

**Note.- One-way ANOVA and post-hoc Tukey multiple comparison were performed to compare fresh-brackish and marine basal resources . For non-parametric data, an extension of Kruskall-Wallis test was used with the post hoc non-parametric Kruskalmc test function. Results were statistical significant when $p < 0.05$.**

## 3. Designing strategies for EHS monitoring

Different approaches for EHS or monitoring program design are always related to the cost-benefit of the involved activities. As previously mentioned, a research-question approach prior to analysis is ideal for EHS. It is not always feasible to measure an enormous amount of biotic and abiotic environmental compartments to estimate EHS. Therefore, for this study a scheme (Fig 3) was designed in order to simplify the cost-benefit of the different approaches.




**Note.- to our knowledge, the most (financially) feasible pathway is printed in black, and toward the lighter attenuation up to white as the least (financially) feasible.**

**Fig 3.** Cost-benefit scheme based on the different approaches to estimate environmental health status (EHS) in marine studies. Budgets (€ · area · yr) were calculated using: metals (e.g. potentially harmful), micro-nutrients and fatty acids (non-harmful or beneficial) and stable isotopes as chemical analysis costs; and per sampling area and year. Marine species drawings were made by the Peruvian artist Samantha Scavino, 2018.

The budget was estimated using the following equation:

$$\text{Budget } (€ \cdot \text{area} \cdot \text{yr}) \text{ estimation} = \text{n x (environ. compart) x n x } (t_{species}) \text{ x R x C.C.A x T.L}_{(f)} + ((n) x S_{Cam}))$$

where:

environ. compart: environmental compartments (species and/or food sources);
$t_{species}$: tissues on species;
$S_{Cam}$: sampling campaigns; it is considered 150 € per sampling collection (incl. materials, carburant,…)
n: number of environ. compart, or $t_{species}$ or $S_{Cam}$.
R: number of replicates.
C.C.A: cost per chemical analysis: 10 € per metal or stable isotope analysis; 20 euros per fatty acid profiling.
$T.L_{(f)}$: time-lapse factor, 12 for monthly; 4 for seasonal; 1 for inter-annual evaluations.

The single species use is the most optimal approach (1 740 € · area · yr), but a substantial ecological knowledge of the species under study (e.g. potential bioindicator; *A. purpuratus*) is required. The species could be analysed by using its different compartments (e.g. gills, hepatopancreas, kidney…) to give a wide spectrum of tissue-specific contaminant accumulation. It is noteworthy to mention that only relevant and specific contaminants must be considered in relation to previous studies, in most cases: potentially harmful metals (Hg, Cd, As,…) due to their high persistence and for the food safety approach. Study areas should be separated by enough distances (not only few miles) to disentangle population mixing. These distances will allow to compare different environmental conditions and anthropogenic pressures.

A multi-species (n=6) approach based on ecologically and economically important species requires a medium budget (~ 5 700 € · area · yr). The budget is mainly related to the measurement costs (~ 20 € per sample) of biomarkers such as fatty acids. The advantage of measuring fatty acids is its dual use as tracer and biomarker, and as beneficial compound for nutritional approaches. Seasons but also low-and-high rain periods are considered in this approach based on the impact of the river discharges (incl. other environmental factors) on the seafood safety and quality.

Ecosystem-based approach requires a large budget (~ 11 940 € · area · yr) investment, this is due to the high number of environmental compartments to be sampled, and the number of sampling (~ 6) expeditions in order to collect all samples per region. In order to have a robust picture of the ecosystem tropho-dynamics and functionality in Peruvian marine ecosystems, a minimum number (n= ~23; 20 marine species and at least 3 food sources i.e. POM, seston,…) of species (or trophic levels) is required for the analysis. This number of species is related to the high biodiversity (and abundance) found in the study locations along the Peruvian coast. The budget of 11 940 € · area · yr is only considering metals and stable isotopes to be analyzed. In case of the nutritional approach, e.g. to search for new marine foods, the amount would reach up to a ~2-fold higher cost due to the fatty acid analysis. Regional and seasonal samplings are a priority for numerous species (i.e. multi-species or ecosystem) approaches, this is due to the already high budget per sampling action. In addition, this sampling method can answer the research questions related to the effect of climate and oceanographic characteristics (i.e. ongoing Climate Change, El Niño), which is stronger at the regional level.

## 4. Advantages and disadvantages of *in-situ* studies

Recently, research studies in environmental toxicology are mostly focused on controlled conditions, either in laboratories or mesocosms (Nilsen et al. 2019; Pansch and Hiebentahl, 2019). These methodologies from high to intermediate controlled experiments are useful to achieve specific research questions. The aim of these studies are to determine specific response-variable effects, which is very common in ecotoxicology (Metian et al. 2008a; b; c). *In -situ* studies could reflect what is actually ongoing in certain ecosystems, and all variables count, however sometimes is not feasible to measure most of them (Crane et al. 2007). Up-to-date techniques which are mostly based on multi-parameter measurements could disentangle some of the variables that can act as cofounding variables (Viarengo and Canesi, 1991; Sardenne et al. 2017).

In developing countries such as Peru, *in-situ* studies are a priority due to the lack of baseline information or studies in natural ecosystems, e.g. marine ecosystems (Barriga-Sánchez and Pariasca, 2018; Vallejos, 2015; IMARPE, 2019; SANIPES, 2019). These *in-situ* study approaches are necessary to understand the ecosystem functionality, subsequently *ex-situ* experiments could be conducted with the knowledge obtained from the field. Peruvian marine ecosystems have been monitored since 1954 by the Consejo de Investigaciones Hidrobiológicas (CIH) (Chavez et al. 2008). So far, oceanographic and sanitary measurements have been reported in Peru, however a more research-based approach could be implemented in order to improve the scientific analysis of these environmental variables (IMARPE, 2019; SANIPES, 2019; pers. comm.). Currently, scientific knowledge in marine science is increasing in Peru, especially in oceanography due to the

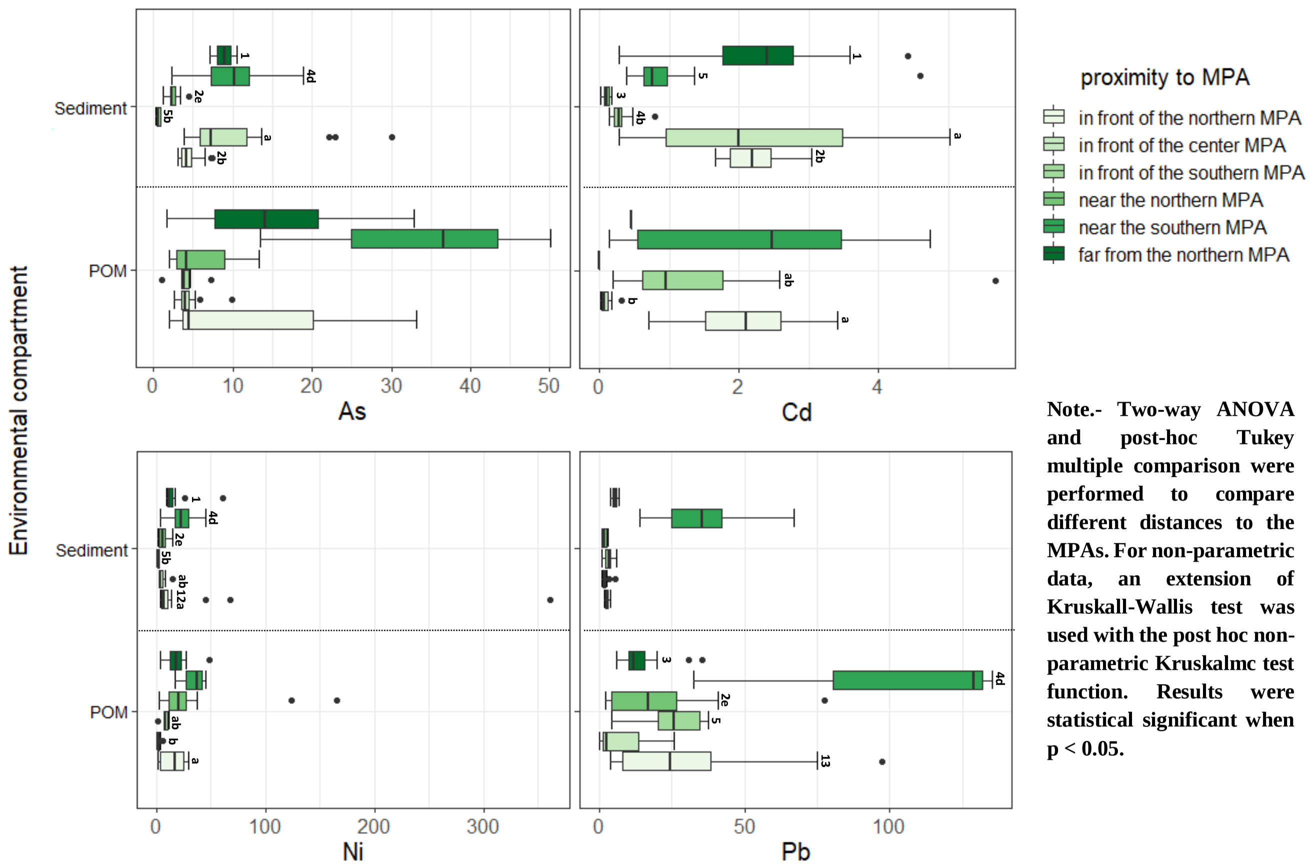
Peruvian anchovy industry and its major threats, e.g. El Niño and ongoing Climate Change (Shen et al. 2017; IMARPE, 2019; Moron et al. 2019). The development of scientific information is also growing in Peru because of the extra funding from the Consejo Nacional De Ciencia y Tecnologia (Concytec) of Peru. Concytec provides funding for relevant research topics for the development of Peru (Melgar-Sasieta et al. 2018), such as the study at hand.

In the present study, only *in-situ* data were considered which had advantages and disadvantages. The fact that the sampling campaigns were performed under different El Niño intensity events (2016, 2017) and no Niño year (2018) was substantially positive to answer the research questions. We could observe *in-situ* the normal conditions and real El Niño scenarios during the sampling periods. Sampling was performed in Dec-April, periods where the El Niño was at its major intensity (Takahashi, 2016; 2017). Moreover, the applied methodology for marine species collection or harvesting with semi-autonomous diving was the most appropriate method. We could observe what was going on in these different environments, e.g. the massive mortality of Peruvian scallops in the southern location of Sechura Bay because of the expansion of the water plume from the Virrilla Estuary (pers. obs.).

To our knowledge and experience, *in-situ* studies also help to get more involved with the study area, including the local population and authorities. Moreover, when the research focusses on contaminants and health risks due to seafood consumption, it is essential to work on a local scale to provide feedback to the local population. We got close to the coastal populations by 1) involving them in the sampling expeditions, 2) and through the social and nutritional survey which was performed and included in **Chapter 4**. A total of 764 people was interviewed and evaluated in terms of anthropometrics and feeding habits. The evaluation was performed to estimate their preferences for seafood consumption. Pollution by nearby mining and oil activities (e.g. Savia; Vale, Shougang,…) was their main concern when we mentioned that we were analyzing contaminants in marine organisms (unpublished results).

The first year sampling focused only on the north of Peru, the location in front of Illescas Reserved Zone (IRZ) was considered as our "control" site. However, during the field work we could observed that major industrial activities were situated around that Reserved Zone. Moreover, during water collection, oil traces were observed along the water surface and sub-surface of the sampling locations (pers. obs.). As a result, more research questions raised, and new study areas, e.g. Paracas Bay and Punta San Juan were added in the following sampling years, 2017 and 2018. Both study areas are in the vicinity of marine reserves or protected areas (MPAs), e.g. Paracas National Reserve and the Punta San Juan Reserve (i.e. part of the Peruvian Guano Islands, Isles and Capes National Reserve (RNSIIPG)).

Fig 4. shows that near to the southern MPA the highest POM and sediment metal concentration were measured, followed by the location in front of the northern MPA. The location near the northern MPA also exhibited slightly higher As and Pb concentrations in POM compared to some of the other locations (Fig 4). Overall, in front of (1-5 miles) and in the vicinity of (up to 12 miles) the MPAs a similar degree of metal contamination was observed, with slightly lower levels in the sampling locations further away. This pattern means that environmental contamination and stressors are reaching the MPAs and their surrounding areas. MPAs management in Peru should be re-formulated in terms of possible direct and indirect environmental impacts in their vicinity, from the south to the north.




**Note.-** **Two-way ANOVA and post-hoc Tukey multiple comparison were performed to compare different distances to the MPAs. For non-parametric data, an extension of Kruskall-Wallis test was used with the post hoc non-parametric Kruskalmc test function. Results were statistical significant when $p < 0.05$.**

**Fig 4.** Metal concentrations (µg/g dwt.) in POM and sediment in the proximity to the MPAs. The northern MPA is Illescas Reserved Zone, the center MPA is the Paracas National Reserve and the southern MPA is the Punta San Juan Reserve. In front, near and far distances refer to 1-5, 10-12 to up to 25 miles, respectively from the MPA. Different numbers indicate significant differences within distances in the north (1,2,3) and in the south (4,5), and different letters for significant differences between regions within front (a,b,c) and near (d,e) locations.

*In-situ* studies could be a useful tool and can be adapted to evaluate current "pop-up" research questions. Nevertheless, we believe that a combination of *in-situ* and *ex-situ* (lab and/or mesocosm conditions) is the most appropriate approach to disentangle cofounding variables in ecotoxicology and adjacent fields. Moreover, this *in and ex-situ* approach is the most convenient to evaluate unsteady environments, such as the NHCS that is notable affected by El Niño phenomenon . In **Chapter 3**, a battery of multi-parameters was applied on *A. purpuratus*: more than 25 chemicals were measured from its tissues and food sources (e.g. POM) which increased the complexity of the data analysis. One type (i.e. metals) of chemicals could not answer one of the main research question, e.g. Is the El Niño event affecting *A. purpuratus*' condition? This research question could only be answered by using fatty acids as biomarkers.

In case that an *ex-situ* component is logistically (~ financially) feasible, it should be performed to address specific research questions that are complementary to the *in-situ* collected data. Pectinidae species (e.g. *Pecten maximus*, *Comptopallium radula*, *Chlamys nobilis*, among others) have been studied for their metal uptake and kinetics using highly sensitive radiotracer techniques in lab conditions (Metian et al. 2008a; b; c; Pan & Wang, 2008). It is urgent to perform such studies on the Peruvian scallop *A. purpuratus* to better quantify and understand their metal bioaccumulation.

# 5. Future perspectives for marine research in Peru

## *5.1 Improving feeding practices and nutrition of Peruvians*

Health risk assessments give insights and useful information in order to estimate the health status, risks and habits of certain populations (Oremus et al. 2011). **Chapter 2 and 4** mainly focused on determining health risks (incl. health benefits in **Chapter 4**) linked to the consumption of Peruvian marine species. Both chapters used risk indices to estimate the possible adverse effect of metal ingestion through the consumption of edible, potentially-edible and non-edible species. In human health, a very precautionary approach must be considered in order to give preventive information to the decision makers (Lipfert et al. 2005).

**Chapter 2** used the maximum level (MAL) of metal in edible species for index calculations, instead of average values as was the case in **Chapter 4**. In **Chapter 2** it was concluded that for example the snail *Bursa ventricosa* could only be consumed at a rate of 0.02 kg/week, a 10-fold lower value than the calculated amount (0.2 kg/week) with the average metal concentrations of **Chapter 4**. These amounts are the safe amounts that can be consumed (per week) before a possible adverse (e.g. cancer development,…) effect can happen. A total of n=84 samples of *B. ventricosa*' edible tissues was analyzed and used for indices estimation in **Chapter 4**, while only MAL (n=1) was used in **Chapter 2**. It is noteworthy to mention that **Chapter 4** also included more precise ingestion rates for the study area, based from the frequency feeding questionnaires (FFQ; incl. anthropometrics), while **Chapter 2** used ingestion rates estimated from the seafood item available for a Peruvian person from the Food Balance Sheets (FBS) (FAO, 2016) (see Addendum section of Chapter 2).

When the estimated ingestion rates based on FFQ and FBS were compared, we found considerably differences: mollusks are ingested (or supplied) up to 35 times more (in accordance to the FBS) than the ~0.4 g/day estimated value for snail consumption using FFQ. An over-estimation could be also observed for crustaceans, up to 7-fold more elevated ingestions were calculated using FBS. However, octopus (i.e. cephalopods) consumption was under-estimated using FBS, with 0.8 g/day compared to an average of 5.0 g/day, based on FFQ. Fish consumption was compared between the national estimated consumption (~14.5 kg/yr or 39.7 g/day) and our FFQ results, a same range of 36-42 g/day was found for both estimations (PRODUCE, 2019). It is worth noting that FBS estimations and national estimated consumptions are based on the entire Peruvian (population) regions, while our FFQ were conducted in coastal (e.g. southern and northern) populations, which also explain part of the differences.

In conclusion, a good prior analysis is required to conclude and properly address actual risks. In this case MAL values would give the most protective advise to the Peruvian authorities or decision makers, i.e. SANIPES, PRODUCE, among others (see Fig 6), however this risk assessment could be over-estimated when FBS data (i.e. food item supply or availability) is used. Individual intake levels (incl. anthropometrics) from nutritional and social surveys (e.g. FFQ) for specific populations gives the most accurate information to determine the actual risks for seafood consumption in Peru. A probabilistic model (e.g. Monte Carlo simulation) that integrates the specific-individual intake and seafood nutrient/contaminant could be also an interesting methodology to determine the risk and benefit for Peruvian seafood consumption, this approach could be applied in future studies (Sioen et al. 2007; Seynaeve & Verbeke, 2017).

Further studies and interventions on improving feeding practices and nutrition of the local population on the long term are needed in Peru (see Fig 5). Chronic malnutrition in children (< 5 year) and chronic diseases in adults are still severe problems in Peru (Majluf et al. 2017; Torres-Roman et al. 2017; INEI, 2018). Risk estimations are valuable information but the *posteriori* actions are the important steps to tackle and attenuate the real problem, this can be realized 1) by providing seafood quality properties and education to researchers, doctors, fishermen and teachers at the local scale, and 2) by increasing awareness on food safety and food quality guidelines for seafood at the national government level. The combined work of public health experts and marine biologists could lead to actions (e.g. human health evaluations) at local and national level (Fig 5).

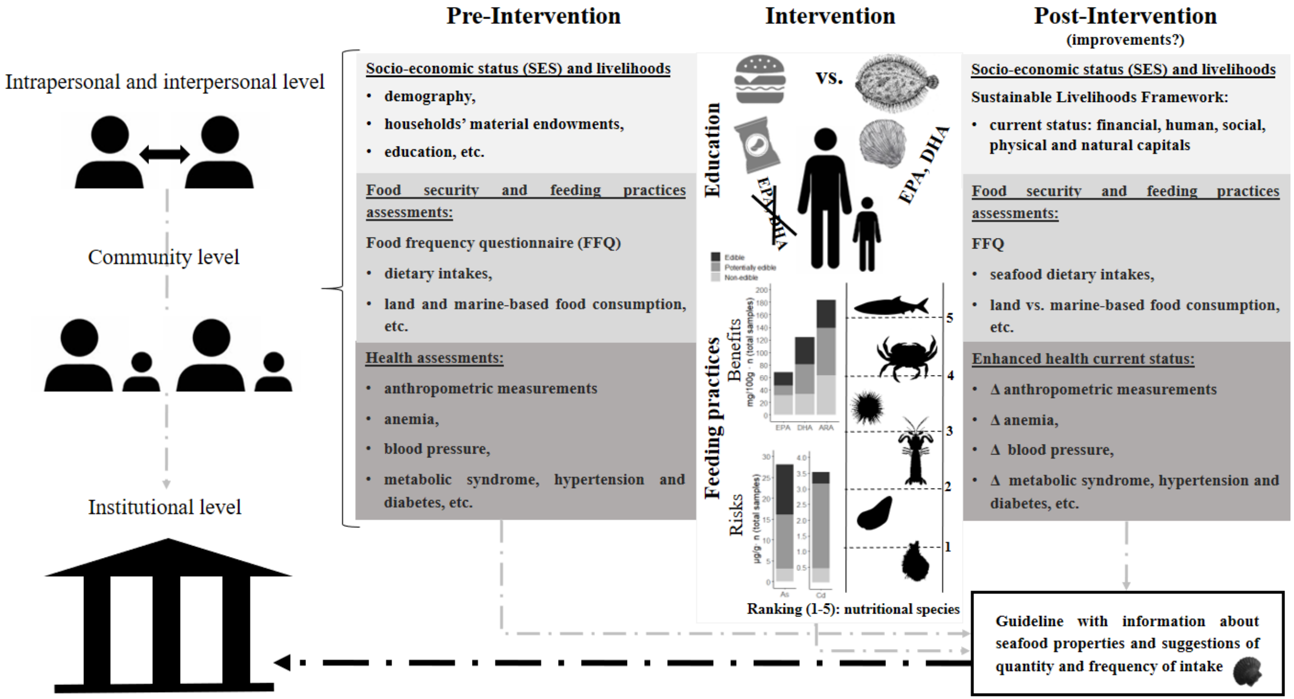
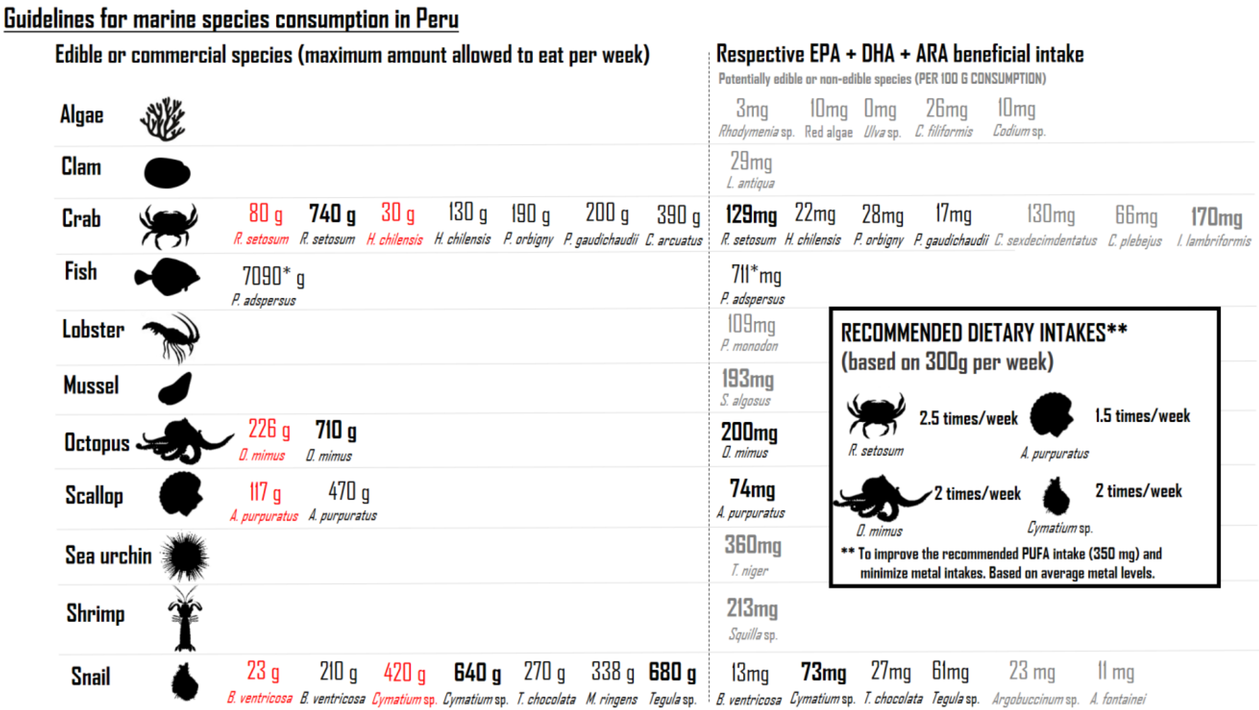



**Fig 5.** Scheme of interventions for improving feeding practices and nutrition for Peruvians.

Seafood consumption, feeding practices, perceptions on fish quality, health related topics (e.g., malnutrition and chronic diseases), factors that could contribute to the family interest and acceptance of other foods should be addressed (Katz, 2013; Laraia et al. 2013). The use of a socio-ecological framework to structure our results according to their potential sphere of influence could be implemented: i) at intrapersonal level, e.g. with issues related to knowledge and skills, ii) at interpersonal level e.g. factors influencing the social network or the interaction between community members, and family, iii) at community level, e.g. information available at a health care level or from programme promoters and iv) at institutional level, e.g. availability of actors and programmes and delivered interventions in the study area and v) at policy level, e.g. general mandates or governmental sectoral policies (incl. human health care projects) (Fig 5). As a result, all actors will be involved for the development of the guidelines for seafood consumption in Peruvian populations. Fig 6. shows a preliminary guidelines for seafood consumption based on the results obtained in this study (WFP, 2018).




**Species and maximum allowed amounts to eat per week that are printed in red were calculated using maximum metal levels (MAL), while the printed in black were calculated with average metal levels. Potentially edible and non-edible species and their respective EPA + DHA + ARA intakes that are printed in grey are based on the consumption of 100 g. (*) The flatfish *P. adspersus*' maximum amount to eat per week and respective beneficial intake were only based on inorganic-As concentrations, Cd was BDL for this species. The highest calculated values (i.e. maximum allowed amount and/or respective intake) were printed in bold.**

**Fig 6.** Guidelines for consumption of seafood from Peruvian marine domain.

## *5.2 New marine foods for alternative diets*

Beneficial compounds are also significantly present in marine species. **Chapter 4** addressed this counterpart to the risk assessments, and provides first insights into which species from the Peruvian marine ecosystem contained substantial polyunsaturated fatty acids (PUFAs) and micro-nutrient concentrations. Species with high amounts (on average; mg/100g) of 180, 100 and 40 for EPA, DHA and ARA, respectively were found along the Peruvian coast. Noting that part of these concentrations (e.g. sometimes the highest) were found in marine species that are characterized as potentially-edible and non-edible species for Peruvian consumers, suggests that consumption of at least these potentially-edible species could be promoted (Lavallée & Michele 2012; Moscoso, 2012; Uribe, 2013; Carbajal et al. 2017, 2018; IFOP, 2019; IMARPE, 2019; PRODUCE, 2019).

Food resources from land or sea ecosystems are getting depleted worldwide. The food industry has exponentially increased in recent decades because of the need to produce enormous amounts of food (Assadourian, 2010; Parodi et al. 2018). Therefore the use of alternative diets must be considered due to this non-sustainable scenario (Parodi et al. 2018). The overexploitation of resources, especially marine-based is also the reason of the abrupt decline of the biomass of some species (Rao 2002; Morato et al. 2006). The over-fishing of a few marine species as human food sources and under-utilization (e.g. discards) of others is the cause of this environmental degradation, and it has significantly been registered in fisheries historical records (e.g. Peruvian anchovy *Engraulis ringens*) (Boerema & Gulland 1973).

New marine foods (i.e. potentially and non-edible) offer possibilities to be used as alternative diets, which is the main conclusion of **Chapter 4**. However, further studies must be conducted on food processing of different marine species (i.e. sea urchin, crabs, algae…) to make them an attractive food for human consumption, as this will determine their potentiality as nutritive commercial products. Physical and chemical processes could be applied on marine products to change texture and taste, and simultaneously to reduce their content of any toxic elements (e.g. metals or other contaminants) (Hajeb et al. 2014; Piras et al. 2016). A research question approach should be applied to find the most suitable process to minimize the metal contamination in these new marine foods. In addition, it is necessary to try to give them the best sensorial properties for human consumption.

Next to their shape and best properties, the method of preparation/cooking will be important as well. Increasing temperature by baking and steaming could considerable decreased the Pb and Cd concentration in fish (Atta et al. 1997). While cooking and boiling could eliminate As up to 95% and up to 50%, respectively in algae (Hajeb et al. 2014). Preparation and proper cleaning of edible tissues (e.g. remove of kidney, hepatopancreas) also considerably reduced the metal contents in mollusk bivalves (Bach et al. 2014). On the other hand, ethylenediaminetetraacetic acid (EDTA) and cysteine are chemicals (i.e. chelating agents) with the highest potential application of the industrial removal of toxic elements (Hajeb and Jinap 2012; Hajeb et al. 2014). Raw Mackerel fish fillets were dipped in the mixed solution (i.e. cysteine, EDTA, hydrochloric acid, sodium hydroxide, and salt) for different durations and time. The optimum conditions for up to 91% Hg reduction were achieved using response surface methodology (RSM) at cysteine concentration of

1.25%, EDTA of 275 mg/L, NaCl of 0.5%, pH of 3.75, and exposure time of 18 min (Hajeb and Jinap 2012).

From the present study, the most promising new marine foods were the crabs *Cycloxanthops sexdecimdentatus* and *Cancer plebejus*, because of their beneficial chemical profile and relatively clean of potentially harmful (e.g. As, Cd) metals. The sea urchin *Tetrapigus niger*, the crab *Inachoides lambriformis*, the mussel *Semimytilus algosus*, mantis shrimp *Squilla* sp. and the highly abundant squat lobster *Pleuroncodes monodon* contained high PUFA concentrations but also Cd concentrations, which made everything a bit more complex to select them as suitable food (Fig 7).

When these new marine foods are compared to land-based (meat) foods, the EPA + DHA concentrations are mostly higher, up to ~190-fold (see Fig 7). Their use as edible species could be possible after testing by an appropriate process for metal depuration and/or sequestration. It is worth to mention that the previous physical (e.g. thermal treatment) and chemical processes could also alter the beneficial compounds, e.g. PUFAs (Piras et al. 2016). Subsequently the new marine foods could be tested as safe and acceptable (i.e. certified) for human consumption. Ecosystem-based management studies must be conducted prior to their exploitation in order to avoid and repeat previous experiences of high resource exploitation (e.g. as most big fisheries from the last decades).

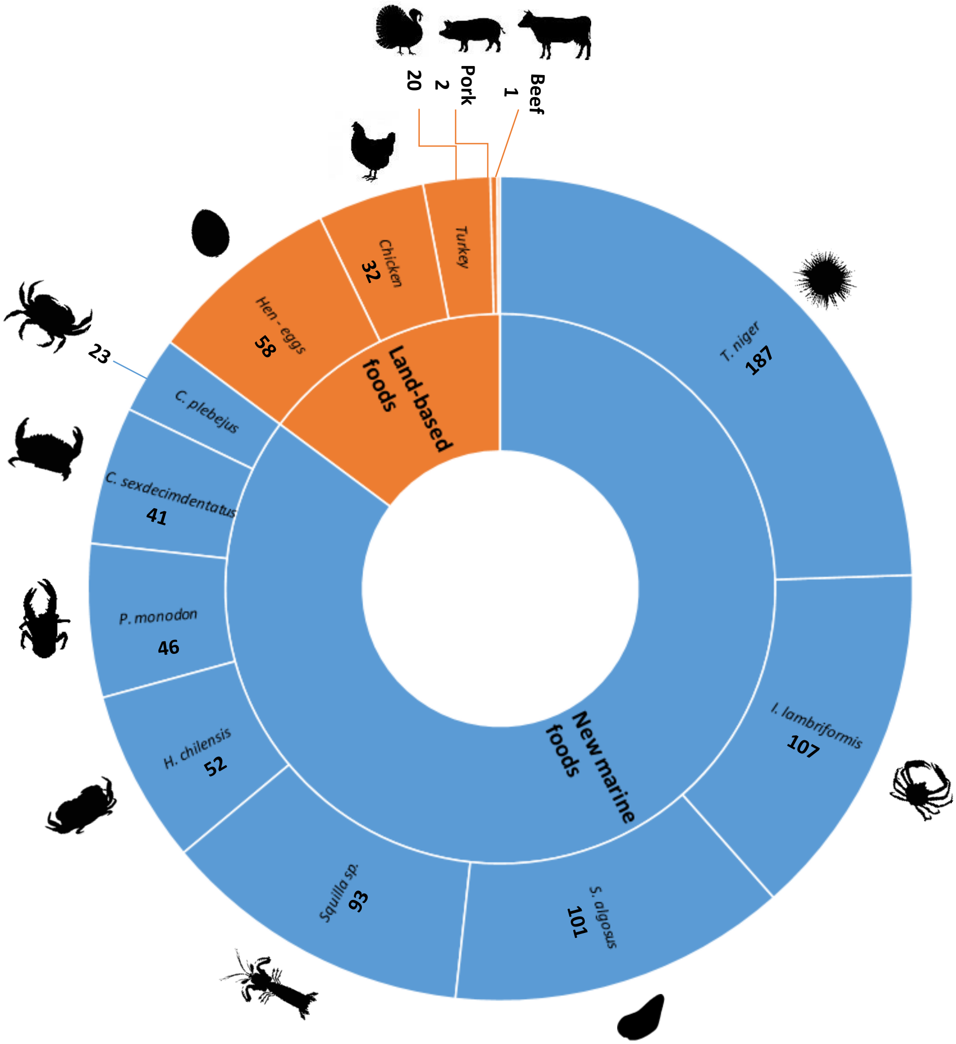



**Fig 7.** EPA + DHA concentrations (mg/100g wwt.) for the new marine foods and land-based foods. Land-based EPA and DHA concentrations were based on Tacon & Metian, (2013).

## *5.3 Bio-monitoring and management study programs in Peru*

Metal analysis in the monitoring program of mollusk bivalves (PCBM) in Peru is conducted semi-annually (Loaiza et al. 2015). This monitoring is mainly focused on the analysis of *A. purpuratus*' edible tissues, and in some cases water and sediment are also analyzed and reported (SANIPES, 2019). In accordance to the last reports (i.e. 2017-2018), more commercial species have been added for metal screening of their edible tissues, e.g. limpet *Fissurella* sp., mussel *Aulacomya atra,* sea urchin *Loxechinus albus*, among others.

Nevertheless, the metal contamination studies and/or reports are hard to find in the Organismo Nacional de Sanidad Pesquera (SANIPES), and no scientific documents have been published to our knowledge. (see SANIPES, 2019). The recent modifications of the PCBM have been influenced by serious concerns due to Cd contamination in aquatic and/or terrestrial foodstuffs from Peru, as well as the presence of other metals, such as Hg and As in marine environments (Loaiza et al. 2015; 2018; 2020; Vallegos, 2015; Barriga-Sánchez and Pariasca, 2018; DIGESA, 2018).

**Chapter 3** gives an example of how to develop a research study using the main species (*A. purpuratus*) of the monitoring program of SANIPES. In this study, scallop *A. purpuratus* was used as a potential bioindicator species in order to monitor Peruvian marine ecosystems. Ecosystems, such as Sechura Bay and Paracas Bay with relevant Peruvian scallop aquaculture were sampled and studied during crucial periods (i.e. El Niño events) by Peruvian marine researchers. A short-period (2 yrs.) bio-monitoring was planned and implemented successfully. The main conclusion from that study was that the metal contamination and El Niño effect could differently change the biochemical composition of *A. purpuratus*. As previously mentioned, the budget to expend for this type of study or monitoring was about 1 740 € · area · yr, considered as 'optimal' (see Fig 3).

The ideal is that preliminary (or historical) monitoring results or studies are analyzed by the authorities (e.g. SANIPES. IMARPE) in order to determine a potential bioindicator species for Peruvian marine waters. Subsequently, priority should be given to these species and more importance must be given to those that provide a good food quality and safety (incl. future foods). As result, a dual approach of data and effort (~financial) could be performed by using multiple bioindicator species that are also important for human nutrition. An ecosystem-based management study could be built from this multi(bioindicator)-species approach when food sources (plankton, seston, sediments,…) and other species are added in accordance to their trophic position (Fig 3).

**Chapter 5** focused on the study of different Peruvian marine ecosystems, however only the benthic communities (with the exception of the squat lobster *P. monodon*) were analyzed in this chapter. The benthic-pelagic component was missing in the food web analysis of **Chapter 5**. In Peru, studies on habitat benthic-pelagic coupling are lacking, except for the recent tropho-dynamic study of Espinoza et al. (2017). A Chilean study in nearby areas of Peru concluded that benthic-pelagic trophic interactions are highly important in the NHCS ecosystems, this is due to the upwelling-governed marine system (Docmac et al. 2017).

The main reason for the lack of benthic-pelagic studies is that investigations mainly focus on single-species because of their economic and individual importance and are therefore never interconnected, e.g. numerous oceanographic ‘pelagic’ studies on *E. ringens*, vs. numerous studies on *A. purpuratus* ecology exist (Mendo et al. 2016; Moron et al. 2019). In Fig 8. a trophic food web of a Peruvian marine ecosystem was built (i.e. modified from the SIAR-modelled ‘global’ matrix from Chapter 5) to understand the interactions of the Peruvian scallop *A. purpuratus* with other functional or taxonomic groups and species. This more complex and ‘complete’ food web highlighted again the importance of *A. purpuratus*, but also other species such as the macroalgae *C. filiformis*, considered as invasor species in Peruvian scallop culture (IMARPE, 2019). *C. filiformis* seems to be highly important (up to 78% contribution) on the diet of herviborous gastropods and sea urchins (Fig 8, and Table 1; supplementary material).

This food web also revealed the high contribution (~ 60%) of scallops and filter-feeders on the diet of sea stars and the octopod (see Fig 8). For predatory gastropods and crabs, the scallop is also the most important diet, leaving the sea stars and sea urchins as the least contributors (≤ 10%) to their diet. The only studied predatory benthic fish, *P. adspersus* exhibited more preferences for crabs and detritivorous species than the filter-feeding (incl. scallops) species in its diet (Fig 8, and Table 1; supplementary material). It is interesting this ‘big picture’ of this Peruvian benthic ecosystem, however the bentho-pelagic interconnection part of this food web was not included, further studies including new models (e.g. ECOSIM model,...) need to integrate the different compartments (incl. contaminants) of the ecosystem under study.

Peruvian marine ecosystems are still rich and abundant in living and non-living resources. This positive aspect could also be a threat for the conservation of these ecosystems. Over-exploitation of Peruvian domain resources is likely to happen when no precautionary management plan is considered and installed. Previous experiences from Peruvian anchovy and Peruvian scallop are *"lesson-learnt"* of the intensive and non-planned exploitation of marine resources. In the meantime, these species were also highly impacted by the 1972 El Niño: Peruvian anchovy, and by El Niño 82/83 and 97/98: Peruvian scallop, resulting in the collapse of both fisheries. This complex NHCS productive ecosystem is well-known because it is hardly affected by the mentioned climate variabilities (e.g. El Niño, among others). Therefore, scientific ecosystem-based research is urgently needed in this changing and unpredictable ecosystems. We could conclude that the Peruvian scallop is a promising bioindicator species for El Niño and contamination scenarios, this species can be used to predict and understand what is ongoing in Peruvian marine environments.

Metal contamination is a serious concern in coastal and land ecosystems of Peru, as indicated by the environmental metal levels reported in the present study. Anthropogenic activities, specifically mining could be the major vector of metal contamination in fresh, brackish and marine ecosystems of Peru. At the same time, marine species from the NHCS-driven ecosystem could also become the most important source of beneficial elements/compounds for Peruvian populations. We could confirm this because we found substantial contents of PUFAs and micro-nutrients (Fe, Cu, Zn, Mn) in marine species (edible or not edible) from different regions of Peru. However, caution is necessary since the marine ecosystems are also affected by anthropogenic activities (e.g. mining), and some of these marine resources are also full of potentially harmful metals (e.g. Cd). A national plan and integration of information from the Peruvian institutions (e.g. IMARPE, SANIPES, PRODUCE,…) should be implemented in order to protect and preserve Peruvian marine ecosystems (e.g. pelagic-benthic coupling approach) as one entity. Marine living resources could be in danger in the near future, for their over-exploitation and depletion, as well as they can become un-utilized resources due to the elevated human risks that could result from consuming them.

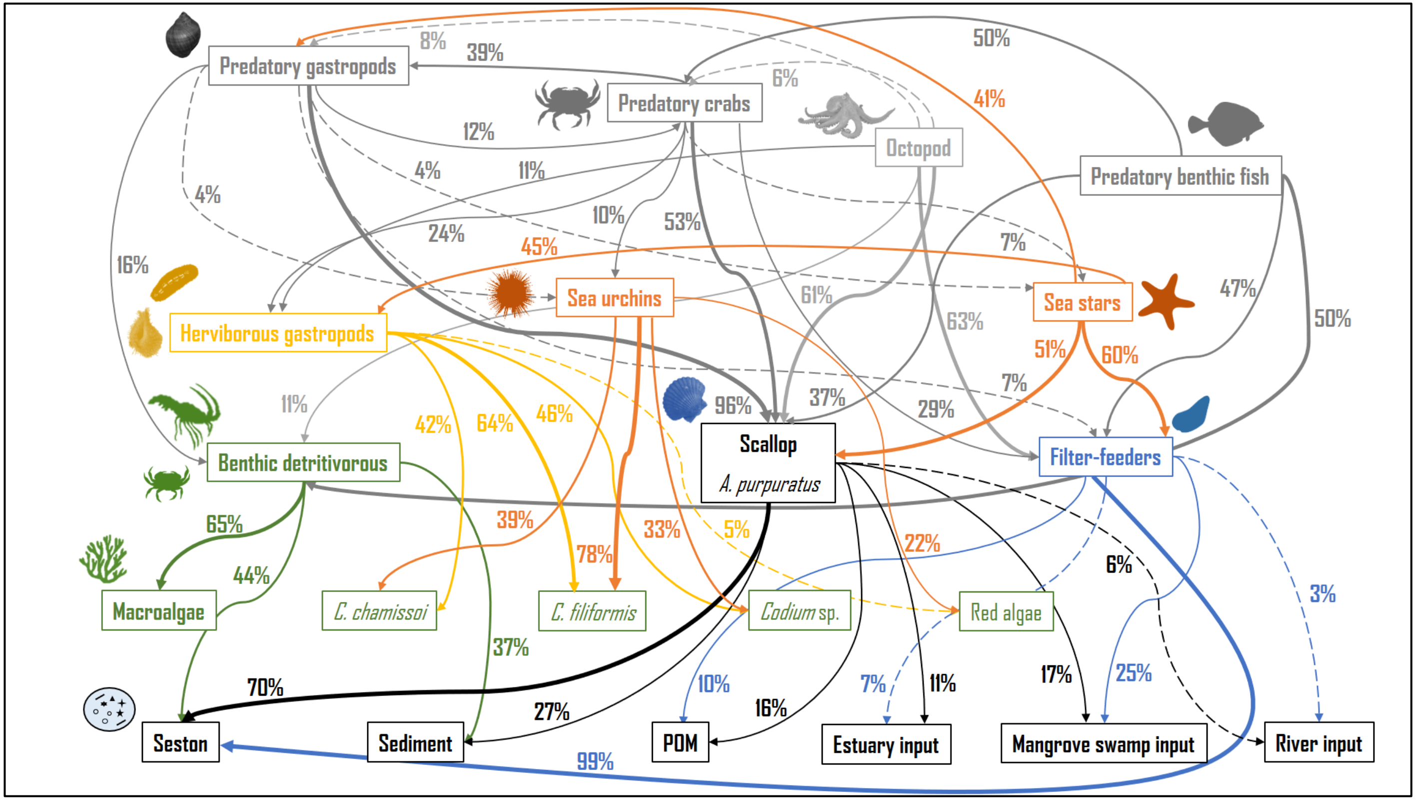



**Fig 8.** Food web structure of benthic community in a Peruvian marine ecosystem by functional and taxonomic groups, and species.

**Note.- Different colours were used for each functional or taxonomic groups, black for scallop and food sources. The diet contributions (%) per species are related to the thickness of the line. The minimum (<10%) diet contributions are showed in dashed lines. Results are based on SIAR-mixing ('global') model. The species per functional or taxonomic group are described in Table 1; supplementary material.**

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# ADDENDUM I

Supplementary material to Chapter 2

**Table 1.** Minimum (MIL), average (AVL) and maximum (MAL) levels of inorganic As in muscle (incl. edible tissue for *A. purpuratus*), respective EDI, weekly intake, %PTWI and amount (kg) of muscle to reach the PTWI of six shellfish species, and from Sechura Bay and front Illescas Reserved Zone

| | | | | **Metal (µg/g wwt)** | | **EDI** | | **Weekly intake (µg/week)** | | **%PTWI** | | **Amount (kg) of muscle to reach the PTWI** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | **Women (50 kg)** | **Men (70 kg)** | **Women (50 kg)** | **Men (70 kg)** |
| Location | Species | | | January (low-raining season) | March (high-raining season) | January (low-raining season) | March (high-raining season) | January (low-raining season) | March (high-raining season) | January (low-raining season) | March (high-raining season) | January (low-raining season) | | March (high-raining season) | |
| Illescas Reserved Zone | *Octopus mimus* ♂ | muscle | MIL | 0.38 | 0.37 | 0.005 | 0.005 | 2.04 | 1.97 | 0.24 | 0.23 | 1.84 | 2.58 | 1.90 | 2.67 |
| | | | AVL | 0.91 | 0.99 | 0.012 | 0.013 | 4.89 | 5.30 | 0.58 | 0.63 | 0.77 | 1.08 | 0.71 | 0.99 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MAL | 1.99 | 2.67 | 0.025 | 0.034 | 10.7 | 14.3 | 1.27 | 1.70 | 0.35 | 0.49 | 0.26 | 0.37 |
| *Octopus mimus* ♀ | muscle | MIL | 0.46 | 0.24 | 0.006 | 0.003 | 2.49 | 1.29 | 0.30 | 0.15 | 1.51 | 2.11 | 2.91 | 4.08 |
| | | AVL | 1.12 | 0.51 | 0.014 | 0.007 | 6.02 | 2.76 | 0.72 | 0.33 | 0.62 | 0.87 | 1.36 | 1.91 |
| | | MAL | 2.03 | 0.74 | 0.026 | 0.009 | 10.9 | 4.00 | 1.30 | 0.47 | 0.34 | 0.48 | 0.95 | 1.33 |
| *Romaleon setosum* ♂ | muscle | MIL | 0.42 | | 0.020 | | 8.35 | | 0.99 | | 1.66 | 2.32 | | |
| | | AVL | 0.78 | | 0.036 | | 15.3 | | 1.82 | | 0.90 | 1.26 | | |
| | | MAL | 1.64 | | 0.077 | | 32.4 | | 3.86 | | 0.43 | 0.60 | | |
| *Hepatus chilensis* ♂ | muscle | MIL | | 0.29 | | 0.013 | | 5.66 | | 0.67 | | | 2.44 | 3.42 |
| | | AVL | | 1.23 | | 0.058 | | 24.3 | | 2.89 | | | 0.57 | 0.80 |
| | | MAL | | 1.91 | | 0.090 | | 37.8 | | 4.50 | | | 0.37 | 0.51 |
| *Hepatus chilensis* ♀ (no ovi) | muscle | MIL | | 0.32 | | 0.015 | | 6.32 | | 0.75 | | | 2.19 | 3.06 |
| | | AVL | | 1.09 | | 0.051 | | 21.6 | | 2.57 | | | 0.64 | 0.90 |
| | | MAL | | 2.53 | | 0.119 | | 50.0 | | 5.95 | | | 0.28 | 0.39 |
| *Hepatus chilensis* ♀ (ovi) | muscle | MIL | | 0.51 | | 0.024 | | 10.1 | | 1.20 | | | 1.38 | 1.93 |
| | | AVL | | 0.95 | | 0.045 | | 18.8 | | 2.24 | | | 0.73 | 1.03 |
| | | MAL | | 1.95 | | 0.092 | | 38.6 | | 4.59 | | | 0.36 | 0.50 |
| *Bursa ventricosa* | muscle | MIL | 3.27 | 1.48 | 0.759 | 0.343 | 319 | 144 | 37.9 | 17.2 | 0.21 | 0.30 | 0.47 | 0.66 |
| | | AVL | 6.92 | 6.56 | 1.605 | 1.523 | 674 | 640 | 80.3 | 76.1 | 0.10 | 0.14 | 0.11 | 0.15 |
| | | MAL | 10.1 | 9.83 | 2.349 | 2.280 | 987 | 958 | 117 | 114 | 0.07 | 0.10 | 0.07 | 0.10 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Cymatium sp.* | muscle | MIL | | 0.17 | | 0.039 | | 16.4 | | 1.95 | | | 4.16 | 5.83 |
| | | | AVL | | 0.33 | | 0.077 | | 32.2 | | 3.83 | | | 2.12 | 2.97 |
| | | | MAL | | 0.65 | | 0.152 | | 63.7 | | 7.58 | | | 1.07 | 1.50 |
| | *Argopecten purpuratus* | muscle | MIL | 0.06 | 0.05 | 0.014 | 0.012 | 5.99 | 4.91 | 0.71 | 0.58 | 11.4 | 16.0 | 13.9 | 19.4 |
| | | | AVL | 0.07 | 0.06 | 0.017 | 0.014 | 6.97 | 5.96 | 0.83 | 0.71 | 9.78 | 13.7 | 11.5 | 16.0 |
| | | | MAL | 0.09 | 0.07 | 0.022 | 0.015 | 9.16 | 6.41 | 1.09 | 0.76 | 7.45 | 10.4 | 10.6 | 14.9 |
| | | edible tissue* | MIL | 0.18 | 0.14 | 0.042 | 0.032 | 17.8 | 13.3 | 2.12 | 1.59 | 3.83 | 5.36 | 5.12 | 7.17 |
| | | | AVL | 0.20 | 0.16 | 0.046 | 0.036 | 19.2 | 15.3 | 2.29 | 1.82 | 3.55 | 4.97 | 4.46 | 6.25 |
| | | | MAL | 0.22 | 0.17 | 0.052 | 0.040 | 21.8 | 16.7 | 2.60 | 1.99 | 3.12 | 4.37 | 4.09 | 5.73 |
| Southern location | *Octopus mimus* ♂ | muscle | MIL | | 0.21 | | 0.003 | | 1.13 | | 0.13 | | | 3.34 | 4.67 |
| | | | AVL | | 0.59 | | 0.008 | | 3.18 | | 0.38 | | | 1.18 | 1.66 |
| | | | MAL | | 0.92 | | 0.012 | | 4.95 | | 0.59 | | | 0.76 | 1.06 |
| | *Octopus mimus* ♀ | muscle | MIL | | 0.37 | | 0.005 | | 2.00 | | 0.24 | | | 1.88 | 2.63 |
| | | | AVL | | 0.52 | | 0.007 | | 2.80 | | 0.33 | | | 1.34 | 1.88 |
| | | | MAL | | 0.73 | | 0.009 | | 3.91 | | 0.47 | | | 0.96 | 1.35 |
| | *Romaleon setosum* | muscle | MIL | 0.94 | 0.65 | 0.044 | 0.031 | 18.6 | 12.9 | 2.21 | 1.53 | 0.74 | 1.04 | 1.07 | 1.50 |
| | | | AVL | 2.69 | 0.89 | 0.127 | 0.042 | 53.2 | 17.6 | 6.33 | 2.10 | 0.26 | 0.36 | 0.78 | 1.10 |
| | | | MAL | 8.94 | 1.08 | 0.420 | 0.051 | 177 | 21.4 | 21.0 | 2.55 | 0.08 | 0.11 | 0.65 | 0.91 |
| | *Romaleon setosum* ♀ (no ovi) | muscle | MIL | 0.23 | | 0.011 | | 4.60 | | 0.55 | | 3.01 | 4.21 | | |
| | | | AVL | 0.57 | | 0.027 | | 11.2 | | 1.34 | | 1.23 | 1.72 | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MAL | 0.73 | | 0.034 | | 14.4 | | 1.71 | | 0.96 | 1.34 | | |
| *Romaleon setosum* ♀ (ovi) | muscle | MIL | 0.43 | | 0.020 | | 8.55 | | 1.02 | | 1.62 | 2.26 | | |
| | | AVL | 0.87 | | 0.041 | | 17.2 | | 2.04 | | 0.81 | 1.13 | | |
| | | MAL | 2.00 | | 0.094 | | 39.6 | | 4.71 | | 0.35 | 0.49 | | |
| *Hepatus chilensis* ♀ (no ovi) | muscle | MIL | 0.37 | 0.38 | 0.017 | 0.018 | 7.28 | 7.59 | 0.87 | 0.90 | 1.90 | 2.66 | 1.82 | 2.55 |
| | | AVL | 0.47 | 0.73 | 0.022 | 0.035 | 9.23 | 14.5 | 1.10 | 1.73 | 1.50 | 2.10 | 0.95 | 1.34 |
| | | MAL | 0.64 | 0.97 | 0.030 | 0.046 | 12.7 | 19.1 | 1.51 | 2.28 | 1.09 | 1.52 | 0.72 | 1.01 |
| *Hepatus chilensis* ♀ *(ovi)* | muscle | MIL | 0.42 | | 0.020 | | 8.29 | | 0.99 | | 1.67 | 2.33 | | |
| | | AVL | 0.65 | | 0.031 | | 12.9 | | 1.53 | | 1.07 | 1.50 | | |
| | | MAL | 1.08 | | 0.051 | | 21.3 | | 2.54 | | 0.65 | 0.91 | | |
| *Bursa ventricosa* | muscle | MIL | 0.39 | 0.53 | 0.090 | 0.124 | 37.6 | 52.0 | 4.48 | 6.19 | 1.81 | 2.54 | 1.31 | 1.84 |
| | | AVL | 0.70 | 1.40 | 0.162 | 0.324 | 68.2 | 136 | 8.12 | 16.2 | 1.00 | 1.40 | 0.50 | 0.70 |
| | | MAL | 1.03 | 3.69 | 0.238 | 0.856 | 99.9 | 360 | 11.9 | 42.8 | 0.68 | 0.96 | 0.19 | 0.27 |
| *Cymatium sp.* | muscle | MIL | | 0.23 | | 0.054 | | 22.8 | | 2.71 | | | 3.00 | 4.20 |
| | | AVL | | 0.49 | | 0.114 | | 47.9 | | 5.70 | | | 1.42 | 1.99 |
| | | MAL | | 0.94 | | 0.218 | | 91.7 | | 10.9 | | | 0.74 | 1.04 |
| *Argopecten purpuratus* | muscle | MIL | 0.04 | 0.06 | 0.010 | 0.014 | 4.35 | 6.06 | 0.52 | 0.72 | 15.7 | 21.9 | 11.2 | 15.7 |
| | | AVL | 0.06 | 0.07 | 0.013 | 0.016 | 5.41 | 6.86 | 0.64 | 0.82 | 12.6 | 17.7 | 9.94 | 13.9 |
| | | MAL | 0.06 | 0.08 | 0.014 | 0.018 | 6.00 | 7.72 | 0.71 | 0.92 | 11.4 | 15.9 | 8.83 | 12.4 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | edible tissue* | MIL | 0.11 | 0.14 | 0.026 | 0.032 | 11.1 | 13.3 | 1.32 | 1.58 | 6.14 | 8.60 | 5.14 | 7.20 |
| | | | AVL | 0.17 | 0.16 | 0.039 | 0.038 | 16.5 | 15.8 | 1.96 | 1.88 | 4.15 | 5.80 | 4.32 | 6.05 |
| | | | MAL | 0.20 | 0.20 | 0.047 | 0.047 | 19.7 | 19.7 | 2.34 | 2.34 | 3.47 | 4.86 | 3.47 | 4.86 |
| Northern location | *Bursa ventricosa* | muscle | MIL | 1.90 | 0.66 | 0.440 | 0.154 | 185 | 64.8 | 22.0 | 7.71 | 0.37 | 0.52 | 1.05 | 1.47 |
| | | | AVL | 3.60 | 1.20 | 0.835 | 0.278 | 351 | 117 | 41.7 | 13.9 | 0.19 | 0.27 | 0.58 | 0.82 |
| | | | MAL | 6.31 | 1.73 | 1.463 | 0.402 | 615 | 169 | 73.2 | 20.1 | 0.11 | 0.16 | 0.40 | 0.57 |
| | *Cymatium sp.* | muscle | MIL | 0.13 | 0.17 | 0.030 | 0.040 | 12.6 | 17.0 | 1.50 | 2.02 | 5.41 | 7.57 | 4.01 | 5.62 |
| | | | AVL | 0.15 | 0.24 | 0.034 | 0.057 | 14.5 | 23.8 | 1.72 | 2.84 | 4.71 | 6.59 | 2.86 | 4.00 |
| | | | MAL | 0.20 | 0.30 | 0.046 | 0.068 | 19.3 | 28.8 | 2.30 | 3.42 | 3.53 | 4.94 | 2.37 | 3.32 |
| | *Argopecten purpuratus* | muscle | MIL | 0.05 | 0.06 | 0.011 | 0.014 | 4.50 | 5.76 | 0.54 | 0.69 | 15.2 | 21.2 | 11.9 | 16.6 |
| | | | AVL | 0.06 | 0.09 | 0.015 | 0.022 | 6.29 | 9.13 | 0.75 | 1.09 | 10.9 | 15.2 | 7.47 | 10.5 |
| | | | MAL | 0.07 | 0.12 | 0.017 | 0.028 | 6.93 | 11.8 | 0.83 | 1.41 | 9.83 | 13.8 | 5.77 | 8.08 |
| | | edible tissue* | MIL | 0.13 | 0.15 | 0.030 | 0.035 | 12.6 | 14.8 | 1.50 | 1.76 | 5.41 | 7.57 | 4.62 | 6.47 |
| | | | AVL | 0.16 | 0.21 | 0.038 | 0.049 | 15.8 | 20.8 | 1.89 | 2.47 | 4.31 | 6.03 | 3.28 | 4.60 |
| | | | MAL | 0.19 | 0.28 | 0.044 | 0.066 | 18.6 | 27.7 | 2.21 | 3.30 | 3.67 | 5.14 | 2.46 | 3.45 |
| | *Argopecten purpuratus* (transplanted experiment) | muscle | MIL | | 0.09 | | 0.020 | | 8.30 | | 0.99 | | | 8.21 | 11.5 |
| | | | AVL | | 0.10 | | 0.023 | | 9.67 | | 1.15 | | | 7.05 | 9.87 |
| | | | MAL | | 0.12 | | 0.029 | | 12.0 | | 1.43 | | | 5.68 | 7.95 |
| | | edible tissue* | MIL | | 0.17 | | 0.040 | | 16.8 | | 2.00 | | | 4.07 | 5.69 |
| | | | AVL | | 0.20 | | 0.046 | | 19.5 | | 2.32 | | | 3.51 | 4.91 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MAL | 0.22 | 0.050 | 21.2 | 2.52 | | 3.22 | 4.50 |

**(*) Edible tissue is considered as adductor muscle + gonad in *A. purpuratus*. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**

**Table 2.** Minimum (MIL), average (AVL) and maximum (MAL) levels of Cd in muscle (incl. edible tissue for *A. purpuratus*), respective EDI, weekly intake, %PTWI and amount (kg) of muscle to reach the PTWI of six shellfish species, and from Sechura Bay and front Illescas Reserved Zone

| | | | | **Metal (µg/g wwt)** | | **EDI (ug/kg/day)** | | **Weekly intake (ug/week)** | | **%PTWI** | | **Amount (kg) of edible tissue to reach PTWI** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | **Women (50 kg)** | **Men (70 kg)** | **Women (50 kg)** | **Men (70 kg)** |
| Location | Species | | | January (low-raining season) | March (high-raining season) | January (low-raining season) | March (high-raining season) | January (low-raining season) | March (high-raining season) | January (low-raining season) | March (high-raining season) | January (low-raining season) | | March (high-raining season) | |
| Illescas Reserved Zone | *Octopus mimus* ♂ | muscle | MIL | 0.02 | 0.04 | 0.0002 | 0.0005 | 0.10 | 0.22 | 0.07 | 0.15 | 6.80 | 9.52 | 3.03 | 4.24 |
| | | | AVL | 0.04 | 0.06 | 0.0005 | 0.0008 | 0.20 | 0.33 | 0.13 | 0.22 | 3.32 | 4.65 | 2.07 | 2.89 |
| | | | MAL | 0.06 | 0.09 | 0.0007 | 0.0011 | 0.31 | 0.47 | 0.20 | 0.32 | 2.19 | 3.07 | 1.42 | 1.98 |
| | *Octopus mimus* ♀ | muscle | MIL | 0.03 | 0.02 | 0.0004 | 0.0003 | 0.15 | 0.14 | 0.10 | 0.09 | 4.35 | 6.08 | 4.84 | 6.78 |
| | | | AVL | 0.08 | 0.05 | 0.0006 | 0.0007 | 0.26 | 0.29 | 0.17 | 0.19 | 2.62 | 3.66 | 2.33 | 3.26 |
| | | | MAL | 0.05 | 0.18 | 0.0010 | 0.0023 | 0.43 | 0.95 | 0.28 | 0.63 | 1.58 | 2.21 | 0.71 | 0.99 |
| | *Romaleon setosum* ♂ | muscle | MIL | 0.01 | | 0.0005 | | 0.21 | | 0.14 | | 12.0 | 16.8 | | |
| | | | AVL | 0.03 | | 0.0012 | | 0.52 | | 0.35 | | 4.76 | 6.67 | | |
| | | | MAL | 0.08 | | 0.0037 | | 1.56 | | 1.04 | | 1.58 | 2.22 | | |
| | *Hepatus chilensis* ♂ | muscle | MIL | | 0.10 | | 0.0049 | | 2.06 | | 1.37 | | | 1.20 | 1.68 |
| | | | AVL | | 1.43 | | 0.0673 | | 28.3 | | 18.9 | | | 0.09 | 0.12 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | MAL | | 4.79 | | 0.2251 | | 94.6 | | 63.0 | | | 0.03 | 0.04 |
| | *Hepatus chilensis* ♀ *(no ovi)* | muscle | MIL | | 0.29 | | 0.0135 | | 5.66 | | 3.77 | | | 0.44 | 0.61 |
| | | | AVL | | 1.27 | | 0.0598 | | 25.1 | | 16.7 | | | 0.10 | 0.14 |
| | | | MAL | | 1.94 | | 0.0914 | | 38.4 | | 25.6 | | | 0.06 | 0.09 |
| | *Hepatus chilensis* ♀ *(ovi)* | muscle | MIL | | 0.60 | | 0.0282 | | 11.8 | | 7.89 | | | 0.21 | 0.29 |
| | | | AVL | | 1.62 | | 0.0763 | | 32.1 | | 21.4 | | | 0.08 | 0.11 |
| | | | MAL | | 3.45 | | 0.1620 | | 68.1 | | 45.4 | | | 0.04 | 0.05 |
| | *Bursa ventricosa* | muscle | MIL | 0.64 | 0.65 | 0.1476 | 0.1516 | 62.0 | 63.7 | 41.3 | 42.5 | 0.20 | 0.27 | 0.19 | 0.27 |
| | | | AVL | 1.42 | 1.95 | 0.3292 | 0.4534 | 138 | 190 | 92.2 | 127 | 0.09 | 0.12 | 0.06 | 0.09 |
| | | | MAL | 3.12 | 5.43 | 0.7245 | 1.2600 | 304 | 529 | 203 | 353 | 0.04 | 0.06 | 0.02 | 0.03 |
| | *Cymatium sp.* | muscle | MIL | | 0.17 | | 0.0389 | | 16.3 | | 10.9 | | | 0.75 | 1.04 |
| | | | AVL | | 0.20 | | 0.0467 | | 19.6 | | 13.1 | | | 0.62 | 0.87 |
| | | | MAL | | 0.26 | | 0.0605 | | 25.4 | | 16.9 | | | 0.48 | 0.67 |
| | *Argopecten purpuratus* | muscle | MIL | 0.10 | 0.08 | 0.0237 | 0.0188 | 9.96 | 7.9 | 6.64 | 5.28 | 1.22 | 1.71 | 1.54 | 2.15 |
| | | | AVL | 0.17 | 0.13 | 0.0386 | 0.0297 | 16.2 | 12.5 | 10.8 | 8.31 | 0.75 | 1.05 | 0.98 | 1.37 |
| | | | MAL | 0.23 | 0.15 | 0.0539 | 0.0356 | 22.7 | 14.9 | 15.1 | 9.96 | 0.54 | 0.75 | 0.82 | 1.14 |
| | | edible tissue* | MIL | 0.18 | 0.25 | 0.0409 | 0.0569 | 17.2 | 23.9 | 11.4 | 15.9 | 0.71 | 0.99 | 0.51 | 0.71 |
| | | | AVL | 0.30 | 0.39 | 0.0693 | 0.0905 | 29.1 | 38.0 | 19.4 | 25.3 | 0.42 | 0.59 | 0.32 | 0.45 |
| | | | MAL | 0.38 | 0.66 | 0.0889 | 0.1524 | 37.4 | 64.0 | 24.9 | 42.7 | 0.33 | 0.46 | 0.19 | 0.27 |
| Southern location | *Octopus mimus* ♂ | muscle | MIL | | 0.02 | | 0.0002 | | 0.09 | | 0.06 | | | 7.34 | 10.3 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AVL | | 0.03 | | 0.0004 | | 0.16 | | 0.11 | | | 4.19 | 5.87 |
| | | MAL | | 0.04 | | 0.0005 | | 0.20 | | 0.13 | | | 3.37 | 4.71 |
| *Octopus mimus* ♀ | muscle | MIL | | 0.02 | | 0.0002 | | 0.08 | | 0.05 | | | 8.15 | 11.41 |
| | | AVL | | 0.02 | | 0.0003 | | 0.13 | | 0.09 | | | 5.09 | 7.12 |
| | | MAL | | 0.03 | | 0.0004 | | 0.16 | | 0.11 | | | 4.11 | 5.76 |
| *Romaleon setosum* | muscle | MIL | 0.08 | 0.16 | 0.0037 | 0.0077 | 1.56 | 3.22 | 1.04 | 2.15 | 1.58 | 2.21 | 0.77 | 1.07 |
| | | AVL | 0.12 | 0.24 | 0.0057 | 0.0111 | 2.39 | 4.66 | 1.59 | 3.11 | 1.04 | 1.45 | 0.53 | 0.74 |
| | | MAL | 0.18 | 0.37 | 0.0086 | 0.0172 | 3.59 | 7.23 | 2.39 | 4.82 | 0.69 | 0.96 | 0.34 | 0.48 |
| *Romaleon setosum* ♀*(no ovi)* | muscle | MIL | 0.03 | | 0.0013 | | 0.53 | | 0.35 | | 4.70 | 6.58 | | |
| | | AVL | 0.07 | | 0.0031 | | 1.31 | | 0.87 | | 1.89 | 2.65 | | |
| | | MAL | 0.12 | | 0.0054 | | 2.28 | | 1.52 | | 1.08 | 1.51 | | |
| *Romaleon setosum* ♀ *(ovi)* | muscle | MIL | 0.03 | | 0.0013 | | 0.53 | | 0.35 | | 4.68 | 6.56 | | |
| | | AVL | 0.11 | | 0.0051 | | 2.16 | | 1.44 | | 1.15 | 1.60 | | |
| | | MAL | 0.39 | | 0.0182 | | 7.66 | | 5.11 | | 0.32 | 0.45 | | |
| *Hepatus chilensis* ♀*(no ovi)* | muscle | MIL | 0.25 | 0.33 | 0.0117 | 0.0157 | 4.92 | 6.59 | 3.28 | 4.39 | 0.50 | 0.70 | 0.37 | 0.52 |
| | | AVL | 0.52 | 0.84 | 0.0243 | 0.0395 | 10.2 | 16.6 | 6.80 | 11.1 | 0.24 | 0.34 | 0.15 | 0.21 |
| | | MAL | 0.71 | 1.69 | 0.0336 | 0.0794 | 14.1 | 33.4 | 9.40 | 22.2 | 0.18 | 0.25 | 0.07 | 0.10 |
| *Hepatus chilensis* ♀ *(ovi)* | muscle | MIL | 0.24 | | 0.0112 | | 4.72 | | 3.15 | | 0.52 | 0.73 | | |
| | | AVL | 0.41 | | 0.0195 | | 8.19 | | 5.46 | | 0.30 | 0.42 | | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | MAL | 0.59 | | 0.0276 | | 11.6 | | 7.73 | | 0.21 | 0.30 | | |
| | *Bursa ventricosa* | muscle | MIL | 0.18 | 0.20 | 0.0420 | 0.0464 | 17.7 | 19.5 | 11.8 | 13.0 | 0.69 | 0.97 | 0.62 | 0.87 |
| | | | AVL | 0.28 | 0.40 | 0.0650 | 0.0924 | 27.3 | 38.8 | 18.2 | 25.9 | 0.45 | 0.62 | 0.31 | 0.44 |
| | | | MAL | 0.37 | 0.88 | 0.0862 | 0.2044 | 36.2 | 85.8 | 24.2 | 57.2 | 0.34 | 0.47 | 0.14 | 0.20 |
| | *Cymatium sp.* | muscle | MIL | | 0.09 | | 0.0200 | | 8.38 | | 5.59 | | | 1.45 | 2.03 |
| | | | AVL | | 0.15 | | 0.0352 | | 14.8 | | 9.84 | | | 0.82 | 1.15 |
| | | | MAL | | 0.30 | | 0.0695 | | 29.2 | | 19.5 | | | 0.42 | 0.58 |
| | *Argopecten purpuratus* | muscle | MIL | 0.19 | 0.14 | 0.0442 | 0.0334 | 18.6 | 14.0 | 12.4 | 9.36 | 0.66 | 0.92 | 0.87 | 1.21 |
| | | | AVL | 0.27 | 0.20 | 0.0618 | 0.0466 | 26.0 | 19.6 | 17.3 | 13.0 | 0.47 | 0.66 | 0.62 | 0.87 |
| | | | MAL | 0.34 | 0.26 | 0.0787 | 0.0612 | 33.1 | 25.7 | 22.0 | 17.1 | 0.37 | 0.52 | 0.47 | 0.66 |
| | | edible tissue* | MIL | 0.38 | 0.28 | 0.0881 | 0.2476 | 37.0 | 104 | 24.7 | 69.3 | 0.33 | 0.46 | 0.12 | 0.16 |
| | | | AVL | 0.48 | 0.66 | 0.1119 | 0.0655 | 47.0 | 27.5 | 31.3 | 18.3 | 0.26 | 0.36 | 0.44 | 0.62 |
| | | | MAL | 0.58 | 1.07 | 0.1336 | 0.1531 | 56.1 | 64.3 | 37.4 | 42.9 | 0.22 | 0.30 | 0.19 | 0.27 |
| Northern location | *Bursa ventricosa* | muscle | MIL | 0.38 | 0.13 | 0.0877 | 0.0304 | 36.8 | 12.8 | 24.5 | 8.52 | 0.33 | 0.46 | 0.95 | 1.33 |
| | | | AVL | 0.64 | 0.42 | 0.1486 | 0.0968 | 62.4 | 40.7 | 41.6 | 27.1 | 0.20 | 0.27 | 0.30 | 0.42 |
| | | | MAL | 1.08 | 0.79 | 0.2513 | 0.1828 | 106 | 76.8 | 70.4 | 51.2 | 0.12 | 0.16 | 0.16 | 0.22 |
| | *Cymatium sp.* | muscle | MIL | 0.05 | 0.12 | 0.0108 | 0.0267 | 4.54 | 11.2 | 3.03 | 7.49 | 2.68 | 3.76 | 1.08 | 1.52 |
| | | | AVL | 0.09 | 0.18 | 0.0209 | 0.0419 | 8.76 | 17.6 | 5.84 | 11.7 | 1.39 | 1.95 | 0.69 | 0.97 |
| | | | MAL | 0.20 | 0.25 | 0.0462 | 0.0575 | 19.4 | 24.1 | 12.9 | 16.1 | 0.63 | 0.88 | 0.50 | 0.71 |
| | *Argopecten purpuratus* | muscle | MIL | 0.19 | 0.12 | 0.0442 | 0.0270 | 18.6 | 11.3 | 12.4 | 7.56 | 0.66 | 0.92 | 1.07 | 1.50 |
| | | | AVL | 0.22 | 0.17 | 0.0519 | 0.0392 | 21.8 | 16.5 | 14.5 | 11.0 | 0.56 | 0.78 | 0.74 | 1.03 |
| | | | MAL | 0.27 | 0.23 | 0.0629 | 0.0532 | 26.4 | 22.4 | 17.6 | 14.9 | 0.46 | 0.65 | 0.54 | 0.76 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | edible tissue* | MIL | 0.36 | 0.32 | 0.0833 | 0.0736 | 35.0 | 30.9 | 23.3 | 20.6 | 0.35 | 0.49 | 0.39 | 0.55 |
| | | AVL | 0.50 | 0.43 | 0.1164 | 0.0991 | 48.9 | 41.6 | 32.6 | 27.8 | 0.25 | 0.35 | 0.29 | 0.41 |
| | | MAL | 0.69 | 0.60 | 0.1593 | 0.1401 | 66.9 | 58.9 | 44.6 | 39.2 | 0.18 | 0.25 | 0.21 | 0.29 |
| *Argopecten purpuratus* | muscle | MIL | | 0.19 | | 0.0450 | | 18.9 | | 12.6 | | | 0.64 | 0.90 |
| (transplanted experiment) | | AVL | | 0.22 | | 0.0508 | | 21.4 | | 14.2 | | | 0.57 | 0.80 |
| | | MAL | | 0.25 | | 0.0576 | | 24.2 | | 16.1 | | | 0.50 | 0.70 |
| | edible tissue* | MIL | | 0.40 | | 0.0937 | | 39.4 | | 26.2 | | | 0.31 | 0.43 |
| | | AVL | | 0.56 | | 0.1303 | | 54.7 | | 36.5 | | | 0.22 | 0.31 |
| | | MAL | | 0.81 | | 0.1884 | | 79.1 | | 52.8 | | | 0.15 | 0.22 |

**(*) Edible tissue is considered as adductor muscle + gonad in *A. purpuratus*. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**

**Table 3.** Average concentrations (µg/g wwt) of Cr (VI), Ni and Pb in muscle (incl. edible tissue for *A. purpuratus*) and respective risk indices of six shellfish species, and from Sechura Bay and front Illescas Reserved Zone

| | | | | | | | | | Amount (kg) of edible tissue to reach | | | | | | Amount (kg) of edible tissue to reach | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cr(VI) | THQ | TR | Ni | THQ | TR | %PTWI | Women (50 kg) | Men (70 kg) | Pb | THQ* | TR | %PTWI$_3$ | Women (50 kg)* | Men (70 kg)* |
| IRZ1 | *O. mimus* ♂ | 0.37 | 0.001573 | **0.0000024** | 0.09 | 0.000056 | **0.0000017** | 0.02 | 20.1 | 28.1 | 0.02 | 0.019347 | 0.0000000 | 0.05 | 9.05 | 12.7 |
| | *O. mimus* ♀ | 0.41 | 0.001738 | **0.0000026** | 0.09 | 0.000058 | **0.0000017** | 0.02 | 19.4 | 27.1 | 0.02 | 0.020659 | 0.0000000 | 0.05 | 8.47 | 11.9 |
| | *R. setosum* ♂ | 1.02 | 0.016055 | **0.0000241** | 0.68 | 0.001592 | **0.0000477** | 0.64 | 2.59 | 3.62 | 0.02 | 0.021430 | 0.0000000 | 0.20 | 8.17 | 11.4 |
| | *B. ventricosa* | 0.65 | 0.050574 | **0.0000759** | 0.87 | 0.010082 | **0.0003025** | 4.03 | 2.01 | 2.82 | 0.02 | 0.023494 | 0.0000000 | 1.09 | 7.45 | 10.4 |
| | *A. purpuratus* | | | | | | | | | | 0.08 | 0.052668 | 0.0000002 | 3.67 | 2.22 | 3.10 |
| SL1 | *R. setosum* ♂ | 0.16 | 0.002537 | **0.0000038** | 0.22 | 0.000507 | **0.0000152** | 0.20 | 8.11 | 11.4 | 0.01 | 0.019683 | 0.0000000 | 0.09 | 17.8 | 24.9 |
| | *R. setosum* ♀ | 0.13 | 0.002036 | **0.0000031** | 0.07 | 0.000174 | **0.0000052** | 0.07 | 23.7 | 33.1 | 0.03 | 0.052473 | 0.0000000 | 0.25 | 6.67 | 9.34 |
| | *R. setosum* ♀ *(ovi)* | 0.07 | 0.001042 | **0.0000016** | 0.03 | 0.000063 | **0.0000019** | 0.03 | 65.8 | 92.1 | 0.01 | 0.018171 | 0.0000000 | 0.09 | 19.3 | 27.0 |
| | *H. chilensis* ♀ | 0.12 | 0.001857 | **0.0000028** | 0.08 | 0.000194 | **0.0000058** | 0.08 | 21.2 | 29.7 | 0.02 | 0.045218 | 0.0000000 | 0.21 | 7.74 | 10.8 |
| | *H. chilensis* ♀ *(ovi)* | 0.19 | 0.003015 | **0.0000045** | 0.07 | 0.000175 | **0.0000053** | 0.07 | 23.5 | 32.9 | 0.02 | 0.039252 | 0.0000000 | 0.18 | 8.9 | 12.5 |
| | *B. ventricosa* | 0.19 | 0.014870 | **0.0000223** | 0.02 | 0.000240 | **0.0000072** | 0.10 | 84.4 | 118 | 0.01 | 0.007477 | 0.0000000 | 0.59 | 13.8 | 19.3 |
| | *A. purpuratus* | | | | | | | | | | 0.07 | 0.046249 | 0.0000001 | 3.22 | 2.52 | 3.53 |
| NL1 | *B. ventricosa* | 0.13 | 0.010058 | **0.0000151** | 0.08 | 0.000900 | **0.0000270** | 0.36 | 22.6 | 31.6 | 0.01 | 0.008615 | 0.0000000 | 0.68 | 12.0 | 16.7 |
| | *Cymatium sp.* | 0.06 | 0.004304 | **0.0000065** | 0.02 | 0.000183 | **0.0000055** | 0.07 | 111 | 155 | 0.02 | 0.010716 | 0.0000000 | 0.85 | 9.61 | 13.5 |
| | *A. purpuratus* | | | | | | | | | | 0.03 | 0.020174 | 0.0000001 | 1.40 | 5.78 | 8.10 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IRZ2 | *O. mimus* ♂ | 0.04 | 0.000164 | 0.0000002 | 0.16 | 0.000105 | **0.0000031** | 0.04 | 10.7 | 15.0 | 0.01 | 0.014471 | 0.0000000 | 0.04 | 12.1 | 16.9 |
| | *O. mimus* ♀ | 0.01 | 0.000053 | 0.0000001 | 0.04 | 0.000026 | 0.0000008 | 0.01 | 43.2 | 60.5 | 0.01 | 0.014317 | 0.0000000 | 0.04 | 12.2 | 17.1 |
| | *H.chilensis* ♂ | 0.04 | 0.000676 | 0.0000010 | 0.18 | 0.000426 | **0.0000128** | 0.17 | 9.66 | 13.5 | 0.02 | 0.042310 | 0.0000000 | 0.20 | 8.27 | 11.6 |
| | *H. chilensis* ♀ *(no ovi)* | 0.04 | 0.000630 | 0.0000009 | 0.09 | 0.000216 | **0.0000065** | 0.09 | 19.1 | 26.7 | 0.02 | 0.038915 | 0.0000000 | 0.18 | 8.99 | 12.6 |
| | *H.chilensis* ♀ *(ovi)* | 0.03 | 0.000475 | 0.0000007 | 0.10 | 0.000230 | **0.0000069** | 0.09 | 17.9 | 25.0 | 0.02 | 0.040926 | 0.0000000 | 0.19 | 8.55 | 12.0 |
| | *B. ventricosa* | 0.05 | 0.004061 | **0.0000061** | 0.09 | 0.001077 | **0.0000323** | 0.43 | 18.8 | 26.4 | 0.01 | 0.007701 | 0.0000000 | 0.61 | 13.4 | 18.7 |
| | *Cymatium sp* | 0.10 | 0.007406 | **0.0000111** | 0.35 | 0.004012 | **0.0001204** | 1.60 | 5.06 | 7.08 | 0.01 | 0.008326 | 0.0000000 | 0.66 | 12.4 | 17.3 |
| | *A. purpuratus* | | | | | | | | | | 0.12 | 0.081802 | 0.0000002 | 5.69 | 1.43 | 2.00 |
| SL2 | *O. mimus* ♂ | 0.01 | 0.000059 | 0.0000001 | 0.02 | 0.000014 | 0.0000004 | 0.01 | 82.3 | 115 | 0.01 | 0.006897 | 0.0000000 | 0.02 | 25.4 | 35.5 |
| | *O. mimus* ♀ | 0.02 | 0.000089 | 0.0000001 | 0.03 | 0.000020 | 0.0000006 | 0.01 | 55.1 | 77.1 | 0.01 | 0.011488 | 0.0000000 | 0.03 | 15.2 | 21.3 |
| | *R. setosum* | 0.02 | 0.000303 | 0.0000005 | 0.05 | 0.000111 | **0.0000033** | 0.04 | 36.9 | 51.7 | 0.01 | 0.021653 | 0.0000000 | 0.10 | 16.2 | 22.6 |
| | *H. chilensis* ♀ *(no ovi)* | 0.04 | 0.000551 | 0.0000008 | 0.13 | 0.000301 | **0.0000090** | 0.12 | 13.7 | 19.1 | 0.02 | 0.041916 | 0.0000000 | 0.20 | 8.35 | 11.7 |
| | *B. ventricosa* | 0.14 | 0.010930 | **0.0000164** | 0.45 | 0.005206 | **0.0001562** | 2.08 | 3.90 | 5.46 | 0.01 | 0.006028 | 0.0000000 | 0.48 | 17.1 | 23.9 |
| | *Cymatium sp.* | 0.03 | 0.002476 | **0.0000037** | 0.04 | 0.000498 | **0.0000150** | 0.20 | 40.7 | 57.0 | 0.01 | 0.007191 | 0.0000000 | 0.57 | 14.3 | 20.0 |
| | *A. purpuratus* | | | | | | | | | | 0.12 | 0.080661 | 0.0000002 | 5.61 | 1.45 | 2.02 |
| NL2 | *B. ventricosa* | 0.05 | 0.003917 | **0.0000059** | 0.20 | 0.002365 | **0.0000710** | 0.95 | 8.58 | 12.0 | 0.02 | 0.008906 | 0.0000000 | 0.70 | 11.6 | 16.2 |
| | *Cymatium sp.* | 0.07 | 0.005760 | **0.0000086** | 0.23 | 0.002649 | **0.0000795** | 1.06 | 7.66 | 10.7 | 0.01 | 0.007243 | 0.0000000 | 0.57 | 14.2 | 19.9 |
| | *A. purpuratus* | | | | | | | | | | 0.04 | 0.029729 | 0.0000001 | 2.07 | 3.92 | 5.49 |

**(*) For Pb: THQ was calculated as C/MRL; and only the $PTWI_3$ was used to estimate the amount (kg) of edible tissue to reach PTWI. TR and THQ that exceed the target cancer risk (0.000001) and the target hazard quotient (1) threshold are printed in bold. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**

**Table 4.** Average concentrations (µg/g wwt) of Mn, Fe, Cu and Zn in muscle (incl. edible tissue for *A. purpuratus*) and respective risk indices of six shellfish species, and from Sechura Bay and front Illescas Reserved Zone

| | | | | | | | Amount (kg) of edible tissue to reach PTWI | | | | | Amount (kg) of edible tissue to reach PTWI | | | | | Amount (kg) of edible tissue to reach PTWI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Species** | **Mn** | **THQ** | **Fe** | **THQ** | **%PTWI** | **Women (50 kg)** | **Men (70 kg)** | **Cu** | **THQ** | **%PTWI** | **Women (50 kg)** | **Men (70 kg)** | **Zn** | **THQ** | **%PTWI** | **Women (50 kg)** | **Men (70 kg)** |
| IRZ1 | *O. mimus* ♂ | 0.30 | 0.000028 | 21.2 | 0.000387 | 0.034 | 13.2 | 18.5 | 4.18 | 0.001336 | 0.011 | 41.9 | 58.6 | 13.5 | 0.000577 | 0.058 | 7.76 | 10.9 |
| | *O. mimus* ♀ | 0.33 | 0.000030 | 22.9 | 0.000419 | 0.037 | 12.2 | 17.1 | 5.68 | 0.001815 | 0.015 | 30.8 | 43.1 | 14.9 | 0.000634 | 0.036 | 7.06 | 9.89 |
| | *R. setosum* ♂ | 0.36 | 0.000121 | 64.0 | 0.004298 | 0.376 | 4.38 | 6.13 | 8.86 | 0.010416 | 0.083 | 19.8 | 27.7 | 39.9 | 0.006249 | 0.625 | 2.63 | 3.69 |
| | *B. ventricosa* | 0.66 | 0.001101 | 70.0 | 0.023094 | 2.021 | 4.02 | 5.62 | 5.85 | 0.033932 | 0.271 | 29.9 | 42.0 | 15.2 | 0.011750 | 1.175 | 6.91 | 9.67 |
| | *A. purpuratus* | 2.10 | 0.003487 | 70.2 | 0.023275 | 2.037 | 3.99 | 5.58 | 1.85 | 0.010702 | 0.086 | 94.8 | 133 | 18.4 | 0.014210 | 1.421 | 5.71 | 8.00 |
| SL1 | *R. setosum* ♂ | 0.10 | 0.000033 | 8.93 | 0.000600 | 0.053 | 31.4 | 43.9 | 6.78 | 0.007974 | 0.064 | 25.8 | 36.1 | 46.5 | 0.007284 | 0.728 | 2.26 | 3.16 |
| | *R. setosum* ♀ | 0.08 | 0.000028 | 8.33 | 0.000560 | 0.049 | 33.6 | 47.1 | 4.94 | 0.005811 | 0.046 | 35.4 | 49.6 | 34.6 | 0.005429 | 0.543 | 3.03 | 4.24 |
| | *R. setosum* ♀ *(ovi)* | 0.08 | 0.000025 | 6.11 | 0.000410 | 0.036 | 45.9 | 64.2 | 5.02 | 0.005899 | 0.047 | 34.9 | 48.8 | 39.3 | 0.006157 | 0.616 | 2.67 | 3.74 |
| | *H. chilensis* ♀ | 0.14 | 0.000047 | 13.0 | 0.000874 | 0.076 | 21.5 | 30.2 | 2.40 | 0.002827 | 0.023 | 72.8 | 102 | 28.2 | 0.004413 | 0.441 | 3.73 | 5.22 |
| | *H. chilensis* ♀ *(ovi)* | 0.15 | 0.000049 | 18.0 | 0.001211 | 0.106 | 15.5 | 21.7 | 4.10 | 0.004818 | 0.039 | 42.7 | 60.0 | 35.1 | 0.005497 | 0.550 | 2.99 | 4.19 |
| | *B. ventricosa* | 0.27 | 0.000445 | 18.6 | 0.006174 | 0.540 | 15.0 | 21.0 | 3.46 | 0.020071 | 0.161 | 50.6 | 71.0 | 12.3 | 0.009538 | 0.954 | 8.51 | 11.9 |
| | *A. purpuratus* | 2.58 | 0.004274 | 41.3 | 0.013678 | 1.197 | 6.78 | 9.50 | 1.70 | 0.009862 | 0.079 | 103 | 144 | 16.0 | 0.012394 | 1.239 | 6.55 | 9.17 |
| NL1 | *B. ventricosa* | 0.30 | 0.000502 | 34.2 | 0.011335 | 0.992 | 8.19 | 11.5 | 3.86 | 0.022362 | 0.179 | 45.4 | 63.5 | 12.9 | 0.009958 | 0.996 | 8.15 | 11.4 |
| | *Cymatium sp.* | 0.72 | 0.001187 | 9.38 | 0.003107 | 0.272 | 30.0 | 41.8 | 1.37 | 0.007962 | 0.064 | 128 | 179 | 12.2 | 0.009396 | 0.940 | 8.64 | 12.1 |
| | *A. purpuratus* | 1.96 | 0.003239 | 28.0 | 0.009284 | 0.812 | 9.99 | 14.0 | 1.68 | 0.009747 | 0.078 | 104 | 146 | 15.6 | 0.012066 | 1.207 | 6.73 | 9.42 |
| IRZ2 | *O. mimus* ♂ | 0.18 | 0.000017 | 3.15 | 0.000057 | 0.005 | 89.0 | 125 | 3.13 | 0.000999 | 0.008 | 56.0 | 78.4 | 10.4 | 0.000445 | 0.044 | 10.1 | 14.1 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *O. mimus* ♀ | 0.20 | 0.000018 | 2.07 | 0.000038 | 0.003 | 135 | 189 | 3.49 | 0.001116 | 0.009 | 50.1 | 70.1 | 10.5 | 0.000449 | 0.045 | 9.96 | 14.0 |
| | *H.chilensis* ♂ | 0.16 | 0.000053 | 13.3 | 0.000895 | 0.078 | 21.0 | 29.4 | 4.01 | 0.004710 | 0.038 | 43.7 | 61.2 | 29.7 | 0.004662 | 0.466 | 3.53 | 4.94 |
| | *H. chilensis* ♀ *(no ovi)* | 0.13 | 0.000045 | 11.2 | 0.000754 | 0.066 | 24.9 | 34.9 | 4.52 | 0.005312 | 0.042 | 38.7 | 54.2 | 27.5 | 0.004314 | 0.431 | 3.82 | 5.34 |
| | *H.chilensis* ♀ *(ovi)* | 0.14 | 0.000048 | 13.3 | 0.000890 | 0.078 | 21.1 | 29.6 | 5.20 | 0.006109 | 0.049 | 33.7 | 47.5 | 26.8 | 0.004198 | 0.420 | 3.92 | 5.49 |
| | *B. ventricosa* | 0.31 | 0.000508 | 22.1 | 0.007334 | 0.642 | 12.7 | 17.7 | 6.12 | 0.035488 | 0.284 | 28.6 | 40.0 | 13.4 | 0.010384 | 1.038 | 7.82 | 11.0 |
| | *Cymatium sp* | 0.39 | 0.000640 | 22.3 | 0.007388 | 0.646 | 12.6 | 17.6 | 0.45 | 0.002615 | 0.021 | 388 | 543 | 10.6 | 0.008162 | 0.816 | 9.95 | 13.9 |
| | *A. purpuratus* | 2.12 | 0.003512 | 35.4 | 0.011723 | 1.026 | 7.91 | 11.1 | 1.50 | 0.008677 | 0.069 | 117 | 164 | 18.5 | 0.014321 | 1.432 | 5.67 | 7.94 |
| SL2 | *O. mimus* ♂ | 0.16 | 0.000015 | 2.50 | 0.000046 | 0.004 | 112 | 157 | 2.92 | 0.000932 | 0.007 | 60.0 | 84.0 | 10.3 | 0.000439 | 0.044 | 10.2 | 14.3 |
| | *O. mimus* ♀ | 0.20 | 0.000018 | 3.38 | 0.000062 | 0.005 | 82.7 | 116 | 2.95 | 0.000943 | 0.008 | 59.3 | 83.0 | 11.4 | 0.000484 | 0.048 | 9.24 | 13.0 |
| | *R. setosum* | 0.08 | 0.000028 | 7.18 | 0.000483 | 0.042 | 39.0 | 54.6 | 5.07 | 0.005959 | 0.048 | 34.5 | 48.3 | 29.4 | 0.004610 | 0.461 | 3.57 | 5.00 |
| | *H. chilensis* ♀ *(no ovi)* | 0.17 | 0.000058 | 16.4 | 0.001104 | 0.097 | 17.0 | 23.9 | 4.30 | 0.005058 | 0.040 | 40.7 | 57.0 | 30.8 | 0.004826 | 0.483 | 3.41 | 4.77 |
| | *B. ventricosa* | 0.38 | 0.000624 | 18.8 | 0.006225 | 0.545 | 14.9 | 20.9 | 4.02 | 0.023294 | 0.186 | 43.6 | 61.0 | 15.7 | 0.012173 | 1.217 | 6.67 | 9.34 |
| | *Cymatium sp.* | 0.44 | 0.000723 | 9.25 | 0.003066 | 0.268 | 30.3 | 42.4 | 2.25 | 0.013028 | 0.104 | 77.9 | 109 | 13.3 | 0.010306 | 1.031 | 7.88 | 11.0 |
| | *A. purpuratus* | 2.53 | 0.004184 | 38.2 | 0.012649 | 1.107 | 7.34 | 10.3 | 1.17 | 0.006773 | 0.054 | 150 | 210 | 18.7 | 0.014436 | 1.444 | 5.62 | 7.87 |
| NL2 | *B. ventricosa* | 0.30 | 0.000502 | 20.2 | 0.006680 | 0.584 | 13.9 | 19.5 | 3.75 | 0.021741 | 0.174 | 46.7 | 65.4 | 12.4 | 0.009619 | 0.962 | 8.44 | 11.8 |
| | *Cymatium sp.* | 0.72 | 0.001187 | 9.57 | 0.003170 | 0.277 | 29.3 | 41.0 | 0.86 | 0.005000 | 0.040 | 203 | 284 | 15.2 | 0.011753 | 1.175 | 6.91 | 9.67 |
| | *A. purpuratus* | 1.96 | 0.003239 | 30.7 | 0.010188 | 0.891 | 9.11 | 12.8 | 1.01 | 0.005862 | 0.047 | 173 | 242 | 17.0 | 0.013117 | 1.312 | 6.19 | 8.67 |

**Note: %PTWI values and amount of edible tissue for Cr and Mn were not calculated due to PTWIs have not been established by the JECFA. THQ that exceed the target hazard quotient (1) threshold are printed in bold. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**

## Materials and methods (*addendum*)

Ingestion rate estimations for Peruvians

The frequency of seafood consumption for Peruvians was estimated from food supplies availability (FSA) of the Food Balance Sheets (FBS) of the Food and Agriculture Organization of the United Nations (FAO) (FAO, 2016). The FBS provide a comprehensive picture of the pattern of a country's food supply during a specified reference period. The food balance sheet shows for each food item, i.e. each primary commodity and a number of processed commodities potentially available for human consumption - the sources of supply and its utilization. The total quantity of foodstuffs produced in a country added to the total quantity imported, and adjusted to any change in stocks that may have occurred since the beginning of the reference period, this gives the supply available during that period. On the utilization side, a distinction is made between the quantities exported, fed to livestock, used for seed, put to manufacture for food use and non-food uses, losses during storage and transportation, and food supplies availability (FSA) for human consumption. The per capital supply of each such food item available for human consumption is then obtained, by dividing the respective quantity by the related data on the population actually partaking of it. Data on per capital food supplies are expressed in terms of quantity and - by applying appropriate food composition factors for all primary and processed products - also in terms of caloric value and protein and fat content (FAO, 2019).

Then the kg/capita/year per group of species was transformed to g/person/day with the following formula:

$$IR(species\ group) = \frac{FSA}{365}\,x\ 1000$$

where

IR: the daily ingestion rate (g/person/day) based on FBS (reference period: year 2013) per species group of shellfish; and

FSA: represents the food supplies availability (kg/capita/year).

# ADDENDUM II

Supplementary material to Chapter 3

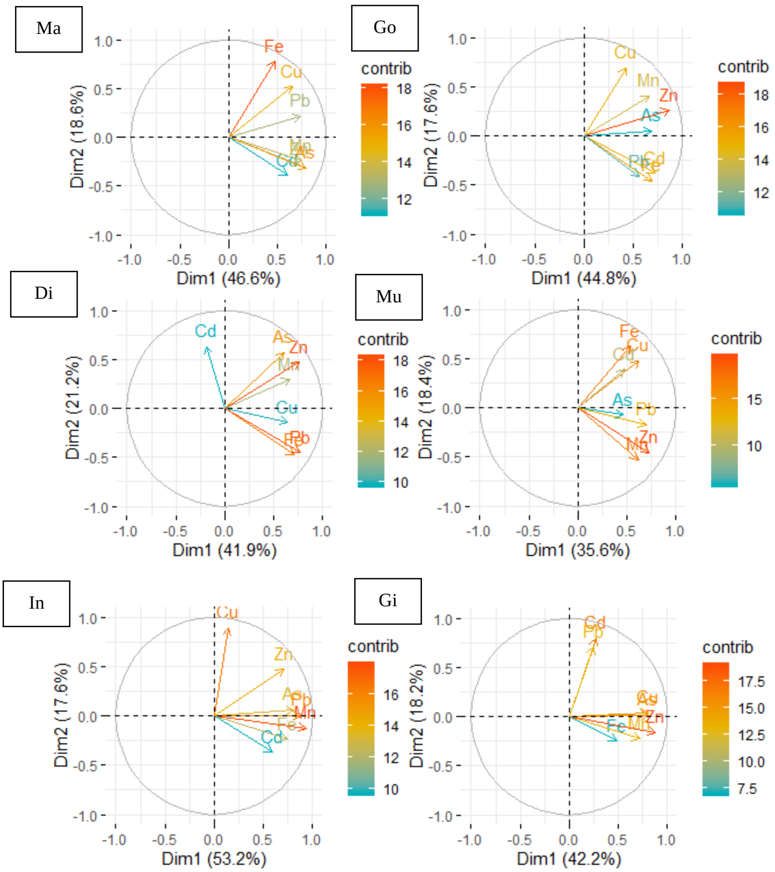



**Fig 7.** PCA results for metal concentration in *A. purpuratus* tissues (Ma: mantle; Go: gonad, Di: digestive gland; Mu: muscle, In: intestine and Gi: gills).

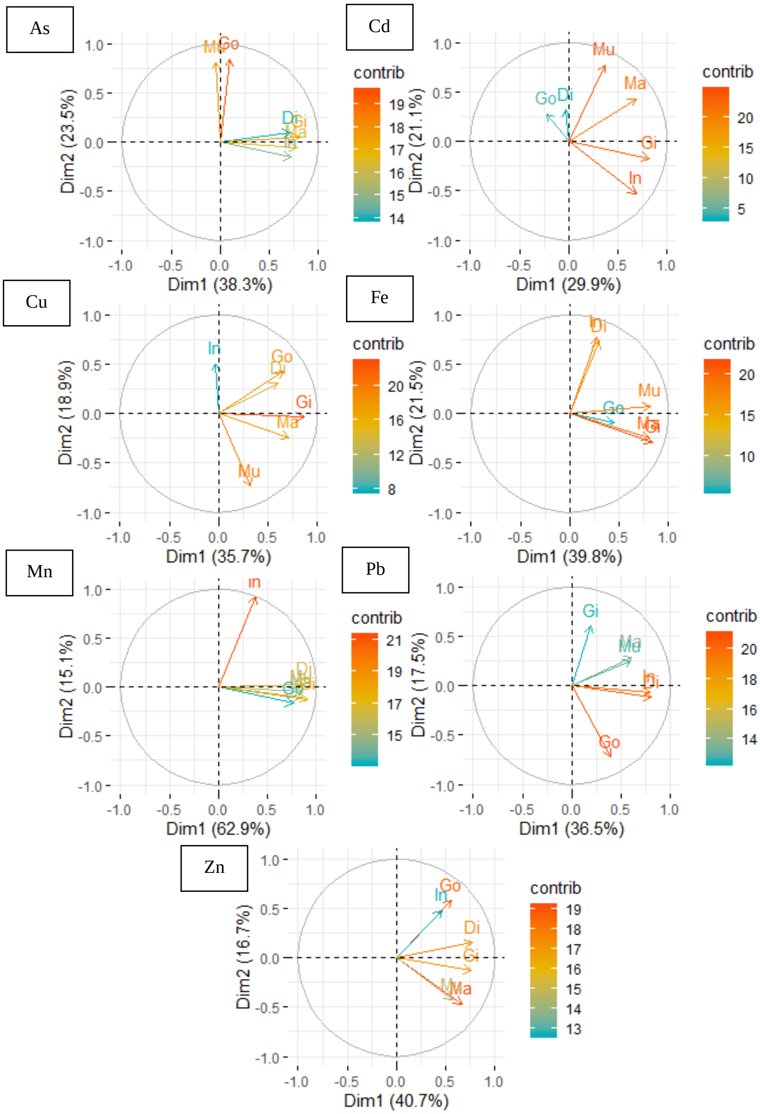
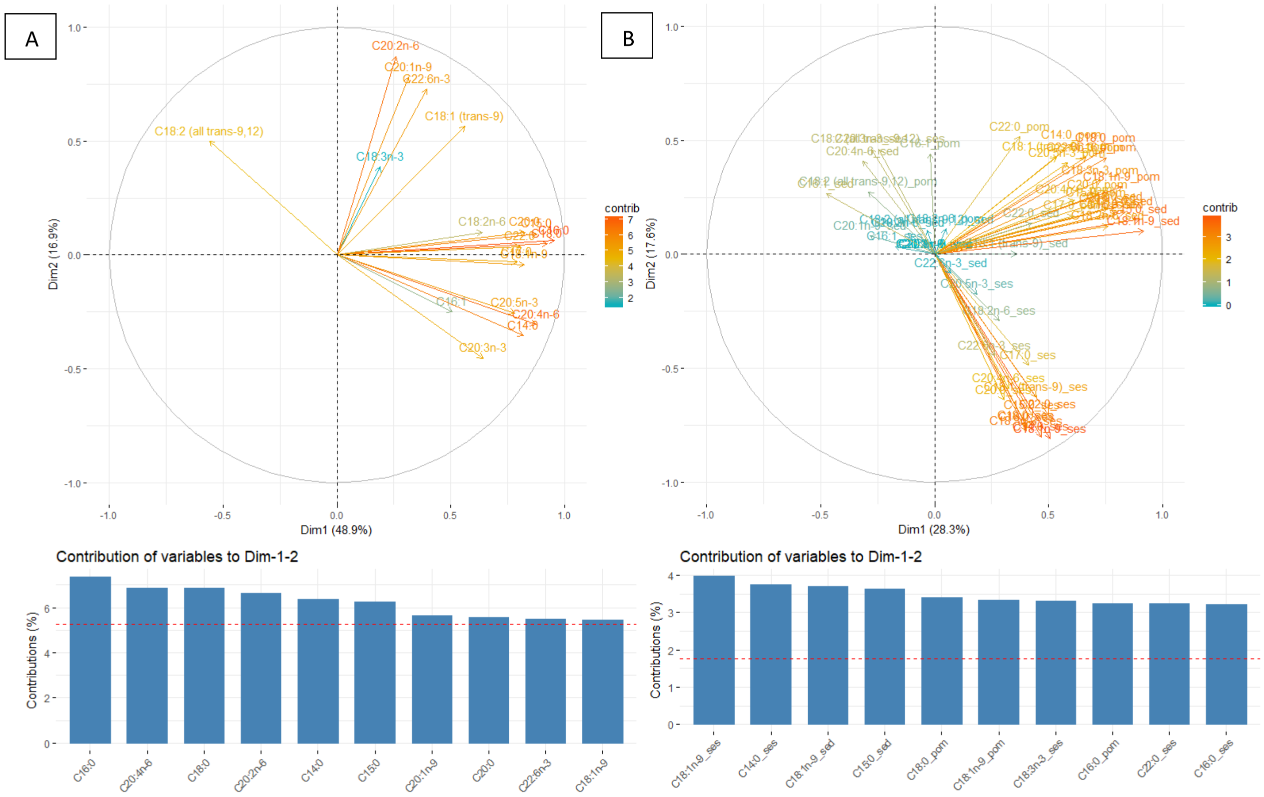



**Fig 8.** PCA results for metals concentrations (As, Cd, Cu, Fe, Mn, Pb and Zn) in *A. purpuratus* tissues (Ma: mantle; Go: gonad, Di: digestive gland; Mu: muscle, In: intestine and Gi: gills).



A
B
Contribution of variables to Dim-1-2
Contribution of variables to Dim-1-2
Dim1 (48.9%)
Dim2 (16.9%)
Dim1 (28.3%)
Dim2 (17.6%)
contrib
Contributions (%)
C18:2 (all trans-9,12)
C20:2n-6
C20:1n-9
C22:6n-3
C18:1 (trans-9)
C18:3n-3
C18:2n-6
C20:5n-3
C20:4n-6
C14:0
C20:3n-3
C16:1
C16:0
C20:4n-6
C18:0
C20:2n-6
C14:0
C15:0
C20:1n-9
C20:0
C22:6n-3
C18:1n-9
C18:1n-9_ses
C14:0_ses
C18:1n-9_sed
C15:0_sed
C18:0_pom
C18:1n-9_pom
C18:3n-3_ses
C16:0_pom
C22:0_ses
C16:0_ses
C22:0_pom
C18:1n-9_sed
C22:1n-3_sed
C20:5n-3_ses
C18:2n-6_ses
C17:0_ses

| | *1* | *2* | *3* | *4* | *5* |
|---|---|---|---|---|---|
| **Fatty acids analyzed in *A. purpuratus* muscle and in food sources** | **C14:0** (tetradecanoic acid) | **C15:0** (pentadecanoic acid) | **C16:0** (hexadecanoic acid) | **C16:1** (palmitoleic acid) | **C17:0** (heptadecanoic acid) |
| | *6* | *7* | *8* | *9* | *10* |
| | **C18:0** (octadecanoic acid) | **C18:1 (trans-9)** (trans-9-octadecenoic acid) | **C18:1n-9** (cis-9-octadecenoic acid) | **C18:2 (all trans-9,12)** (all trans-9,12-octadecadienoic acid) | **C18:2n-6 LA** (linoleic acid) |
| | *11* | *12* | *13* | *14* | *15* |
| | **C20:0** (eicosanoic acid) | **C18:3n3 ALA** (α-linolenic acid) | **C20:1** (paullinic acid) | **C20:2** (eicosadienoic acid) | **C22:0** (docosanoic acid) |
| | *16* | *17* | *18* | *19* | |
| | **C20:3n3** (all cis-11,14,17-eicosatrienoic acid) | **C20:4n6 ARA** (cis-5,8,11,14-eicosatetraenoic acid) | **C20:5n3 EPA** (cis-5,8,11,14,17-eicosapentaenoic acid) | **C22:6n3 DHA** (cis-4,7,10,13,16,19-docosahexanoic acid) | |

**(*) Chemical or common name are used in accordance to the fatty acid relevance for marine food and nutrition.**

**Fig 9.** PCA results for FA in (A) *A. purpuratus* muscle and (B) food sources (ses: seston; pom: POM; sed: sediment).

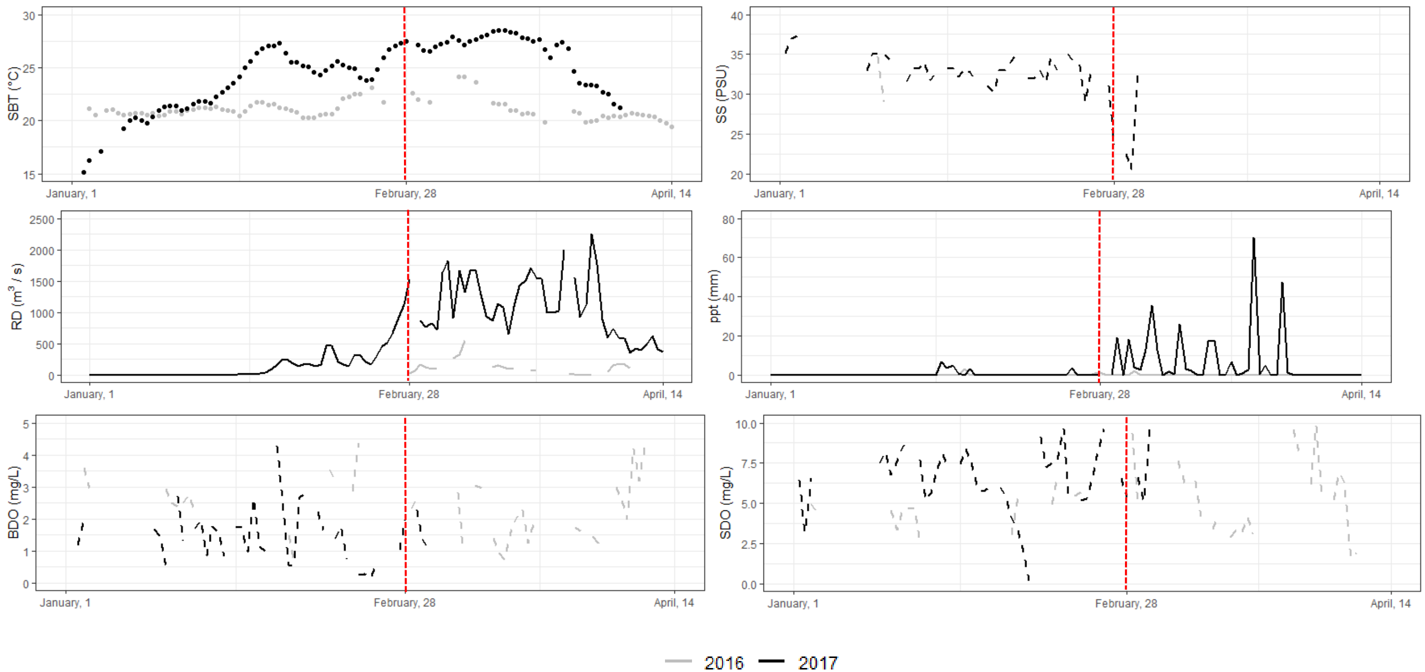



**Fig 10.** Daily sea bottom temperature (SBT), surface salinity (SS), river discharge (RD), precipitation (ppt) and bottom (BDO) and surface (SDO) dissolved oxygen in Southern location (SL), Sechura Bay 2016 – 2017. Red dash line ( ) indicates the beginning of the high-rain period.

**Table 2.** Total FA (ΣFA; ng/g fdwt) concentrations (mean ± S.D; n=3) in alive and moribund *A. purpuratus* from SL and NL, 2017 and stable isotope $\delta^{15}N$ (‰; dwt) signatures (mean ± S.D; n=3) for *A. purpuratus* from IRZ, SL, NL, 2016-2017. January (1S) and March (2S) 2016, and January (3S) and March (4S) 2017 as sampling months.

| **Year** | **Sampling period** | **Location** | **Individuals condition** | **ΣFA** | **$\delta^{15}N$** |
|---|---|---|---|---|---|
| 2016 | 1S | IRZ | alive | - | 6.96 ± 0.19 |
| | | SL | | - | 7.50 ± 0.17 |
| | | NL | | - | 8.27 ± 0.13 |
| | 2S | IRZ | alive | - | 7.82 ± 0.08 |
| | | SL | | - | 8.05 ± 0.25 |
| | | NL | | - | 8.45 ± 0.16 |
| | | NLt | | - | 7.98 ± 0.09 |
| 2017 | 3S | IRZ | alive | - | 7.20 ± 0.08 |
| | | SL | | - | 7.59 ± 0.20 |
| | | NL | | - | 7.77 ± 0.28 |
| | 4S | IRZ | alive | - | 8.02 ± 0.16 |
| | | SL | alive | 11131 ± 935 | 9.32 ± 0.29 |
| | | | moribund | 15595 ± 716* | |
| | | NL | alive | 10764 ± 919 | 7.76 ± 0.21 |
| | | | moribund | 13163 ± 1916 | |

**NLt: *A. purpuratus* individuals from the transplanted culture from SL to NL.**
*** Significant differences among alive and moribund *A. purpuratus* individuals per location.**

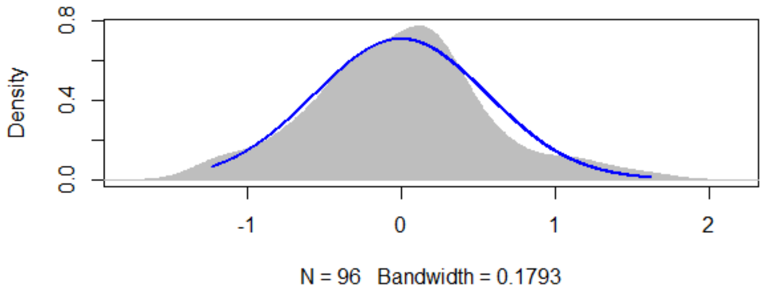
## Materials and methods

Data analysis *(addendum)*

Two-way ANOVA and post-hoc Tukey multiple comparison were performed on the important variables (determined by the PCA) using location (IRZ, SL, NL, PL1, PL2) and sampling period (1S, 2S, 3S and 4S, which are January, 2016, March, 2016, January, 2017 and March, 2017) as fixed factors. For non-parametric data, an extension of Kruskall-Wallis test was used with the post hoc non-parametric Kruskalmc test function. Results were statistically significant when $p < 0.05$.

Effect of month and location on Mn concentrations in gills (as example of parametric test):

```
Shapiro-Wilk normality test

data:  residulogMnGi
W = 0.98474, p-value = 0.3316
```

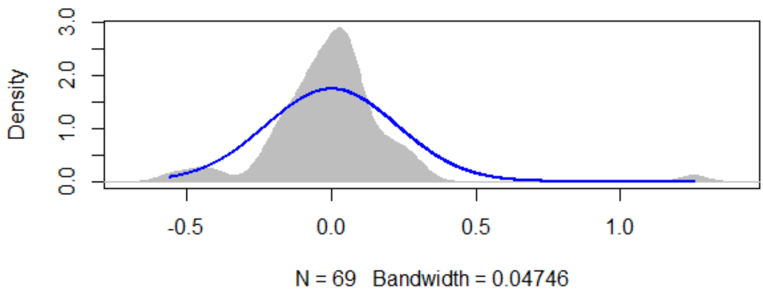



```
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group 15   1.414 0.1612
      80

Anova Table (Type II tests)

Response: log(Mn)
               Sum Sq Df F value    Pr(>F)
month          14.809  3 13.0840 4.916e-07 ***
location       18.326  4 12.1433 9.219e-08 ***
month:location 17.443  8  5.7791 8.229e-06 ***
Residuals      30.183 80
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> CLDMnGi<-cld(LSMnGi,alpha=.05, Letters=letters,adjust="tukey
")
month location   lsmean        SE df lower.CL upper.CL .group
 1S    IRZ      2.202663 0.2507629 80 1.421794 2.983532  a
 2S    NL       2.206537 0.2507629 80 1.425668 2.987406  a
 3S    SL       2.328772 0.2507629 80 1.547903 3.109641  a
 3S    IRZ      2.335528 0.2507629 80 1.554659 3.116397  a
 4S    NL       2.459610 0.2507629 80 1.678741 3.240479  a
```

```
 2S     IRZ       2.736161 0.2507629  80 1.955292 3.517030  a
 2S     SL        2.740063 0.2507629  80 1.959194 3.520932  a
 3S     PL2       2.820920 0.2507629  80 2.040051 3.601789  a
 3S     NL        2.895010 0.2507629  80 2.114141 3.675879  ab
 3S     PL1       2.900567 0.2507629  80 2.119698 3.681436  ab
 4S     SL        2.995542 0.2507629  80 2.214673 3.776411  ab
 1S     SL        3.080437 0.2507629  80 2.299568 3.861306  ab
 4S     IRZ       3.253741 0.2507629  80 2.472872 4.034610  ab
 1S     NL        3.395447 0.2507629  80 2.614578 4.176316  ab
 4S     PL2       4.177115 0.2507629  80 3.396246 4.957984   bc
 4S     PL1       5.192973 0.2507629  80 4.412104 5.973842    c
 1S     PL1         nonEst         NA  NA        NA        NA
 2S     PL1         nonEst         NA  NA        NA        NA
 1S     PL2         nonEst         NA  NA        NA        NA
 2S     PL2         nonEst         NA  NA        NA        NA
```

Effect of month and location on Zn concentrations in sediment (as example of non-parametric test):

```
Shapiro-Wilk normality test

data:  residulogZn_sed
W = 0.80826, p-value = 4.704e-08
```




```
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group 11  1.4621 0.1717
      57
```

```
> SRHZn_sed
               Df  Sum Sq       H p.value
month           3  1302.5   3.236 0.35665
location        2 22049.8  54.782 0.00000
month:location  6   967.1   2.403 0.87918
Residuals      57  3050.5
```

```
> kruskalmc(Zn_sed, month)
Multiple comparison test after Kruskal-Wallis
p.value: 0.05
Comparisons
        obs.dif critical.dif difference
1S-2S  1.229167     18.18626      FALSE
1S-3S  9.729167     18.18626      FALSE
1S-4S  1.202206     18.43625      FALSE
2S-3S  8.500000     17.64326      FALSE
2S-4S  2.431373     17.90084      FALSE
3S-4S 10.931373     17.90084      FALSE
```

```
> kruskalmc(Zn_sed, location)
Multiple comparison test after Kruskal-Wallis
p.value: 0.05
Comparisons
        obs.dif critical.dif difference
IRZ-NL 22.59091     14.17638       TRUE
IRZ-SL 21.60870     14.01467       TRUE
NL-SL  44.19960     14.32301       TRUE
```

```
> kruskalmc(data_1S$Zn_sed,data_1S$location)
Multiple comparison test after Kruskal-Wallis
p.value: 0.05
Comparisons
         obs.dif critical.dif difference
IRZ-NL  5.833333     7.357134      FALSE
IRZ-SL  4.333333     6.580421      FALSE
NL-SL  10.166667     7.357134       TRUE
```

```
> kruskalmc(data_2S$Zn_sed,data_2S$location)
Multiple comparison test after Kruskal-Wallis
p.value: 0.05
Comparisons
       obs.dif critical.dif difference
IRZ-NL       6     7.378741      FALSE
IRZ-SL       6     7.378741      FALSE
NL-SL       12     7.378741       TRUE
```

```
> kruskalmc(data_3S$Zn_sed,data_3S$location)
Multiple comparison test after Kruskal-Wallis
p.value: 0.05
Comparisons
         obs.dif critical.dif difference
IRZ-NL  6.666667     7.378741      FALSE
IRZ-SL  4.666667     7.378741      FALSE
NL-SL  11.333333     7.378741       TRUE
```

```
> kruskalmc(data_4S$Zn_sed,data_4S$location)
Multiple comparison test after Kruskal-Wallis
p.value: 0.05
Comparisons
       obs.dif critical.dif difference
IRZ-NL     6.0     6.979591      FALSE
IRZ-SL     5.5     7.320256      FALSE
NL-SL     11.5     7.320256       TRUE
```

```
> kruskalmc(data_IRZ$Zn_sed,data_IRZ$month)
Multiple comparison test after Kruskal-Wallis
p.value: 0.05
Comparisons
        obs.dif critical.dif difference
1S-2S  4.333333     10.77064      FALSE
1S-3S 12.166667     10.77064       TRUE
1S-4S  1.833333     10.77064      FALSE
2S-3S  7.833333     10.77064      FALSE
2S-4S  6.166667     10.77064      FALSE
3S-4S 14.000000     10.77064       TRUE
```

```
> kruskalmc(data_SL$Zn_sed,data_SL$month)
Multiple comparison test after Kruskal-Wallis
p.value: 0.05
Comparisons
        obs.dif critical.dif difference
1S-2S  3.833333     10.33084      FALSE
1S-3S  7.666667     10.33084      FALSE
1S-4S 13.033333     10.83507       TRUE
2S-3S  3.833333     10.33084      FALSE
2S-4S  9.200000     10.83507      FALSE
3S-4S  5.366667     10.83507      FALSE
```

```
> kruskalmc(data_NL$Zn_sed,data_NL$month)
Multiple comparison test after Kruskal-Wallis
p.value: 0.05
Comparisons
        obs.dif critical.dif difference
1S-2S  3.666667    11.058498      FALSE
1S-3S  8.500000    11.058498      FALSE
1S-4S  1.166667    11.058498      FALSE
2S-3S 12.166667     9.891022       TRUE
2S-4S  2.500000     9.891022      FALSE
3S-4S  9.666667     9.891022      FALSE
```

# ADDENDUM III

## Supplementary material to Chapter 4

**Table 3.** Sampling year and period, region, species, taxonomic group, categorized (use), sample number (n), total length and total weight (mean ± S.D) of Peruvian marine species

| Sampling year | Sampling period | Region | Species | Taxonomic group | Categorized (use) | n | Total length (cm) | Total weight (g) |
|---|---|---|---|---|---|---|---|---|
| 2016 | low-rain | North | *Argopecten purpuratus* | scallop | edible | 199 | 5.23 ± 6.28 | 24.28 ± 9.31 |
| | | | *Bursa ventricosa* | snail | | 54 | 5.30 ± 0.47 | 27.40 ± 8.10 |
| | | | *Cymatium* sp. | snail | | 18 | 7.45 ± 0.50 | 40.44 ± 10.94 |
| | | | *Octopus mimus* | cephalopod | | 10 | 9.09 ± 1.66 | 315.10 ± 97.92 |
| | | | *Romaleon setosum* | crab | | 26 | 11.29 ± 1.17 | 76.07 ± 142.87 |
| | | | *Hepatus chilensis* | crab | potentially edible | 15 | 6.22 ± 0.55 | 50.63 ± 12.37 |
| 2016 | high-rain | North | *Argopecten purpuratus* | scallop | edible | 191 | 5.69 ± 9.27 | 32.78 ± 15.69 |
| | | | *Bursa ventricosa* | snail | | 67 | 5.17 ± 0.51 | 22.73 ± 4.88 |
| | | | *Cymatium* sp. | snail | | 31 | 7.78 ± 1.46 | 63.99 ± 30.16 |
| | | | *Octopus mimus* | cephalopod | | 25 | 9.99 ± 1.76 | 386.40 ± 181.58 |
| | | | *Romaleon setosum* | crab | | 4 | 10.62 ± 0.95 | 233.25 ± 60.98 |
| | | | *Hepatus chilensis* | crab | potentially edible | 38 | 6.17 ± 0.66 | 51.05 ± 14.52 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2017 | low-rain | North | *Argopecten purpuratus* | scallop | edible | 96 | 5.75 ± 14.12 | 38.64 ± 29.34 |
| | | | *Bursa ventricosa* | snail | | 18 | 5.88 ± 0.45 | 36.54 ± 12.67 |
| | | | *Callinectes arcuatus* | crab | | 1 | 7.00 | 22.77 |
| | | | *Chitonidae* sp. | chiton | | 4 | 1.37 ± 0.17 | 0.46 ± 0.17 |
| | | | *Cymatium* sp. | snail | | 7 | 9.99 ± 1.84 | 100.71 ± 48.41 |
| | | | *Octopus mimus* | cephalopod | | 14 | 10.77 ± 2.16 | 401.96 ± 261.56 |
| | | | *Platixanthus orbigny* | crab | | 6 | 9.08 ± 1.01 | 156.55 ± 65.81 |
| | | | *Platymera gaudichaudii* | crab | | 4 | 11.20 ± 0.24 | 151.09 ± 20.64 |
| | | | *Pteria sterna* | oyster | | 6 | 3.57 ± 0.79 | 8.97 ± 3.83 |
| | | | *Romaleon setosum* | crab | | 6 | 4.74 ± 0.67 | 20.09 ± 5.74 |
| | | | *Sea cucumber* | sea cucumber | | 7 | 3.86 ± 1.44 | 5.36 ± 7.38 |
| | | | *Solenosteira gatesi* | snail | | 6 | 4.98 ± 0.29 | 13.23 ± 2.52 |
| | | | *Thaisella chocolata* | snail | | 6 | 6.32 ± 0.39 | 46.68 ± 6.16 |
| | | | *Cycloxanthops sexdecimdentatus* | crab | potentially edible | 10 | 3.57 ± 0.76 | 12.73 ± 6.96 |
| | | | *Hepatus chilensis* | crab | | 27 | 6.52 ± 0.94 | 60.29 ± 22.31 |
| | | | *Isognomon sp* | oyster | | 6 | 2.68 ± 0.37 | 2.06 ± 0.59 |
| | | | *Semimytilus algosus* | mussel | | 18 | 5.01 ± 0.54 | 6.24 ± 2.04 |
| | | | *Tegula* sp. | snail | | 6 | 2.02 ± 0.24 | 8.20 ± 0.93 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *Triumphis distorta* | snail | | 6 | 6.16 ± 0.35 | 50.78 ± 10.09 |
| | | | *Caulerpa filiformis* | algae | | 6 | .. | .. |
| | | | *Eurypanopeus transversus* | crab | | 2 | 9.81 ± 0.39 | 163.44 ± 8.77 |
| | | | *Moreirodromia sarraburei* | crab | non-edible | 3 | 2.38 ± 0.15 | 10.77 ± 2.48 |
| | | | *Pilumnoides perlatus* | crab | | 12 | 1.61 ± 0.25 | 2.11 ± 0.95 |
| | | | *Tetrapigus niger* | sea urchin | | 7 | 3.06 ± 0.75 | 41.11 ± 15.34 |
| | | | *Argopecten purpuratus* | scallop | | 146 | 6.62 ± 2.97 | 55.15 ± 10.32 |
| | | | *Thaisella chocolata* | snail | edible | 13 | 53.74 ± 10.77 | 36.83 ± 20.28 |
| 2017 | low-rain | Center | *Romaleon setosum* | crab | | 8 | 99.93 ± 11.39 | 194.67 ± 63.64 |
| | | | *Hepatus chilensis* | crab | potentially edible | 9 | 73.98 ± 4.98 | 79.29 ± 16.82 |
| | | | *Tegula* sp. | snail | | 6 | 28.46 ± 4.85 | 9.43 ± 4.73 |
| | | | *Argopecten purpuratus* | scallop | | 79 | 5.62 ± 12.67 | 34.33 ± 20.34 |
| | | | *Bursa ventricosa* | snail | | 30 | 4.86 ± 1.16 | 22.07 ± 12.36 |
| | | | *Callinectes arcuatus* | crab | | 1 | 7.34 | 23.99 |
| 2017 | high-rain | North | *Chitonidae* sp. | chiton | edible | 6 | 1.42 ± 0.14 | 0.46 ± 0.08 |
| | | | *Cymatium* sp. | snail | | 16 | 7.85 ± 1.99 | 60.06 ± 52.65 |
| | | | *Lepadidae* sp. | barnacle | | 6 | 5.26 ± 0.48 | 2.03 ± 0.42 |
| | | | *Malea ringes* | snail | | 6 | 10.69 ± 0.66 | 166.16 ± 30.19 |

| | | | | | |
|---|---|---|---|---|---|
| *Octopus mimus* | cephalopod | | 11 | 10.57 ± 2.32 | 464.62 ± 275.01 |
| *Panulirus sp.* | lobster | | 6 | 18.64 ± 0.87 | 200.45 ± 31.75 |
| *Platixanthus orbigny* | crab | | 1 | 8.38 | 195.28 |
| *Pteria sterna* | oyster | | 12 | 3.42 ± 0.83 | 11.02 ± 9.28 |
| *Sea cucumber* | sea cucumber | | 6 | 3.51 ± 0.65 | 3.86 ± 1.23 |
| *Semele corrugata* | clam | | 5 | 4.30 ± 0.89 | 20.51 ± 12.68 |
| *Solenosteira gatesi* | snail | | 6 | 6.00 ± 0.18 | 25.72 ± 1.09 |
| *Tagelus dombeii* | clam | | 13 | 5.79 ± 2.61 | 15.80 ± 17.82 |
| *Thaisella chocolata* | snail | | 6 | 6.03 ± 0.46 | 35.29 ± 12.37 |
| *Chione compta* | clam | | 8 | 3.77 ± 0.77 | 22.23 ± 13.57 |
| *Cycloxanthops sexdecimdentatus* | crab | | 13 | 3.03 ± 0.83 | 9.10 ± 6.4 |
| *Hepatus chilensis* | crab | | 3 | 6.65 ± 0.49 | 54.93 ± 22.96 |
| *Squilla* sp. | shrimp | potentially edible | 9 | 12.57 ± 1.71 | 20.59 ± 8.21 |
| *Tegula* sp. | snail | | 12 | 1.61 ± 0.69 | 4.11 ± 3.69 |
| *Turritella gonostoma* | snail | | 6 | 10.65 ± 0.89 | 40.69 ± 9.29 |
| *Ulva* sp. | algae | | 6 | .. | .. |
| *Caulerpa filiformis* | algae | non-edible | 6 | .. | .. |
| *Inachoides lambriformis* | crab | | 13 | 0.98 ± 0.26 | 0.67 ± 0.54 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *Nassarius dentifer* | snail | | 6 | 1.30 ± 0.30 | 0.60 ± 0.39 |
| | | | *Tetrapigus niger* | sea urchin | | 6 | 2.85 ± 0.72 | 20.70 ± 8.25 |
| 2017 | high-rain | Center | *Argopecten purpuratus* | scallop | edible | 186 | 6.15 ± 4.72 | 42.83 ± 9.82 |
| | | | *Thaisella chocolata* | snail | | 12 | 69.55 ± 6.76 | 77.56 ± 33.45 |
| | | | *Cymatium* sp. | snail | | 6 | 88.25 ± 7.97 | 76.71 ± 22.85 |
| | | | *Chondracanthus chamissoi* | algae | | 6 | .. | .. |
| | | | *Hepatus chilensis* | crab | potentially edible | 10 | 75.26 ± 4.88 | 83.6 ± 16.63 |
| | | | *Tegula* sp. | snail | | 46 | 23.92 ± 4.03 | 8.69 ± 2.78 |
| | | | *Semimytilus algosus* | mussel | | 28 | 54.75 ± 4.49 | 8.09 ± 1.31 |
| | | | *Cycloxanthops sexdecimdentatus* | crab | | 7 | 54.05 ± 5.00 | 41.02 ± 12.51 |
| | | | *Codium* sp. | algae | non-edible | 6 | .. | .. |
| | | | *Caulerpa filiformis* | algae | | 12 | .. | .. |
| 2018 | high-rain | South | *Argopecten purpuratus* | scallop | edible | 5 | 7.39 ± 1.25 | 99.6 ± 37.73 |
| | | | *Bursa ventricosa* | snail | | 2 | 6.98 ± 0.71 | 54.00 ± 5.66 |
| | | | *Cancer porteri* | crab | | 21 | 9.74 ± 0.73 | 161.53 ± 47.76 |
| | | | *Fisurella* sp. | limpet | | 4 | 5.30 ± 1.08 | 21.25 ± 10.35 |
| | | | *Lessonia* sp. | algae | | 6 | .. | .. |
| | | | *Lexochinus albus* | sea urchin | | 5 | 8.10 ± 1.71 | 215.80 ± 141.95 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *Macrocystis pyrifera* | algae | | 5 | .. | .. |
| | *Octopus mimus* | cephalopod | | 1 | 11.35 | 499.00 |
| | *Oliva peruviana* | snail | | 10 | 3.57 ± 0.25 | 9.60 ± 1.65 |
| | *Paralichtys adspersus* | fish | | 6 | 29.68 ± 3.70 | 302.17 ± 102.58 |
| | *Platymera gaudichaudii* | crab | | 1 | 9.83 | 111.00 |
| | *Romaleon setosum* | crab | | 19 | 11.19 ± 1.06 | 271.74 ± 75.33 |
| | *Semele corrugata* | clam | | 9 | 7.33 ± 0.35 | 114.67 ± 22.36 |
| | *Tagelus dombeii* | clam | | 10 | 15.49 ± 0.97 | 57.20 ± 8.95 |
| | *Thaisella chocolata* | snail | | 10 | 7.89 ± 0.44 | 108.10 ± 15.12 |
| | *Xanthochorus cassidiformis* | snail | | 4 | 4.57 ± 0.71 | 18.25 ± 7.50 |
| | *Aeneator fontainei* | snail | | 10 | 5.41 ± 0.25 | 18.70 ± 1.71 |
| | *Argobuccinum (Priene) scabrum* | snail | | 13 | 4.57 ± 0.32 | 18.85 ± 5.48 |
| | *Hepatus chilensis* | crab | | 2 | 7.38 ± 0.94 | 94.00 ± 45.26 |
| | *Leukoma antiqua* | clam | potentially edible | 2 | 5.02 ± 1.20 | 45.50 ± 37.48 |
| | *Pleuroncodes monodon* | lobster | | 10 | 3.38 ± 0.45 | 0.93 ± 0.24 |
| | *Red algae* | algae | | 6 | .. | .. |
| | *Rhodymenia* sp. | algae | | 7 | .. | .. |

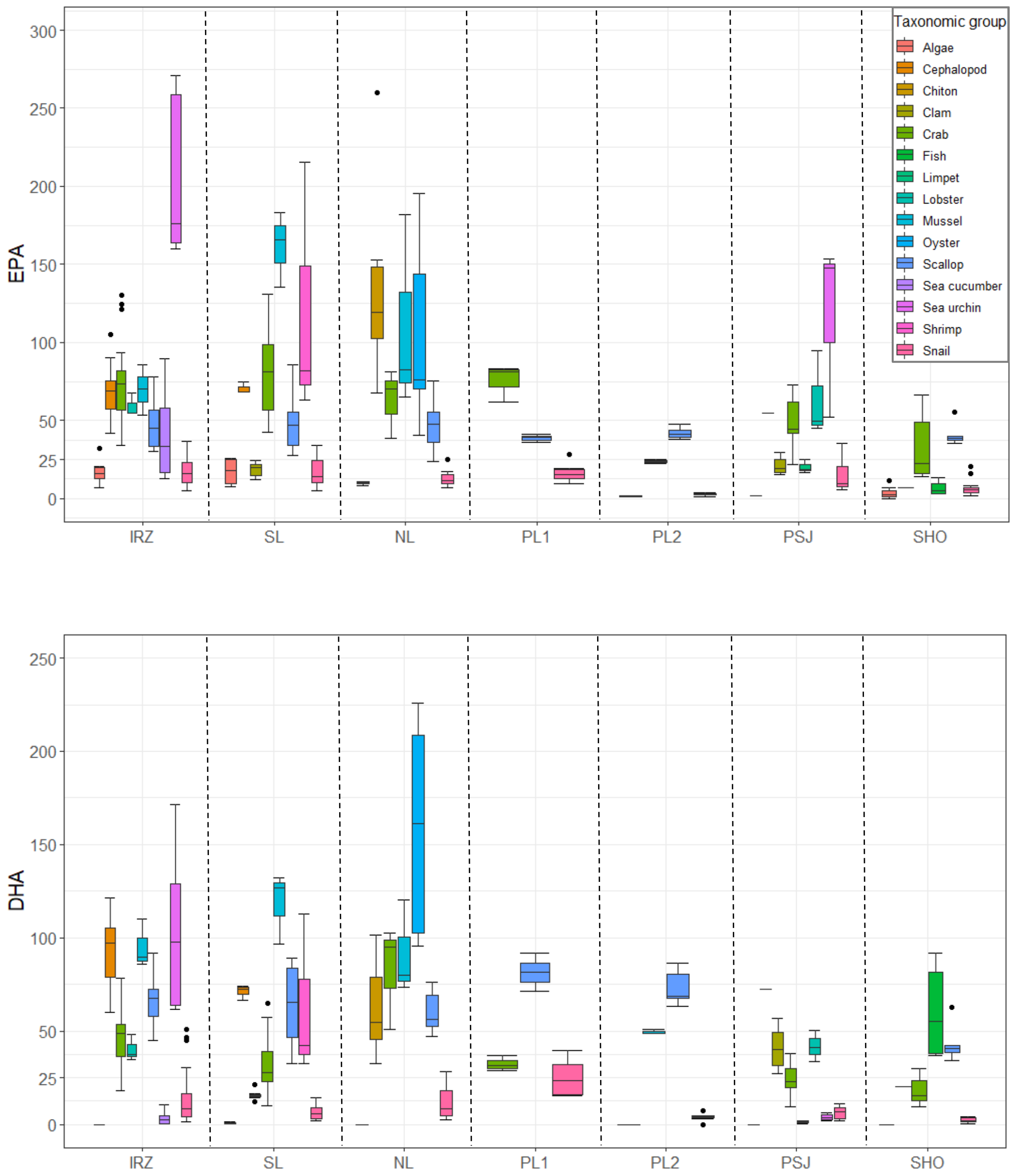
| | | | | | |
|---|---|---|---|---|---|
| *Tegula* sp. | snail | | 17 | 1.86 ± 0.82 | 13.37 ± 4.97 |
| *Ulva sp.* | algae | | 6 | .. | .. |
| *Albunea lucasia* | crab | non-edible | 10 | 4.64 ± 0.15 | 56.40 ± 3.78 |
| *Cancer plebejus* | crab | | 9 | 8.43 ± 1.87 | 89.23 ± 36.38 |
| *Pinnaxodes chilensis* | crab | | 4 | 1.46 ± 0.34 | 4.00 ± 1.42 |
| *Tetrapigus niger* | sea urchin | | 10 | 6.52 ± 1.24 | 80.10 ± 25.90 |

**Total length: Molluscans and barnacle – shell length; Crabs – cephalothorax width; Cephalopods - mantle length; Shrimp, lobster, fish and sea cucumber - total length; Sea urchin - Height length**

**Edible tissues: scallop, snail, chiton, barnacle, sea cucumber and limpet as muscle; cephalopod as muscle arms; crab as soft tissue; oyster, mussel and sea urchin (*T. niger*) as soft tissue (incl. viscera), sea urchin (*L. albus*) as gonads; algae as thallus; lobster and shrimp as muscle tail; clam as muscle+mantle+syphon+foot (with exception of *C. compta* as only muscle); and small species: *I. lambriformis, P. chilensis, Pilumnoides perlatus and P. monodon* as *toto*.**

**Table 4.** Body weight, height measurement, BMI, seafood frequency consumption and ingestion rates of coastal populations from nearby areas of Sechura and Paracas - Peru

| **Location** | **Region** | **Gender** | **n** | **Weight (kg)** | **Height (cm)** | **BMI (kg/m$^2$)** | ***Consumption of seafood at home (g/year)*** | | | | | ***Consumption of seafood outside home (g/year)*** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | scallop | snail | crab | octopus | fish | scallop | snail | crab | octopus | fish |
| Sechura | | | | | | | | | | | | | | | | |
| (and nearby areas) | North | male (m) | 190 | 69.0 | 1.62 | **24.84** | 1681.3 | 195.1 | 138.0 | 3200.0 | 28062.5 | 1450.0 | 166.7 | 129.8 | 1453.1 | 3056.3 |
| | | female (f) | 192 | 62.6 | 1.55 | 26.01 | 1487.4 | 195.9 | 144.0 | 2791.6 | 26172.6 | 1380.0 | 169.8 | 139.1 | 1591.6 | 3082.1 |
| | | m + f | | | | | 1584.8 | 195.5 | 141.0 | 2996.9 | 27122.5 | 1415.2 | 168.2 | 134.4 | 1522.0 | 3069.1 |
| | | m + f | | | | **Ingestion rate (g/day)** | **4.1** | **0.5** | **0.4** | **6.2** | **41.4** | | | | | |
| Paracas | | | | | | | | | | | | | | | | |
| (and nearby areas) | Center-south | male (m) | 196 | 70.62 | 1.64 | 26.37 | 2968.0 | 281.5 | 116.2 | 2557.2 | 27798.4 | 925.0 | 98.2 | 139.7 | 500.5 | 1007.9 |
| | | female (f) | 186 | 65.14 | 1.56 | 26.92 | 3557.3 | 136.8 | 100.1 | 2069.3 | 22841.9 | 1054.9 | 65.3 | 159.5 | 465.5 | 1289.5 |
| | | m + f | | | | | 3255.0 | 210.9 | 108.4 | 2319.7 | 25385.0 | 988.2 | 82.2 | 149.3 | 483.5 | 1145.0 |
| | | m + f | | | | **Ingestion rate (g/day)** | **5.8** | **0.4** | **0.4** | **3.8** | **36.3** | | | | | |


Taxonomic group
Algae
Cephalopod
Chiton
Clam
Crab
Fish
Limpet
Lobster
Mussel
Oyster
Scallop
Sea cucumber
Sea urchin
Shrimp
Snail
EPA
DHA
IRZ
SL
NL
PL1
PL2
PSJ
SHO

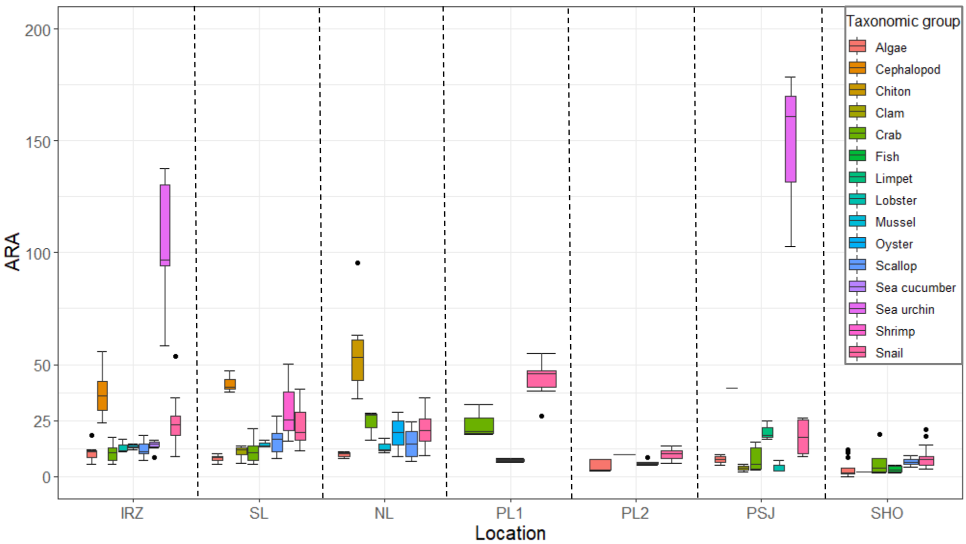



**Fig 5.** EPA, DHA, ARA concentrations (mg/100g wwt.) in different group of species from north: Illescas Reserved Zone (IRZ), Sechura Bay (SL, NL), center-south: Paracas Bay (PL1, PL2), and south: Marcona (PSJ, SHO) 2016 – 2018. Wet weight or fresh weight as wwt. was considered for the nutritional and food safety approach.


60
40
20
10
5
0
Cu
IRZ
SL
NL
PL1
PL2
PSJ
SHO
3000
2000
1000
500
250
0
Fe
Taxonomy group
Algae
Barnacle
Cephalopod
Chiton
Clam
Crab
Fish
Limpet
Lobster
Mussel
Oyster
Scallop
Sea cucumber
Sea urchin
Shrimp
Snail

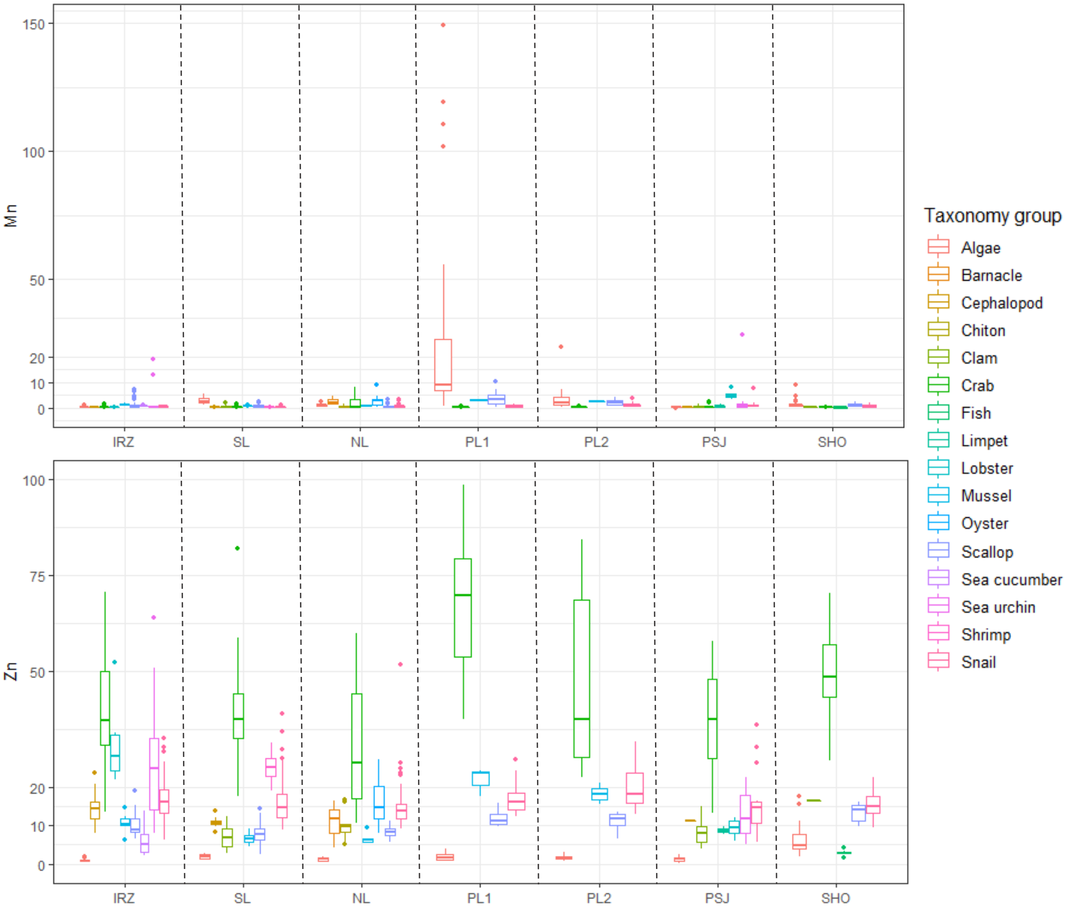
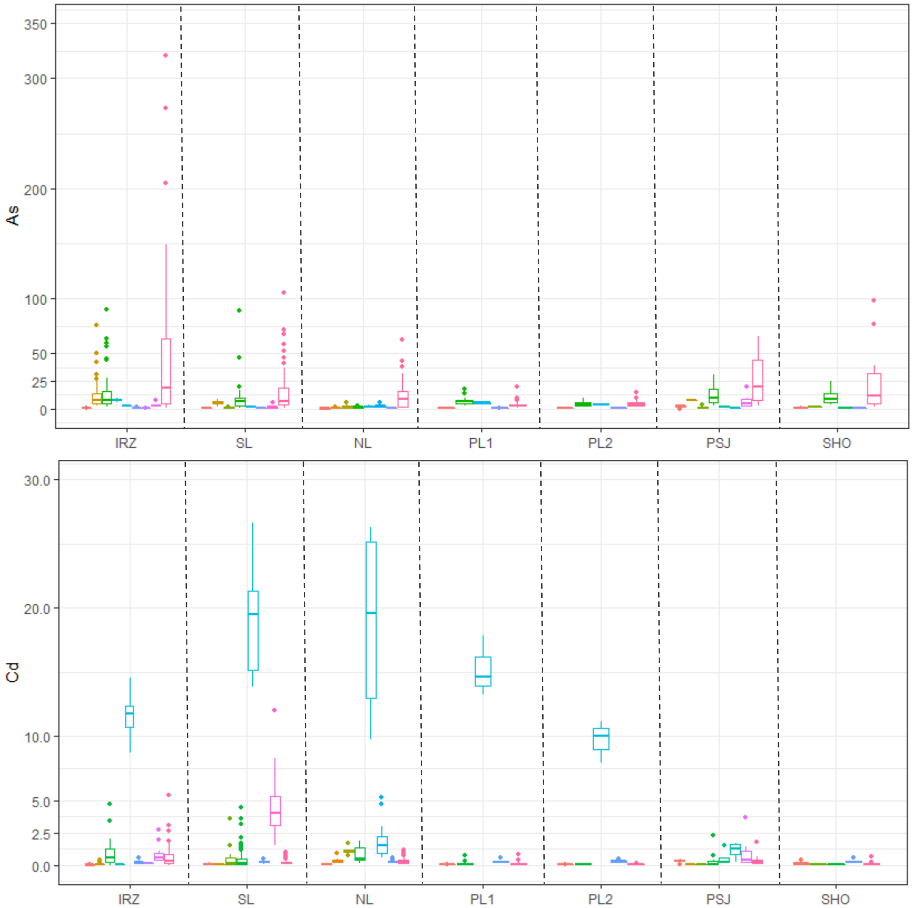



**Fig 6.** Metal (micro-nutrient) concentrations (µg/g wwt.) in different group of species from north: Illescas Reserved Zone (IRZ), Sechura Bay (SL, NL), center-south: Paracas Bay (PL1, PL2), and south: Marcona (PSJ, SHO) 2016 – 2018. Wet weight or fresh weight as wwt. was considered for the nutritional and food safety approach.


As
350
300
200
100
50
25
0
IRZ
SL
NL
PL1
PL2
PSJ
SHO
Cd
30.0
20.0
10.0
5.0
2.5
0.0
IRZ
SL
NL
PL1
PL2
PSJ
SHO

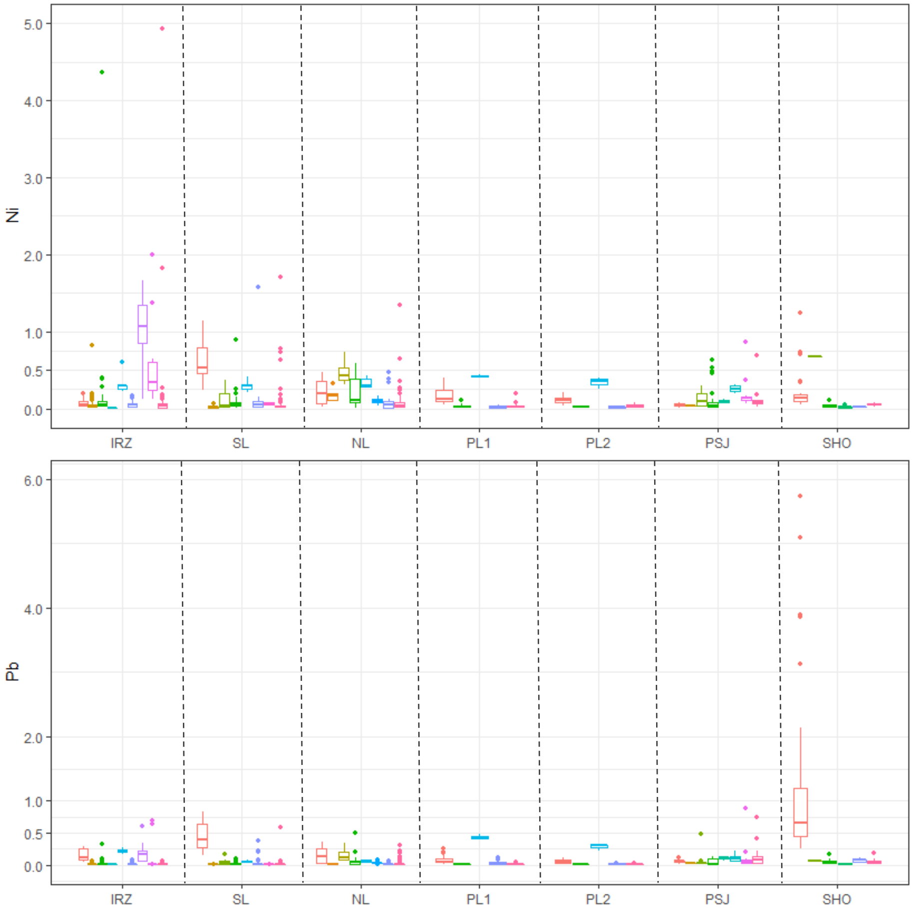



**Fig 7.** Metal concentrations (µg/g wwt.) in different group of species from north: Illescas Reserved Zone (IRZ), Sechura Bay (SL, NL), center-south: Paracas Bay (PL1, PL2), and south: Marcona (PSJ, SHO) 2016 – 2018. Wet weight or fresh weight as wwt. was considered for the nutritional and food safety approach.

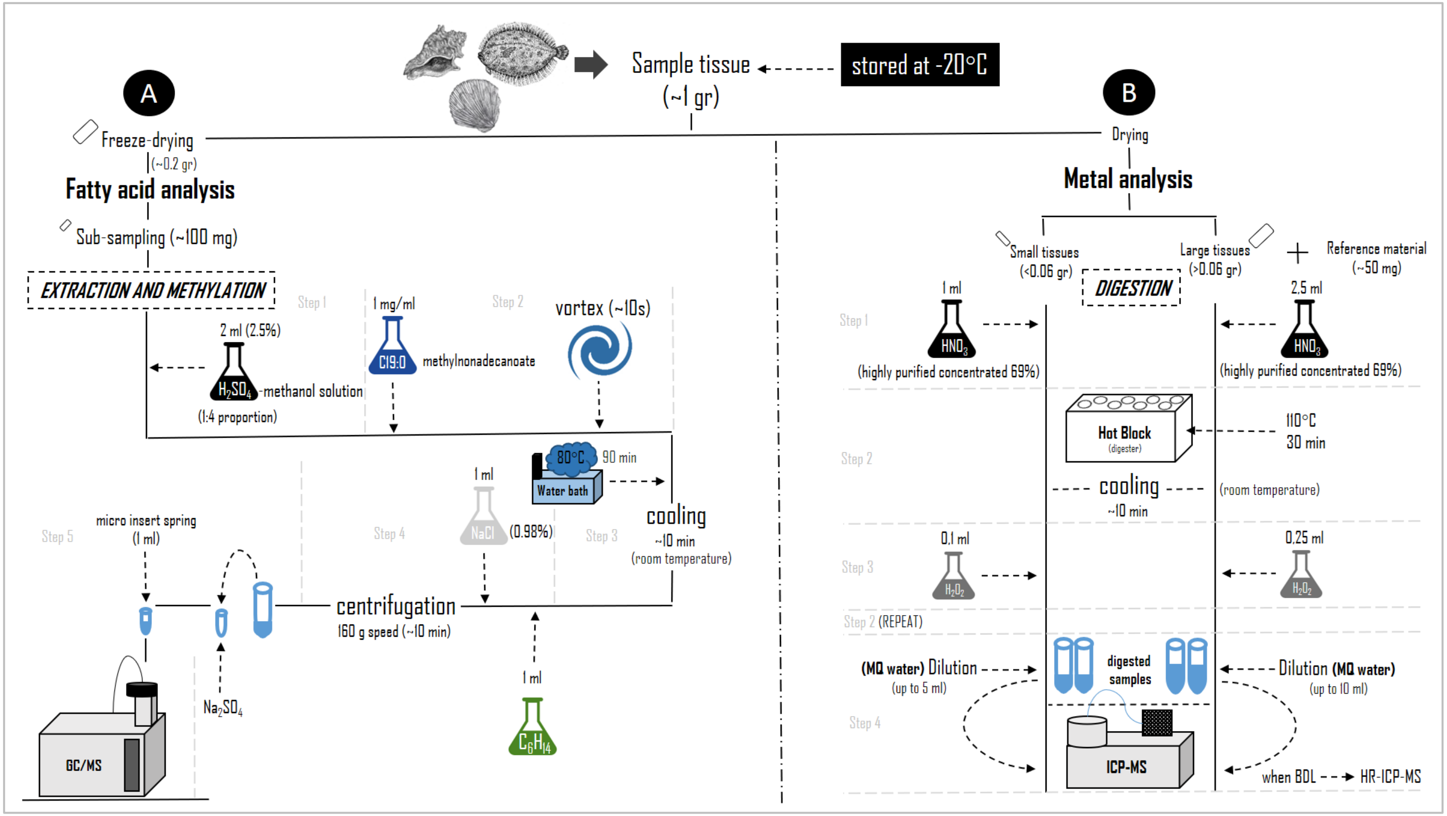



**Fig 8.** Process scheme for (A) fatty acid analysis (FAMEs extraction) and for (B) metal analysis of marine species. Reference material was treated and processed as small tissues for metal analysis.

**Table 5.** Certified (CC) and measured (MC) concentrations (µg/g dwt. ± S.D) for standard reference materials (SRM) for mussel tissues (2976, National Institute of Standards and Technology, NIST).

| Metal | Replicates (n) | Certified concentrations (CC) | Measured concentrations (MC) | Percentage of recovery (%) (CC/MC x 100) |
|---|---|---|---|---|
| Mn | 71 | 33.00 ± 2.00 | 37.47 ± 4.71 | 113.52 |
| Fe | 71 | 171.00 ± 4.90 | 169.11 ± 22.07 | 98.89 |
| Ni | 71 | 0.93 ± 0.12 | 1.04 ± 0.49 | 111.37 |
| Cu | 70 | 4.02 ± 0.33 | 4.29 ± 0.58 | 106.52 |
| Zn | 71 | 137.00 ± 13.00 | 142.46 ± 18.72 | 103.98 |
| As | 71 | 13.30 ± 1.80 | 13.73 ± 1.82 | 103.21 |
| Cd | 71 | 0.82 ± 0.16 | 0.87 ± 0.11 | 105.04 |
| Pb | 71 | 1.19 ± 0.18 | 1.30 ± 0.33 | 108.91 |

**Table 6.** Chemical and nutritional composition of Peruvian marine species for NSSs.

| Species | Categorize (use) | n | Mn | Fe | Ni | Cu | Zn | As | Cd | Pb | *ARA* | *EPA* | *DHA* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***A. purpuratus*** | edible | **138** | 1.28 ± 0.15 | 7.80 ± 0.64 | 0.07 ± 0.02 | 0.21 ± 0.01 | 9.32 ± 0.23 | 0.69 ± 0.03 | 0.27 ± 0.01 | 0.03 ± 0.01 | 12.52 ± 0.81 | 46.12 ± 3.91 | 63.30 ± 4.65 |
| ***B. ventricosa*** | | **86** | 0.52 ± 0.03 | 30.51 ± 2.94 | 0.15 ± 0.07 | 6.34 ± 0.47 | 16.34 ± 0.51 | 34.44 ± 3.38 | 0.69 ± 0.08 | 0.02 ± 0.01 | 23.36 ± 1.20 | 14.04 ± 1.03 | 5.23 ± 1.24 |
| ***Cymatium* sp.** | | **60** | 0.55 ± 0.04 | 10.33 ± 0.71 | 0.09 ± 0.04 | 1.84 ± 0.30 | 14.05 ± 0.49 | 4.45 ± 0.59 | 0.15 ± 0.02 | 0.02 ± 0.01 | 21.95 ± 1.91 | 13.94 ± 1.48 | 14.62 ± 1.46 |
| ***T. chocolata*** | | **43** | 1.18 ± 0.09 | 12.28 ± 1.08 | 0.03 ± 0.01 | 13.88 ± 1.31 | 16.87 ± 0.57 | 20.08 ± 3.72 | 0.12 ± 0.02 | 0.02 ± 0.01 | 17.17 ± 3.30 | 10.97 ± 2.32 | 17.12 ± 3.92 |
| ***R. setosum*** | | **55** | 0.17 ± 0.03 | 13.42 ± 5.10 | 0.14 ± 0.08 | 6.35 ± 0.46 | 43.18 ± 2.10 | 10.02 ± 1.69 | 0.12 ± 0.02 | 0.02 ± 0.01 | 14.11 ± 0.69 | 80.55 ± 2.98 | 33.64 ± 3.05 |
| ***P. gaudichaudii*** | | **5** | 0.14 ± 0.02 | 11.59 ± 2.89 | 0.04 ± 0.01 | 6.81 ± 1.20 | 39.50 ± 3.83 | 9.94 ± 3.32 | 0.57 ± 0.23 | 0.02 ± 0.01 | 3.17 | 21.95 | 9.41 |
| ***P. orbigny*** | | **7** | 0.16 ± 0.02 | 8.18 ± 1.47 | 0.04 ± 0.01 | 10.33 ± 1.41 | 46.19 ± 3.01 | 41.56 ± 13.10 | 0.45 ± 0.07 | 0.02 ± 0.01 | 5.56 ± 0.22 | 62.57 ± 9.77 | 34.56 ± 7.24 |
| ***C. porteri*** | | **6** | 0.14 ± 0.03 | 16.30 ± 2.32 | 0.04 ± 0.01 | 11.55 ± 2.01 | 49.37 ± 2.88 | 11.01 ± 2.99 | 0.08 ± 0.03 | 0.05 ± 0.02 | 2.41 ± 0.63 | 17.72 ± 2.52 | 12.73 ± 1.63 |
| ***O. mimus*** | | **62** | 0.24 ± 0.02 | 6.68 ± 1.22 | 0.06 ± 0.02 | 4.37 ± 0.33 | 13.48 ± 0.44 | 11.15 ± 1.59 | 0.06 ± 0.01 | 0.02 ± 0.01 | 37.96 ± 1.89 | 67.79 ± 3.34 | 88.98 ± 4.05 |
| ***P. adspersus*** | | **6** | 0.10 ± 0.02 | 4.30 ± 0.94 | 0.03 ± 0.01 | 0.19 ± 0.03 | 2.95 ± 0.35 | 1.10 ± 0.21 | - | 0.02 ± 0.01 | 3.28 ± 0.63 | 6.70 ± 1.86 | 60.30 ± 10.46 |
| ***H. chilensis*** | Potentially-edible | **86** | 0.21 ± 0.02 | 13.23 ± 1.00 | 0.09 ± 0.01 | 5.92 ± 0.36 | 43.61 ± 1.87 | 10.37 ± 0.86 | 0.93 ± 0.11 | 0.03 ± 0.01 | 11.23 ± 1.05 | 69.77 ± 6.32 | 40.73 ± 9.74 |
| ***S. algosus*** | | **24** | 1.44 ± 0.18 | 49.71 ± 7.21 | 0.34 ± 0.02 | 0.96 ± 0.07 | 11.03 ± 1.24 | 3.10 ± 0.27 | 15.55 ± 1.18 | 0.18 ± 0.03 | 12.68 ± 0.72 | 91.30 ± 17.51 | 88.64 ± 8.63 |
| ***Tegula* sp.** | | **41** | 0.61 ± 0.19 | 38.71 ± 10.31 | 0.11 ± 0.03 | 8.89 ± 1.07 | 21.99 ± 1.03 | 4.08 ± 0.58 | 0.21 ± 0.05 | 0.08 ± 0.03 | 21.63 ± 1.31 | 23.38 ± 1.41 | 7.86 ± 4.94 |
| ***C. sexdecimdentatus*** | | **26** | 0.24 ± 0.03 | 13.79 ± 1.67 | 0.06 ± 0.01 | 4.32 ± 0.62 | 46.59 ± 3.58 | 3.59 ± 0.51 | 0.06 ± 0.01 | 0.03 ± 0.01 | 8.13 ± 1.07 | 88.17 ± 8.43 | 33.22 ± 2.01 |
| ***Squilla* sp.** | | **9** | 0.19 ± 0.02 | 11.22 ± 0.88 | 0.07 ± 0.01 | 6.07 ± 0.88 | 24.98 ± 1.29 | 2.22 ± 0.68 | 5.10 ± 1.09 | 0.01 ± 0.01 | 30.54 ± 10.23 | 120.16 ± 47.91 | 62.67 ± 25.30 |
| ***Ulva* sp.** | | **12** | 1.02 ± 0.12 | 49.09 ± 11.68 | 0.13 ± 0.02 | 0.37 ± 0.06 | 4.59 ± 1.78 | 0.22 ± 0.03 | 0.11 ± 0.02 | 0.22 ± 0.07 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| ***A. scabrum*** | | **9** | 0.71 ± 0.13 | 62.10 ± 13.85 | 0.08 ± 0.01 | 10.28 ± 3.19 | 10.08 ± 0.88 | 17.73 ± 6.70 | 0.11 ± 0.02 | 0.09 ± 0.02 | 12.50 ± 2.59 | 8.53 ± 1.75 | 2.24 ± 0.47 |
| ***L. antiqua*** | | **2** | 0.76 ± 0.35 | 21.93 ± 0.10 | 0.39 ± 0.30 | 0.31 ± 0.02 | 12.24 ± 4.22 | 1.56 ± 0.14 | 0.13 ± 0.04 | 0.05 ± 0.02 | 2.16 | 7.00 | 20.24 |
| ***Rhodymenia* sp.** | | **7** | 0.99 ± 0.04 | 160.77 ± 26.04 | 0.11 ± 0.01 | 1.09 ± 0.11 | 4.51 ± 0.41 | 0.49 ± 0.04 | 0.06 ± 0.01 | 1.00 ± 0.21 | 1.63 ± 0.16 | 7.93 ± 1.69 | 0.00 ± 0.00 |
| **Red algae** | | **6** | 3.38 ± 1.29 | 955.41 ± 374.55 | 0.47 ± 0.19 | 7.47 ± 3.07 | 10.01 ± 1.28 | 1.16 ± 0.20 | 0.16 ± 0.02 | 3.46 ± 0.81 | 1.64 ± 0.18 | 1.46 ± 0.21 | 0.00 ± 0.00 |
| ***A. fontainei*** | | **6** | 1.66 ± 0.13 | 69.36 ± 13.94 | 0.06 ± 0.01 | 8.80 ± 2.59 | 12.77 ± 0.48 | 20.58 ± 4.07 | 0.09 ± 0.02 | 0.04 ± 0.01 | 4.90 ± 0.70 | 4.81 ± 0.41 | 1.46 ± 0.24 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***P. monodon*** | | **6** | 5.13 ± 0.68 | 52.37 ± 5.83 | 0.27 ± 0.02 | 7.45 ± 1.53 | 9.39 ± 0.99 | 0.89 ± 0.14 | 1.15 ± 0.24 | 0.12 ± 0.03 | 4.04 ± 1.54 | 62.95 ± 15.80 | 41.86 ± 4.91 |
| ***T. niger*** | non-edible | **19** | 3.84 ± 1.79 | 245.49 ± 118.90 | 0.46 ± 0.12 | 1.13 ± 0.18 | 22.26 ± 3.55 | 5.49 ± 1.00 | 1.05 ± 0.21 | 0.14 ± 0.07 | 119.95 ± 13.89 | 172.84 ± 24.19 | 66.95 ± 22.22 |
| ***C. filiformis*** | | **42** | 15.21 ± 5.58 | 213.61 ± 41.02 | 0.33 ± 0.04 | 0.58 ± 0.11 | 1.71 ± 0.13 | 0.47 ± 0.03 | 0.06 ± 0.01 | 0.25 ± 0.04 | 9.53 ± 0.81 | 15.82 ± 2.07 | 0.26 ± 0.12 |
| ***I. lambriformis*** | | **6** | 5.87 ± 0.86 | 49.00 ± 5.72 | 0.48 ± 0.04 | 1.80 ± 0.21 | 13.79 ± 1.19 | 1.95 ± 0.29 | 1.54 ± 0.09 | 0.06 ± 0.01 | 24.04 ± 3.83 | 63.19 ± 12.76 | 82.89 ± 15.99 |
| ***Codium* sp.** | | **16** | 3.07 ± 0.81 | 22.25 ± 1.56 | 0.12 ± 0.01 | 0.12 ± 0.02 | 1.27 ± 0.14 | 1.05 ± 0.07 | 0.06 ± 0.01 | 0.04 ± 0.01 | 7.62 ± 0.05 | 1.83 ± 0.06 | 0.00 ± 0.00 |
| ***C. plebejus*** | | **6** | 0.18 ± 0.05 | 5.91 ± 1.54 | 0.06 ± 0.03 | 5.77 ± 1.12 | 31.42 ± 4.87 | 8.92 ± 2.84 | 0.13 ± 0.06 | 0.02 ± 0.00 | 3.27 ± 0.16 | 42.47 ± 0.76 | 19.95 ± 0.67 |

**n= number of replicates for metal analysis (from 1-59 for fatty acid analysis)**

# ADDENDUM IV

## Supplementary material to Chapter 5

**Table 2.** Sampling year and period, region, species, taxonomic group, sample number (n), total length and total weight (mean ± S.D) of Peruvian marine species

| Sampling year | Sampling period | Region | Species | Taxonomic group | n | Total length (cm) | Total weight (g) |
|---|---|---|---|---|---|---|---|
| 2016 | low-rain | North | *Argopecten purpuratus* | scallop | 199 | 5.23 ± 6.28 | 24.28 ± 9.31 |
| | | | *Bursa ventricosa* | snail | 54 | 5.30 ± 0.47 | 27.40 ± 8.10 |
| | | | *Cymatium* sp. | snail | 18 | 7.45 ± 0.50 | 40.44 ± 10.94 |
| | | | *Octopus mimus* | cephalopod | 10 | 9.09 ± 1.66 | 315.10 ± 97.92 |
| | | | *Hepatus chilensis* | crab | 15 | 6.22 ± 0.55 | 50.63 ± 12.37 |
| | | | *Romaleon setosum* | crab | 26 | 11.29 ± 1.17 | 76.07 ± 142.87 |
| 2016 | high-rain | North | *Argopecten purpuratus* | scallop | 191 | 5.69 ± 9.27 | 32.78 ± 15.69 |
| | | | *Bursa ventricosa* | snail | 67 | 5.17 ± 0.51 | 22.73 ± 4.88 |
| | | | *Cymatium* sp. | snail | 31 | 7.78 ± 1.46 | 63.99 ± 30.16 |
| | | | *Octopus mimus* | cephalopod | 25 | 9.99 ± 1.76 | 386.40 ± 181.58 |
| | | | *Hepatus chilensis* | crab | 38 | 6.17 ± 0.66 | 51.05 ± 14.52 |
| | | | *Romaleon setosum* | crab | 4 | 10.62 ± 0.95 | 233.25 ± 60.98 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Argopecten purpuratus* | scallop | 96 | 5.75 ± 14.12 | 38.64 ± 29.34 |
| | | | *Bursa ventricosa* | snail | 18 | 5.88 ± 0.45 | 36.54 ± 12.67 |
| | | | *Cymatium* sp. | snail | 7 | 9.99 ± 1.84 | 100.71 ± 48.41 |
| | | | *Thaisella chocolata* | snail | 6 | 6.32 ± 0.39 | 46.68 ± 6.16 |
| | | | *Tegula* sp. | snail | 6 | 2.02 ± 0.24 | 8.20 ± 0.93 |
| | | | *Octopus mimus* | cephalopod | 14 | 10.77 ± 2.16 | 401.96 ± 261.56 |
| | | | *Cycloxanthops sexdecimdentatus* | crab | 10 | 3.57 ± 0.76 | 12.73 ± 6.96 |
| | | | *Hepatus chilensis* | crab | 27 | 6.52 ± 0.94 | 60.29 ± 22.31 |
| 2017 | low-rain | North | *Platixanthus orbigny* | crab | 6 | 9.08 ± 1.01 | 156.55 ± 65.81 |
| | | | *Platymera gaudichaudii* | crab | 4 | 11.20 ± 0.24 | 151.09 ± 20.64 |
| | | | *Romaleon setosum* | crab | 6 | 4.74 ± 0.67 | 20.09 ± 5.74 |
| | | | *Semimytilus algosus* | mussel | 18 | 5.01 ± 0.54 | 6.24 ± 2.04 |
| | | | *Pteria sterna* | oyster | 6 | 3.57 ± 0.79 | 8.97 ± 3.83 |
| | | | *Tetrapigus niger* | sea urchin | 7 | 3.06 ± 0.75 | 41.11 ± 15.34 |
| | | | Sea cucumber | sea cucumber | 7 | 3.86 ± 1.44 | 5.36 ± 7.38 |
| | | | *Chitonidae* sp. | chiton | 4 | 1.37 ± 0.17 | 0.46 ± 0.17 |
| | | | *Caulerpa filiformis* | algae | 6 | .. | .. |
| 2017 | low-rain | Center | *Argopecten purpuratus* | scallop | 146 | 6.62 ± 2.97 | 55.15 ± 10.32 |
| | | | *Thaisella chocolata* | snail | 13 | 53.74 ± 10.77 | 36.83 ± 20.28 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Tegula* sp. | snail | 6 | 28.46 ± 4.85 | 9.43 ± 4.73 |
| | | | *Hepatus chilensis* | crab | 9 | 73.98 ± 4.98 | 79.29 ± 16.82 |
| | | | *Romaleon setosum* | crab | 8 | 99.93 ± 11.39 | 194.67 ± 63.64 |
| | | | *Argopecten purpuratus* | scallop | 79 | 5.62 ± 12.67 | 34.33 ± 20.34 |
| | | | *Bursa ventricosa* | snail | 30 | 4.86 ± 1.16 | 22.07 ± 12.36 |
| | | | *Cymatium* sp. | snail | 16 | 7.85 ± 1.99 | 60.06 ± 52.65 |
| | | | *Thaisella chocolata* | snail | 6 | 6.03 ± 0.46 | 35.29 ± 12.37 |
| | | | *Tegula* sp. | snail | 12 | 1.61 ± 0.69 | 4.11 ± 3.69 |
| | | | *Octopus mimus* | cephalopod | 11 | 10.57 ± 2.32 | 464.62 ± 275.01 |
| | | | *Cycloxanthops sexdecimdentatus* | crab | 13 | 3.03 ± 0.83 | 9.10 ± 6.4 |
| | | | *Inachoides lambriformis* | crab | 13 | 0.98 ± 0.26 | 0.67 ± 0.54 |
| 2017 | high-rain | North | *Hepatus chilensis* | crab | 3 | 6.65 ± 0.49 | 54.93 ± 22.96 |
| | | | *Platixanthus orbigny* | crab | 1 | 8.38 | 195.28 |
| | | | *Tetrapigus niger* | sea urchin | 6 | 2.85 ± 0.72 | 20.70 ± 8.25 |
| | | | Sea cucumber | sea cucumber | 6 | 3.51 ± 0.65 | 3.86 ± 1.23 |
| | | | *Pteria sterna* | oyster | 12 | 3.42 ± 0.83 | 11.02 ± 9.28 |
| | | | *Semele corrugata* | clam | 5 | 4.30 ± 0.89 | 20.51 ± 12.68 |
| | | | *Tagelus dombeii* | clam | 13 | 5.79 ± 2.61 | 15.80 ± 17.82 |
| | | | *Squilla* sp. | shrimp | 9 | 12.57 ± 1.71 | 20.59 ± 8.21 |
| | | | *Panulirus sp.* | lobster | 6 | 18.64 ± 0.87 | 200.45 ± 31.75 |

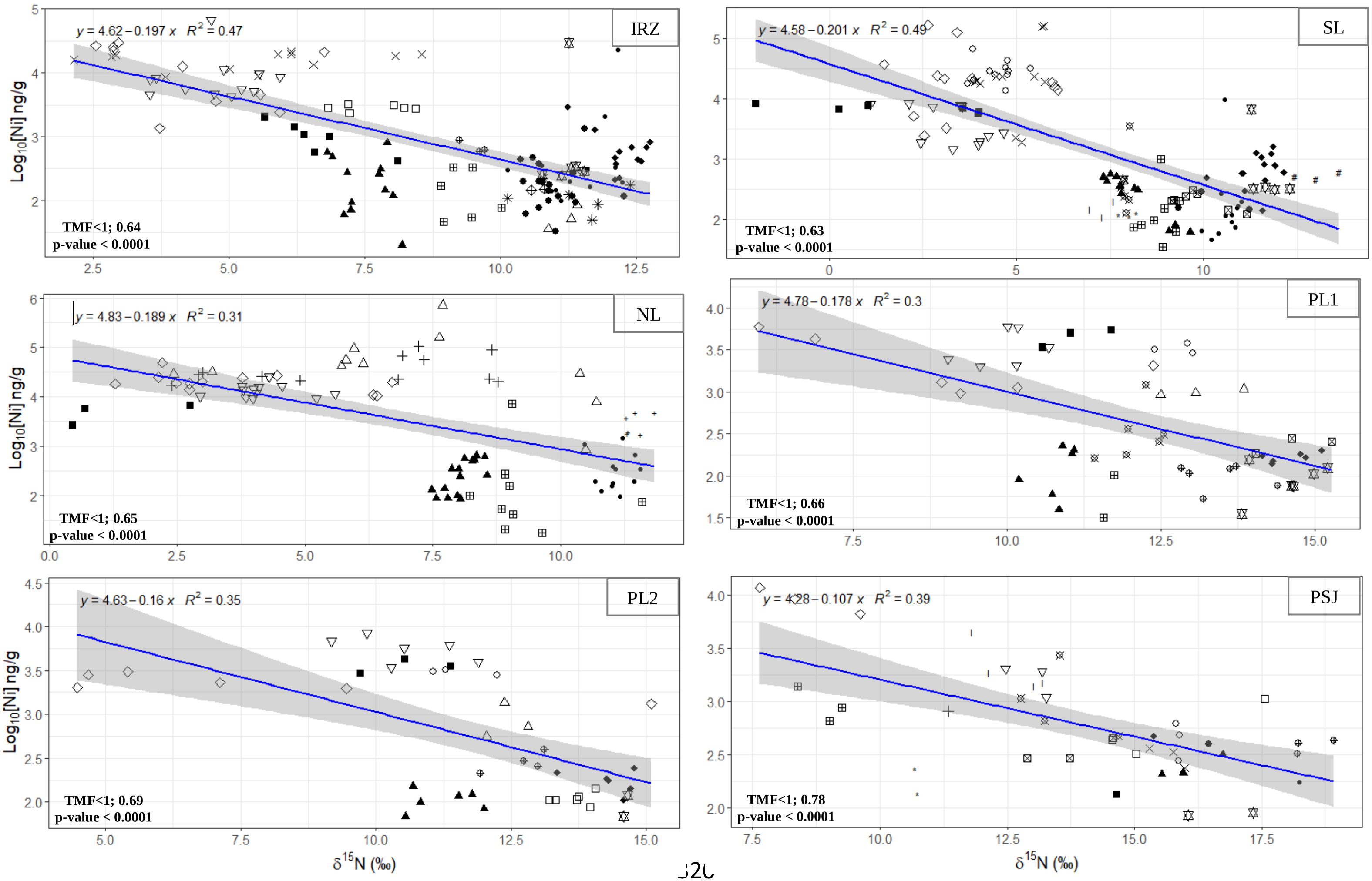
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Chitonidae* sp. | chiton | 6 | 1.42 ± 0.14 | 0.46 ± 0.08 |
| | | | *Ulva* sp. | algae | 6 | .. | .. |
| | | | *Caulerpa filiformis* | algae | 6 | .. | .. |
| 2017 | high-rain | Center | *Argopecten purpuratus* | scallop | 186 | 6.15 ± 4.72 | 42.83 ± 9.82 |
| | | | *Cymatium* sp. | snail | 6 | 88.25 ± 7.97 | 76.71 ± 22.85 |
| | | | *Thaisella chocolata* | snail | 12 | 69.55 ± 6.76 | 77.56 ± 33.45 |
| | | | *Tegula* sp. | snail | 46 | 23.92 ± 4.03 | 8.69 ± 2.78 |
| | | | *Cycloxanthops sexdecimdentatus* | crab | 7 | 54.05 ± 5.00 | 41.02 ± 12.51 |
| | | | *Hepatus chilensis* | crab | 10 | 75.26 ± 4.88 | 83.60 ± 16.63 |
| | | | *Semimytilus algosus* | mussel | 28 | 54.75 ± 4.49 | 8.09 ± 1.31 |
| | | | *Caulerpa filiformis* | algae | 12 | .. | .. |
| | | | *Chondracanthus chamissoi* | algae | 6 | .. | .. |
| | | | *Codium* sp. | algae | 6 | .. | .. |
| 2018 | high-rain | South | *Argopecten purpuratus* | scallop | 5 | 7.39 ± 1.25 | 99.60 ± 37.73 |
| | | | *Aeneator fontainei* | snail | 10 | 5.41 ± 0.25 | 18.70 ± 1.71 |
| | | | *Argobuccinum* sp. | snail | 13 | 4.57 ± 0.32 | 18.85 ± 5.48 |
| | | | *Bursa ventricosa* | snail | 2 | 6.98 ± 0.71 | 54.00 ± 5.66 |
| | | | *Oliva peruviana* | snail | 10 | 3.57 ± 0.25 | 9.60 ± 1.65 |
| | | | *Thaisella chocolata* | snail | 10 | 7.89 ± 0.44 | 108.10 ± 15.12 |

| | | | | |
|---|---|---|---|---|
| *Tegula* sp. | snail | 17 | 1.86 ± 0.82 | 13.37 ± 4.97 |
| *Xanthochorus cassidiformis* | snail | 4 | 4.57 ± 0.71 | 18.25 ± 7.50 |
| *Octopus mimus* | cephalopod | 1 | 11.35 | 499.00 |
| *Albunea lucasia* | crab | 10 | 4.64 ± 0.15 | 56.40 ± 3.78 |
| *Cancer plebejus* | crab | 9 | 8.43 ± 1.87 | 89.23 ± 36.38 |
| *Cancer porteri* | crab | 21 | 9.74 ± 0.73 | 161.53 ± 47.76 |
| *Hepatus chilensis* | crab | 2 | 7.38 ± 0.94 | 94.00 ± 45.26 |
| *Pagurus sp.* | crab | 18 | | 6.50 ± 5.82 |
| *Pinnaxodes chilensis* | crab | 4 | 1.46 ± 0.34 | 4.00 ± 1.42 |
| *Platymera gaudichaudii* | crab | 1 | 9.83 | 111.00 |
| *Romaleon setosum* | crab | 19 | 11.19 ± 1.06 | 271.74 ± 75.33 |
| *Lexochinus albus* | sea urchin | 5 | 8.10 ± 1.71 | 215.80 ± 141.95 |
| *Tetrapigus niger* | sea urchin | 10 | 6.52 ± 1.24 | 80.10 ± 25.90 |
| *Heliaster helianthus* | sea star | 10 | 6.69 ± 1.07 | 196.8 ± 50.91 |
| *Patiria chilensis* | sea star | 10 | 1.84 ± 0.43 | 5.00 ± 1.64 |
| *Stichaster striatus* | sea star | 10 | 8.86 ± 1.01 | 91.3 ± 11.39 |
| *Phymanthea pluvia* | anemone | 5 | 4.06 ± 1.14 | 43.00 ± 32.62 |
| *Leukoma antiqua* | clam | 2 | 5.02 ± 1.20 | 45.50 ± 37.48 |
| *Semele corrugata* | clam | 9 | 7.33 ± 0.35 | 114.67 ± 22.36 |

| | | | | |
|---|---|---|---|---|
| *Tagelus dombeii* | clam | 10 | 15.49 ± 0.97 | 57.20 ± 8.95 |
| *Pleuroncodes monodon* | lobster | 10 | 3.38 ± 0.45 | 0.93 ± 0.24 |
| *Paralichtys adspersus* | fish | 6 | 29.68 ± 3.70 | 302.17 ± 102.58 |
| *Fissurella* sp. | limpet | 4 | 5.30 ± 1.08 | 21.25 ± 10.35 |
| *Lessonia* sp. | algae | 6 | .. | .. |
| *Macrocystis pyrifera* | algae | 5 | .. | .. |
| Red algae | algae | 6 | .. | .. |
| *Rhodymenia* sp. | algae | 7 | .. | .. |
| *Ulva* sp. | algae | 6 | .. | .. |

**Total length: Molluscans and barnacle – shell length; Crabs – cephalothorax width; Cephalopods - mantle length; Shrimp, lobster, fish and sea cucumber - total length; Sea urchin and Anemone - Height length; Sea star – Arm length.**

**Sample tissues: scallop, snail, chiton, barnacle, sea cucumber, limpet and anemone as muscle; cephalopod as muscle arms; crab as soft tissue; oyster, mussel and sea urchin (*T. niger*) as soft tissue (incl. viscera), sea urchin (*L. albus*) and sea star as gonads; algae as thallus; lobster as muscle tail; clam as muscle+mantle+syphon+foot (with exception of *C. compta* as only muscle); shrimp as muscle tail; and small species: *I. lambriformis*, *Pa. chilensis*, *Pi. chilensis*, *P. monodon* and *Pagurus* sp. as *toto*.**



IRZ
y = 4.62 − 0.197 x R² = 0.47
TMF<1; 0.64
p-value < 0.0001
SL
y = 4.58 − 0.201 x R² = 0.49
TMF<1; 0.63
p-value < 0.0001
NL
y = 4.83 − 0.189 x R² = 0.31
TMF<1; 0.65
p-value < 0.0001
PL1
y = 4.78 − 0.178 x R² = 0.3
TMF<1; 0.66
p-value < 0.0001
PL2
y = 4.63 − 0.16 x R² = 0.35
TMF<1; 0.69
p-value < 0.0001
PSJ
y = 4.28 − 0.107 x R² = 0.39
TMF<1; 0.78
p-value < 0.0001
Log10[Ni] ng/g
δ15N (‰)

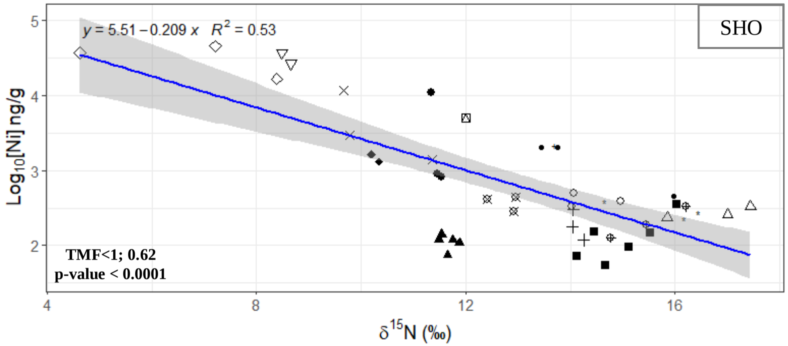
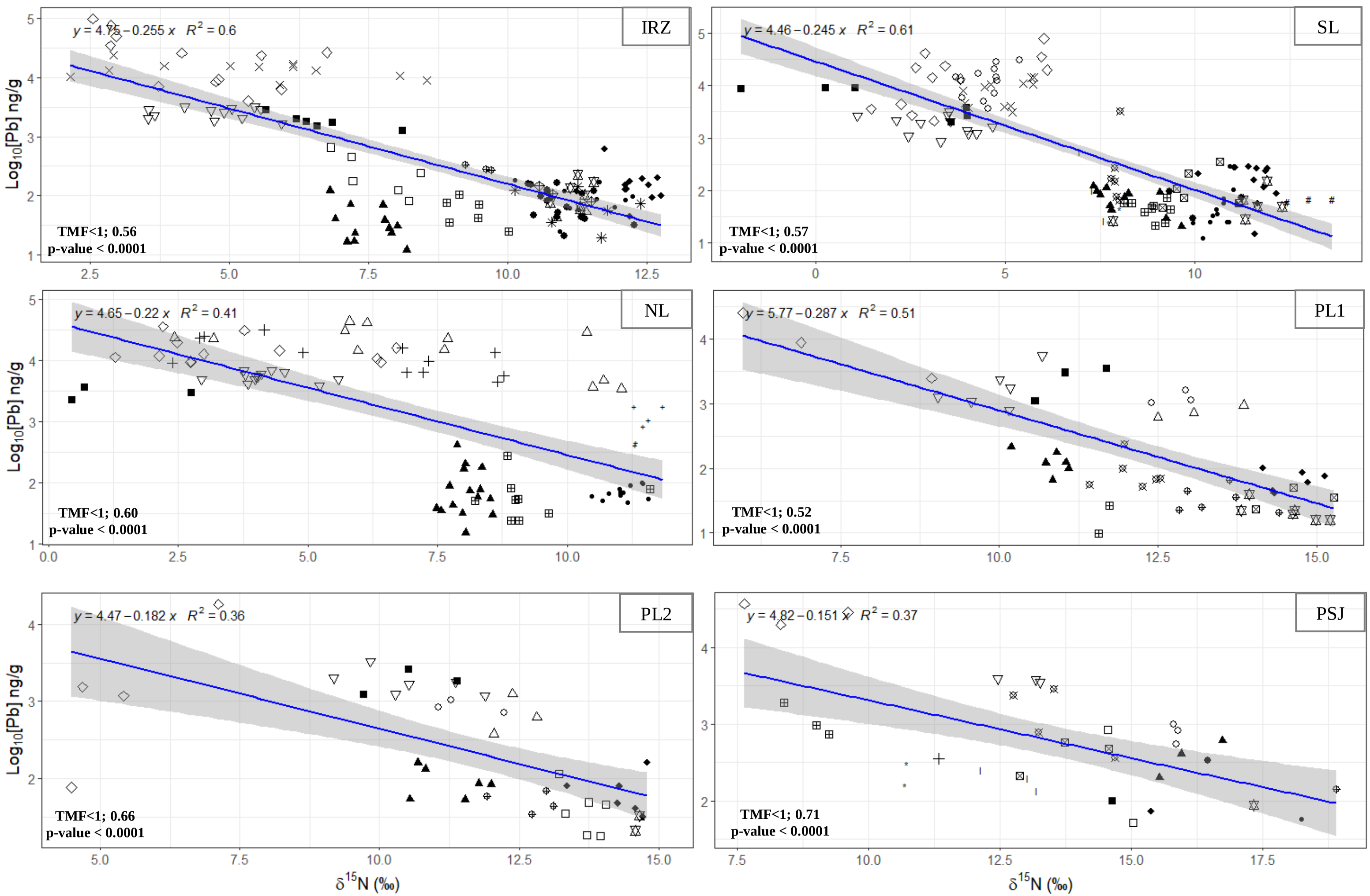



**Fig 9.** Relationship between δ $^{15}$N values and log-transformed concentrations (dwt.) of Ni in food sources and marine species from all locations of Peru.

**Species symbol per location are showed in Fig 5 (IRZ, SL, NL), Fig 6 (PL1, PL2) and Fig 7 (PSJ, SHO) from Chapter 5. Metal concentrations per taxonomic group can be found in Chapter 4; addendum section. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**


IRZ
y = 4.75 − 0.255 x R² = 0.6
TMF<1; 0.56
p-value < 0.0001
SL
y = 4.46 − 0.245 x R² = 0.61
TMF<1; 0.57
p-value < 0.0001
NL
y = 4.65 − 0.22 x R² = 0.41
TMF<1; 0.60
p-value < 0.0001
PL1
y = 5.77 − 0.287 x R² = 0.51
TMF<1; 0.52
p-value < 0.0001
PL2
y = 4.47 − 0.182 x R² = 0.36
TMF<1; 0.66
p-value < 0.0001
PSJ
y = 4.82 − 0.151 x R² = 0.37
TMF<1; 0.71
p-value < 0.0001
Log10[Pb] ng/g
δ15N (‰)

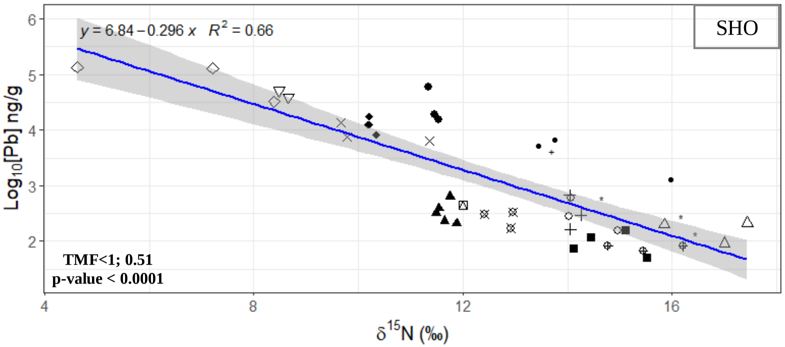
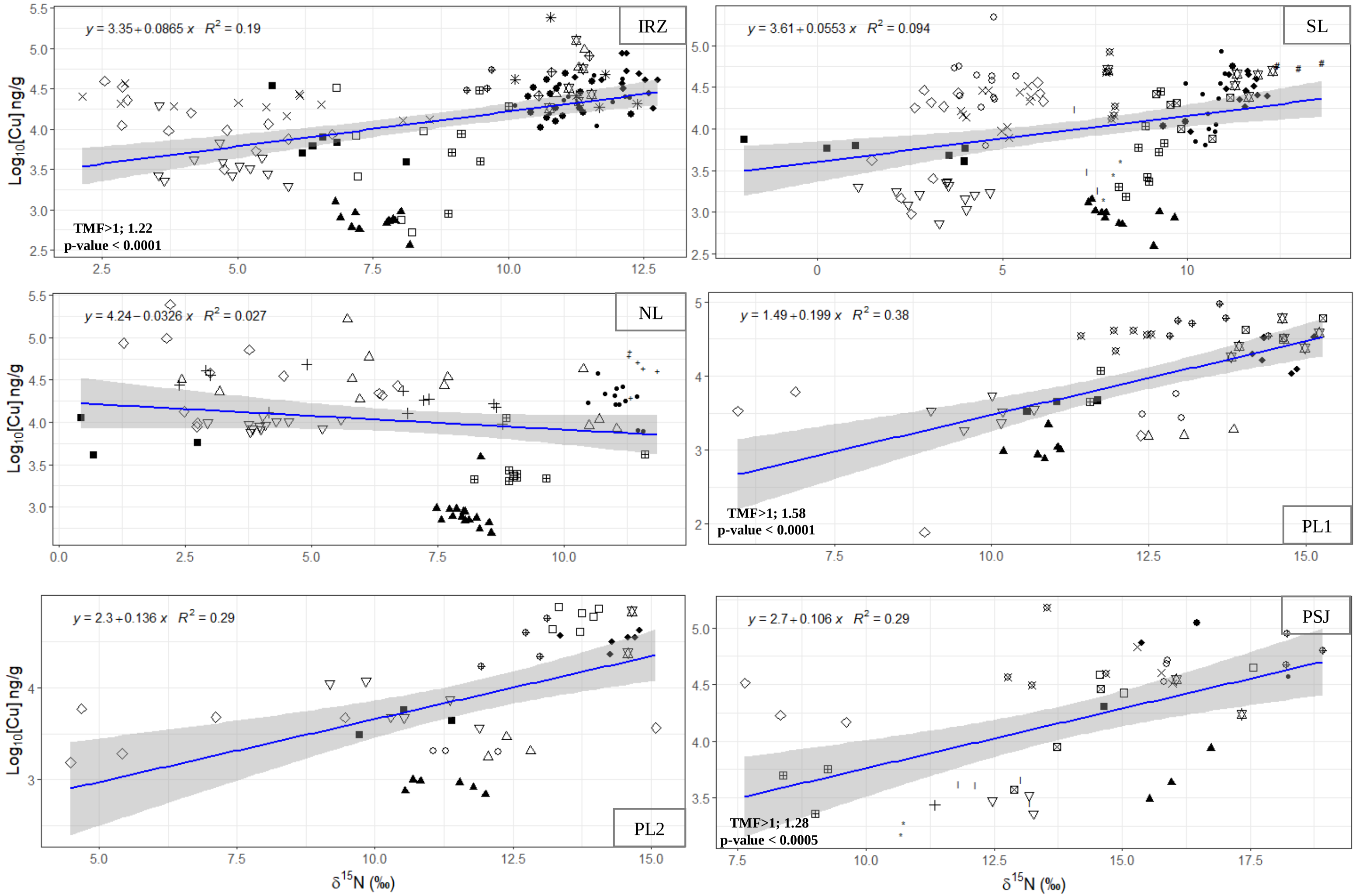



**Fig 10.** Relationship between δ $^{15}$N values and log-transformed concentrations (dwt.) of Pb in food sources and marine species from all locations of Peru.

**Species symbol per location are showed in Fig 5 (IRZ, SL, NL), Fig 6 (PL1, PL2) and Fig 7 (PSJ, SHO) from Chapter 5. Metal concentrations per taxonomic group can be found in Chapter 4; addendum section. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**



IRZ
y = 3.35 + 0.0865 x  $R^2$ = 0.19
TMF>1; 1.22
p-value < 0.0001
SL
y = 3.61 + 0.0553 x  $R^2$ = 0.094
NL
y = 4.24 − 0.0326 x  $R^2$ = 0.027
PL1
y = 1.49 + 0.199 x  $R^2$ = 0.38
TMF>1; 1.58
p-value < 0.0001
PL2
y = 2.3 + 0.136 x  $R^2$ = 0.29
PSJ
y = 2.7 + 0.106 x  $R^2$ = 0.29
TMF>1; 1.28
p-value < 0.0005
$Log_{10}$[Cu] ng/g
$\delta^{15}N$ (‰)

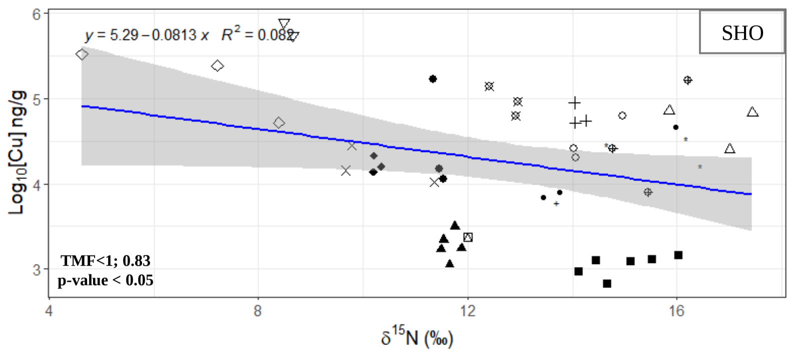
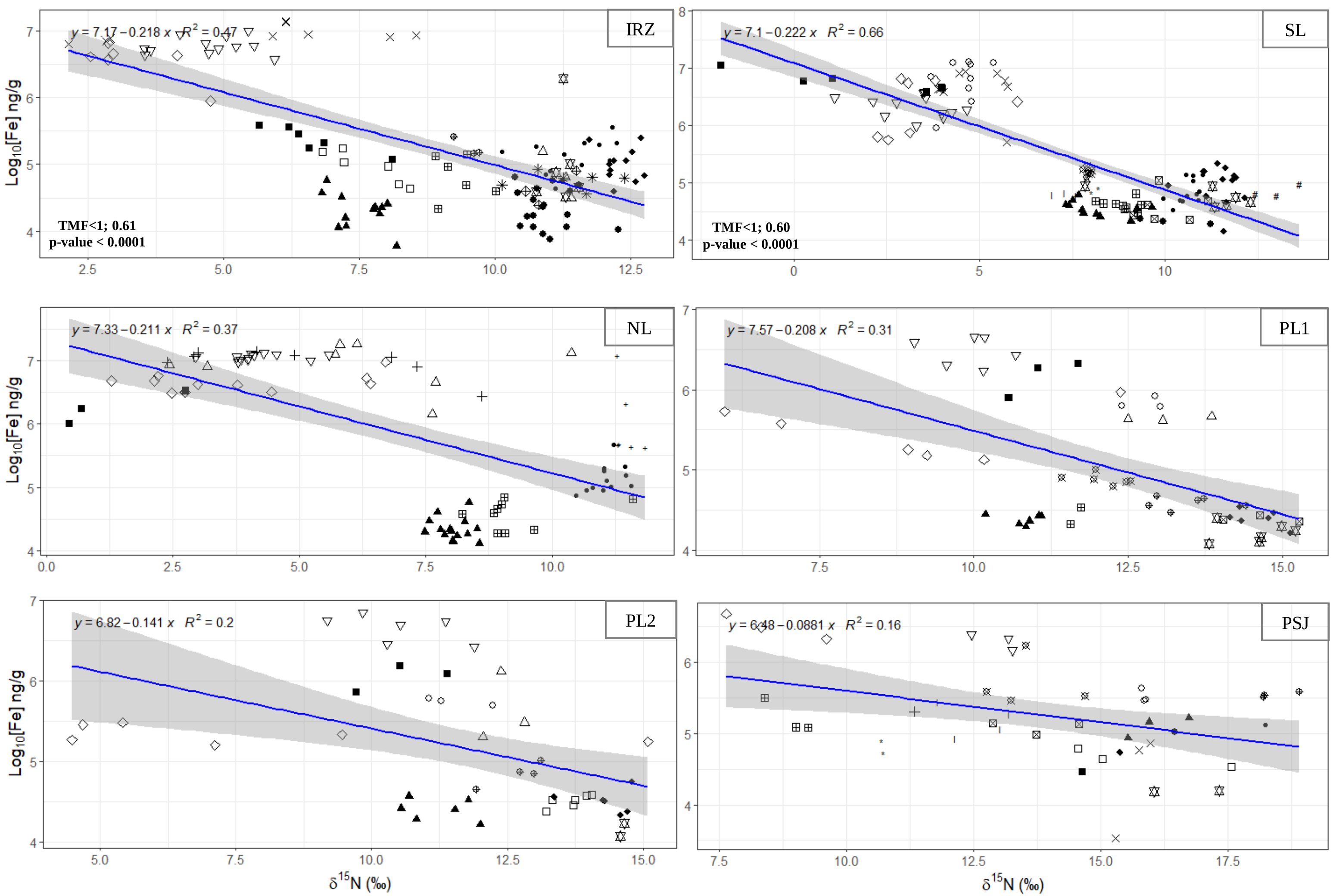



**Fig 11.** Relationship between $\delta^{15}N$ values and log-transformed concentrations (dwt.) of Cu in food sources and marine species from all locations of Peru.

**Species symbol per location are showed in Fig 5 (IRZ, SL, NL), Fig 6 (PL1, PL2) and Fig 7 (PSJ, SHO) from Chapter 5. Metal concentrations per taxonomic group can be found in Chapter 4; addendum section. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**


IRZ
y = 7.17 − 0.218 x   R² = 0.47
TMF<1; 0.61
p-value < 0.0001
SL
y = 7.1 − 0.222 x   R² = 0.66
TMF<1; 0.60
p-value < 0.0001
NL
y = 7.33 − 0.211 x   R² = 0.37
PL1
y = 7.57 − 0.208 x   R² = 0.31
PL2
y = 6.82 − 0.141 x   R² = 0.2
PSJ
y = 6.48 − 0.0881 x   R² = 0.16
Log10[Fe] ng/g
δ15N (‰)

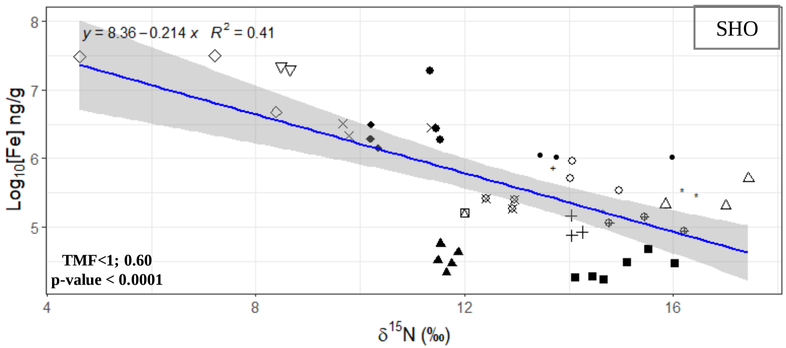
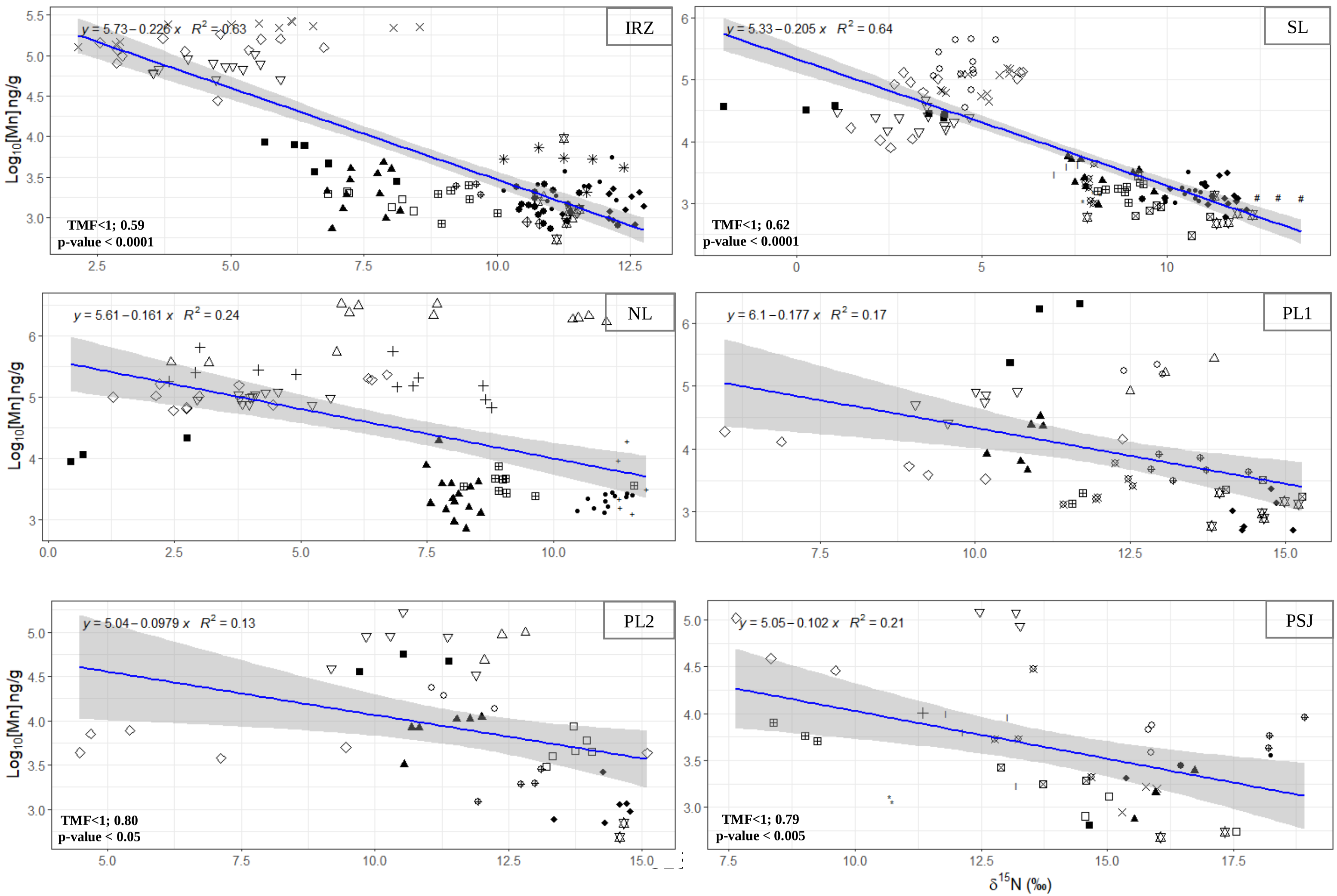



**Fig 12.** Relationship between $\delta^{15}N$ values and log-transformed concentrations (dwt.) of Fe in food sources and marine species from all locations of Peru.

**Species symbol per location are showed in Fig 5 (IRZ, SL, NL), Fig 6 (PL1, PL2) and Fig 7 (PSJ, SHO) from Chapter 5. Metal concentrations per taxonomic group can be found in Chapter 4; addendum section. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**


IRZ
$y = 5.73 - 0.226\,x$ $R^2 = 0.63$
TMF<1; 0.59
p-value < 0.0001
SL
$y = 5.33 - 0.205\,x$ $R^2 = 0.64$
TMF<1; 0.62
p-value < 0.0001
NL
$y = 5.61 - 0.161\,x$ $R^2 = 0.24$
PL1
$y = 6.1 - 0.177\,x$ $R^2 = 0.17$
PL2
$y = 5.04 - 0.0979\,x$ $R^2 = 0.13$
TMF<1; 0.80
p-value < 0.05
PSJ
$y = 5.05 - 0.102\,x$ $R^2 = 0.21$
TMF<1; 0.79
p-value < 0.005
$Log_{10}[Mn]$ ng/g
$\delta^{15}N$ (‰)

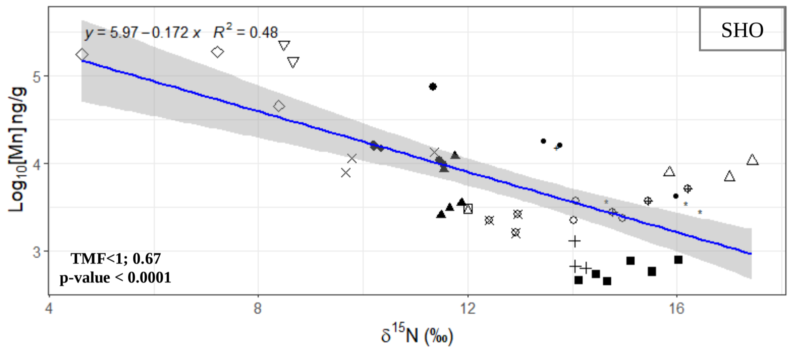
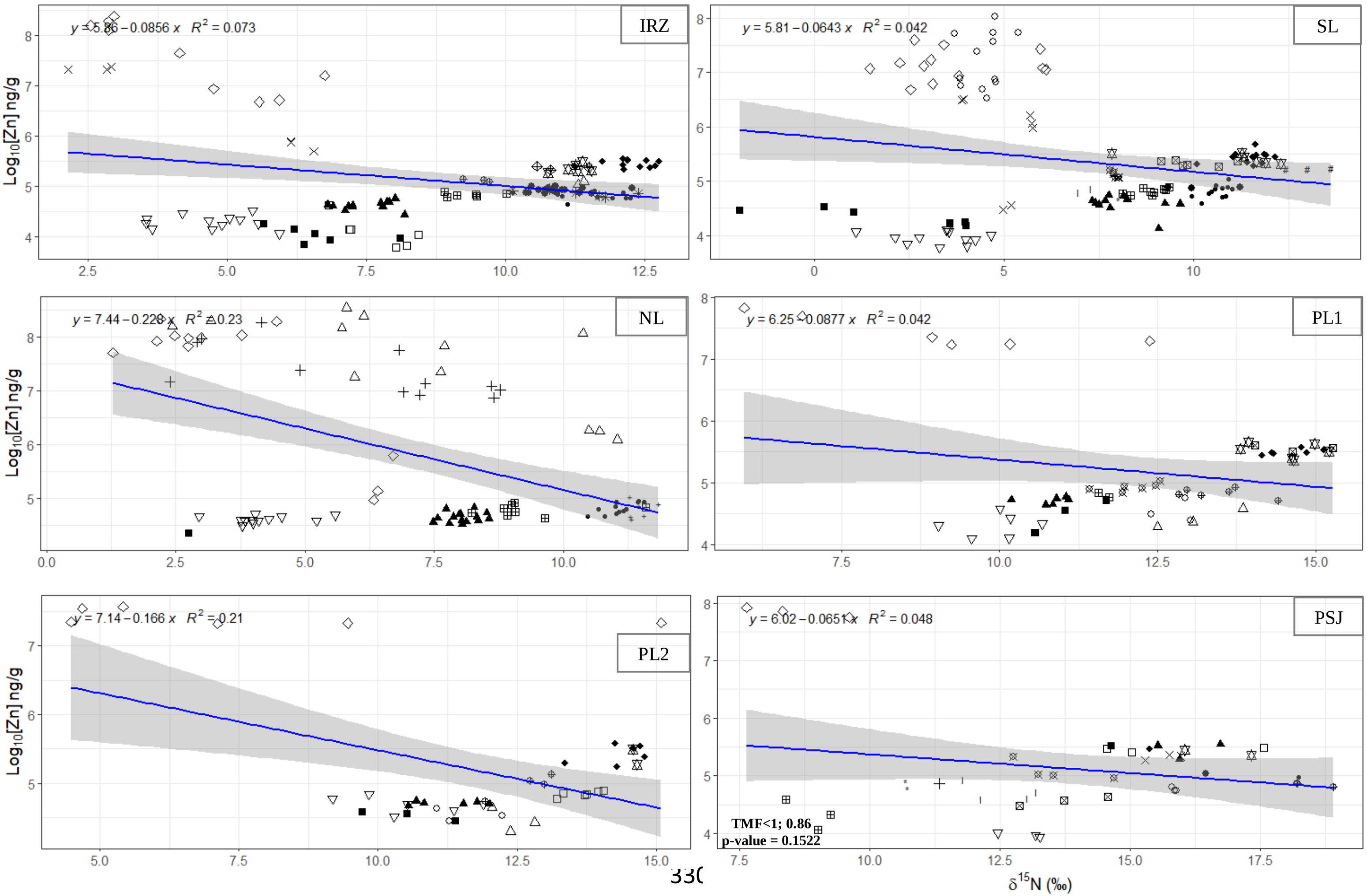



**Fig 13.** Relationship between δ [15]N values and log-transformed concentrations (dwt.) of Mn in food sources and marine species from all locations of Peru.

**Species symbol per location are showed in Fig 5 (IRZ, SL, NL), Fig 6 (PL1, PL2) and Fig 7 (PSJ, SHO) from Chapter 5. Metal concentrations per taxonomic group can be found in Chapter 4; addendum section. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**



IRZ
y = 5.86 − 0.0856 x R² = 0.073
SL
y = 5.81 − 0.0643 x R² = 0.042
NL
y = 7.44 − 0.228 x R² = 0.23
PL1
y = 6.25 − 0.0877 x R² = 0.042
PL2
y = 7.14 − 0.166 x R² = 0.21
PSJ
y = 6.02 − 0.0651 x R² = 0.048
TMF<1; 0.86
p-value = 0.1522
Log10[Zn] ng/g
δ15N (‰)

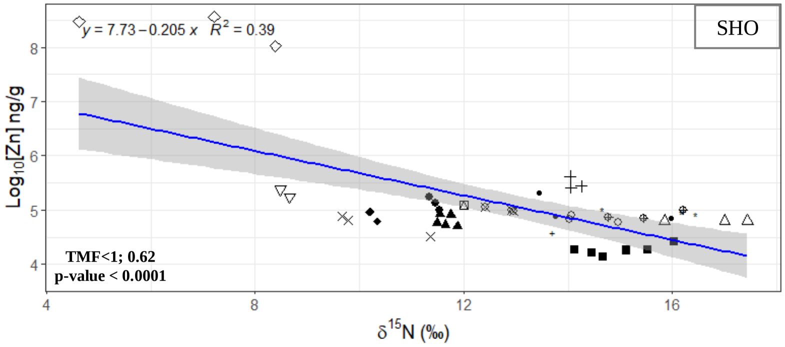
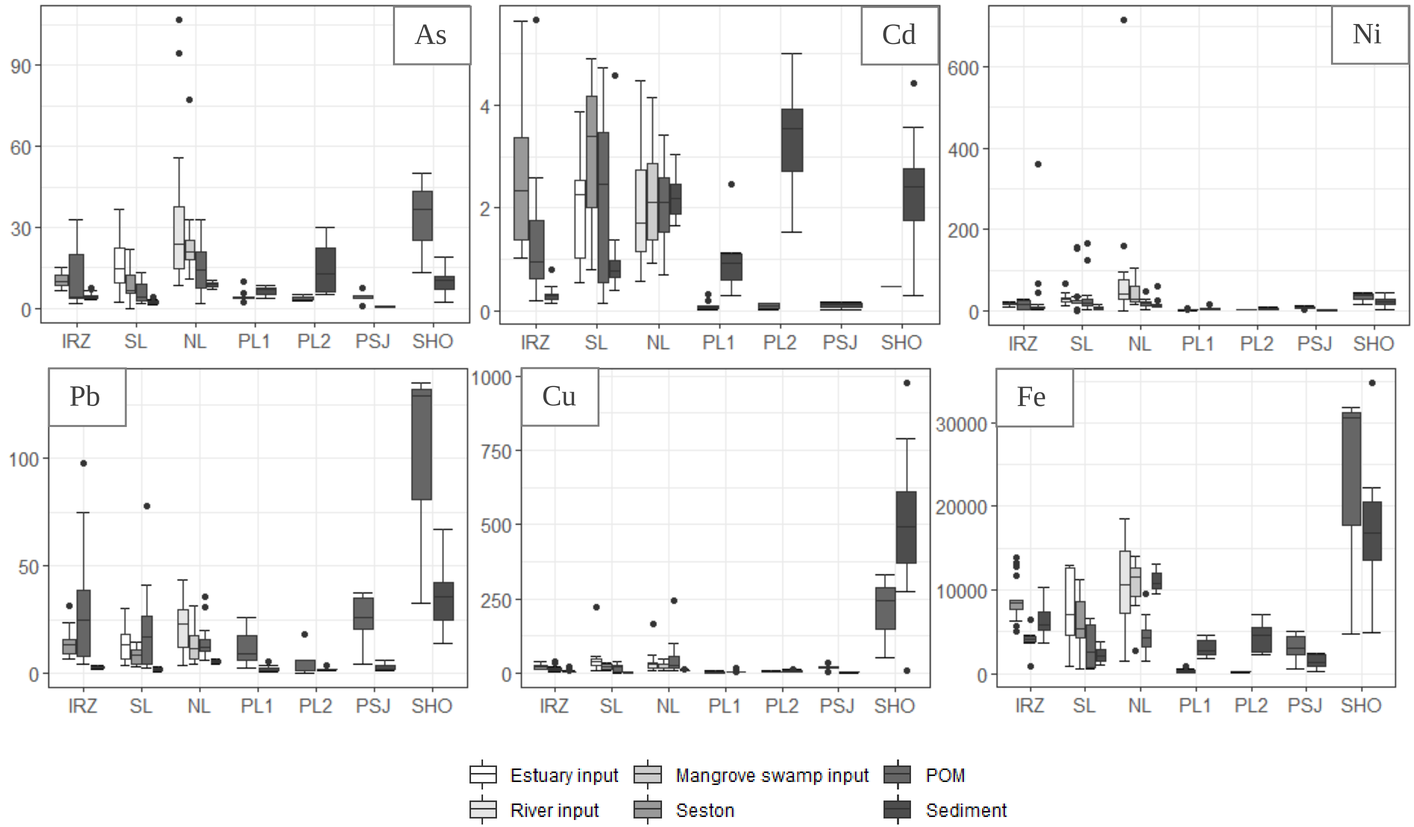



**Fig 14.** Relationship between $\delta^{15}N$ values and log-transformed concentrations (dwt.) of Zn in food sources and marine species from all locations of Peru.

**Species symbol per location are showed in Fig 5 (IRZ, SL, NL), Fig 6 (PL1, PL2) and Fig 7 (PSJ, SHO) from Chapter 5. Metal concentrations per taxonomic group can be found in Chapter 4; addendum section. A factor (ratio) of 1:5.5 was estimated (using 7720 measurements) for the conversion from wet (wwt) to dry (dwt) weight in muscle tissue.**


As
Cd
Ni
Pb
Cu
Fe
IRZ
SL
NL
PL1
PL2
PSJ
SHO
Estuary input
River input
Mangrove swamp input
Seston
POM
Sediment

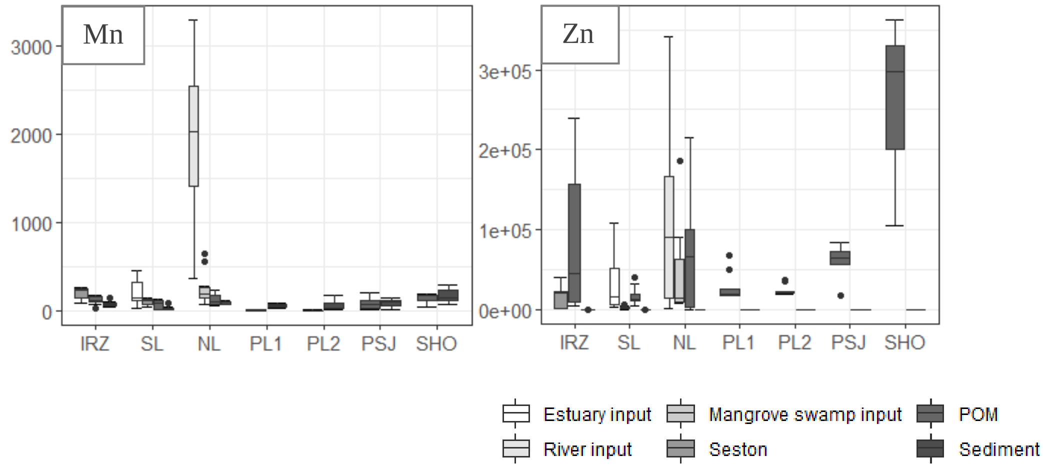



**Fig 15.** Metal concentrations (µg/g dwt.) in food sources (n=3-8 replicates per location) from north: Illescas Reserved Zone (IRZ), Sechura Bay (SL, NL), center-south: Paracas Bay (PL1, PL2), and south: Marcona (PSJ, SHO) 2016 – 2018.

**Table 2.** Certified (CC) and measured (MC) concentrations (μg/g dwt. ± S.D) for sediment certified reference materials (CRM).

| Metal | Certified reference material (CRM) | Replicates | Certified concentrations (CC) | Measured concentrations (MC) | Percentage of recovery (CC/MC x100%) |
|---|---|---|---|---|---|
| **Mn** | **BCR-320R- Channel Sediment** | 9 | 910.00 ± 50.00 | 979.48 ± 55.99 | 107.63 |
| **Fe** | | 9 | 25700.00 ± 1300.00 | 21744.03 ± 727.14 | 84.61 |
| **Ni** | | 9 | 27.10 ± 2.20 | 32.82 ± 3.70 | 121.10 |
| **Cu** | | 9 | 46.30 ± 2.90 | 49.63 ± 3.47 | 107.19 |
| **Zn** | | 9 | 319.00 ± 20.00 | 344.25 ± 19.33 | 107.91 |
| **As** | | 9 | 21.70 ± 2.00 | 24.30 ± 1.81 | 111.97 |
| **Cd** | | 9 | 2.64 ± 0.18 | 2.83 ± 0.19 | 107.06 |
| **Pb** | | 9 | 85.00 ± 5.00 | 77.51 ± 4.70 | 91.18 |
| **Fe** | **BCR-277R- Estuarine Sediment*** | 8 | 25700.00 ± 1300.00 | 28195.10 ± 633.25 | 109.71 |
| **Ni** | | 8 | 130.00 ± 8.00 | 70.62 ± 1.79 | **54.32** |
| **Cu** | | 8 | 63.00 ± 7.00 | 39.47 ± 1.26 | **62.64** |
| **Zn** | | 8 | 178.00 ± 20.00 | 106.24 ± 3.00 | **59.68** |
| **As** | | 8 | 18.30 ± 1.80 | 12.87 ± 0.44 | **70.29** |
| **Cd** | | 8 | 0.61 ± 0.07 | 0.40 ± 0.04 | **65.40** |

**(*) Recoveries in bold were used in order to calculate the final concentrations.**

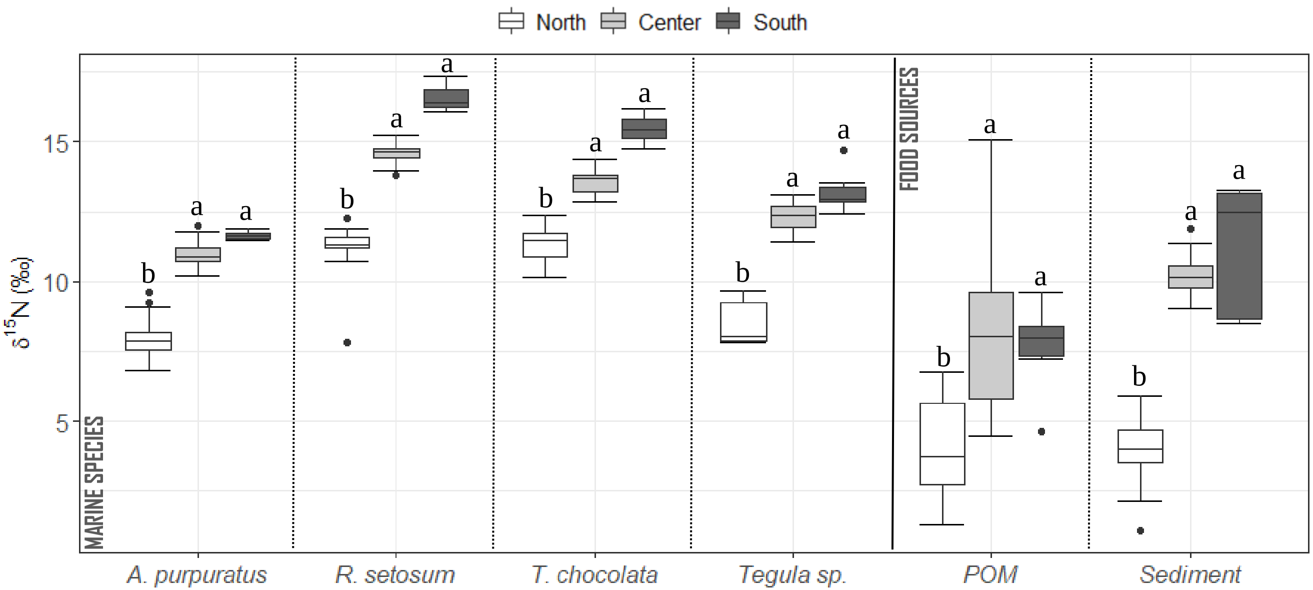



**Fig 16.** $\delta^{15}N$ of marine species and food sources per region. North (5 ºS), center (13 ºS) and south (15º S). Different letters indicate significant differences between regions within marine species or food sources.

# ADDENDUM V

Supplementary material to Chapter 6

**Table 1.** Functional or taxonomic groups, sample number (n), subgroup and species for the SIAR-mixing model (‘global’) of the Peruvian marine ecosystem.

| Functional or taxonomic group | n | Subgroup | Species |
|---|---|---|---|
| Scallop | **56** | | *Argopecten purpuratus* |
| Filter-feeders | 15 | Mussel | *Semimytilus algosus* |
| | 7 | Clam | *Semele corrugata* |
| | 6 | Clam | *Tagelus dombeii* |
| | 2 | Clam | *Leukoma antiqua* |
| | 6 | Oyster | *Pteria sterna* |
| **n total** | **36** | | |
| Benthic detritivorous | 9 | Crab | *Cycloxanthops sexdecimdentatus* |
| | 3 | Crab | *Pagurus* sp. |
| | 3 | Crab | *Platixanthus orbigny* |
| | 3 | Lobster | *Panulirus* sp. |
| **n total** | **18** | | |
| Herviborous gastropods | 26 | Snail | *Tegula* sp. |
| | 6 | Chiton | *Chitonidae* sp. |
| **n total** | **32** | | |

| | | | |
|---|---|---|---|
| Predatory gastropods | 38 | Snail | *Bursa ventricosa* |
| | 26 | Snail | *Cymatium* sp. |
| | 21 | Snail | *Thaisella chocolata* |
| | 6 | Snail | *Argobuccinum* sp. |
| | 3 | Snail | *Aeneator fontainei* |
| | 3 | Snail | *Oliva peruviana* |
| | 3 | Snail | *Xanthochorus cassidiformis* |
| **n total** | **100** | | |
| Predatory crabs | 23 | Crab | *Romaleon setosum* |
| | 37 | Crab | *Hepatus chilensis* |
| | 3 | Crab | *Albunea lucasia* |
| | 3 | Crab | *Cancer plebejus* |
| | 3 | Crab | *Cancer porteri* |
| | 1 | Crab | *Platymera gaudichaudii* |
| **n total** | **70** | | |
| Predatory benthic fish | **6** | Flatfish | *Paralichtys adspersus* |
| Octopod | **22** | Octopus | *Octopus mimus* |
| Sea urchins | 9 | | *Tetrapigus niger* |
| | 3 | | *Lexochinus albus* |
| **n total** | **12** | | |
| Sea stars | 3 | | *Heliaster helianthus* |
| | 3 | | *Patiria chilensis* |
| | 3 | | *Stichaster striatus* |

| | | |
|---|---|---|
| **n total** | **9** | |
| Macroalgae | 21 | *Caulerpa filiformis* |
| | 6 | *Chondracanthus chamissoi* |
| | 6 | *Codium* sp. |
| | 6 | *Red Algae* |
| | 4 | *Ulva* sp. |
| | 3 | *Lessonia* sp. |
| | 3 | *Macrocystis pyrifera* |
| **n total** | **49** | |

**(*) Functional groups are based on ecological information (incl. pers. obs.) and Taylor et al. 2008.**

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## Peer reviewed articles:

**Loaiza, I.**, De Troch, M., & De Boeck, G. (2018). Potential health risks via consumption of six edible shellfish species collected from Piura-Peru. *Ecotoxicology and environmental safety*, *159*, 249-260

**Loaiza, I.**, Pillet, M., De Boeck, G., & De Troch, M. (2019). Peruvian scallop *Argopecten purpuratus*: From a key aquaculture species to a promising bioindicator species. *Chemosphere*, 124767

## Articles in preparation:

**Loaiza, I.**, De Troch, M. & De Boeck, G., (under review). Marine species as safe source of LC-PUFA and micronutrients: insights in new promising marine food in Peru.

**Loaiza I.**, De Boeck G., Alcazar J., Campos D., Cárdenas-Alayza S., Ganoza M., Gómez-Sanchez M., Miglio M. and De Troch M.(in prep.). Peruvian marine ecosystems: trophic interactions and metal transfer in aquatic food webs.

Lydie I.E. Couturier; Philippe Soudant; **Iván Loaiza**; Teresa Amaro; Suzanne Budge; Elisabeth Costa; Marleen DeTroch; Valeria DiDato; Patrick Fink; Carolina Giraldo; Fabienne Le Grand; Margaux Mathieu-Resuge; Loic Michel; Peter Nichols; Chris Parrish; Fany Sardenne; Marie Vagner; Fabrice Pernet (in prep.) Fatty acid analyses in aquatic trophic ecology, aquaculture and nutrition: state of the art and best practices.

**Other publications:**

Waller, C. L., Griffiths, H. J., Waluda, C. M., Thorpe, S. E., **Loaiza, I**., Moreno, B., ... & Hughes, K. A. (2017). Microplastics in the Antarctic marine system: an emerging area of research. *Science of the Total Environment*, *598*, 220-227.

Waller, C., Griffiths, H. J., Waluda, C. M., Thorpe, S. E., **Loaiza, I**., Moreno, B., ... & Hughes, K. A. (2018). Microplastics in the Southern Ocean.

**Previous publication:**

**Loaiza, I.**, Hurtado, D., Miglio, M., Orrego, H., & Mendo, J. 2015. Tissue-specific Cd and Pb accumulation in Peruvian scallop (*Argopecten purpuratus*) transplanted to a suspended and bottom culture at Sechura Bay, Peru. *Marine pollution bulletin, 91*(2), 429-440.

**Supervision of students:**

***Bachelor theses***

Determinación de plomo (Pb) y cadmio (Cd) mediante el uso del bivalvo (*Tagelus dombeii*) en aguas de la bahía de Sechura, Piura – 2017. - *Antonio Díaz Bello*

Bioacumulación de cadmio y cobre en Concha de abanico *Argopecten purpuratus* en las Bahías de Paracas y Sechura, Perú. – *Mariano Cabanillas Torpoco*

The pollution response of marine invertibrates: combination of field and lab experiment – *Siebe Vandecasteele*

***Master thesis***

Biomagnificación de metales en predadores de Concha de abanico (*A. purpuratus)*, en concesiones de acuicultura en Bahía de paracas. – *Maria Cristina Miglio Toledo*

***Bachelor thesis projects***

Bach. thesis project: Bioacumulación de plomo y arsénico en Concha de abanico (*Argopecten purpuratus*) en las Bahías de Paracas y Sechura, Verano 2017 – *Abigail Chuzón Pacheco*

Bach. thesis project: Trofodinámica de la comunidad bentónica asociada al cultivo de fondo de *Argopecten purpuratus* (Lamarck, 1819) "concha de abanico" mediante análisis de isótopos estables en la Bahía de sechura durante las precipitaciones de verano 2016-2017 – *Diego Nahuel Campos*

**Project coordinator:**

**TRACEseafood:** Traceability and trophodynamics of seafood species from the Punta San Juan (PSJ) marine protected area (Peru).

**DEPURAproject:** Depuration of mollusc bivalves for human consumption in Peru.

**SINAPSIS 2019** (as part of the Committee Organizer)**:** Fourth Meeting of Peruvian Scientists in Europe SINAPSIS 2019. Gent, Belgium.

**Conference presentations:**

***Oral presentations***

**Loaiza I.**, De Boeck G. and De Troch M. Marine species from Peru: a safe solution to improve the intake of polyunsaturated fatty acids and first insight of new alternative diets. XVIII Congreso Latinoamericano de Ciencias del Mar (COLACMAR). Mar de Plata, Argentina. December, 2019.

**Loaiza I.**, Cárdenas-Alayza S., Miglio M., Ganoza M., Campos D., Millet M., De Boeck G. and De Troch M. MACOPSproject: from PhD project to networking opportunity and development of new guidelines for a better sanitary monitoring program in Peru. Oceans of Opportunities, Rivers of Ideas **(**OORI) 2018. Brussels, Belgium. December, 2018.

**Loaiza I**., De Boeck G. and De Troch M. Benefits and risks of seafood consumption in Peru: quantitative analysis of fatty acid and micro-contaminant intake. LIPIDS IN THE OCEAN conference. Brest, France. November, 2018

**Loaiza I**., De Boeck G. and De Troch M. Beneficios y riesgos por consumo de productos pesqueros del Perú: análisis cuantitativo de la ingesta de ácidos grasos y micro-contaminantes. Third Meeting of Peruvian Scientists in Europe SINAPSIS 2018. Barcelona, Spain. October, 2018

**Loaiza I**., De Boeck G. and De Troch M. Macrobenthic communities associated to the Peruvian scallop *Argopecten purpuratus* culture (MACOPS): structural and functional diversity, feeding ecology and contaminant exposure. Marine Symposium Section - Marine Biology Research Group 16MBSS 2018**.** Gent, Belgium. May, 2018

**Loaiza I**., De Boeck G. and De Troch M. Comunidades macrobénticas marinas asociadas al cultivo de la concha de abanico Argopecten purpuratus: diversidad funcional y estructural, ecología de alimentación y exposición a contaminantes. Second meeting of Peruvian Scientists in Europe SINAPSIS 2017. Berlin, Germany. October 2017.

**Loaiza I**., De Troch., De Boeck. 2016. Organ distribution and food safety aspects of trace metals in Peruvian scallop *Argopecten purpuratus* and macrobenthic associated community from Sechura Bay and Illescas Reserved Zone, Piura – Peru. V Congreso Nacional de Ciencias Marinas (CONCIMAR). Chiclayo, Peru. November 2016

***Poster presentations***

**Loaiza I**., De Boeck G., De Troch. 2016. First insigths in the trophic interactions and metal biomagnitication in benthic food webs from Sechura Bay and Illescas Reserved Zone, Piura – Peru. V Congreso Nacional de Ciencias Marinas (CONCIMAR). Chiclayo, Peru. November 2016

**Loaiza I**., De Boeck G., De Troch. 2016. Macrobenthic communities associated to the Peruvian scallop *Argopecten purpuratus* culture (MACOPS): structural and functional diversity, feeding ecology and contaminant exposure. Biology Research Day 2016 (BRD). Antwerp, Belgium. October 2016.

**Relevant work during the PhD**:

**March 2019 - Present. PROGRAMA NACIONAL DE INNOVACIÓN EN PESCA Y ACUICULTURA (PNIPA). Consultant as Peer reviewer (PET).** Reviewer of project applicants about the feasibility of new aquaculture species in Peru: mussels, algae, oysters, among others, as well as the implementation of new processing, food safety and environmental concerns in Fisheries and Aquaculture.

**March 2014 - Present. UNIVERSIDAD CIENTIFICA DEL SUR (UCSUR). Associate Professor.** Faculty of Marine Biology. Teaching: Fisheries and Environmental impacts.

**December, 2019. Organismo Nacional de Sanidad Pesquera (SANIPES). Senior Consultant.** Teaching and consulting in the workshop and training programme: “Aplicación de metodologías *Research-oriented*, para la evaluación sanitaria de ecosistemas acuáticos, recursos pesqueros y su cadena productiva: Estrategia de muestreo basado en *Research-question approach, data management*, *BIG Data análisis*, diseño de estudios de riesgo y *Scientific writing*”.