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THE EARLY LIFE GROWTH OF ANCHOVETA (*Engraulis ringens*) AND THE GROWTH-MORTALITY HYPOTHESIS IN THE HUMBOLDT CURRENT SYSTEM OFF CHILE.

José Francisco Cerna Troncoso

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Doctor by the Universitat de les Illes Balears

Ackowledgments

Dedicated to my Dear wife Cecilia and my children Paula, Pamela and Camilo, and in memory of my mother Lucila, father José Gregorio, and my father-in-law and friend Sergio Ramon.

Abstract

The population structure, growth pattern and the relationship between growth and survival of anchoveta (*Engraulis ringens*) in the Humboldt Current System (HCS) off Chile were studied using morphometry and microstructure analysis of sagittal otoliths.

The phenotypical variability in otolith shape of anchoveta was analyzed among three zones, i.e., Northern zone I (18-24 °S); North-central zone II (26-30 °S) and Southern zone III (33-42 °S) off the Chilean coast, using generalized Additive Models (GAM) to analyze shape indices and canonical discriminant analysis to analyze Elliptical Fourier Descriptors (EFDs) of otolith outline. The Form factor and ellipticity indices varied significantly among the three zones, whereas roundness, circularity and rectangularity indices only showed differences between zones I and III. The EFDs showed the identifying at least two completely independent demographic units (I and & III) of anchoveta off the Chilean coast, lower discriminating power between zone I and II, suggests some level of mixing between these zones. The results support the hypothesis that juveniles and adults of anchoveta have remained segregated throughout their entire or at least a fraction of their life-cycles, mainly between the extreme northward and southward zones.

The spatial and temporal variation of the early growth patterns (EGP) of anchoveta, were analyzed and compared among three geographic areas, i.e., Northern zone (18-24 °S); North-central zone (26-30 °S) and Southern zone (33-42 °S) off the Chilean coast over three years (i.e., 2015, 2016 and 2019). EGP were determined through otolith microstructure analysis for juveniles ranging from 2.0 to 12.0 cm in total length (TL), corresponding to ages from 28 to 187 days. The comparative growth analysis between the three study areas for the 2015, 2016 and 2019 cohorts showed significant differences in the Laird-Gompertz growth parameters and the absolute growth rate at the inflection point (0.67-1.11 mmd^{-1}). A generalized additive model (GAM) was used to analyze changes in mean daily increment width profiles (IW) as related to the area, monthly cohort, sea surface temperature (SST) and Chlorophyll concentration (Chloa) as predictive variables. The GAM did not show significant difference in mean IW between northern and north-central areas (7.9±3.7 vs 8.3± 4.1μm), although they significantly varied with respect the southern area, where the lower values of IW were found (5.6±3.0 μm). The GAM showed higher IW partial residuals when SST was above 14°C and Chloa concentration was lower than 2 mgm^{-3}. Overall results showed a high plasticity in the early growth pattern of *E. ringens*, indicating interannual and spatial variability, with SST playing a pivotal role across a wide latitudinal gradient off Chilean coast.

The growth-mortality hypothesis was tested in spring cohorts of anchoveta in four different years (2014, 2019, 2020 and 2021) in Northern Chile (18-24°S) in the HCS. Linear mixed-effect models (LMMs) were used to compare the variation of otolith radii-at-age (RAI) and increment width (IW), as proxy of fish size and growth rate of survivors (pre-recruit and recruits) with an initial population of larvae hatched in spring. Spline smoothed profiles of RAI and IW showed an increasing pattern from a common origin, after which a clear divergent tendency appeared, where survivors always showed larger and wider RAI and IWs for the first 20 days, when comparing with larvae, for the two cohorts evaluated (2014 and 2019). LMMs showed that both RAI and IW were significant bigger and higher for recruits than larvae, irrespective of years. Overall results showed evidence that survivors were bigger and grew faster than members of the original population, giving support for the growth–mortality hypothesis for this small pelagic fish in the HCS. In addition, other distinctive finding was that older juvenile (recruits), were larger and grew faster than young juveniles (pre-recruits), when were larvae and transforming juveniles.

Resum

Es va estudiar l'estructura poblacional, el patró de creixement i la relació creixement-sobrevivència de l'anchoveta (*Engraulis ringens*) al Sistema del Corrent de Humboldt (HCS) davant de Xile mitjançant morfometria i anàlisi de microestructura d'otòlits sagitals.

La variabilitat fenotípica en la forma otòlit de l'anxova es va analitzar entre tres zones, és a dir, la zona nord I (18-24 °S); Zona central nord II (26-30 °S) i zona sud III (33-42 °S) davant de la costa xilena, utilitzant Models Additius Generalitzats (GAM) per analitzar índexs de forma i anàlisi discriminant canònic per analitzar Descriptors El·líptics de Fourier (EFDs) del contorn de l'otòlit. Els índexs factor de forma i el·lipticitat van variar significativament entre les tres zones, mentre que els índexs rodonesa, circularitat i rectangularitat només van mostrar diferències entre les zones I i III. Els EFD van mostrar l'existència d'almenys dues unitats demogràfiques completament independents (I i III) davant de la costa xilena, encara que el menor poder discriminant entre la zona I i II suggereix algun nivell de barreja entre aquestes zones.

Es va analitzar i comparar la variació espacial i temporal dels patrons de creixement primerenc (EGP) de l'ampitxeta entre tres àrees geogràfiques, és a dir, zona nord (18-24 °S); Zona centre-nord (26-30 °S) i zona sud (33-42 °S) davant Xile per a les cohorts 2015, 2016 i 2019. Els EGP es van determinar mitjançant l'anàlisi de la microestructura dels otòlits per a juvenils de 2,0 a 12,0 cm de longitud total (LT), corresponents a edats de 28 a 187 dies. L'anàlisi de creixement comparatiu entre les tres zones d'estudi per a les cohorts va mostrar diferències significatives als paràmetres de creixement de Laird-Gompertz i la taxa de creixement absoluta al punt d'inflexió (0,67-1,11 mmd-1). Es va utilitzar un model additiu generalitzat (GAM) per analitzar els canvis als perfils d'amplada d'increment diari mitjà (IW) en relació amb la zona, la cohort mensual, la temperatura de la superfície del mar (SST) i la concentració de clorofil·la (Chloa) com a variables predictives. El GAM no va mostrar diferència significativa en IW mitjana entre les àrees nord i centre-nord (7.9±3.7 vs 8.3±4.1μm), encara que sí que va variar significativament respecte a l'àrea sud, on es van trobar els menors valors d'IW (5.6±3.0 μm). El GAM va mostrar més residus parcials d'IW quan la SST va estar per sobre de 14°C i la concentració de Chloa va ser menor a 2 mgm^{-3}. Els resultats generals van mostrar una alta plasticitat en el patró de creixement primerenc d'E. ringens, cosa que indica una variabilitat interanual i espacial, amb la TSM exercint un paper fonamental en un ampli gradient latitudinal davant de la costa xilena.

La hipòtesi de creixement-mortalitat es va provar en cohorts primaverals d'ampitxeta en quatre anys diferents (2014, 2019, 2020 i 2021) al nord de Xile (18-24°S) a l'HCS. Es van utilitzar models lineals d'efectes mixtos (LMM) per comparar la variació dels radis per edat (RAI) i l'amplada d'increment (IW) dels otòlits, com a proxy de la mida dels peixos i la taxa de creixement dels supervivents (Prereclutes i reclutes) amb una població inicial de larves. Els perfils suavitzats spline de RAI i IW van mostrar un patró creixent a partir d'un origen comú, després d'això va aparèixer una clara tendència divergent, on els supervivents sempre van mostrar RAI i IW més grans i més amples durant els primers 20 dies, en comparació amb les larves, per a dues cohorts avaluades. (2014 i 2019). Els LMM van mostrar que tant RAI com IW eren significativament més grans i més alts per als reclutes que per a les larves, independentment dels anys. Els resultats generals van mostrar evidència que els supervivents eren més grans i creixien més ràpid que els membres de la població original, cosa que recolza la hipòtesi de creixement-mortalitat per a aquest petit peix pelàgic a l'HCS. A més, una altra troballa distintiva va ser que els juvenils més vells (reclutes), eren més grans i creixien més ràpid que els juvenils joves (prereclutes), quan eren larves i juvenils en transformació.

Resumen

Se estudió la estructura poblacional, el patrón de crecimiento y la relación crecimiento-sobrevivencia de la anchoveta (*Engraulis ringens*) en el Sistema de la Corriente de Humboldt (HCS) frente a Chile mediante morfometría y análisis de microestructura de otolitos sagitales.

La variabilidad fenotípica en la forma de los otolitos de anchoveta se analizó entre tres zonas, i.e., zona norte I (18-24 °S); Zona centro-norte II (26-30 °S) y zona sur III (33-42 °S) frente a la costa chilena, utilizando Modelos Aditivos Generalizados (GAM) para analizar índices de forma y análisis discriminante canónico para analizar Descriptores Elípticos de Fourier (EFDs) del contorno del otolito. Los índices factor de forma y elipticidad variaron significativamente entre las tres zonas, mientras que los índices redondez, circularidad y rectangularidad solo mostraron diferencias entre las zonas I y III. Los EFDs mostraron la existencia de al menos dos unidades demográficas completamente independientes (I y III) frente a la costa chilena, aunque el menor poder discriminante entre la zona I y II, sugiere algún nivel de mezcla entre estas zonas.

Se analizó y comparó la variación espacial y temporal de los patrones de crecimiento temprano (EGP) de la anchoveta entre tres áreas geográficas, a saber, zona norte (18-24 °S); Zona centro-norte (26-30 °S) y zona sur (33-42 °S) frente a Chile para las cohortes 2015, 2016 y 2019. Los EGP se determinaron mediante el análisis de la microestructura de los otolitos para juveniles de 2,0 a 12,0 cm de longitud total (LT), correspondientes a edades de 28 a 187 días. El análisis de crecimiento comparativo entre las tres zonas de estudio para las cohortes mostró diferencias significativas en los parámetros de crecimiento de Laird-Gompertz y la tasa de crecimiento absoluta en el punto de inflexión (0,67-1,11 mmd-1). Se utilizó un modelo aditivo generalizado (GAM) para analizar los cambios en los perfiles de ancho de incremento diario promedio (IW) en relación con la zona, la cohorte mensual, la temperatura de la superficie del mar (SST) y la concentración de clorofila (Chloa) como variables predictivas. El GAM no mostró diferencia significativa en IW promedio entre las áreas norte y centro-norte (7.9±3.7 vs 8.3± 4.1 μm), aunque sí varió significativamente con respecto al área sur, donde se encontraron los menores valores de IW (5.6±3.0 μm). El GAM mostró mayores residuos parciales de IW cuando la SST estuvo por encima de 14°C y la concentración de Chloa fue menor a 2 mgm^{-3}. Los resultados generales mostraron una alta plasticidad en el patrón de crecimiento temprano de *E. ringens*, lo que indica una variabilidad interanual y espacial, con la TSM desempeñando un papel fundamental en un amplio gradiente latitudinal frente a la costa chilena.

La hipótesis de crecimiento-mortalidad se probó en cohortes primaverales de anchoveta en cuatro años diferentes (2014, 2019, 2020 y 2021) en el norte de Chile (18-24°S) en el HCS. Se utilizaron modelos lineales de efectos mixtos (LMM) para comparar la variación de los radios por edad (RAI) y el ancho de incremento (IW) de los otolitos, como proxy del tamaño de los peces y la tasa de crecimiento de los sobrevivientes (Pre-reclutas y reclutas) con la población inicial de larvas. Los perfiles suavizados spline de RAI y IW mostraron un patrón creciente a partir de un origen común, después de lo cual apareció una clara tendencia divergente, donde los sobrevivientes siempre mostraron RAI y IW más grandes y más anchos durante los primeros 20 días, en comparación con las larvas, para dos cohortes evaluadas. (2014 y 2019). Los LMM mostraron que tanto RAI como IW fueron significativamente más grandes y más altos para los reclutas que para las larvas, independientemente de los años. Los resultados generales mostraron evidencia de que los sobrevivientes eran más grandes y crecían más rápido que los miembros de la población original, lo que respalda la hipótesis de crecimiento-mortalidad para este pequeño pez pelágico en el HCS. Además, otro hallazgo distintivo fue que los juveniles más viejos (reclutas), eran más grandes y crecían más rápido que los juveniles jóvenes (pre-reclutas), cuando eran larvas y juveniles en transformación.

List of publications

The present Thesis is a compendium of the following peer-reviewed publications:

Cerna F, Saavedra-Nievas J, Plaza G, Niklitschek E, Morales-Nin B. 2019. Ontogenetic and intraspecific variability in otolith shape of anchoveta (*Engraulis ringens*) used to identify demographic units in the Pacific southeast off Chile. **Marine and Freshwater Research**, 70: 1794–1804. https://doi.org/10.1071/MF18278.

Cerna, F. y Plaza, G. 2016. Daily growth patterns of juveniles and adults of the Peruvian anchovy (*Engraulis ringens*) in northern Chile. **Marine and Freshwater Research**, 67, 899–91. https://www.publish.csiro.au/MF/MF15032

Cerna, F, Gómez, M, Moyano, G, Plaza, G, Morales-Nin, B. 2022. Spatial and inter-annual changes in the growth patterns of young-of-year anchovy in a high productive ecosystem. **Fisheries Research**, 249, 106236. https://doi.org/10.1016/j.fishres.2022.106236.

This paper was submitted to the Frontier Marine Science
Cerna, F, Plaza, G, Morales-Nin, B. 2023. Testing the growth-mortality hypothesis in the anchoveta (*Engraulis ringens*) in the Humboldt Current system using otolith microstructure analysis. **Frontier in Marine Science**. Received on: 06 Apr 2023, Manuscript ID: 1201690.

The original manuscripts are compiled in the Annex.

List of publications not included but related with this doctoral thesis:

Plaza, G., **Cerna, F.**, Landaeta, M.F., Hernández, A., Contreras, J.E. 2018. Daily growth patterns across developmental stages and age-at-recruitment of the anchoveta (*Engraulis ringens*): Evidence from inter-annual monitoring of otolith microstructure analysis. Journal of Fish Biology, 93:370–381. https://doi.org/10.1111/jfb.13773

Garcés, C., Niklitschek, E.J., Plaza, G., **Cerna, F.**, Leisen, M., Toledo, P., Barra, F. 2019. Anchoveta *Engraulis ringens* along the Chilean coast: Management units, demographic units and water masses: Insights from multiple otolith‐based approaches. Fisheries Oceanography, 28: 735–750. DOI: https://10.1111/fog.12455

Hernández, A., Plaza, G., Gutiérrez, J., **Cerna, F.**, Niklitschek, E. 2020. Spatiotemporal analysis of the daily growth traits of the prerecruits of a small pelagic fish in response to environmental drivers. Fisheries Oceanography. DOI: https://doi.org/10.1111/fog.12489

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Chapter 1: General Introduction

1.1. Background on HCS and anchoveta

Small pelagic fishes are boom-and-bust species that play important roles in a wide range of marine ecosystems (Checkley et al., 2009; Peck et al., 2021). They play an important ecological role as they are in an intermediate trophic position, linking plankton to higher trophic levels and top predators (as marine mammals and seabirds) (Moyano et al., 2022). Engraulids and other small pelagic fishes are classified as "r" strategy species, with a short life and fast growth (Pianka, 1970), as well as survival strongly determined by environmental drivers along with ecological processes (e.g., predation). Mortality occurs mainly during the early life period, where the higher mortality is expected to occur (Pikitch et al., 2012; Peck et al., 2013; Vasbinder and Ainsworth, 2020). To partly overcome the high natural mortalities, small pelagic fishes have developed a partial spawning strategy, combined with long spawning seasons (Millan, 1999; Claramunt et al., 2019; Vega-Cendejas et al., 1997). However, such a reproductive strategy has been developed at the expense of producing small eggs that lead to small larvae at hatching, due to a short embryonic process of only a few days, with a yolk sac that barely guarantees a tiny margin of endogenous feeding (Peck et al., 2013). Despite all these disadvantages, a small fraction of the progeny survives and complete their life cycle, which is enough to reach high levels of abundance, which in many cases allows not only to sustain large fisheries but also a significant part of the food webs in the ecosystems where they inhabit, particularly in Eastern boundary upwelling systems (EBUS).

EBUS are one of the most productive oceanic systems supporting about one-fifth of the ocean wild fish harvests (Pauly and Christensen, 1995), and within the EBUS, the Humboldt Current System (HCS) is the most productive biome. HCS originates from the west drift flow that collides with the South American coast, originating three surface currents, two to the north: the oceanic and coastal Humboldt current; and one to the south: the Hornos cape current. Between the oceanic current and the coastal Humboldt current, the current goes down towards the pole and the Peru-Chile countercurrent, flow from Peru towards the north and center of Chile (Robles, 1979; Bernal et al., 1982; Fonseca, 1989). These current systems off Chile and Peru are characterized by permanent upwelling processes that occur along the entire Chilean coast. During upwelling, high amounts of nutrients are produced in abundance near the coast, while the temperature and oxygen decrease; chlorophyll concentrations remain relatively low during the onset of the upwelling event, then increase during wind relaxation, when nutrients decrease and oxygen and temperature increase. This is similar to the pattern off Oregon and California, where a front move offshore and/or toward the equator during the upwelling event and migrates back toward the coast and/or poleward during relaxation. Therefore, the combined action of coastal upwelling and the transport of nutrients from the sub-Antarctic region by the Humboldt current give rise to the Chile-Peru marine system being one of the most productive on the planet (Bernal et al., 1983). Like other EBUS, there is a low diversity of pelagic species in the HCS, with a high abundance, but at the same time highly variable. This variability is generally associated with both the intensity of fishing exploitation and changes in environmental conditions (Csike and Sharp, 1985; Cañon, 1986; Yañez et al., 1986; 1988; Bernal, 1990; Yañez et al., 1992).

The anchoveta is a key species in the HCS, where is an important food source for seabirds, marine mammals, and other larger fishes (e.g., Jordan 1967; George-Nascimento et al., 1985; Simeone et al., 2003; Hückstädt et al., 2007). Anchoveta is also an important fishing resource, with an important economic value for Peru and Chile along the HCS. In Chile, the maximum

landing was 2 million tons in 1994, decreasing in the following years showing a high interannual variability, that in the current century have oscillated between 600 thousand to 1.5 million tons/year (Aranis et al., 2015). It is known that the interannual variations of the biomass of the exploited populations are determined by the recruitment or year-class strength, even more so in short-lived pelagic species such as the anchoveta, in which the recruitment determines the abundance of the resource susceptible to be captured in the following months. Recruitment variability still is the single least understood problem in fisheries science (Houde, 1987). Recruitment variability, that determines the stochastic fluctuations in biomass and abundance, seems to be fundamentally driven by fluctuations in the environment. Variations in wind force and temperature that determine the primary productivity seem to be the main factors responsible for the variation in recruitment (Cury and Roy, 1989; Backun, 1996). Predicting recruitment is complex and requires identifying the biotic and abiotic factors that drive it. An important part of the complexity of predicting recruitment lies in having knowledge of the environment, the limits of population distribution, migratory, reproductive, individual growth patterns, and ecological interactions, that allow characterizing the population dynamics (Brosset et al. 2019). Recruitment is observed massively between November and March, in a size range between 6 and 12 cm in total length, with individuals with sizes less than 6 cm being classified as pre-recruits (Fisher, 1958; 1963; Martínez et al., 2007).

The anchoveta is distributed throughout the entire HCS from the equator to the southern fjords off Chile. On the coast in front of Chile since the 60's there are three main administrative fishing stocks, the northern zone from Arica-Antofagasta, the central-northern zone from Caldera to Coquimbo, the southern zone from Valparaíso to Valdivia (Figure 1.1). The reproductive period extends mainly from winter to spring, but it can vary between areas, for example, in the north, where the sea temperature is higher, spawning lasts until late summer (Hernández-Santoro et al., 2019). The mean size at maturity can vary between 11.5 and 12.5 cm TL depending on the fishing area (Cubillos et al. 1999; Canales and Leal, 2009, Böhm et al., 2022). The interannual changes in the body weight-fecundity relationship are related to the temperatures of the environment, which affects the egg size. The anchoveta accommodate their offspring characteristics according to the prevalent environment (e.g., egg size, fecundity) (Claramunt et al. 2012). In the anchoveta from the Chilean coast the egg size, larval length at hatching and yolk sac volume of recently hatched larvae, increase with latitude (Llanos-Rivera and Castro 2006). The size of oocyte is smaller and fecundity is higher in the northern population compared with the population off central Chile (Leal et al. 2009).

Currently, key questions about anchoveta such as whether these administrative stocks corresponded to independent demographic units, which are the natural limits of their distribution, remain unresolved. Genetic studies up to the beginning of the 2000's showed the existence of a single stock or population of anchoveta on the Chilean coast from Iquique (20°13'S) to Talcahuano (36°41'S) (Ferrada et al., 2002), considered as a panmictic single unit (Herrera, 2019). Later, a couple of studies, using the parasite method, suggested the occurrence of two ecological populations of anchovy off the coast of Chile, one from Arica to Coquimbo (18°24'S - 29°) and the second one between Valparaíso and Valdivia (32°02'S-39°38'S) (Valdivia et al., 2007; George-Nascimento and Moscoso, 2013). Therefore, the anchoveta stock structure off the Chilean coast is still an unresolved matter requiring to be revisited using new ecological markers to reach more reliable conclusions from a more holistic point of view (Cadrin et al. 2014). Addressing this goal is important to contribute to reducing uncertainty in

stock assessment models and consequently in the fishery management of this important resource.
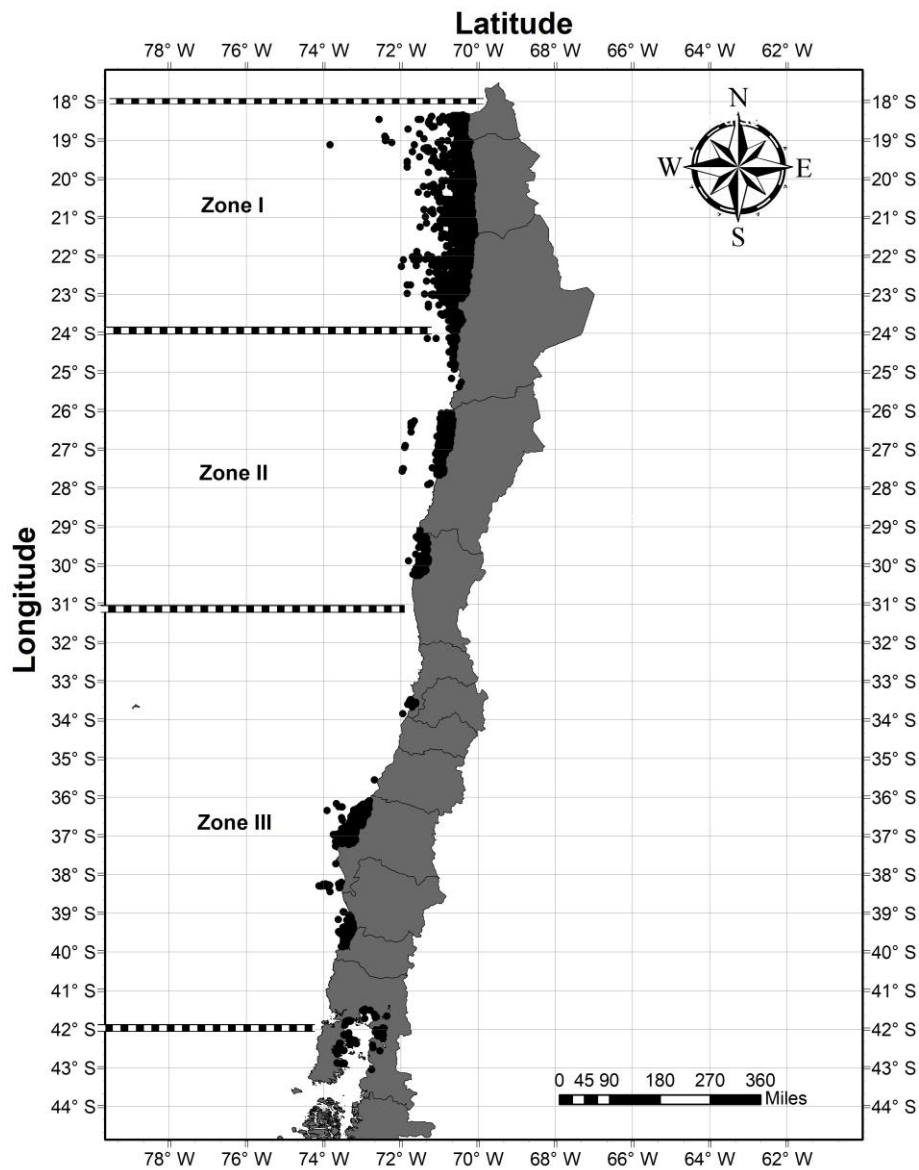
Figure 1.1. The administrative fishing stocks zones of anchoveta (*Engraulis ringens*) along the Chilean coast demarcate with dashed lines. The black dots show the fishing hauls from 2019 to 2022.

In addition to the population structure, there are other gaps in knowledge on life history traits of the anchoveta. Although biological aspects such as areas and spawning periods and the mean size at maturity have been widely studied in all areas of the Chilean coast (e.g., Claramunt et al., 2007; Claramunt et al., 2012; Hernandez-Santoro et al., 2013; Cubillos et al., 1999; Canales and Leal, 2009; Moreno and Claramunt, 2022), other life history traits are less known. Most of the studies on growth and age determination have been carried out using annual growth rings,

which have reported maxim age of about 4 years old (Aguayo, 1976; Morales-Nin, 1989; Cubillos and Arancibia, 1993; Canales and Leal, 2009). Contrary, the larval and juvenile stages are less known, although daily growth is very important to understand the dynamics of the survival patterns that support the variations in the strength of the year classes. It should be noted that recent studies have shown that early somatic growth is a concomitant variable (indicator) that reflects the physiological condition of the population under certain intrinsic and extrinsic drivers. (Morrongiello et al. 2014; Enberg et al. 2012). However, it is reasonable to infer that these early growth variations could also be associated with spatial patterns dependent on environmental conditions. Therefore, it is essential to develop research using otolith microstructure to fill these information gaps.

Regarding the effects of growth variability on the population dynamics, daily growth patterns, estimated by reading daily otolith microincrements, in larval and/or juvenile fish collected from spawning and/or nursery areas are a powerful tool for ecological issues, such as the biger-is-better mechanism and growth-mortalily relationship. Findings from specialized studies in the northern hemisphere have shown that growth and survival are directly related during the early stages of fish. By growing faster, larvae reach larger sizes at any age, which increases the chances of survival compared to smaller, slower-growing specimens (Anderson 1988; Takasuka et al., 2004a; Takasuka et al., 2004b; Alemany et al., 2006; Meekan et al., 2006; Plaza and Ishida, 2008; Robert et al., 2007; Satoh et al., 2013; Shoji et al., 2006; Tanaka et al., 2006). The survival of new recruits is a fundamental factor on the population dynamics and might depend on the characteristics of a determined stock. Although, the growth mortality hypothesis has been tested in a number of fishes, including some engraulids (Tanaka et al. 2022), it remains untested for the Anchoveta in the HCS.

The sustainable management of an exploited fish requires the knowledge of the population structure and its geographical boundaries, the biological characteristics and population dynamics. Despite the great relevance of anchoveta, both in ecological terms as a fisheries resource, still several gaps remain unresolved such as the numbers and boundaries of demographic units, the variability of life history traits as the somatic growth. The growth rate is a key trait for survival mainly in the early life stage before first year old. In this sense, the knowledge of the growth's environmental drivers is relevant for understanding the population dynamics of this important ecological and fisheries species.

1.2   Hypothesis of PhD Thesis

Hypothesis 1: Population structure
The anchoveta has an extended spatial distribution along the Chilean coast from north to south. Under such a scenario, it is highly reasonable to expect spatial segregation of population, which can trigger differences in some ecological markers, such otolith shape.

Hypothesis 2: Early growth and environmental drivers
The growth of juvenile anchoveta (*Engraulis ringens*) varies with temperature and the phytoplankton biomass present in its habitat in temporal and spatial terms, i.e., year and population, respectively. This growth variability   determines the survival of the early stages of development in the Humboldt Current system off the coast of Chile.

Hypothesis 2: Growth-survival relationship
Larger and older survivors of the anchoveta were always those that grew faster when they were larvae in Humboldt Current System.

1.3   Objectives of PhD Thesis

Based on the uncertainty on the stock structure of this important species and the lack of detailed knowledge on anchoveta growth depending on its development phase and the geographical area considered, the main purpose of this thesis was to determine of the demographic units and the growth pattern of anchoveta (*Engraulis ringens*) in the cost off Chile, the main growth drivers and growth effect on the survival of juveniles. The overall findings aimed to contribute to the definition of the stock structure of this relevant species in ecological terms and as a valuable exploited resource. Therefore, the following objectives were developed:

The first objective was to determinate the population structure of anchoveta on the coast of Chile, in order to identify the numbers of demography units.

The second objective was to determine the growth pattern of young of the year (YOY) in different years and three areas of the Chilean coast (Zones I, II and III), analyzing the effect of the temperature and chlorophyll-*a* as drivers of growth variation.

Finally, the third objective was testing the growth-mortality hypothesis in the anchoveta of the Humboldt Current, comparing the otolith-radii-at-age and daily increment width, as proxy of fish size and growth rate, respectively, between survivors and members of an original larval population.

1.4   Structure of the PhD Thesis

The present thesis is organized into six chapters as follows: Chapter 1 is the thesis' general introduction where the state-of-the-art, hypothesis, main and specifics objectives of the study, and references on the subject of the thesis are reviewed. Chapters 2, 3 and 4 are compilations of four scientific papers. Chapters 2 and 3 have already been published in peer-reviewed Journals: two papers in Marine and Freshwater Research and one paper in Fisheries Research. A fourth manuscript (Chapter 4) has been submitted to Frontiers Marine Science and its currently under review. The original content of the papers has been homogenized on each chapter (letter type and size, line spacing, figures and tables numbering, etc.) in order to facilitate the reading process. Chapter 5 corresponds to the general and integrated discussion, and finally Chapter 6 presents the general conclusions of the PhD Thesis.

The references have been included in each Chapter to provide more clear support to the hypothesis and conclusions presented.

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Chapter 2: Demographic units of anchoveta in the Pacific southeast of Chile.

Ontogenetic and intraspecific variability in otolith shape of anchoveta (*Engraulis ringens*) used to identify demographic units in the Pacific southeast off Chile

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Abstract

The phenotypical variability in otolith shape of anchoveta (*Engraulis ringens*) was analyzed among three zones i.e., zone I (Northern zone); zone II (North-central) and zone III (Southern) off the Chilean coast, using juvenile and adult fish. Generalized Additive Models (GAM) were used to analyze shape indices and canonical discriminant analysis was used to analyze Elliptical Fourier harmonics. The Form factor and ellipticity indices varied significantly among the three zones, whereas roundness, circularity and rectangularity indices only showed differences between zones I and III. Fourier reconstructed outlines for five ontogenetic stages suggested important differences among sampling zones, which were larger for sampling zone III, where at the same fish length, otoliths were smaller than those sampled in zones I and II, at least at the pre-recruit stage. Elliptical Fourier descriptors showed significant differences among the three units, with total percentage of correct classifications for juveniles of 89 and 74% for raw data and cross-validated cases, respectively, whereas for adult fish were over 85% and around 65%, respectively. The results support the hypothesis that juveniles and adults of anchoveta have remained segregated throughout their entire or at least a fraction of their life-cycles, mainly between the extreme northward and southward zones.

Keywords: Anchoveta, Stock, GAM Otolith shape indices, Elliptical Fourier analysis, Chile.

2.1   Introduction

A very complex issue in fisheries management is the uncertainty in the number, distribution and connectivity of demographic units (stocks) (Begg et al. 1999; Taylor and Dizon 1999, Luck et al. 2003, Kerr et al. 2014). In practice, it is not possible to estimate the productive surplus allowable to be harvested with acceptable precision and accuracy, without knowing the effective distribution, abundance, degree of reproductive isolation, self-recruitment potential and dependence on immigration of a management unit (Secor 2014). Although in only some overexploited fisheries control measures have been taken to achieve their recovery, presumably due to the poor match between management units and biological stocks (demographic units) (Hutching et al. 2010; Murawski 2010; Petitgas et al. 2010).

Otolith morphology and, particularly, shape analysis have been widely used in fisheries science to determine demographic units (Campana and Casselman 1993; Torres et al. 2000; Begg and Brown 2000; Pothin et al. 2006; Canas et al. 2012). Otoliths are pairs of calcified structures, located in the inner ear of fishes, whose main functions are balance and hearing (Campana 1999). Although otolith shape is species specific (Hecht and Appelbaum 1982; Gaemers 1984), environmental variability (biotic and abiotic) may leads to intraspecific differences among geographical regions, probably mediated through differences in individual growth rates (Vignon 2012). The use of otolith shape analysis to differentiate demographic units is possible

and adequate when enough and prolonged spatial isolation leads to detectable and consistent differences in otolith shape among demographic units (Neilson et al. 1985; Bird et al. 1986; Campana and Casselman 1993; Begg and Brown 2000; Turan 2000; Turan et al. 2006).

Otolith shape analysis includes shape indices (circularity, rectangularity, ellipticity, eccentricity, roundness), shape factors (Pothin et al. 2006), and otolith outline analyses, commonly based on elliptic Fourier and Wavelet's transforms(Bird et al. 1986; Smith 1992; Campana and Casselman 1993; Begg and Brown 2000; Bergenius et al. 2005; Parisi-Baradad et al. 2005; Pothin et al. 2006; Turan et al. 2006).These approaches have been benefited from an increasing technological ability to produce reliable 2D digital images in most fishery laboratories. Furthermore, otolith shape analysis is far less time consuming and less costly than other techniques used for stock discrimination, such as otolith micro-chemistry and otolith micro-structure analysis (Chittaro et al. 2006). Consequently, otolith shape analysis can be used as either a complementary or an effective alternative to other techniques, particularly for long-term monitoring of the degree of separation/mixing of units previously established using multiple approaches.

Anchoveta (*Engraulis ringens*) is distributed from northern Peru (4°30´S) to southern Chile (42°30´S). Two main population units have been identified within this range: one off the northern-central coast of Peru and the other off southern Peru and northern Chile (Pauly and Tsukayama 1987). The Northern unit supports an important fishery activity throughout its distribution (i.e., 16°00'S-24º00'S), representing a substantial fraction of the global anchoveta fisheries (Bakun and Weeks 2008; Schreiber and Halliday 2013). Despite the tremendous ecological role anchoveta plays in the Humboldt Current System (Chavez et al. 2003; Espinoza and Bertrand 2008; Karstensen and Ulloa 2008), and its huge contribution to regional economies from the 1950s to date, limited efforts have been conducted to enhance our knowledge about is population structure (Galleguillos *et al.* 1996; Ferrada et al. 2002; Chávez *et al*. 2007; Valdivia et al. 2007; Rojas 2010; George-Nascimento and Moscoso 2013), and/or the degree of coherence between management and demographic units.

The efforts to identify the number, distribution and connectivity of demographic units within Chilean waters have been limited until now. Genetic studies have failed to reject the null hypothesis of panmixia and have suggested the existence of a single evolutionary unit along the Chilean (Galleguillos et al. 1996; Ferrada et al. 2002) and Peruvian (Rojas 2010) coasts. Parasitological studies (Valdivia et al. 2007; George-Nascimento and Moscoso 2013), on the other hand, have indicated the possible existence of, at least, two separate demographic units, one in the north, and another in southern Chile. Therefore, the aim of the present work was to investigate the spatial, temporal and ontogenetic variability in the shape sagittal otoliths of anchoveta to contribute to reveal the population structure of this important fisheries resource.

2.2 Materials and methods

Study area

The study area was divided in three sampling zones that correspond at the three main fishing areas off Chile: zone I: Arica to Antofagasta (18°24'- 26°00' S); zone II: Caldera to Coquimbo (26°01'-32°16'S); and zone III: Valparaiso to Valdivia (32°17'-41°77'S) (Figure 2.1).

However, there is oceanographic and topographic traits to justify this sampling design. At first, there a coastal transition zone (CTZ) defined between 34 and 39ºS (Morales et al. 2010), which can act as natural boundary. In the northern zones (I & II) there is not a strict topographic boundary between them, but there is a critical latitude described around Mejillones and Moreno Bays (~ 22.8°S) (Letelier et al. 2012). Furthermore, each of these zones has discreet catch and spawning areas, where catches and biological activity (e.g. spawning) is almost inexistent.

The anchoveta fishery in zone I is concentrated from Arica (18ºS) to Antofagasta (26ºS) (Böhm et al., 2018). The spatial distribution of spawning in this area is concentrated in two recurring focus, one from Arica (18°24'S) to Punta Patache (21ºS), and another secondary focus located between Copaca (22°20'S) and Mejillones (23°S) (Angulo et al., 2018). Historically, the zone II (Caldera-Coquimbo) has had lower landing than that zone I, concentrated mainly in Caldera (26-28ºS) and Coquimbo (29-30ºS) (Böhm et al., 2018). In these areas, the spawning is distributed from Chañaral (16°10'S) to Tongoy (30°10'S) (Reyes et al., 2018). In the III zone the anchoveta is distributed from 36º to 40ºS (Aranis et al. 2018), and the spawning occurs mainly between 35º and 40ºS (Cubillos et al. 2017).

Sampling and preparation of otoliths

A total of 940 juveniles and adult anchoveta were collected between December 2015 and October 2016 at each of the three sampling zones (Table 2.1). Juvenile fish corresponded to specimens <12 cm total length (TL), sampled during the main recruitment period (December to February) from annual scientific surveys conducted during this period by the Chilean Instituto de Fomento Pesquero (IFOP). Samples of adult fish corresponded to individuals ≥12 cm TL, sampled during the main spawning period (August to October) from annual scientific surveys conducted during this period by IFOP. A complementary sample of adults was available from the 2013 annual reproductive survey and was included in the present study for an inter-annual comparison between 2016, a year with a strong ENSO ("El NIÑO") event, and a normal year without ENSO (2013). The total length to the nearest cm, and total weight (g) were measured and sagittal otoliths extracted under a stereomicroscope. Otoliths were cleaned in distilled water just after the extraction, eliminating remaining tissue from the macula and the vestibule with fine tweezers (Secor *et al*. 1992). Once cleaned, the otolith pairs were dried and kept dry in Eppendorff tubes.

Digital images of each left otolith were acquired with the software Image-Pro Plus (*MediaCybernetics*) which also binarized the images and calculated otolith surface area (A_0), perimeter (P_0), Feret length (F_L, longest caliper length of otolith) and Feret width (F_W, smalest caliper length of otolith). These morphometric variables were corrected to avoid the effect of fish size and the allometry that occurs during otolith growth (Lombarte and Lleonart 1993) using the method proposed by Lleonart et al. (2000) where the morphometric measures, used

as dependent variables, were related to fish total length as independent variable, and then used to calculate five shape indices: form factor, roundness, circularity, rectangularity and ellipticity (Pothin *et al*. 2006). Finally, approximately 1000 Cartesian coordinates of each otolith outline were extracted using the "getaoipoints" macro of the Image-Pro Plus Software, which were exported to a text file for further Elliptic Fourier analysis.
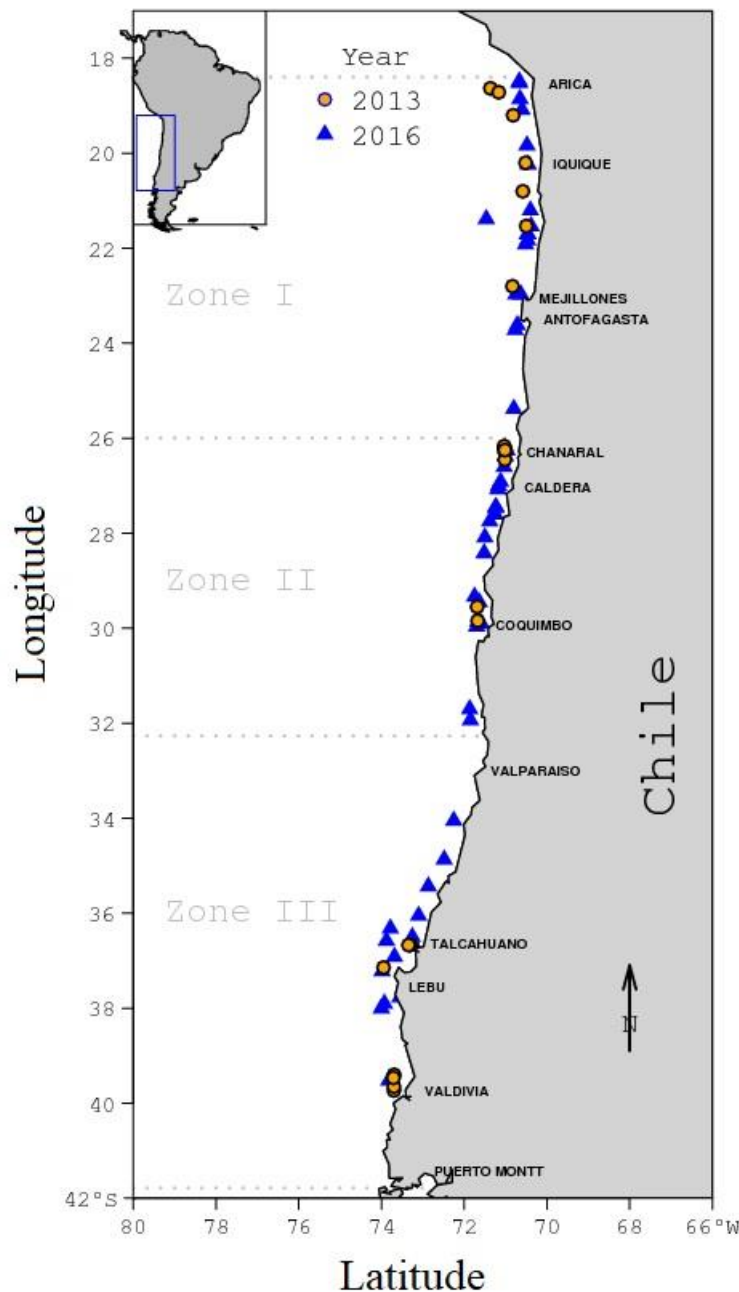
Figure 2.1. The anchoveta (*Engraulis ringens*) study area along the Chilean coast. The symbols show the fishing hauls in each year, and the dashed lines demarcate the study area.

Table 2.1. Number (n) of sagittal otoliths of the anchoveta (*Engraulis ringens*) used in morphological analysis, classified by ontogenetic stages, area and locality of landing origin off Chile, for the years 2016 and 2013. Range of fish length (cm TL) are also included for reference.

Ontogenic Stage	Zone		2016				2013		
				Length of fish (cm)				Length of fish (cm)	
			n	min	-	max	n	min	-	max
Juveniles	I	Arica-Iquique	50	6.5	-	11.5				
		Antofagasta	92	4.0	-	9.5				
	II	Caldera	103	9.0	-	11.5				
		Coquimbo	69	6.5	-	11.5				
	III	Valparaíso	126	2.0	-	9.5				
		Valdivia	49	2.0	-	6.0				
Adults	I	Arica-Iquique	51	12.0	-	14.0	32	14.0	-	14.5
		Antofagasta	46	12.0	-	14.0	18	14.0	-	14.5
	II	Caldera	53	12.0	-	14.0	20	14.0	-	14.5
		Coquimbo	55	12.0	-	14.0	30	14.0	-	14.5
	III	Valparaíso	45	12.0	-	14.0	21	14.0	-	14.5
		Valdivia	50	12.0	-	14.0	29	14.0	-	14.5

Analysis of otolith shape indices

Descriptive analyses of shape indices were organized in six groups of length intervals linked to ontogenetic stages: two intervals of pre-recruit 1 (4.0-5.5 cm TL) and pre-recruit 2 (6.0-7.5 cm TL), recruits (8.0-9.5 cm TL), two recruit group: recruit 1 (8.0-9.5 cm TL) and recruit 2 (10.0-11.0 cm TL), two adults: from 2016 (13.5-14.0 cm TL) and 2013 (14.0-14.5 cm LT).

The relationship between shape indices and both sampling zone and fish length (as a covariate) was modeled and analyzed through a Generalized Additive Model (GAM) using the R package "mgcv" (Wood 2006) Both Gaussian and gamma distribution models were compared using Akaike Information Criterion (AIC) (Akaike 1973) to select the most informative representation of the distribution data. Explanatory variable effects were then evaluated, on the selected model, through deviance analysis (Venables and Ripley 2002). The general equation of evaluated models is as follows:

$$SI_i = \varphi\big(\beta_0 + s(TL_i) + Z_{ij}\big)$$

where φ is the link function which links the mean to the model, *SI* correspond to the shape indices, β_0 is the intercept of model, TL is total fish length, *s* is the smoothing function and Z (zone) is a dummy variable that represents the origin of the sample with *j=1,2* and *i* the *i-esimo* fish.

Analysis of otolith outline

The elliptic Fourier analysis is a method which allows describing a closed curve, characterized by equidistant (x, y) coordinates such as the otolith contour, through the infinite summation of ellipses with different amplitudes and angles. The objective of the analysis is, however, the description of the otolith outline using the minimum number of ellipses. Each ellipse of the

Fourier analysis is called an Elliptical Fourier Descriptors (EFD) or simply an "harmonic", since its functional form (sines and cosines). Because the shape described by the EFDs is sensitive to the orientation, size and starting point of the ordered pairs (x, y), a normalization process was performed following Kuhl and Giardina (1982). Although normalization reduced bias in EFDs due to correlation with other morphological variables, such as fish length, to avoid any undesired effect of fish size upon otolith outlines, the analysis was carried out by fish size classes. Hence separated elliptical Fourier analyses were performed for fish ranging from 6.5 and 9.5 cm TL, and from 13.5 and 14.0 cm TL, for juveniles and adults, respectively, collected in 2016, whereas EFDs were obtained for adult fish ranging from 14.0 and 14.5 cm TL in 2013. A total of 200 EFDs was initially calculated for each otolith according to the algorithm implemented by Claude (2008) in the R package. After a power analysis was performed, only 20 harmonics were sufficient to explain 95% of the variance in otolith outlines. The first EFDs, which corresponded to the ellipse, used to normalize the data was discarded for further analyses.

Stepwise canonical discriminant analysis (CDA) was applied to determine whether otoliths collected in different sampling zones could be distinguished based on the 20 selected EFDs. CDA is a standard method where data belonging to known groups (sampling zones) are used to find linear combinations of descriptors that maximize the Wilks lambda (λ) (Ramsay and Silverman 2005; Pothin et al. 2006). The Wilks λ is the ratio between the intra-group variance and the total variance, and provides an objective means of calculating the chance-corrected percentage of agreement between real and predicted groups' membership. Wilks λ values range from 0 to 1: the closer λ is to 0, the better the discriminating power of the CDA (Lord et al. 2012).

2.3   Results

Basic otolith shape indices

The area, perimeter, ferret length and ferret width, showed differences among length-groups (ontogenetic state). Overall, shape indices showed a latitudinal gradient for all ontogenetic stages, *i.e.,* the ellipticity and circularity were higher in the sampling zone I, and lower in sampling zones II and III. On the other hand, rectangularity, form factor and roundness were higher in the zone III respect to the other zones, at least for pre-recruits and recruits. In adults, mean shape indices show closer values between zones I and II, than between them and zone III (Table 2.2).

Roundness and form factor decreased with fish length increased, whereas the opposite occurred with circularity and ellipticity, which tended to increase with fish length. A GAM analysis of the relationship between shape indices and fish length (year 2016) across sampling zones showed an overall trend towards a continuous change of the otolith shape with the fish length, at least, until 14 cm TL. Within this general pattern, more pronounced changes with size were noticeable between 4 and 8 cm TL, and slight evidence of stabilization between 8 and 10 cm TL fish (Figure 2.2).

Table 2.2. Mean basic morphometric variables and shape indices of sagittal otoliths of pre-recruits, recruits and adults of the anchoveta (*Engraulis ringens*) by sampling zone. Zone I: Arica to Antofagasta, zone II: Caldera to Coquimbo, zone III: Valparaíso to Valdivia. In brackets: length range in cm of each ontogenetic stage. s.d.= 1 standard deviation.

Ontogenetic state		Area (mm²)		Perimeter (mm)		Feret length (mm)		Feret width (mm)		Ellipticity		Rectangularity		Circularity		Form factor		Roundness	
		Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Pre-Recruit 1	Zone I	0,41	0,10	2,49	0,35	0,94	0,14	0,61	0,06	0,22	0,03	0,71	0,01	15,39	0,72	0,82	0,04	0,58	0,04
(4.0 - 5.5)	Zone II																		
	Zone III	0,18	0,09	1,50	0,35	0,54	0,14	0,42	0,08	0,12	0,03	0,75	0,02	13,30	0,46	0,95	0,03	0,75	0,06
Pre-Recruit 2	Zone I	0,97	0,16	4,16	0,44	1,59	0,17	0,88	0,07	0,30	0,02	0,70	0,01	17,95	1,00	0,70	0,04	0,48	0,03
(6.0 - 7.5)	Zone II	1,00	0,22	4,16	0,53	1,60	0,22	0,90	0,08	0,28	0,03	0,70	0,02	17,50	0,91	0,72	0,04	0,50	0,03
	Zone III	0,71	0,22	3,31	0,66	1,26	0,26	0,77	0,12	0,25	0,04	0,73	0,01	15,68	1,07	0,80	0,06	0,56	0,06
Recruit 1	Zone I	1,30	0,18	4,99	0,44	1,91	0,17	0,98	0,07	0,33	0,02	0,70	0,01	19,24	0,98	0,65	0,03	0,45	0,03
(8.0 - 9.5)	Zone II	1,66	0,30	5,60	0,59	2,20	0,26	1,08	0,08	0,35	0,03	0,70	0,02	19,01	1,15	0,66	0,04	0,43	0,03
	Zone III	1,22	0,14	4,66	0,36	1,81	0,15	0,96	0,05	0,32	0,03	0,72	0,01	17,82	0,90	0,71	0,03	0,48	0,03
Recruit 2	Zone I	2,53	1,01	6,93	1,35	2,80	0,66	1,28	0,22	0,38	0,04	0,70	0,01	19,65	0,58	0,64	0,02	0,40	0,03
(10.0 - 11.5)	Zone II	2,12	0,25	6,45	0,49	2,59	0,20	1,18	0,07	0,38	0,02	0,71	0,02	19,66	1,30	0,64	0,04	0,40	0,02
	Zone III																		
Adults 2016	Zone I	3,72	0,25	8,73	0,41	3,59	0,15	1,49	0,06	0,41	0,02	0,69	0,02	20,54	1,46	0,61	0,04	0,37	0,02
(13.5 - 14.0)	Zone II	3,48	0,32	8,61	0,44	3,50	0,20	1,46	0,07	0,41	0,02	0,68	0,02	21,36	1,28	0,59	0,03	0,36	0,02
	Zone III	3,53	0,38	8,69	0,54	3,47	0,21	1,48	0,09	0,40	0,02	0,69	0,02	21,48	1,33	0,59	0,04	0,37	0,02
Adults 2013	Zone I	3,66	0,26	8,66	0,39	3,59	0,15	1,48	0,06	0,42	0,02	0,69	0,02	20,50	1,42	0,62	0,04	0,36	0,02
(14.0 - 14.5)	Zone II	3,44	0,26	8,53	0,52	3,48	0,16	1,44	0,07	0,42	0,02	0,69	0,02	21,17	1,73	0,60	0,05	0,36	0,02
	Zone III	0,41	0,10	2,49	0,35	0,94	0,14	0,61	0,06	0,22	0,03	0,71	0,01	15,39	0,72	0,82	0,04	0,58	0,04

The GAM deviance analysis showed sampling zone and fish length explained over 88% of the deviance in all shape indices except rectangularity, whose explained deviance was only 62.8% (Table 2.3). Considering fish length covariance, there were significant differences for all indices between sampling zones I and III, II and III. However, significant differences between zones I and II were limited to form factor and ellipticity.

Otolith outline

A two-functions discriminant model fit using otolith EFDs of juvenile fish (6.5-9.5 cm TL), explained 79 and 21% of variance, with canonical correlations of 0.88 and 0.69, respectively. Only the first discriminant function presented a significant λ-Wilks value of 0.117 (P<0.0001). The lower λ-Wilks of the second function (0.521, P<0.2), suggested overlap between zones regarding this function. These canonical discriminant functions produced an accurate self-classification of 89% of the samples, where the zone III had accurate classification in the 95% of the cases. The total percentage of correct classifications of cross-validated grouped cases in their original group was 74% and in zones I and III were over 76% (Table 2.4; Figure 2.3a).
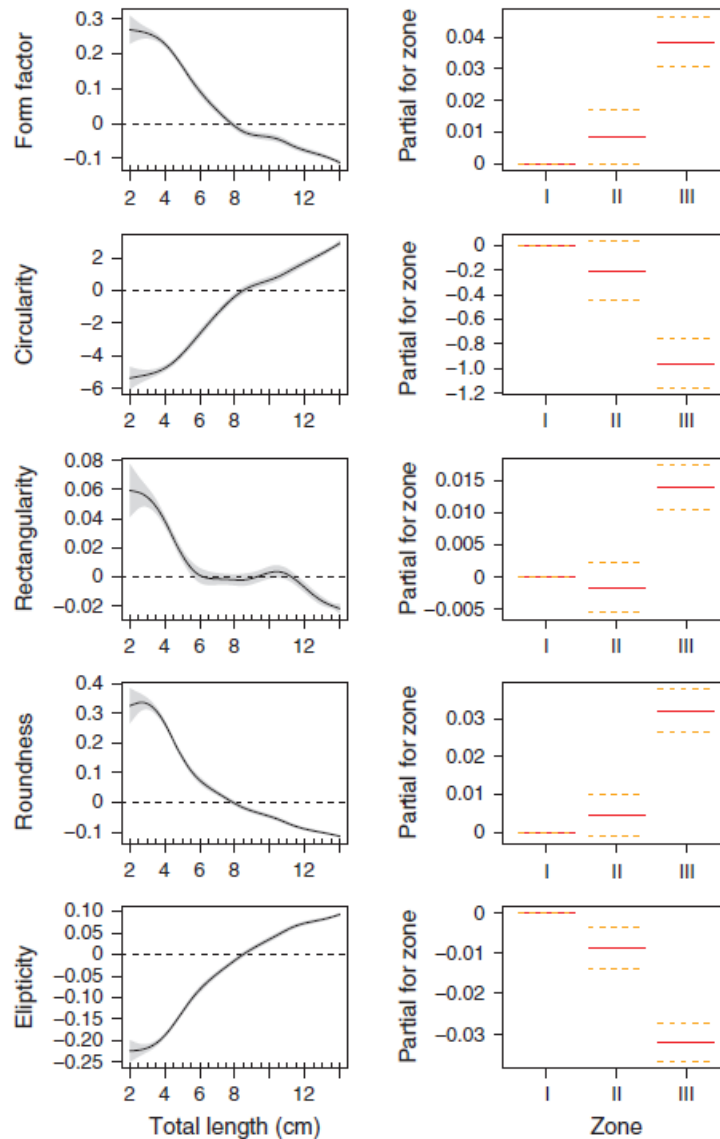
Figure 2.2. GAM modeling for form factor, roundness, ellipticity, circularity and rectangularity as a nonlinear function of the total length of the anchoveta (*Engraulis ringens*) for zone I (Arica-Antofagasta), zone II (Caldera-Coquimbo) and zone III (Valparaíso-Valdivia) in the coast off Chile. The graphs on the right show the influence of the zone factor.

Table 2.3. Deviance analysis from generalized additive models (GAM) for each shape index by sampling zone. Zone I: Arica to Antofagasta; zone II: Caldera to Coquimbo and zone III: Valparaíso to Valdivia (Chile).

Indices	Factor	Estimation	S.E	t value	P value		Indices	Factor	Estimation	S.E	t value	P value	
Form factor	Intercept	0.684	0.003	245.29	<2e-16	***	Redondez	Intercept	0.466	0.002	235403	<2e-16	***
	Zone II	0.009	0.004	2.03	0.043	*		Zone II	0.004	0.003	1578	0.115	
	Zone III	0.039	0.004	9.85	<2e-16	***		Zone III	0.032	0.003	11356	<2e-16	***
	Log Like	1427.9						Log Like	1666.2				
	Deviance %	91.1						Deviance %	94.8				
Circularity	Intercept	1.892	0.076	248892.00	<2e-16	***	Ellipticity	Intercept	0.334	0.002	197.86	< 2e-16	***
	Zone II	-0.205	0.120	-1714.00	0.087	.		Zone II	-0.009	0.003	-3.34	0.001	***
	Zone III	-0.961	0.101	-9531.00	<2e-16	***		Zone III	-0.032	0.002	-13.49	< 2e-16	***
	Log Like	-1165.2						Log Like	1824.933				
	Deviance %	88.8						Deviance %	95.2				
Rectangularity	Intercept	0.705	0.001	567309.00	< 2e-16	***							
	Zone II	-0.002	0.002	-1051.00	0.293								
	Zone III	0.013	0.002	7558.00	1.2E-13	***							
	Log Like	2058.3											
	Deviance %	62.8											

The two-functions discriminant model fit using otolith FDs of 2016 adults (13.5 to 14.0 cm TL), explained 67 and 33% of variance, with canonical correlations of 0.78 and 0.57, respectively. The first discriminant function was significant with λ-Wilks of 0.222 (P<0,000). λ-Wilks of the second function was higher 0.568, at the edge of significance (P<0.053). Classification reached an overall accuracy of 85%, with all sampling zones exhibiting crude accuracies >81%. The total percentage of correct classifications of cross-validated grouped cases in their original group was 64% and especially the zone III with 68% of classification (Table 2.5; Figure 2.3b).

Discriminant functions for 2013 adults explained 63 and 37% of the variance, with canonical correlations of 0.83 and 0.76, respectively. λ-Wilks for the first discriminant function (0.130) was highly significant (P<0.000), while the second function showed a much lower λ-Wilks value (0.425; P = 0.072), indicating a greater overlap between sampling zones. The accuracy for 2013 adults reached 90% of all samples, with all sampling zones reaching accuracies >87% of the cases. The total percentage of correct classifications of cross-validated grouped cases in their original group was 66% and especially the zone III with 70% of classification (Table 2.6; Figure 2.3c).

Fourier reconstructed outlines (from the first 20 EFDs) for the six different ontogenetic stages suggested important differences among sampling zones (Figure 2.4).

Table 2.4. Classification matrix of sagittal otoliths of juvenile anchoveta collected in 2016 from zone I: Arica to Antofagasta; zone II: Caldera to Coquimbo; zone III: Valparaíso to Valdivia in the coast of Chile.

		Zone	Predicted Group			Total
			I	II	III	
Original	number	I	**74**	10		84
		II	5	**35**	1	41
		III	1	1	**41**	43
	percentage	I	**88.1**	11.9		100
		II	12.2	**85.4**	2.4	100
		III	2.3	2.3	**95.3**	100
Cross Validation	number	I	**64**	13	7	84
		II	10	**26**	5	41
		III	3	6	**34**	43
	percentage	I	**76.2**	15.5	8.3	100
		II	24.4	**63.4**	12.2	100
		III	7.0	14.0	**79.1**	100

Table 2.5. Classification matrix of sagitta otoliths of adult anchoveta collected in 2016. Zone I: Arica to Antofagasta; zone II: Caldera to Coquimbo; zone III: Valparaíso to Valdivia in the coast of Chile.

		Zone	Predicted Group			Total
			I	II	III	
Original	number	I	**59**	8	3	70
		II	5	**61**	9	75
		III	4	3	**58**	65
	percentage	I	**84.3**	11.4	4.3	100
		II	6.7	**81.3**	12.0	100
		III	6.2	4.6	**89.2**	100
Cross Validation	number	I	**45**	17	8	70
		II	19	**45**	11	75
		III	10	11	**44**	65
	percentage	I	**64.3**	24.3	11.4	100
		II	25.3	**60.0**	14.7	100
		III	15.4	16.9	**67.7**	100

Table 2.6. Classification matrix of sagittal otoliths of adult anchoveta collected in 2013. Zone I: Arica to Antofagasta; zone II: Caldera to Coquimbo; zone III: Valparaíso to Valdivia in the coast of Chile.

		Zone	Predicted Group			Total
			I	II	III	
Original	number	I	**45**	5		50
		II	3	**44**	3	50
		III	1	3	**46**	50
	percentage	I	**90.0**	10.0	0.0	100
		II	6.0	**88.0**	6.0	100
		III	2.0	6.0	**92.0**	100
Cross Validation	number	I	**33**	9	8	50
		II	12	**31**	7	50
		III	6	9	**35**	50
	percentage	I	**66.0**	18.0	16.0	100
		II	24.0	**62.0**	14.0	100
		III	12.0	18.0	**70.0**	100

2.4 Discussion

The anchovy fishery in Chile for long time was managed using a purely administrative criterion without knowing the population structure of this species. In this context, the present study made a substantive contribution identifying at least two completely independent demographic units (I & III) of anchoveta (*Engraulis ringens*) off the Chilean coast, through of otolith shape analysis, where both basic shape indices and EFDs showed consistent results.

These results match with the stocks or management units used today, which support stock assessment of this species based on separated stocks. However, the lower discriminating power between zone I and II, suggests some level of mixing between these zones, which should be elucidated in further studies in order to specify the exactly limits between the populations to improve the management of this resource. Some of the mean features associated to the extent of these new findings are discussed below.
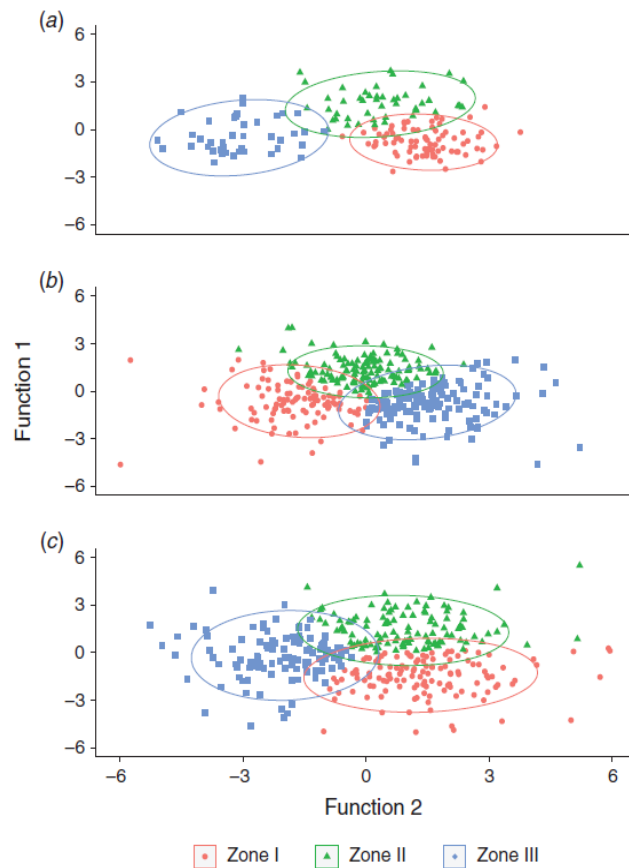
Figure 2.3. Scatterplots of the first and second discriminant function scores for Fourier coefficients of sagittal otolith oulines of anchoveta (*Engraulis ringens*) to a) juveniles for 2016, b) adult 2016 and c) adult 2013. Zone I: Arica to Antofagasta, zone II: Caldera to Coquimbo, zone III: Valparaíso to Valdivia.
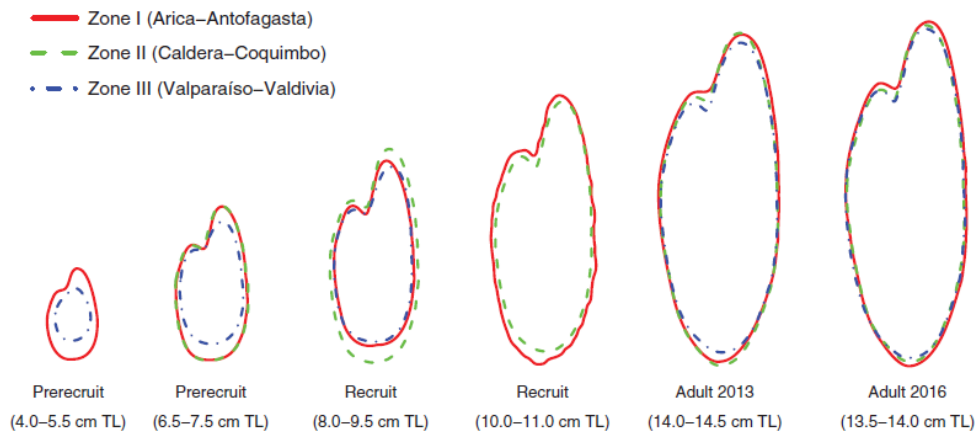
Figure 2.4. Average of otolith shape from elliptical Fourier coefficients for four length ranges, representing three ontogenetic stages of anchoveta (*Engraulis ringens*). Zone I: solid blue line, zone II: broken green line, zone III: dotted red line.

Discriminatory capacity of basic otolith shape indices and EFDs

Otolith shape indices (form factor, circularity, rectangularity, roundness, ellipticity) showed high variability and significant differences among sampling zones. While the magnitude of these morphological differences tended to increase with age and size, we found evidence indicating fish length effects upon shape indices decreased with size, leading to a relative stability in morphological differences among sampling zones after fish reached their sexual maturity (~14 cm TL). Such a condition has been reported for the clupeoid *Strangomera bentincki* by Curin et al. (2012) and to other species as well (e.g., Tuset *et al*. 2003). Consequently, the evaluation of the otolith shape stability is important to determine when the otolith outline is not influenced by fish ontogeny but rather by genetic and environmental variability. Furthermore, it would be very interesting to evaluate if the pattern of the otolith shape stability found in *E. ringens* in the current study are also applicable to other engraulid species. For example, Zengin et al. (2015) and Jemaa et al. (2015), identified population units of *Engraulis encrasicolus*, in the Black and the Mediterranean seas, respectively, although these authors used only immature fish (11-12 cm TL), when the shape or otolith outline are not completed formed yet. In this sense, it is reasonable to infer that results derived from otolith shape analysis of adult fish in the current study are reliable.

A distinctive finding of the current study was the significant discriminatory capacity of EFDs to distinguish anchoveta from the three localities, even when the analysis was carried out in two different years (i.e., 2013 vs 2016). These results support previous findings where a high discriminatory capacity of EFDs have been reported in a several species of teleost fishes as well (i.e., Poper *et al*. 2005; Brophy *et al*. 2016; Afanasyev *et al*. 2017; Duncan *et al*. 2018). Moreover, similar original classification accuracies (>81%) were found for *E. encrasicolus*, when this technique was used to differentiate four populations in the Mediterranean Sea (Jemaa et al., 2015). These results are promissory for fisheries resource for monitoring purposes, because otolith shape is a cost-effective technique, which would continuously update and enhanced due to the permanent progress of image-based platforms.

 A further finding in the current study was the Fourier reconstructed otolith outlines showed differences between zones, that appear to be larger for sampling zone III (Valparaíso-Valdivia), where at the same fish length, otoliths were smaller than those sampled in zones I and II, at least at the pre-recruit stage (< 8 cm TL). The smaller and rounder otoliths of zone III could be associated to growth differences of juveniles, because a recent study reported a very slower growth of pre-recruits in southern zones than northern ones (Cerna and Plaza, 2015).

Population structure and spatial segregation of anchoveta

The marked differences found in otolith morphology among the two extreme localities reported in the present study are strong evidences of an environmental spatial heterogeneity of anchoveta along the Chilean coast. These evidences match well with previous studies, which have proposed that environmental variability can produce important differences in otolith shape (Lombarte and Lleonart 1993; Cardinale et al. 2004; Vignon 2012). Indeed, juveniles and adult fish inhabiting the northward and southward extreme zones analyzed for anchoveta must cope with important environmental differences in biological productivity (e.g. chlorophyll-a), upwelling intensity, temperature and both physical and topographic changes. Important features here are the coastal transition zone (CTZ) defined between 34 and 39ºS (Morales *et al*. 2010).

Although the northern zones (I & II) does not have an strict topographic boundary between them, there is a critical latitude (~ 22.8°S) described around Mejillones and Moreno Bays, which exhibited higher concentrations of chlorophyll, throughout the year, and stronger water retention dynamics related to local topography and particular Ekman transport characteristics of adjacent waters (Letelier et al. 2012). Hence, it is reasonable to hypothesize this critical latitudinal zone could enhance retention of early stages of anchoveta and limit the flow of early juveniles between zones I and II.

Phenotypic differences found among sampling zones were large and consistent between years and trough ontogenetic stages, which is indicate of a prolonged and demographically relevant separation among units (Reiss et al. 2009). However, these differences does not provide direct evidence of genetic (i.e. nearly complete) isolation between sampling areas. Nonetheless, while classical genetic studies carried out for this area and species have failed to reject the conventional hypothesis of panmixia (Galleguillos et al. 1996; Ferrada et al., 2002; Rojas 2010), new evidence contributed by Ferrada et al. (2018) suggest progressive differentiation with distance and might change the current view about the population genetics of anchoveta along the Humbold Current. In this sense, in the current study otolith-based morphometric differences were larger for sampling zone III (Valparaiso-Valdivia), than those sampled in zones I and II. The high differences between zones I with the III, and II with III suggest greater genetic and/or environmental a greater distance between these areas and proximity between zones I and II, which is consistent with the geographic distribution of the species (Castillo et al. 1998; Leiva et al. 2016), as well as with previous parasitological studies (Valdivia et al. 2007; George-Nascimento et al. 2011).

The similar classification accuracies found in 2013 and 2016 provide first evidence that otolith morphological differences among zones were not sensitive to El Niño event, which dominated the oceanographic condition during the 2014-2016 in the southern hemisphere (Vera and Osman 2018). This finding suggests the absence of a generalized movement and mixing of schools in a southward direction, which could be expected as a result of movement of warm-water masses from north to south. Latitudinal stability in fish distribution under El Niño events has been, in fact, observed in recruiting acoustic cruises (Castillo et al. 1998; Leiva et al. 2016), where a change in bathymetric distribution seems to be the dominant response to warm water conditions due to the "El Niño" event, where anchoveta get deeper, being less vulnerable to purse seine fishing gears.

The existence of clear otolith-based morphological differences in both juvenile and adult fish among the two extreme localities supports the hypothesis that individuals have remained segregated throughout their entire or at least a fraction of their life-cycles, supporting the increasing scientific evidence of homing in marine fishes in the last two decades (e.g., Thorrold et al. 2001; Rooker et al. 2008; Solmundsson et al. 2015). Hence, further studies focused in extending both the spatial and temporal scale and also incorporating additional ecological markers (e.g., otolith microchemistry and microstructure) would provide additional insight to reveal homing and level of mixing if any in this important fishery resource in the Humboldt Current System.

Acknowledgements

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Chapter 3: Spatial and inter-annual changes in the growth patterns of anchoveta.

Daily growth patterns of juveniles and adults of the anchoveta (*Engraulis ringens*) in northern Chile

Francisco Cerna, Guido Plaza

The study began with the identification of the growth pattern of anchoveta from the northern zone of Chile. In this study, daily growth patterns of juvenile and adult anchoveta in northern Chile (18°21'-24°00'S) were determined using micro-increments of sagittal otoliths for the recruitment and fishery seasons of 2009 and 2010. A characteristic feature was the existence of very distinctive daily micro-increments, through which a complete sequence of micro-increments was obtained from the primordium to otolith edge for juveniles (7.5–12-cm total length (TL)) and adults (12.5–18.0 cm TL), whose ages were in the range 56–166 and 137–409 days respectively. Hatch dates extended from July 2009 to July 2010, where juveniles recruited to a fishery in a given month were the survivors of a spawning that had occurred approximately 3–4 months before. A Laird–Gompertz model fitted to length-at-age data for juveniles estimated maximum instantaneous growth rates that ranged from 0.98 to 1.57 mmday^{-1}, whereas overall mean individual growth rates back-calculated for the age range of juveniles ranged from 0.65 to 1.02 mmday^{-1}. The von Bertalanffy seasonal parameters estimated through the entire life history showed high growth, with a growth coefficient K=1.1 and mean length at the first year of 16.3 cm TL. Consequently, *E. ringens* in northern Chile seems to maximize growth in the first year of life.

Enhanced growth during the late juvenile and adult stages of *E. ringens* agrees with recent studies in other clupeoids, which have also reported fast growth. In a recent study performed by Namiki et al. (2010), it was possible to age adult fish of the Japanese anchovy (E. japonicus) by counting daily increments in specimens ranging from 8 to 14 cm SL. All aged specimens were 365 days of age (i.e. 1 year), with the largest fish being 329 days of age. Further evidence of fast growth comes from two recent studies using otolith microstructure analysis in *E. encrasicolus* in the Gulf of Biscay (Aldanondo et al. 2011) and in the Adriatic Sea (La Mesa et al. 2009; Durovic et al. 2012). These studies demonstrated that juveniles can attain 6–8 cm in 70–90 days. In the study of Aldanondo et al. (2011) in the Gulf of Biscay, the age of the larger fish (~14 cm SL) was less than 1 year of life. Based on this evidence and the results of high growth for *E. ringens*, some questions arise, specifically, to what extent is the anchoveta a species that maximizes its growth during the first year of life before markedly reducing growth to perhaps maximize reproduction? Alternatively, are the fast growth patterns observed in juveniles and adults in the present study restricted to a particular year, geographical area or hatch month, perhaps linked to the enhanced environmental conditions of growth controllers? Under a fast growth scenario, a considerable proportion of the asymptotic length will probably be reached in only a few months of life, where year-class strength will be the result of survival rates occurring just one season before; this could have a considerable effect on the management of anchovy fisheries.

From this discovery, the following questions also emerged: is this growth pattern common along the entire coast of Chile? and if there are differences in the growth pattern between the different anchovy populations, what are the environmental factors driving the variations in the growth?

Hence, it is worth attempting to address these questions given the commercial and ecological importance of *E. ringens* in the Humboldt Current System.

Spatial and inter-annual changes in the growth patterns of young-of-year anchoveta in a high productive ecosystem

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ABSTRACT

The spatial and temporal variation of the early growth patterns (EGP) of anchoveta (*Engraulis ringens*), were analyzed and compared among three geographic areas, i.e., Northern zone (18-24 °S); North-central zone (26-30 °S) and Southern zone (33-42 °S) off the Chilean coast over three years (i.e., 2015, 2016 and 2019). EGP were determined through otolith microstructure analysis for juveniles ranging from 2.0 to 12.0 cm in total length (TL), corresponding to ages from 28 to 187 days. The comparative growth analysis between the three study areas for the 2015, 2016 and 2019 cohorts showed significant differences in the Laird-Gompertz growth parameters and the absolute growth rate at the inflection point (0.67-1.11 mmd^{-1}). A generalized additive model (GAM) was used to analyze changes in mean daily increment width profiles (IW) as related to the area, monthly cohort, sea surface temperature (SST) and Chlorophyll concentration (Chlo*a*) as predictive variables. The GAM did not show significant difference in mean IW between northern and north-central areas (7.9±3.7 vs 8.3±4.1μm), although they significantly varied with respect the southern area, where the lower values of IW were found (5.6±3.0 μm). The GAM showed higher IW partial residuals when SST was above 14°C and Chlo*a* concentration was lower than 2 mgm^{-3}. Overall results showed a high plasticity in the early growth pattern of *E. ringens,* indicating interannual and spatial variability, with SST playing a pivotal role across a wide latitudinal gradient off Chilean coast.

Keywords: Anchoveta, daily width increment, geographical growth variation, growth-temperature and growth-Chlo*a* relationship

3.1.   Introduction

Engraulids and other small pelagic fishes are classified as "r" strategy species, with a short life and fast growth (Pianka, 1970), as well as survival strongly determined by environmental drivers along with ecological processes (e.g., predation). Mortality occurs mainly during the early life period, where the high mortality is expected to occur (Pikitch et al., 2012; Peck et al., 2013; Vasbinder and Ainsworth, 2020). To partly overcome the high natural mortalities, small pelagic fishes have developed a partial spawning strategy, combined with long spawning seasons (Millan, 1999; Claramunt et al., 2019; Vega-Cendejas et al., 1997). However, such a reproductive strategy has been developed at the expense of producing small eggs that lead to small larvae at hatching, due to a short fertilization process of only a few days, with a yolk sac that barely guarantees a tiny margin of endogenous feeding (Peck et al., 2013). Despite all these disadvantages, a small fraction of the progeny survives and complete their life cycle, which is enough to reach high levels of abundance, which in many cases allows not only to sustain large fisheries but also a significant part of the food webs in the ecosystems where they inhabit.

Engraulids are very important small pelagic fishes in tropical and temperate waters and even in some freshwater ecosystems, where they constitute the food base for several invertebrates, larger piscivorous fish, mammals and birds (Ruiz and Fondacaro, 1997; Pikitch et al., 2012; Plaganyi and Essington, 2014). These tiny fishes, which generally do not exceed 15 cm in length, have huge commercial importance, since they support many fisheries worldwide, especially in high-productivity ecosystems, where anchovies reach biomass levels of hundreds of millions of tons (Pikitch et al., 2012; Fréon et al., 2014). However, it is also true that engraulids undergoes large fluctuation in abundance at various spatial and temporal scales, which makes their evaluation and fisheries management a considerable and permanent challenge, heightened in the current scenario of global climate change (Pikitch et al., 2012). Hence, it is important to conduct basic and applied research to fill the gaps on their life cycles,

which in many cases are not yet completely revealed, particularly during their most vulnerable early phases.

Somatic growth is one of the key life history parameters in aquatic ectothermic organisms because is a concomitant variable (i.e., an output product) that can reflect the condition of a given population. In case of small pelagic fishes, the somatic growth is highly variable, particularly during their early life stages, likely linked to the variability of the main growth controllers, i.e., food availability and water temperature (Shepherd and Cushing, 1980; Kendall and Miller 2009). However, it seems reasonable to infer that growing faster through these vulnerable stages could be advantageous for small pelagic fishes, as reported in some species (e.g., Anderson, 1988; Meekan et al., 2006; Takasuka et al., 2003, 2004b) under the growth-survival paradigm, and its fourth-associated mechanisms. The bigger-is-better mechanism (Leggett and DeBlois, 1994) suggests that larger individuals at age would have a lower probability of mortality than those smaller ones. The growth rate mechanism (Shepherd and Cushing, 1980) proposes that higher growth rates reduce the time during which individuals experience high mortality. The third mechanism suggests that those individuals that make ontogenetic transitions (e.g., yolk sac to first feeding, larvae to juvenile) at earlier ages have a higher probability of survival, and vice versa (i.e., "stage duration" mechanism; Chambers and Leggett, 1987). More recently, Takasuka et al. (2003, 2004ab), proposed the growth-selective predation hypothesis, which states that slower-growing larvae are more vulnerable to predation than faster-growing larvae, through a process that could be predator specific.

In the last decade, some insights have been generated to get a better picture of the mechanisms linked to growth dynamics in the different phases of the life cycle of small pelagic fishes (Peck et al., 2013; Petitgas et al., 2012). For example, some recent studies carried out on the growth of engraulid juveniles using otolith microstructure analysis, in high productivity ecosystems, have reported extremely high growth rates, where the species have reached between 70-80% of the asymptotic length during their first year of life (e.g., *Engraulis encrasicolus*-Aldanondo et al., 2011; *Engraulis japonicus* -Namiki et al., 2010; *Engraulis ringens*-Plaza et al., 2018, Cerna and Plaza, 2016), after which there is a drastic drop in growth presumably linked to reproduction. However, despite these progresses several questions remain unaddressed. e.g., to what extent are these findings the rule for anchovies in these ecosystems? What about the influence of the spatial heterogeneity for a species with an extended latitudinal distribution? Or could such a fast growth only be linked to high cycles of productivity of these anchovy species? It is very important to note that anchovies in high productivity ecosystems undergone large interdecadal fluctuations, alternating cycles of high and low levels of abundance with other sympatric sardine species (e.g., Chavez et al., 2003). Answering these questions is not yet possible for anchovies, because early growth data are not available on a sequential monitoring basis, even for species of commercial importance, where otoliths are collected permanently. Hence, it is very important to continue accumulating information on early growth at different spatial and temporal scales in anchovies, because early growth could be used as health index in these important fisheries resources.

The anchoveta (*Engraulis ringens*) is the most abundant species in the Humboldt Current System (HCS) across the southeastern Pacific Ocean, and supports the largest pelagic fishery in the world (Aranda, 2009; Pikitch et al., 2012; Joo et al., 2015). This engraulid is widely distributed from the 3°S to 42°S, and is divided in four stocks, managed with independent stock assessment procedures and administrative measures, i.e., the North Central Peruvian stock (4-24°S), the southern Peru-northern Chilean stock (16-24°S), the Northern Central Chilean stock (15-24°S), and the most meridional stock with fishery activity from 34°S to the northern area of the Patagonian fjords (33-42°S). The biomass levels of anchovy vary dramatically between stocks during cycles of high productivity, where the approximately 7-17 million tons of biomass in the northern Peruvian stock (Freon et al. 2008; Joo et al. 2015) contrasts with biomass less

than 1 million tons for the southernmost stock in the HCS (Cubillos et al., 2002). To understand what triggers such a large difference in productivity, it is essential to understand the role that environmental drivers play in the different stages of the life cycle of this species. The present study consequently proposes to make a comprehensive analysis of early growth patterns on various temporal and spatial scales for juveniles of *E. ringens*. To achieve this goal, otolith microstructure was revealed for young of the year (YOY) for three years and across three geographical areas off the Chilean coast (i.e., Zones I, II & III; Figure 3.1). In addition, the effect of temperature and Chlorophyll as drivers of early growth variations were examined and tested. The selected zones correspond to independent demographic units as demonstrated by Garcés et al. (2019) and Cerna et al. (2019).

3.2.   Materials and methods

3.2.1 Field sampling

Anchoveta juveniles were collected in the three study areas: zone I (Northern zone; 8-24 °S), zone II (North-central zone; 26-30 °S), and zone III (Southern zone; 33-42 °S) off the Chilean coast (SE Pacific) from the research vessel *Abate Molina* (IFOP) during the annual hydroacoustic cruises. The fish were caught with a mid-water trawl between the sea surface and 59 m. The campaigns were conducted during spring in zone I in 2015, 2016, 2019 and in summer in zone II and III, on 2016, 2017 and 2020, corresponding to the recruitment periods for anchovy in each of the zones. On board, the total length was recorded with a precision of 0.01 mm, the total weight (gr) and the pair of sagittal otoliths of each fish was extracted, which were cleaned, dried and stored for later analysis. The analyzed samples corresponded to a total of 848 specimens, which covered the 2015, 2016 and 2019 cohorts as detailed in Table 3.1.

Table 3.1 Number of sagittal otoliths of young-of the year anchoveta used in the analysis (n), classified by zone, year of birth for winter-spring cohorts of 2015, 2016 and 2019. Mean values, standard deviation (SD) and range for length and age are also given.

Zone	Cohort	Total length (cm)		Age (days)		n
		Mean±sd	Range	Mean±sd	Range	
I Northern	2015	7.8±3.6	4.0 - 11.0	104±73.2	28-145	60
	2016	5.2±2.1	3.5 - 12.0	86±17.7	56-147	70
	2019	5.5±1.1	3.5 - 7.5	66±11.1	45-92	131
Total, Zone I		6.0±2.4	3.5 - 12.0	80±40.1	28-147	261
II North-central	2015	10.2±1.2	6.0 - 12.0	122±20.7	83-172	40
	2016	9.4±1.7	5.5 - 12.0	126±26.1	75-185	36
	2019	7.7±1.3	5.5 - 10.0	91±16.0	63-137	167
Total, Zone II		8.3±1.7	5.5 - 12.0	101±24.0	63-185	243
III Southern	2015	4.1±1.0	2.0 - 7.0	62±18.1	36-129	118
	2016	6.4±4.0	2.0 - 12.0	89±50.1	30-187	60
	2019	6.3±3.4	2.0 - 12.0	92±45.2	35-178	144
Total, Zone III		5.5±3.1	2.0 - 12.0	81±41.2	30-187	322

3.2.2 Otolith preparation

The left sagittal otoliths from pre-recruits, specimens <6 cm TL, were embedded in a small portion of Cristal Bond, after which no additional preparation was required to achieve satisfactory resolution of increments. In the case of juveniles, the left sagittal otoliths were mounted in a small portion of two-part epoxy resin on slide glasses with the sulcus side down and grounded until the primordium became clearly visible, with 800-2000 grit sandpaper and cleaned with 0.5 um alumina polisher. Then, otoliths were flipped and polished following the

same procedures, until revealing the primordia and reach a reliable resolution of daily increments.
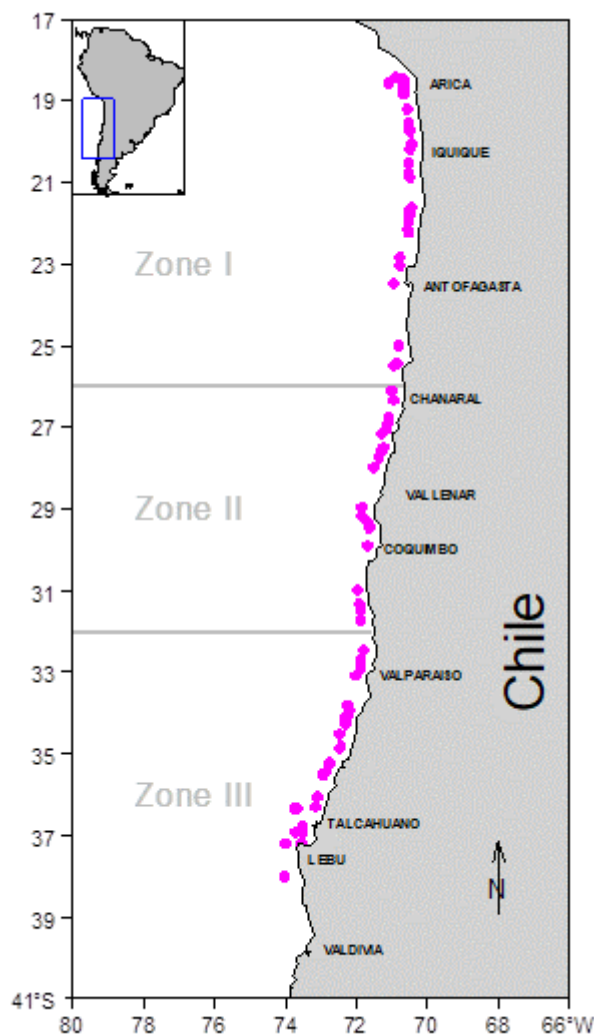
Figure 3.1. Study area that includes Zones I (North), II (Central-north) and III (South), corresponding to the three demographic populations that support independent fish stocks. The points indicate juvenile sampling hauls in the hydroacoustic surveys, where the otoliths were collected.

3.2.3 Microincrement reading procedure

A sequence of 400X magnification images were obtained from each prepared sagittal otolith, using an image analysis system consisting of a triocular light microscope (Olympus), and a digital camera (QImage 5 mega pixels) connected to a desktop computer equipped with Image-Pro Plus 5.1 software (*Mediacibernetycs*). A complementary image with 1000X magnification was taken around the primordium, to ensure a correct identification of the micro-increments in this area

The increments were identified as two successive concentric units (Panella, 1971; Campana, 1992) and were then counted and measured twice for a unique experimented reader across the area of distinctive increments, which in most cases matched to post-rostrum area. All records were performed using the "Caliper Tool" available in the Image-Pro Plus image analyzer

(version 6.0). When counting differed by over 5% between readings, the record was discarded. The daily periodicity of increment formation was validated by Plaza and Cerna (2015).

3.2.4 Identification of hatched cohorts
The hatching dates (HD) of juvenile were back-calculated subtracting the total age in days from the calendar day of sampling. The hatch date was determined for monthly hatched cohorts by zones for 2015, 2016 and 2019 corresponding to the 2016, 2017 and 2020 cruises.

3.2.5 Analysis of length-age data from back calculation
The proportionality between somatic growth and otolith growth was verified using a linear model fitted using maximum likelihood. The otolith radius was measured along the caudal radius. The data on radii-at-age were converted to a length-at-age matrix using the biological intercept method (BIM; Campana, 1990), as follows:

$$L_i = L_c + (R_i - R_c)(L_c - L_0)(R_c - R_0)^{-1}$$

where L_i is total length at age i, L_0 is the larval length at hatching (biological intercept), R_0 is the otolith radius at hatching, L_c is the total length at capture, R_i is the otolith radius at age i and R_c is the postrostral radius (PR) at the time of capture. The biological intercept L_0 was set to 2.4 mm, corresponding to the mean length at hatching reported by Hernandez and Castro (2000) for the northern stock of *E. ringens* off Chilean coast.

The BIM was used to back-calculate fish-growth trajectories from the last 35 days of fish life counted backwards from the date of catch, which corresponded to the lower limit from which the otolith radius and fish length are linearly related for this species.

3.2.6 Growth analysis
The relationship between age and back-calculated length was analyzed using the Laird-Gompertz model (Gompertz, 1825; Laird et al., 1965), using the function "nls", available in the statistical software R version 3.6.3 (Inaka and Gentleman, 1996):

$$L_t = L_\infty \exp\left[-\exp\left(-\alpha\left\{X - X_0\right\}\right)\right]$$

where L_i is the total length at age $\underline{i}$, L_∞ is he asymptotic length, X is the age in days, X_0 is the inflexion point of the curve, and alpha (α) is the instantaneous growth rate at age X_0. From this equation, the absolute growth rate at the inflexion point (GAR_{X0}) was calculated as: $GAR_{X0}= \alpha L_{X0}(\ln L_\infty - \ln L_{X0})$, where L_{X0} is the estimated length at X_0.

The growth parameters derived from the age-length models were compared between zones using the maximum likelihood method developed by Kimura (1980), using the "fishmethods" package in R (Nelson, 2019). The method assumes independence among samples and normality of residuals.

3.2.7 Increment width profiles analysis
The variation in the increment width (IW) was analyzed as a proxy of daily fish growth for the 2015, 2016 and 2019 cohorts for each spatial zone, applying a generalized additive model (GAM). This model considered IW as a response variable, including as explanatory co-variables the age (day on which the increment was formed), and the previous increment width (IWP), to avoid the highly autocorrelation between increments, i.e., daily otolith growth is dependent on the growth of the previous day (Gutiérrez and Morales-Nin, 1986; Hinrichsen et al., 2010; Schismenou et al., 2016). The model also included as an explanatory variable the zone of origin, the cohort, the sea surface temperature (SST), and concentration of chlorophyll-a. The GAM model was fitted using the "mgcv" library (Wood, 2006 under the assumption normal errors and the following formula for the expected value:

$$IW_{ijk} = \varphi = \beta_0 + s(age_{ijk}) + s(IWP_{ijk}) + s(SST_j) + s(Chloa_j) + Zone_j + Cohort_k$$

where φ represents the function that allows to link the mean of IW and the additive predictor (link function); β_0 is the intercept of the model (global mean of the increase); IW_{ijk} corresponds to the width of the microincrements of the i-th fish, in the j-th zone (j = {I, II, III}) for the k-th cohort (k = {2015, 2016, 2019}); age_{ijk} is the day on which the i-th fish increment was formed, in the j-th zone (j = {1, 2, 3}) for the k-th cohort (k = {2015, 2016, 2019}); s is a smoothing function; IWP_{ijk} corresponds to the width of the previous microincrements of the i-th fish, in the j-th zone (j = {I, II, III}) for the k-th cohort (k = {2015, 2016, 2019}). $Zone_j$ represents the zone of origin of the sample (j = {1, 2, 3}); $Cohort_k$ is the birth monthly cohort (k = {2015, 2016, 2019}); SST_j which is the sea surface temperature (°C) corresponding to the month when the increment was formed in the j-th Zone (j = {1, 2, 3}) and $Chloa_j$ (mg/m3) which is the concentration of chlorophyll corresponding to the month when the increment was formed in the j-th Zone for k-th cohort. The SST and Chlo*a* data come from the analysis of satellite images for the first 20 nm from the MODIS-Aqua sensor with a resolution of 4 km (http://oceancolor.gsfc.nasa.gov/). In addition, the SST and Chloa data were compared between zone and month using a two factor ANOVA.

3.3. Results
3.1 Hatch date distributions
The YOY ages ranged from 28 to 187 days for each cohort and zone (Table 3.1). The back calculated hatch dates of *E. ringens* occurred mainly in late winter and early spring, although some summer hatching occurred in the more northward areas. The hatch date monthly frequency within these two seasons differed between zones albeit without a clear interannual trend (Figure 3.2).

Table 3.2 Parameters of the linear relationship between total length and otolith radius of the anchovy for the 2015, 2016 and 2019 cohorts in the three geographic zones. SE= standard error of parameters; R^2 = coefficient of determination; n= number of fish analyzed.

	Parameters	Cohort 2015				Cohort 2016				Cohort 2019			
		Value	SE	t_{value}	P_{value}	Value	SE	t_{value}	P_{value}	Value	SE	t_{value}	P_{value}
Zone I	a	3.52	0.264	13.4	<2e-16	2.61	0.152	17.2	<2e-16	1.45	0.122	11.9	<2e-16
	b	0.006	2e-4	27.9	<2e-16	0.007	1e-4	51.8	<2e-16	0.008	2e-4	34.7	<2e-16
	TL range	4.0-11.0				3.5-12.0				3.5-7.5			
	R^2	0.96				0.99							
	n	220				289							
Zone II	a	3.63	0.216	16.8	<2e-16	3.77	0.206	18.4	<2e-16	3.38	0.110	38.8	<2e-16
	b	0.006	2e-4	24.4	<2e-16	0.006	2e-4	19.7	<2e-16	0.006	2e-4	40.3	<2e-16
	TL range	6.5-12.0				8.5-12.0				5.5-10.0			
	R^2	0.99				0.98							
	n	166				206							
Zone III	a	1.65	0.281	5.9	<2e-16	1.60	0.131	12.3	<2e-16	0.78	0.130	6.0	<2e-16
	b	0.011	7e-4	16.0	<2e-16	0.011	4e-4	28.1	<2e-16	0.013	6e-4	22.8	<2e-16
	TL range	2.0-7.0				2.0-12.0				2.0-12.0			
	R^2	0.99				0.99				0.90			
	n	98				216				84			

3.3.2 Growth analysis

The YOY ranged between 2 and 12 cm TL corresponding to 0.03 and 12.7 g (Table 3.1). The relationship between total length and otolith radius was significantly linear in the three areas and cohorts (P <0.001) and highly correlated (R^2> 0.95), supporting back calculation procedures (Table 3.2). The Laird-Gompertz growth parameters were highly significant (P <0.001), where age explained more than 88% of the length variability (Table 3.3). The estimated age, length and absolute growth rate at the inflection point (GAR$_{X0}$) ranged from 45-60 days, 4-10 cm and 0.8-1.01 mmd^{-1}, respectively for different cohorts and zones (Table 3). The Kimura likelihood ratio test found significant differences in all the parameters between zones, for the three cohorts (Table 3.4).
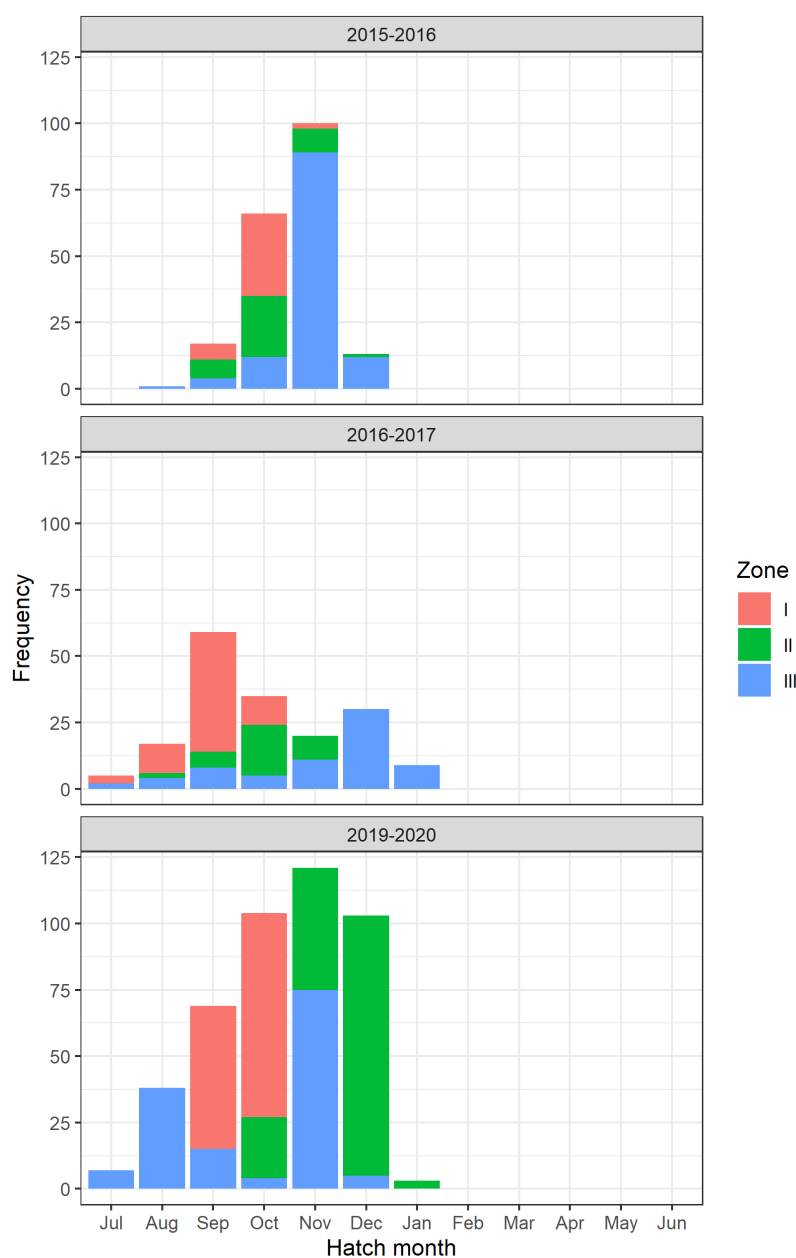
Figure 3.2. Month frequency of hatch dates of anchoveta juveniles for Zone I (Northern); Zone II (North-central); Zone III (Southern) for 2015, 2016 and 2019.

Table 3.3 Growth parameters based on Laird-Gompertz models for anchoveta juveniles (*E. ringens*), in three geographic zones of the Southeastern Pacific off Chile by the winter-spring 2015 cohort. L_∞ = Asymptotic length; α = instantaneous growth rate at age X_0; X_0= Age at the inflection point (days); L_{X0} = total length of the fish at the inflection point; GAR_{X0}= absolute growth rate at X_0 (mm).

Zone	Parameters	Cohort								
		2015			2016			2019		
		Value	SE	P_{value}	Value	SE	P_{value}	Value	SE	P_{value}
I	L_∞	11.15	0.132	<2e-16	11.43	0.026	<2e-16	9,18	0,144	<2e-16
	α	0.021	0.0003	<2e-16	0.024	0.008	<2e-16	0,030	0,008	<2e-16
	X_0	57.04	0.682	<2e-16	54.57	1.193	<2e-16	45,56	1,193	<2e-16
	L_{X0}	4.10			4.20			3,38		
	GAR_{X0}	0.85			1.01			1,01		
	R^2	0.90			0.88			0.89		
	n	8815			1600			8636		
II	L_∞	11.70	0.117	<2e-16	12.97	0.159	<2e-16	11,16	0,075	<2e-16
	α	0.026	0.004	<2e-16	0.020	0.004	<2e-16	0,026	0,0002	<2e-16
	X_0	58.26	0.515	<2e-16	67.07	0.827	<2e-16	55,61	0,31	<2e-16
	L_{X0}	4.31			4.77			4,11		
	GAR_{X0}	1.11			0.93			1,07		
	R^2	0.90			0.96			0.93		
	n	4807			1285			15383		
III	L_∞	7.72	0.112	<2e-16	12.86	0.283	<2e-16	10,33	0,487	<2e-16
	α	0.023	0.004	<2e-16	0.019	0.006	<2e-16	0,022	0,0007	<2e-16
	X_0	50.5	0.693	<2e-16	72.63	1.428	<2e-16	57.68	2,04	<2e-16
	L_{X0}	2.84			4.77			3,80		
	GAR_{X0}	0.67			0.90			0,84		
	R^2	0.89			0.88			0.89		
	n	6720			1949			4661		

Table 3.4 Comparison of Laird-Gompertz growth parameters of juvenile anchoveta (*E. ringens*), applying the Kimura likelihood ratio test, between three zones of the Southeast Pacific off Chile, for the cohorts 2015, 2016 and 2019.

Cohort	Test	Hypothesis	Chi-square	df	P_{value}	Model	SSR
2015	H_0 vs H_1	$L_{\infty1} = L_{\infty2}= L_{\infty3}$	145.1	2	< 2e-16	1	1285
	H_0 vs H_2	$K_1=K_2=K_3$	143.3	2	< 2e-16	2	1285
	H_0 vs H_3	$t_{01}=t_{02}=t_{03}$	26.8	2	< 2e-16	3	1278
2016	H_0 vs H_1	$L_{\infty1} = L_{\infty2}= L_{\infty3}$	18.7	2	< 2e-16	1	5058
	H_0 vs H_2	$K_1=K_2=K_3$	30.3	2	< 2e-16	2	5070
	H_0 vs H_3	$t_{01}=t_{02}=t_{03}$	82.6	2	< 2e-16	3	5126
2019	H_0 vs H_1	$L_{\infty1} = L_{\infty2}= L_{\infty3}$	83.8	2	0.000	1	9950
	H_0 vs H_2	$K_1=K_2=K_3$	83.1	2	0.000	2	9950
	H_0 vs H_3	$t_{01}=t_{02}=t_{03}$	141.7	2	0.000	3	9970

3.3.3 Otolith increment width profile analysis

The IW of juvenile anchoveta ranged from 1.1 to 28.6 μm (7.9±3.7), 1.1 to 29.0 μm (8.3±4.1), 0.9 to 23.2 μm (5.6±3.0), for zones 1, 2 and 3, respectively, corresponding to age ranges 3-40 days, 8-60 days, 19-118 days, respectively. Regardless of the cohort, the increments were significantly wider in zone I and II than in zone III for the first two months of life. The initial IW was higher in zone I during 2015 and 2019. In addition, the maximum growth occurred earlier in zone I than in the other two zones, approximately 40 days after hatching (Figure 3.3).
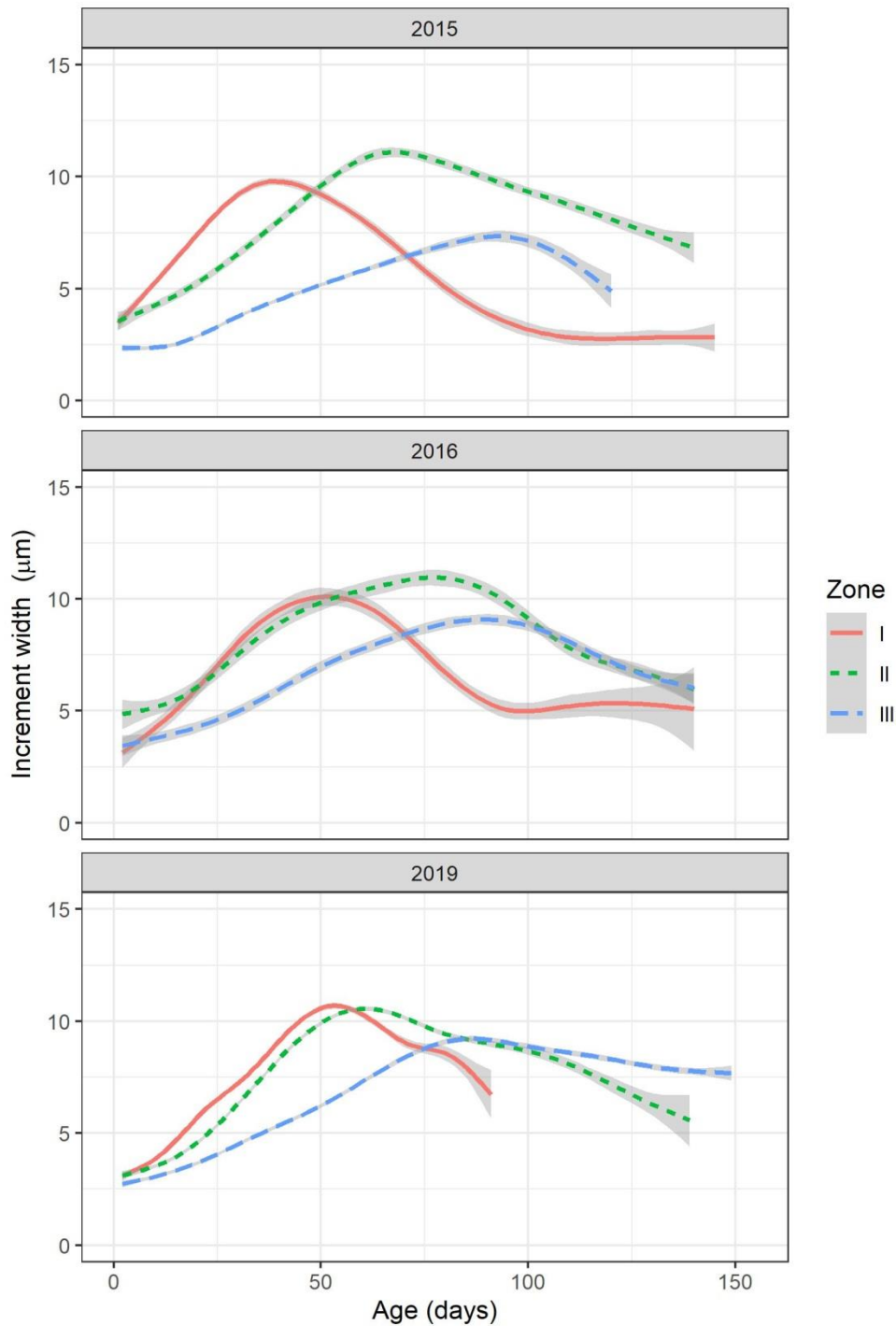
Figure 3.3. Daily increment width profiles with their standard errors by zone and cohort.

3.3.4 Seasonal and spatial fluctuation in SST and Chloa

The combined SST for the study years ranged from 15.4 to 22.7 °C (18.4±1.5), 13.3 to 19.9°C (15.9±1.8), 11.3 to 16.8 °C (13.2±1.2), for zones I, II and III, respectively. STT showed a similar pattern irrespective of zones during the year, with maximum values in summer (Figure 3.4a), but with significant differences between months (F=169.5, P< $2.2e^{-16}$), zones (F=2208.4, P< $2.2e^{-16}$) and month x zone interaction (F=3.7, P< $1.1e^{-07}$). Combined chlorophyll-*a* for the three years ranged from 0.7 to 5.8 mg m^{-3} (2.4±1.1), 0.6 to 3.6 mg m^{-3} (1.3±0.6), 1.0 to 13.9 mg m^{-3} (3.5±2.5) for zones I, II and III, respectively. The highest concentrations were observed in summer (Fig. 4b) with significant differences between months (F=46.5, P< $2.2e^{-16}$), zones (F=219.6, P< $2.2e^{-16}$) and month x zone interaction (F=2.3, P<0.001).
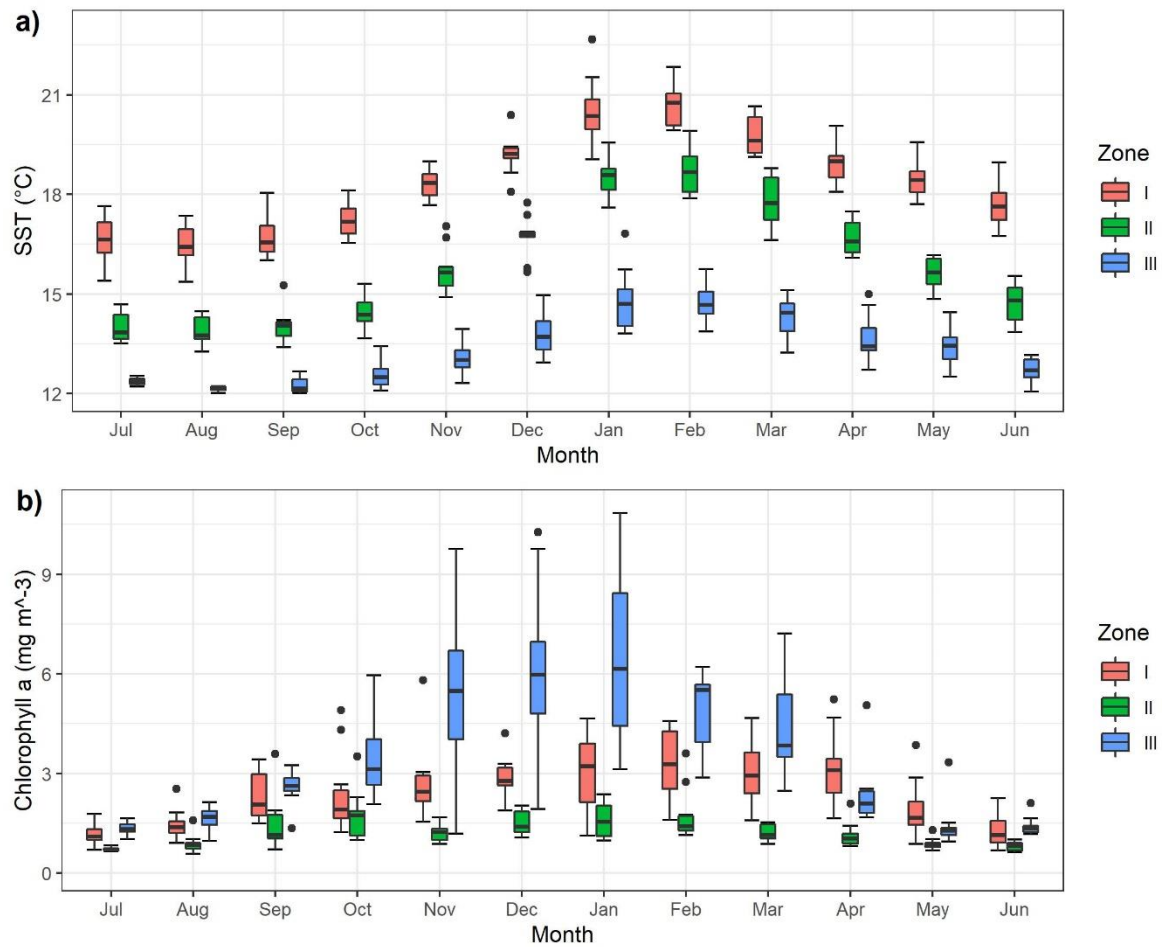
Figure 3.4. (a) Mean monthly sea surface temperature and (b) mean monthly concentration of Chlorophyll a by zone and years (2015, 2016, 2019).

3.3.5 Generalized additive model

GAM showed that zone, cohort, SST, Chlo*a* as predictors accounted for the 81.4% of deviance in IW. Otolith growth indices did not vary between northward zones (I and II), but differences occurred between them and Zone III (Table 3.5; Figure 3.5 a-d). Conversely, interannual differences were detected between the 2019 with the two previous cohorts (Table 5; Fig. 5e). The specific effect of SST and Chlo*a* on IW changes was significant (F=35.09; P<2e-16 and F=7.40; P<2e-16), respectively. The mean curve of IW partial residuals showed that the largest increments (higher growth) matched the period of high temperature, with values between 16 and 19°C. Conversely, narrower increments occur at temperatures between 12 and 14°C (Fig. 5f). For Chlo*a* the partial residual showed a decreasing tendency, where the maximum values occurred at lower concentrations (< 2 mg m-3) and vice versa (Figure 3.5g).

Table 3.5. Summary of generalized additive model (GAM) of increment width versus age of increment formation and the effect of previous formed increment. The model also considers the predictor variables: SST, Chloa as random factors and zone, cohort as fixes factors.

Parametric coefficients			
	Estimate	Std. Error	t value	P_{value}
Intercept	771.5	0.0565	136.55	<2e-16
Zone II	0.0115	0.0333	0.35	0.730
Zone III	-121.9	0.1021	-11.94	<2e-16
Cohorte 2016	-0.0403	0.0381	-1.05	0.293
Cohorte 2019	-0.2332	0.0278	-8.38	<2e-16
Approximate significance of smooth terms			
	df	F	P_{value}
s(Age):Zone I	6.002	178.34	< 2e-16
s(Age):Zone II	7.267	232.084	< 2e-16
s(Age):Zone III	6.638	162.824	< 2e-16
s(IWprev)	8.980	8.253.164	< 2e-16
s(SST)	8.925	35.904	< 2e-16
s(Chloa)	8.851	7.404	4.54e-10
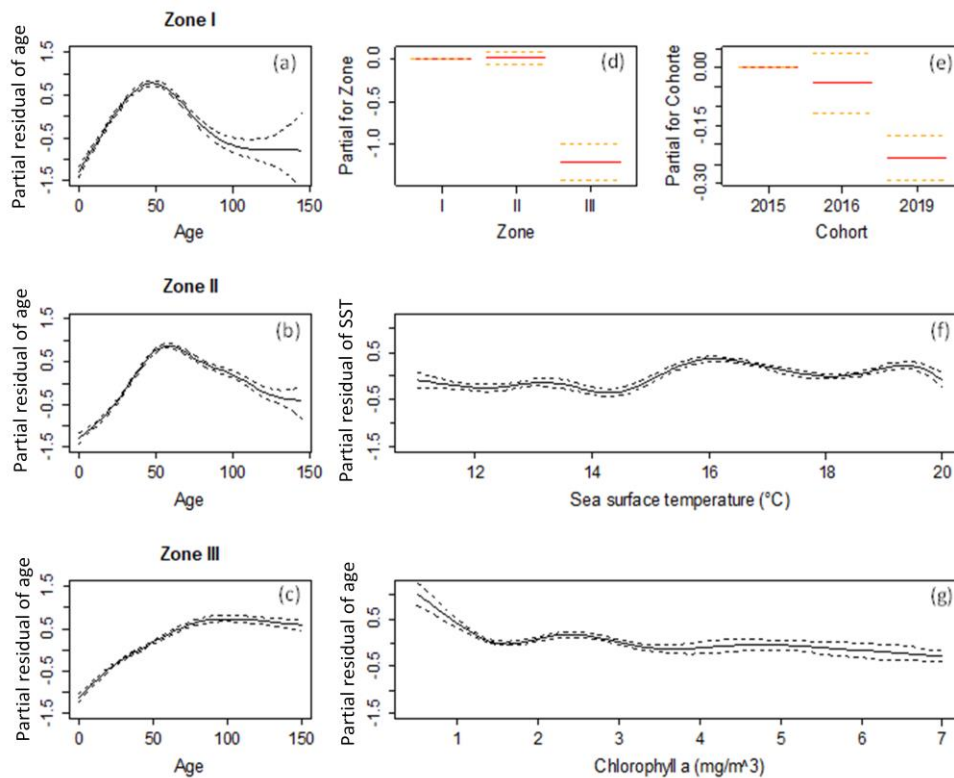
Figure 3.5. Interpolation graphs of age from a generalized additive model (GAM). In the left panels the solid black line denotes the partial residuals of the increment width (IW) for each zone with age as predictor variable (a to c), whereas the doted lines denote 95% confidence intervals. Panels "d" and "e" show the effect of the zone and cohort on IW, respectively whiles panels "f" and "g" illustrate the change of the IW partial residuals in relation to sea-surface temperature (SST) and the chlorophyll *a* concentration (Chlo*a*), respectively.

3.4.   Discussion

3.4.1 Ontogenetic and spatial variations in growth trend

This study found that regardless of geographical area and cohort, anchoveta from the Humboldt Current system had an increasing otolith growth rate as age progressed to a maximum. This pattern associated with the winter and spring cohorts expand the findings reported by Cerna and Plaza (2016), who reported the same pattern for monthly cohorts throughout an annual cycle for this species in the HCS, although varying in magnitude as season progressed. In the present study the plateau was reached at older ages as latitude increased but the decreasing tendency remained. This up and down pattern of IW has been reported in several studies on juveniles cupleoids and other species in various ecosystems (e.g., *Engraulis encrasicolus*-Aldanondo et al., 2011; Schismenou et al., 2014; *Engraulis japonicus* -Namiki et al., 2010; *Engraulis ringens*-Plaza et al., 2018, Cerna and Plaza, 2016; *Sardina pilchardus*- Schismenou et al., 2016; *Trachurus japonicus*-Xie et al., 2005). From these findings and the results of the current study it is reasonable to hypothesize that the shape of IW profile is ontogenetically mediated but modeled by environmental drivers. The increasing phase seems indicative that fish move fast through larval phase and even beyond the transforming stage (i.e., metamorphosis), whereas the decreasing growth phase may be associated to unknown

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physiological causes or alternatively due to species-specific allometric changes in the otolith growth. It is important to highlight that the decreasing phase of IW is in the range of ages where a significant TL-OR relationship was evident and hence IW can be translated proportionally to somatic growth.

The anchoveta juveniles showed significant latitudinal differences in the otolith growth off Chilean coast. Such a consistent pattern was evidenced by the growth parameters derived from age-length relationships fitted to Laird-Gompertz models and when daily increment widths were analyzed using chronological analysis using a GAM model. The growth parameters varied significantly between the three zones for all the cohorts. However, the chronological analysis of the IW only showed differences between the most septentrional zones (I and II) compared to the most meridional zone (III). These differences can be explained because the chronological analysis removes the effect of ontogeny and is independent of seasonality, unlike the approach of length-at-age models that require similar birth cohorts to be compared to reach reliable conclusions. This requirement was overlooked in part in the present study, because most juveniles come from winter and spring hatching seasons.

A second advantage of the chronological analysis of the IW using GAM is the capacity to consider intrinsic and extrinsic factors as independent variables (Hastie and Tibshirani, 1990). Similar approaches were recently used with mixed linear models to study the annual chronology of adult fish (e.g., Morrongelio and Thresher, 2015). However, in the present study a GAM was applied because it was more appropriate to model the non-linear distribution of the chronology of anchovy IW. In addition, serial autocorrelation was addressed by including the previous IW as random factor, following the criteria described by Schismenou et al., (2014, 2016).

It is important to note that both the length-at-age based approach and the chronological growth analysis were consistent in detecting significant differences between zones I and III. From these findings, an immediate question arises, i.e., what are the main environmental drivers of juvenile growth of this forage species in this ecosystem of high productivity, which also vary at smaller local scales?

3.4.2 Potential environmental drivers of early growth

The three years studied showed that temperature is an important environmental driver for the early growth of juveniles of this species, where higher temperatures were associated with high daily growth. Temperature is a main driver of the somatic growth of larvae and juveniles (Shismenou et al., 2014, 2016; Zenitani *et al.*, 2009; Wenger *et al.*, 2016; Gallagher et al., 2005). The positive relationship between growth and temperature has also been found for *Engraulis mordax* larvae from southern California (Methot and Kramer, 1979). Similarly, Namiki et al. (2010), in an experimental analysis, found that a low water temperature (<13-$14°C$) limited the growth of the *Engraulis japonicus* otolith. Other studies based on otolimetry have reported parabolic relationships between temperature and early growth where an optimal temperature for the growth of clupeiforms has been determined (e.g., Takasuka et al., 2007). Hernandez et al. (2020) also reported significant parabolic relationship between SST and recent growth of otoliths of pre-recruits in northern Chile, where an optimal temperature of $18°C$ was determined. The results derived from GAM where a sinusoidal relationship between SST and standardized growth index support the idea of an optimal temperature range where early growth of small pelagic fishes seem to be maximized.

It is important to highlight that an increase in temperature leads to a rise in metabolic rate in teleosts (Hussy et al., 2020), which is evident through the increase in the carbonate bio-mineralization rate in otoliths (i.e., Mosegaard et al., 1988; Wright, 1991; Wright et al., 2001; Morales-Nin, 2000; Yamamoto et al., 1998; Hussy and Mosegaard, 2004), where the crystal precipitation rate in the otolith doubles with an increase of $10°C$, over an optimal temperature range (Hussy and Mosegaard, 2004; Fablet et al., 2011). In the present study, the difference in

SST between zones showed a north-south gradient that could be enough to trigger significant changes in otolith growth and consequently in its somatic growth of this species.

The inverse effect of chlorophyll-*a* on the chronology of anchoveta growth seems somewhat contradictory because an increase in Chlo*a* should be associated with an increase in food availability, as has been shown in several studies in marine ecosystems and in the HCS as well (e.g., Thiel et al., 2007; Huot *et al*., 2007; Wernand et al., 2013). Why then is high productivity not associated with higher growth in the HCS? Are these findings evidence that productivity is not a limiting factor for early growth of this species? Or alternatively there a hidden effect of a concomitant variable positively related with productivity that negatively impacts early growth? Addressing these questions in future studies is very important, because in the last fifteen years there is scientific evidence of a gradual decrease in mean size and weight of *E. ringens* (e.g., Canales et al., 2018), which seems to be indicative of a decrease in the population health maybe related to exploitation patterns as well. On the other hand, it is also necessary to look for alternative answers to explain the inverse association between early growth and productivity for the first 50 days of life of this species. For example, it is reasonable to infer that increased production of food does not imply that it can be available for consumption. Growth and survival depends on the match of more than one factor in the environment, not only productivity (enrichment), but also that food, supply and type, are available to larvae and pre-recruits at the right time (i.e., Lasker events, Lasker, 1981). For this, water stability is decisive because, conditional of low turbulence increase the concentration of food particles (Bakun and Parrish, 1980; Cury and Roy, 1989).

It is well known that the physical conditions of zone I and II off Chilean coasts have moderate and permanent upwelling during winter and spring, with low turbulence and Ekman transport, for a prolonged period (Thiel et al., 2007). This not only facilitates the retention of larvae, phytoplankton and zooplankton, but also provides a permanent stability, which increases the probability of encounters between larvae/juveniles and their prey. So, although Zones I and II on average has less primary production than zone III, the physical conditions of the habitat facilitate the feeding of anchovy, and this may explain the higher growth recorded. Matching with this feature in the Mediterranean anchovy, when the physical forces are relaxed (Ekman transport), the stability of the area favored a rapid growth in post-larvae, although primary production was reduced (see Aldanondo et al., 2010). Conversely, the physical conditions in zone III, where there is strong upwelling, lead to high primary production, followed by calm events that favor retention and feeding, which is continued in a new upwelling event in an intermittent cycle (e.g., Arcos et al., 1996). Such an oceanographic feature probably generates a discontinuity in the availability of food for the early stages of anchovy triggering a lower somatic growth.

An alternative explanation for the inverse relationship between chlorophyll concentration and early growth could be in the trophic behavior of anchoveta because, the engraulids in general have mainly a zooplanktivorous diet in larval stages (e.g., *Engraulis japonicus*, Shahidul and Tanaka 2009; *Engraulis encrasicolus*, Borme et al., 2009; *Engraulis ringens*, Landaeta et al., 2014). For the juvenile and adult phase, a more varied dietary composition has been reported for *E. ringens*, with the presence of phytoplankton and zooplankton in stomach contents (Medina et al. 2015). Recent studies using of C^{13} and N^{14} stable isotopes have shown that anchovy has a much higher trophic position than previously believed (i.e., Espinoza and Bertrand 2008; Pizarro et al. 2019; Castro et al. 2020), because it mainly assimilates zooplankton, euphausiids and other larger prey (Pizarro et al. 2019). Conversely, phytoplankton is assimilated very slowly. Therefore, the lesser influence of chlorophyll concentration on early growth evidenced in the present study matches well with this new paradigm of trophic ecology that supports the existence of a more complex and specialized trophic behavior for this species. Hence, it is very important that future studies examine the preferred type of prey on a spatial

and temporal basis, because irrespective of productivity levels, different compositions of prey species can occur, and some prey may be more preferred or be more advantageous for fast growth than other prey (see Robert et al. 2014).

The HCS is one of the most productive systems in the world, although it is not homogeneous in terms of abundance of pelagic fishes such as anchovy. It is reasonable to infer that these differences could be associated to variable survivorship during the early stages where the higher mortalities are expected to occur (Anderson, 1988). In the present study, the growth of the survivors was mainly influenced by temperature, and the highest growth was found for juveniles from the northern zone (Zone I). This stock has the high fisheries landings, but lower when compared with the north-central stock of Peru. Therefore, it is important to expand this kind of study to this main fish stock, where studies on early life history of anchoveta still do not existent, to verify that high productivity is associated with a higher somatic growth of juveniles. Addressing this goal is worthy because growth and survival during the early stages of teleost fishes seem to be related (i.e., Anderson, 1988; Takasuka et al., 2003; Robert et al., 2007).

Acknowledgements

6. References

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Chapter 4: Testing the growth-mortality hypothesis for anchoveta.

Testing the growth-mortality hypothesis in the anchoveta (*Engraulis ringens*) in the Humboldt Current system using otolith microstructure analysis

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Abstract

The growth-mortality hypothesis was tested in spring cohorts of anchoveta (*Engraulis ringens*) in four different years (2014, 2019, 2020 and 2021) in Northern Chile (18-24°S) in the Humboldt Current system (HCS). Linear mixed-effect models (LMMs) were used to compare the variation of otolith radii-at-age (RAI) and increment width (IW), as proxy of fish size and growth rate of survivors (pre-recruit and recruits) with an initial population of larvae hatched in spring. Spline smoothed profiles of RAI and IW showed an increasing pattern from a common origin, after which a clear divergent tendency appeared, where survivors always showed larger and wider RAI and IWs for the first 20 days, when comparing with larvae, for the two cohorts evaluated (2014 and 2019). LMMs showed that both RAI and IW were significant bigger and higher for recruits than larvae, irrespective of years. Overall results showed evidence that survivors were bigger and grew faster than members of the original population, giving support for the growth–mortality hypothesis for this small pelagic fish in the HCS. In addition, other distinctive finding was that older juvenile (recruits), were larger and grew faster than young juveniles (pre-recruits), when were larvae and transforming juveniles.

Keywords: Anchoveta, otolith, juvenile fish, daily age, growth variation, growth-mortality relationship

4.1. Introduction

Most teleost fishes undergone high mortalities during their early life stages, where only a small fraction survive to adulthood, triggering marked fluctuations in the recruitment of many species, especially in fishes having a broadcast pelagic spawning strategy (Cushing, 1974; Lasker, 1975; Miller et al., 1988; Cury and Roy, 1989; Bakun, 1996; Olsen et al., 2019). To date, several hypotheses have been proposed to explain such a large fluctuation in natural mortalities occurring in the ocean worldwide, and one of them is the so-called "Growth-mortality hypothesis; GMH" proposed by (Anderson, 1988), who predicted higher survival for relatively larger, faster growing individuals, as a combination of feeding and predation-related mechanisms during the developmental stages of fishes.

The GMH unifies in a conceptual framework previous hypothesis that establish that specimens that grow faster require less time to transit through those vulnerable stages (i.e., "the stage duration hypothesis"; Ware, 1975; Houde, 1987; Chambers and Leggett, 1987). Furthermore, if mortality functionally decreases with fish size, then larger individuals at any defined age would have a lower probability of mortality than smaller ones, due to their better ability to detect preys and escape from predators (i.e., "the bigger-is-better hypothesis"; Leggett and DeBlois, 1994; Miller et al., 1988). More recently, the growth-selective predation "hypothesis" was proposed, which states that faster-growing larvae are more likely to survive than slow-growing ones, but that this process may be mediated by the effect of predation. In certain cases, some predators may prefer poorly nourished, slow-growing larvae, but in other cases larger,

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more robust larvae may be easily seen by predators (Takasuka et al., 2003; Takasuka et al., 2007).

To date, several studies have evaluated the GMH, using different approaches, in either field (e.g., Hare and (Hare and Cowen, 1997); (Shahidul Islam et al., 2010)), or experimental conditions (e.g., Meekan et al., 2006). One of the most common approaches is through the comparison of daily growth rates, based on otoliths, between an original larval population and older larvae from the same hatch cohort (i.e., survivors), and through the same temporal window. This approach called "traditional method" has demonstrated for different species and habitats, that in some cases the survivors always were bigger and grew faster at each daily age (e.g. *Engraulidae*, Takasuka et al., 2004a; Takasuka et al., 2004b; Takasuka et al., 2006; *Clupeidae*, Alemany et al., 2006; Meekan et al., 2006; Plaza and Ishida, 2008; Scombridae, Robert et al., 2007; Satoh et al., 2013; Shoji and Tanaka, 2006; Tanaka et al., 2006; Pleuronectidae , Howenkamp, 1992, Joh et al., 2013; Gadidae, Campana, 1996; Meekan and Fortier, 1996; Nielsen and Munk, 2004). These evidences have demonstrated that early growth plays a key pivotal role in explaining variability in size-selective mortality, especially at the larval level in teleost fishes.

Based on all these findings, it is reasonable to ask ourselves to what extent growth is a key driver for survival? Would those fast-growing post-larvae or transforming larvae, which have already succeeded the vulnerable phases, become true survivors until reaching the adulthood? Or are they mostly part of a population that is also destined to perish by natural mortality? These questions could be addressed by comparing the initial growth (i.e., first 30 days) between individuals recently recruited to the adult population, with an initial larval population of the same hatched cohort. If recruits were larger and grew faster when they were larvae, it would be indicative of the existence of a carry-over effect, i.e., the gain in growth obtained during the larval phase would be projected throughout the entire life history. To date, several field studies, using the traditional method, have reported evidence in favor of the bigger is better (B-I-B) mechanism in survivors, composed of young-of the year (YOY) fish in some species (e.g., Nielsen and Munk, 2004; Meekan et al., 2006; Alemany et al., 2006; Robert et al., 2007; Plaza and Ishida., 2008). However, only few studies to date have used recruits or young adults (older survivors) to compare with the original larval population (e.g. Alemany et.al., 2006; Robert et.al, 2007; Shahidul Islam et al., 2010).

Short-lived species, such as engraulids, can be good models to assess the potential existence of a "carry-over effect" within the framework of the GMH, because otoliths from engraulids (i.e. Cotano and Alvarez 2003; Cerna and Plaza, 2016) have daily micro-increments suitable for daily age determination, allowing to obtain reliable back-calculated estimates of hatch dates and therefore of cohort assignment. Hence, in the current study the anchoveta (*Engraulis ringens*) is used to perform a comprehensive analysis of otolith microstructure to compare otolith-radii-at-age and increment width, as proxy of fish size and growth rate, between survivors and members of an original larval population, using linear mixed-effect models. Our aim is to contribute to the knowledge of the population dynamics of this engraulid, a species that plays a key ecological role in the pelagic ecosystem of the Humboldt Current system, supporting an important fishery activity as well (Canales et al., 2020).

4.2. Materials and methods

4.2.1 Field sampling

Pre-recruits and recruits of anchoveta were collected through field campaigns, onboard the research vessel Abate Molina, of the Instituto de Fomento Pesquero (IFOP) carried out on annual basis for the adult sampling during echo surveys in the northern stock of Chile, where higher abundance of this resource occurs. All individuals were collected through mid-water trawls, using a net Engel® with a total stretched length of 97 m, with a mouth circumference of 302.4 m (168 meshes by 1800 mm), 24 mm in size mesh in the tunnel and 13 mm in the codend. Smaller pre-recruited fish are trapped in the codend whose mesh size was 13 mm, which avoided the escape of small fish between 30 and 55 mm in length. The campaigns were conducted during December of 2014, 2019, 2020 and 2021, corresponding to the onset of recruitment periods for anchovy, which extends from December to March of the following year (Figure 1; Toledo and Claramunt 2022; Zunguza et al., 2022). To obtain otolith sections with a high resolution of daily increments several samples must be prepared because   a number of otoliths are destroyed during this process. In addition, some otoliths must be discharged when they do not fit a seasonal hatched cohort, even if the resolution of its micro-increments is suitable for analysis. For this reason, in some years, older juveniles (recruits) were included to complement samples from eco-surveys. These samples of recruits were taken on board of fisheries commercial vessels from January to April in 2015, 2020 and 2022, respectively. The pre-recruits corresponded to early juveniles <60 mm TL (range: 30-55), which appear regularly in the hydro-acoustic surveys with a discrete mode of length distribution, distinguishable from the larger recruits (late juveniles) harvested by the fishery. On board, the total length was recorded with a precision of 0.01 mm, the total weight (gr) and sagittal otoliths of each fish were extracted, cleaned, dried and stored for later analysis. Larval samples were collected in October 2014 and 2019 in northern Chile (22°30'-22°33'S) using a standard Bongo net (60 cm diameter in mouth opening, 300 μm in mesh size), which was towed obliquely from 30 m deep to surface. Samples were preserved just after capture in 96% ethanol after and stored for posterior analysis. Identified anchovy larvae were measured from the snout to the base of the hypural bones, without correcting for shrinkage. The overall analyzed samples corresponded to a total of 764 specimens from four cohorts, and in two of them the original population (larvae) and survivors (recruit) were available, whereas in the other two cohorts only pre-recruit and recruit were available (Figure 4.1).

Table 4.1. Basic statistics of total length (mm) and number of sagittal otoliths of *Engraulis ringens*, analyzed by ontogenetic stage and annual cohorts.

| Ontogenetic stage | Length of fish (mm) | | Number of otolith by cohort | | | | |
	Mean±sd	Range	2014	2019	2020	2021	Total
Larvae	7.3±3.4	2.0-16.3	106	56			162
Pre-recruit	43.4±6.4	30.0-55.0		119	81	52	252
Recruit	96.9±17.2	60.0-120.0	95	98	75	82	350
Total by year			201	273	156	134	764

4.2.2 Otolith preparation

Otoliths from larvae (2 to 16 mm SL) and pre-recruits (30 to 55 mm TL), were embedded in a small portion of Cristal Bond on slide glasses, after which no additional preparation was needed to observe micro- increments. For larger juveniles (60 to 120 mm TL), the left sagittal otoliths were mounted in a small portion of two-part epoxy resin on slide glasses with the sulcus side down and grounded until the primordium became clearly visible, with 800-2000 grit sandpaper and cleaned with 0.5 um alumina polisher. Then, otoliths were flipped and polished following the same procedures, until revealing the primordia and reach a reliable resolution of daily increments.
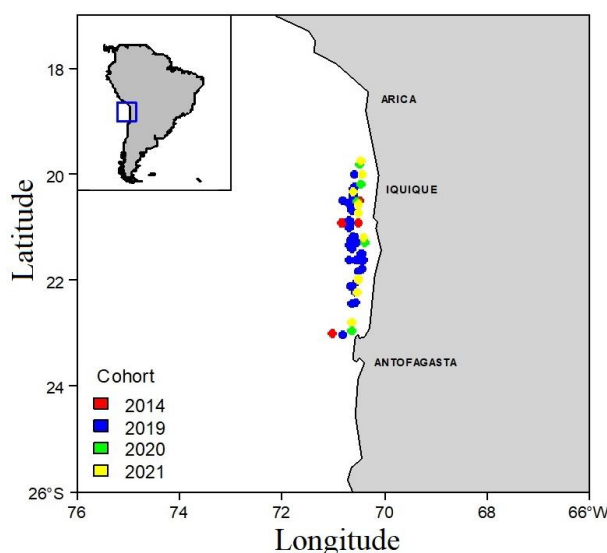
Figure 4.1. Study area in northern Chile. The points indicate sampling hauls in the hydroacoustic surveys, where larvae, pre-recruit and recruits of anchoveta were collected.

2.3 Otolith micro-increment analysis

Digitized images were obtained from each prepared sagittal otolith, using an image analysis system consisting of a triocular light microscope (Olympus), and a digital camera (QImage 5 mega pixels) connected to a desktop computer equipped with Image-Pro Plus 5.1 software (*Mediacibernetycs*). For each larva one image taken at 1000X of magnification was enough to cover the entire sequence of microincrements. For pre-recruit and recruits, a sequence of several images taken at 400X magnification were needed. Complementary images were taken around the primordium at 1000X to ensure a correct identification of the micro-increments in this area. The micro-increments were identified as two successive concentric units (Panella, 1971; Campana and Jones, 1992) and were then counted and measured twice, with an agreement of 95% between readings, by a unique experienced reader across the area of distinctive increments, which in most cases corresponded to post-rostrum area. All records were performed using the "Caliper Tool" available in the Image-Pro Plus image analyzer (version 6.0). When counting differed by over 5% between readings, the record was discarded.

The daily periodicity of micro-increment formation at later stages, for juveniles and young adults, were validated by Plaza and Cerna (2015). However, some missing increments were reported close to the hatch check (HC), when counting and measuring increment widths (IW) and radii-at-age (RAI) under rearing conditions (see Plaza et al., 2019). As there no way to know the amount of missed rings at the individual level to make a correction of IWs and RAI, the first increment formed just after the HC was regarded as the first day of life for survivors (pre-recruits and recruits) in the current field study. The hatching dates (HD) of fish were back-

calculated subtracting the total age in days from the calendar day of sampling. Only individuals hatched in spring season between July to September to 2014, 2019, 2020 and 2021 were combined to either increase sample size and obtain more robust statistical outcomes. Mean increment width and radii-at-age profiles were summarized using a smoothed plot, using the library "ggplot2" available in the R software. The smooth process was based in a GAM model (family = gaussian; link = "identity"), due to the no linear shape visible particularly in pre-recruits and recruits.

4.2.5. Statistical analysis

The traditional method was applied to compare RAI as proxy of fish size-at-age and IW as proxy of daily somatic growth rates during the first twenty days of life. Each cohort was considered separately and comparisons were made for the three life stages considered. The bigger-is-better (B-I-B) and growth rate mechanisms were analyzed. To test for significant differences in RAI and IW between the larval initial population and survivors, linear-mixed effect models (LMMs) with a random slopes and intercepts were used, as follow:

$$\sqrt[4]{RAI_{ijk}} = \alpha_0 + \beta \sqrt{A}_{ijk} OS_{ijk} + \left(\beta \sqrt{A}_{ijk} \right) |Y_{ij}/IDfish) + \varepsilon_{ijk}$$

$$logIW_{ijk} = \alpha_0 + \beta \log(A_{ijk}) * OS_{ijk} + \left(\beta \log(A_{ijk}) | Y_{ij}/IDfish \right) + \varepsilon_{ijk}$$

where: RAI_{ijk} and IW_{ijk} are the radius and increment width, respectively, of fish i to age of formation j and year of birth k; A_{ijk} is the age of formation (covariate); OS_{ijk} in ontogenetic stage; Y_{ij} is the year of birth; IDfish is the identification number of fish; β correspond to the slope of each covariate; α_0 is the intercept that measure the global mean of RAI or IW. The year of birth was included as a random factor because the database was not balanced across years and to model the changes in the individual variability of RAI and IW as year of birth changed. To satisfy model assumptions RAI were quarter-root transformed whereas "OS" and "A" were square-root transformed. In case of-LMMs where IW was depended variables natural log-transformation were applied. Prior to analysis continuous variable were mean-centered to facilitate model convergence and interpretation of interaction terms (i.e., Morrongiello and Thresher 2015). All analyses were performed using the lme4 package (Bates et al., 2013). The models were fitted using restricted maximum likelihood (REML). A Tukey multiple comparison analysis was applied on results of LMMs of RAI and IW using package "multcomp" of R project (Hothorn et al., 2022)

4.3. Results

3.1. Length frequency distribution and age data.

Length frequency distributions by year from the 764 analyzed individuals are shown in Figure 4.2 and summary in the Table 4.1. The daily age at catch ranged from 2 to 32 days (overall mean 8.1±5.3days; CV=0.65), from 40 to 107 days (overall mean 65.9±12.4; CV=0.19) and from 73 to 180 days (overall mean 142.5±40.5; CV=0.28), for larvae, pre-recruits and larger survivors (recruits), respectively.
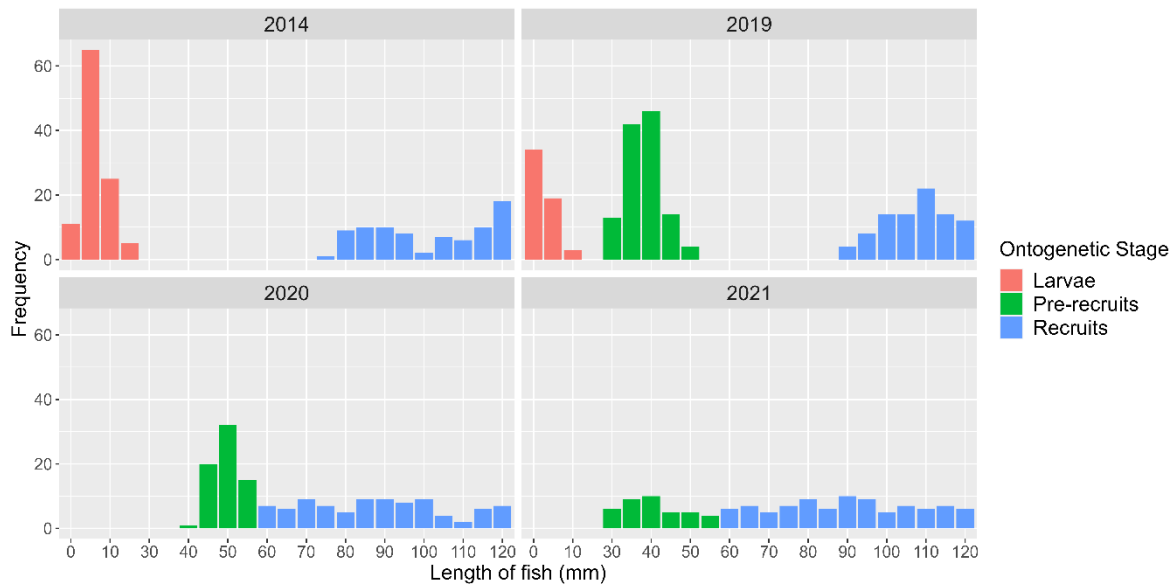
Figure 4.2. Length frequency distribution for two seasonal cohorts of larvae, pre-recruits and recruits of the anchoveta (*Engraulis ringens*) for four spring cohorts in northern of Chile.

4.3.2 Radii-at-age (RAI) and increment width (IW) profiles

GAM smoothed profiles of RAI and IW showed in general an increasing pattern from a common origin, after which a clear divergent tendency appeared, where survivors always showed larger and wider RAI and IWs for the first 20-30 days, compared with larvae (Figure 3). Pooled values of RAI and IWs, for larvae and larger survivors (pre-recruit and recruits) ranged from 2.5 to 1,838.5 µm (overall 461.0±416.2; CV=0. 90) and from 0.22 to 21.7 µm (overall 7.07±3.9; CV=0.55), respectively. Beyond the firth 50-60th days, a marked decrease in IWs occurred until to 150 days of life (Figure 3). At the individual level, the IW profiles showed great variability, but maintaining the increasing trend for the first 20 days in the case of larvae and for the first 50-60 days of life in the case of pre-recruits and recruits (Figure 4). This individual linear trend was modeled through the LMMs (Figure 4.3).
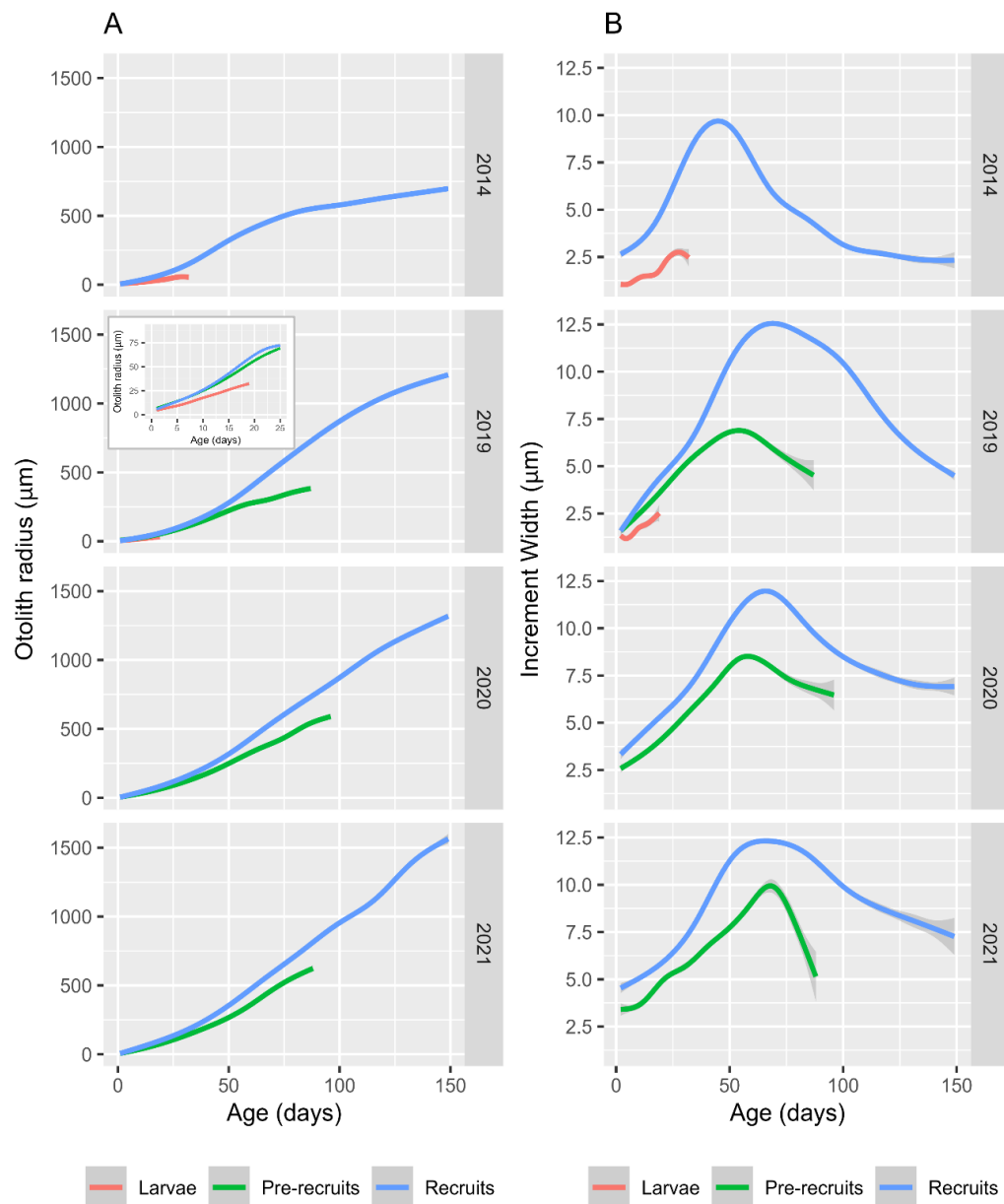
Figure 4.3. GAM smoothed profiles of mean otolith radii-at-age (A) and width-at-age (B) of larvae, pre-recruit and recruits of anchoveta (*Engraulis ringens*) in northern Chile, for spring cohorts of 2014, 2019, 2020 and 2021. The figure of otolith radii-at-age of 2019 was zoom inside the graphic to observe the detail in the early ages.

4.3.2 Linear mixed-effect models

The LMMs for the first 20 days of life showed that fixed effect (ontogenetic stage) combined with random effects (year of birth, and fish) explain the 97.4% of variance of RAI and the 90.6% of variance of IW-at-age. In both models the random effect of the fish ID and the year of birth showed a higher variance than the random effect of the slope and the residual effect of the models, which was indicative that the fish ID and the year were relevant to explain the variations in the RAI and IW response (Table 4.2 and 4.3).

Table 4.2. Summary of estimated parameters for random and fixed effects from linear mixed-effect models, to test for significant differences in radii-at-age, for the first 20th days of life, between larvae, pre-recruit and recruit of *Engraulis ringens* for spring hatched cohorts in northern Chile.

Random effects:				
Groups	**Name**	**Variance**	**Std.Dev.**	**Corr**
IDFish:Year	Intercept	0.01348	0.11608	
	Sqrt (age)	0.00216	0.04652	0.51
Year	Intercept	0.01017	0.10083	
	Sqrt(age)	0.00107	0.03263	0.89
Residual		0.00106	0.03254	

Fixed effects:					
	Estimate	**Std. Error**	**df**	**t value**	**Pvalue**
Intercept	2.37888	0.05080	4.04	46.83	1.11e-06
Square root (age)	0.41143	0.01651	4.06	24.92	1.37e-05
Pre-recruits	-0.09544	0.01049	724.06	-9.10	< 2e-16
Larvae	-0.34068	0.01328	852.80	-25.65	< 2e-16
Sqrt (age)*Pre-recruits	-0.04679	0.00426	662.45	-10.98	< 2e-16
Sqrt (age)*Larvae	-0.17220	0.00575	907.35	-29.96	< 2e-16

Table 4.3. Summary of estimated parameters for random and fixed effects from linear mixed-effect models, to test for significant differences in increment width at age, for the first 20th days of life, between larvae, pre-recruit and recruit of *Engraulis ringens* for spring hatched cohorts in northern Chile.

Random effects:				
Groups	**Name**	**Variance**	**Std.Dev.**	**Corr**
IDFish:Year	Intercept	0.05141	0.2267	
	log(age)	0.01715	0.1310	0.21
Year	Intercept	0.03900	0.1975	
	log(age)	0.00996	0.0998	-1.00
Residual		0.04249	0.2061	

Number of observations: 12,443, groups: ID:Year birth, 733; Year birth, 4

Fixed effects:					
	Estimate	**Std. Error**	**df**	**t value**	**Pvalue**
Intercept	1.30889	0.09953	4.05	13.15	0.00018
log(age)	0.26820	0.05054	4.08	5.31	0.00574
Pre-recruits	-0.24279	0.02036	693.73	-11.92	< 2e-16
Larvae	-0.92784	0.02885	1.189.20	-32.17	< 2e-16
log(age)*Pre-recruits	0.01163	0.01297	646.96	0.90	0.37008
log(age)*Larvae	-0.25587	0.02062	1.459.03	-12.41	< 2e-16

LMMs of RAI showed significant differences between the ontogenetic stages both in intercepts and slope (p<2e-16) (Table 4.2). The recruits showed higher mean predicted RAI than pre-recruits and larvae (Figure 4.4A). LMMs of IW also showed significant differences between ontogenetic stages both in intercept and slope (p<0.001), with the exception of the slope of pre-recruits that did not show significant differences compared to recruits (p=0.370) (Table 4.3). Recruits have higher mean predicted IW than pre-recruits and larvae (Figure 4.4B). The Tukey multiple comparison analysis showed significant differences between the different ontogenetic states (P<2e-16) for the means of otolith radius and daily increments width derived from linear

mixed-effect models (Table 4.4). It is important to note that LMMs converged well for both IW and RAI with showing a normality distributions of residuals values for both variables (Kolmogorov-Smirnov test; $p > 0.05$).
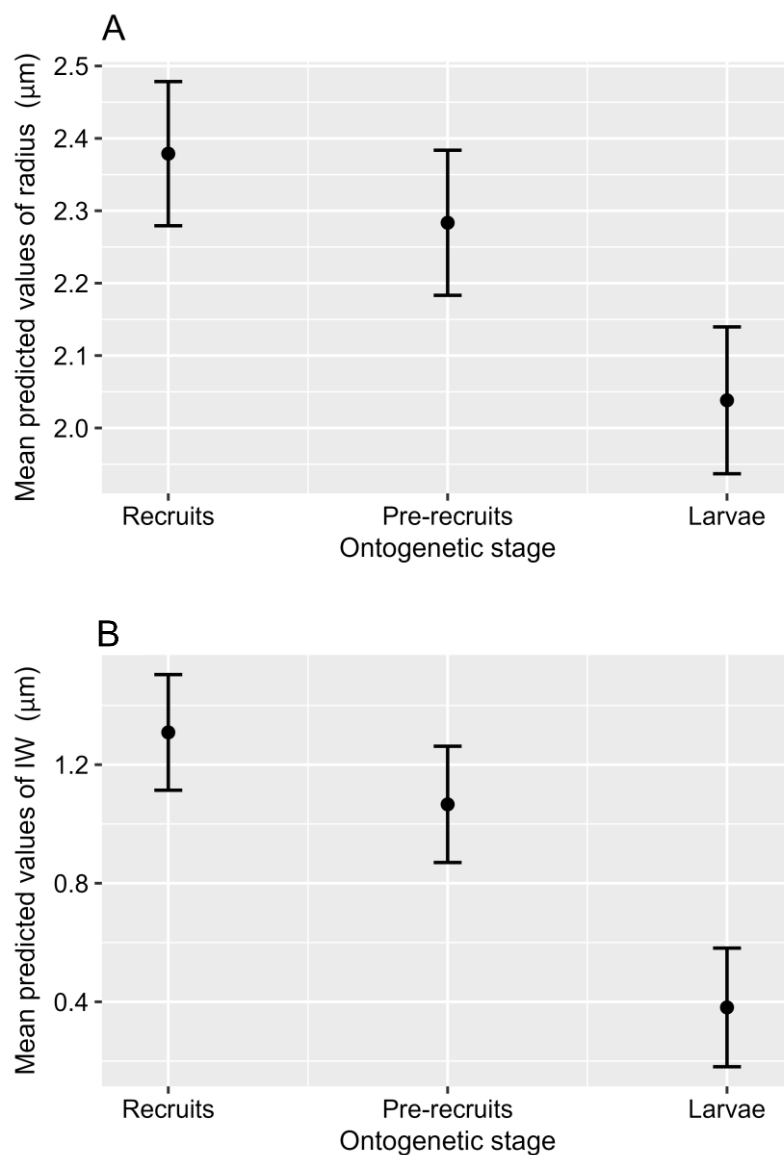
Figure 4.4. Predicted marginal means of log-transformed otolith radius (A) and daily increment width of otoliths (B), derived from linear mixed-effect models for ontogenetic stage of *Engraulis ringens* for four spring cohorts.

Table 4.4. Multiple comparison of means of otolith radius and daily increment width log-transformed derived from linear mixed-effect of three ontogenetic stage of *Engraulis ringens* of Northern zone of Chile.

	Ontogenetic stages	Estimate	Std. Error	z value	Pvalue
Radius	Pre-recruits – Recruits == 0	-0.1792	0,0177	-10.02	<2e-16
	Larvae – Recruits == 0	-1.3139	0,0272	-48.31	<2e-16
	Larvae – Pre-recruits == 0	-1.1367	0,0300	-37.89	<2e-16
Increment Width	Pre-recruits – Recruits == 0	-0.2501	0.0191	-13.08	<2e-16
	Larvae – Recruits == 0	-1.3279	0.0352	-37.68	<2e-16
	Larvae – Pre-recruits == 0	-1.0778	0.0378	-28.49	<2e-16
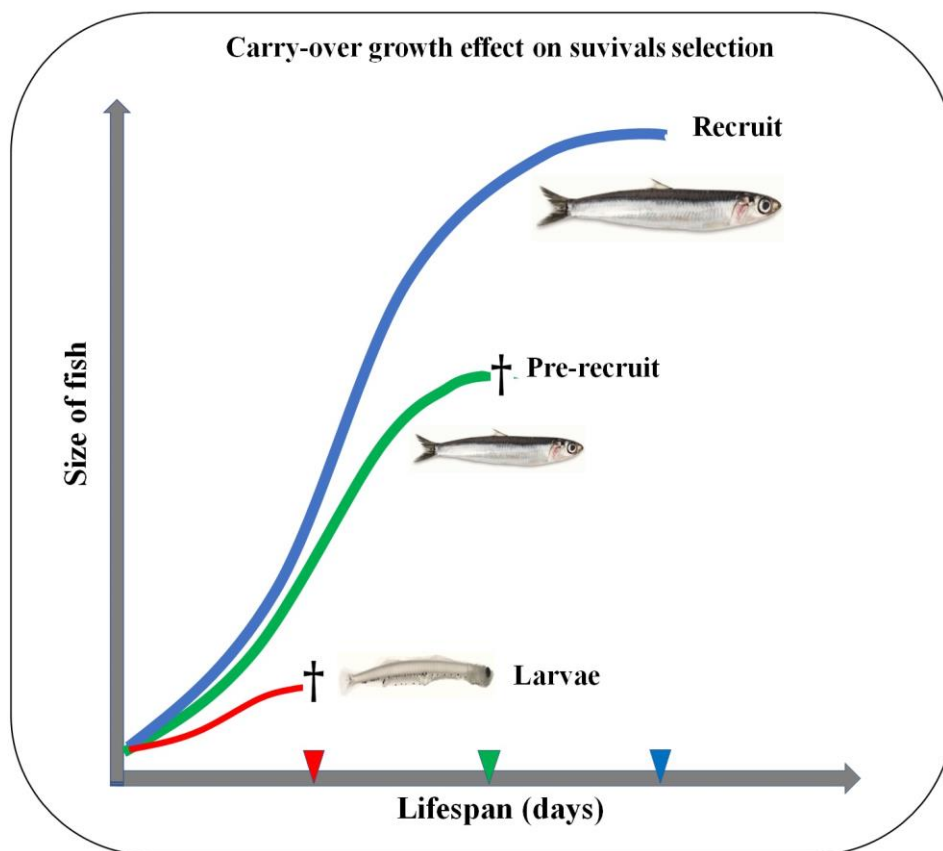
Figure 4.5. Conceptual model to illustrate a "carry-over effect" as fish grow, where always the last survivors were larger when they were larvae and when they were early-juveniles (pre-recruits).

Table 4.5. Summary of otolith-based studies in which the traditional method was used to test the bigger-is-better (BIB) and growth-survivors mechanics (GR), when juveniles were used as survival and larvae as original population. SA corresponds to the age of the oldest survivors in the analyses; Na: not-available data; ne: not evaluated.

| Author	Family	Species	Climate	BIB	GR	Stage	Survivors	SA
Takahashi et al., (2012)	Carangidae	Trachurus japonicus	Temp	+	+	Larvae	YOY	134
Meekan et al. (2006)	Clupeidae	Spratelloides gracilis	Trop	+	+	YOY	YOY	78
Slotte et al. (2019)		Clupea harengus	Temp	ne	-	Larvae	YOY	NA*
Plaza and Ishida (2008)	Engraulidae	Sardinops melanostictus	Subtrop	+	+	Larvae	YOY	80
Takahashi and Watanabe (2004)		Engraulis japonicus	Temp	+	+	Larvae	YOY	80
Nielsen and Munk (2004)	Gadidae	Gadus morhua	Temp	+	+	Larvae	YOY	106
Raventos and Macpherson (2005)	Labridae	Symphodus roissali	Subtr	+	+	Larvae	YOY	70
Searcy and Sponaugle (2001)		Thalassoma bifasciatum	Trop	+	+	Larvae	YOY	65
Shahidul Islam et al. (2010)	Lateolabracidae	Lateolabrax japonicus	Subtr	+	+	Larvae	YOY	60
D'Alessandro et al. (2013)	Lutjanidae	Ocyurus chrysurus	Trop	+	+	Larvae	YOY	>50 days
		Lutjanus synagris	Trop	+	+	Larvae	YOY	NA*
		Lutjanus griseus	Trop	+	+	Larvae	YOY	NA*
	Sphyraenidae	Sphyraena barracuda	Trop	-	-	Larvae	YOY	NA*
Grote et al. (2012)	Merlucciidae	Merluccius paradoxus	Temp	+	ne	Larvae	YOY	152
Sirois and Dodson, 2000	Osmeridae	Osmerus mordax	Temp	+	+	larvae	Early juv	102
Oshima et al. (2010)	Paralichthyidae	Paralichthys olivaceus	Temp	ne	+	Larvae	RSJ	40
May et al. (2020)	Percidae	Sander vitreus	Freshwat	ne	- +	Larvae	YOY	NA*
Vigliola & Meekan (2002)	Pomacentridae	Neopomacentrus filamentosus	Trop	+	ne	Larvae	Post setlers	90
Cotano and Alvarez, 2003	Scombridae	Scomber scombrus	Temp	ne	+	Larvae	YOY	200
Robert et al., 2007		Scomber scombrus	Temp	+	ne	Larvae	YOY	NA*
Murphy et al. (2014)	Sparidae	Pagrus auratus	Temp	+	ne	Larvae	YOY	<5 month

* Maximum age of survivors could not be identified from the results of the cited study

4.4. Discussion

In the present study, mixed effects models showed that the anchovy survivors were always larger and grew faster when they were larvae in the first three weeks of life in the cohorts tested. These results match well with previous field studies, carried out using a comparable methodology with otolith analysis (i.e., the traditional method; Table 4.5), where a positive relationship between growth and survival has been reported when juveniles were used as survivors, in small pelagic fishes and in others teleost as well (Table 4.5) To date some previous works have used older juveniles, presumably closer to what would be the population of definitive survivors, and when this was the case the B-I-B and GR mechanisms were operating in most cases (Table 4.5). From these findings and the results of the current study in anchoveta, it is reasonable to question to what extent this pattern could be a generalization in teleost fishes? Could these patterns could emerge more clearly when similar methodologies are compared in field studies and when older survivors are used in the analyses? Or when the individuals of the original and surviving population come strictly from the same birth cohort? To address these questions, more studies of similar nature are needed for other commercial and no commercial species in different ecosystems.

In the present study, the evidence in favor of the B-I-B and GR mechanisms were consistent for seasonal hatched cohorts over the 4-year study period, despite the fact that early growth patterns of anchovy varied, given the significance of the random effect of year in the LMMs. To date, a few previous studies have evaluated these mechanisms on an interannual scale with old juveniles as survivors (e.g., Cotano and Alvarez, 2003; Robert et al. 2007; Takahashi et al., 2012). Robert et al. (2007), reported significant selection for fast growth when 1 year old

juveniles were compared with late-stage larvae for a 1997 cohort in *Scomber scombrus* of southern Gulf of St. Lawrence, although growth did not differ between survival and members of the original population in other two annual cohorts. Certainly, the information is still too partial to assess these differences between these species and their ecosystems, although some inference can be made for *E. ringens*. The study area for this species is part of the HCS, one of the most productive marine ecosystems in the world (Abrahams et al., 2021; Thiel *et al.*, 2007; Massing et al., 2022). In the particular case of northern Chile, high biological productivity is triggered by upwelling of moderate intensity, but constant throughout the year (Thiel et al., 2007). It could be inferred that the interannual consistency in the survival patterns found for *E. ringens*, is associated to the stable patterns of productivity in the HCS, where food would be granted (e.g., Thiel et al., 2007; Cerna et al., 2022). To expand this kind of studies to other engraulids or clupeids in the HCS and in other Eastern boundary upwelling systems seems a worthy challenge to test this inference.

A distinctive finding for *E. ringens* in the present study was that the older juveniles were larger and grew faster than the pre-recruits (young juveniles), even up to about the second month of life. This was evident when comparing both radii and increment width-at age for the spring cohort of 2020 and 2021, where recruits that survived up to the first 150 days were larger and grew faster for the first 50 days than the pre-recruits, which is strong evidence that such an advantage persists into later life stages driving fish selection, likely related to predation (Leopold et al., 1998; Scharf et al., 2002; López et al., 2012; Takahashi et al., 2022). Therefore, it seems reasonable to infer that this selective advantage could be more critical for small pelagic fishes, because they are forage species that sustain trophic chains in many marine ecosystems. For these species, being bigger and growing faster seems to be a permanent challenge, both to escape from predators and to deal with highly changing environmental conditions. For example, in the case of anchoveta, it is well known that both juveniles and adults are important food sources for seabirds, marine mammals, and other larger fish throughout the HCS (e.g., Jordan 1967; George-Nascimento et al., 1985; Simeone et al., 2003; Hückstädt et al., 2007). Therefore, it would be interesting that further studies can address this hypothesis, by reconstructing growth history from otoliths collected from stomach of predators.

Finally, it is important to underline that the definitive survivors are those that have successfully transited through the larval phase, the early and late juvenile phase, and have subsequently recruited into the adult population. In this context, the present study showed that indeed the oldest survivors analyzed were always the larger and faster growing individuals when they were in the previous stages, in a similar way to the model illustrated in Figure 4.5, which was demonstrated for the 2019 cohort for anchoveta in the current study. Here is important to mention the study of Islam et al. (2010), who also reported for *Lateolabrax japonicus*, that selection for fast growth occurred at every developmental stage, with juveniles being bigger and growing faster than postflexion larvae and so on for flexion and preflexion larvae. All these findings empirically support recent studies using a complementary methodology, based on autocorrelation analysis of daily increment width of otoliths (i.e., Robert et al., 2014; Burns et al., 2021; Pepin et al., 2015; Primo et al., 2021). These studies have shown that the growth gains obtained during the initial phases would be projected into older larval stages. More recently Tanaka et al. (2023) reported similar findings, where serial autocorrelation increased with age for the early post-larval period for three clupeoid species, *Sardinops melanostictus*, *Engraulis japonicus*, and *Etrumeus micropus*. A future attempt to compare both methodologies (auto-correlation vs traditional method) in a same species and in closely-related ones, it would

be an interesting challenge to expand to what extent to be bigger and grow faster could be indeed a definitive and demonstrated advantage for forage species and other teleost fishes.

ACKNOWLEDGEMENTS

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Chapter 5: General discussion

The main aim of this thesis was to provide insights about the anchoveta population dynamics, one of the more important pelagic species off the coast of Chile. The focus was from an ecological point of view, because anchoveta is a key component of the trophic chain, and because it is one of the exploited species with annual catches of over a million tons in Chile. The stock structure, life history traits, and the recruitment process were analyzed and have contributed to the knowledge of the characteristics of the species in Chilean waters. The main findings were:

Population structure

The thesis was mainly aimed to understand the early juvenile growth of anchoveta (*E. ringens*) in the HCS off Chile and testing, from an ecological point of view, if this growth was related to survival in one of the most productive systems in the world. The first step for the study was to establish correctly the structure of the population or demographic units of anchoveta off the coast of Chile. For a long time, the anchoveta fishery in Chile was managed using a purely administrative criterion, without knowing the population structure of the species. In this context, the present study has made a substantive contribution, identifying the existence of at least two completely independent demographic units (I and III) through of otolith shape analysis, where both basic shape indices and EFDs showed consistent results. However, the lower discriminating power between Zones I and II suggests some level of mixing between these zones, which should be elucidated in further studies in order to specify the exact limits between populations to improve the management of this resource.

The marked differences found in otolith morphology between the two extreme localities in the present study, were strong evidence of an environmental spatial heterogeneity of anchoveta along the Chilean coast. This evidence matches well with previous studies, which have proposed that environmental variability can produce important differences in otolith shape (Lombarte and Lleonart, 1993; Cardinale et al., 2004; Vignon, 2012). Indeed, juveniles and adult fish inhabiting the extreme northward and southward zones analyzed (Zone I and III) must cope with important environmental differences in biological productivity (e.g. chlorophyll-a), upwelling intensity, temperature and both physical and topographic variability. An important feature here is the Central Transition Zone (CTZ) defined between 34 and 39°S. In this area of the Humboldt Current System, specifically the region off central-southern Chile (Zone III) has relatively high eddy kinetic energy, generating an extensive coastal transition zone (600 km offshore) in which coastally derived eddies are recurrent features. This energy might promote strong exchanges of water, biogeochemical properties, and plankton between the coastal upwelling band and the adjacent oceanic zone. Strong mesoscale variability in the region of oceanic and coastal water interactions is associated with higher eddy kinetic energy, which forms a coastal transition zone (CTZ), (Morales et al., 2010). This zone is characterized as an area of high productivity but also of high disturbance due to its particular physical conditions.

On the other hand, although the northern zones (Zones I and II) do not have a strict topographic boundary between them, there is a critical latitude (~22.8°S), described around Mejillones and Moreno bays. This latitude is characterized by a high concentrations of chlorophyll *a* throughout the year and stronger water retention dynamics related to local topography(bay) and particular Ekman transport characteristics of adjacent waters (Letelier et al., 2012). Hence, it is

reasonable to hypothesize that this critical latitudinal zone could enhance retention of early stages of anchoveta and limit the flow of early juveniles between Zones I and II. These stock boundaries, derived from the analysis of the shape of the otoliths, are consistent with a study by Garces et al. (2019); these authors analyzed otolith chemical markers (trace elements and stable isotopes) and otolith microstructure for the first 40 days of life in juveniles and adults, showing that there are three separate demographic units in the Chilean coast, Zone I (18–25°S), Zone II (25–32°S) and Zone III (32–42°S). However, these authors identified mixing rates in which the zone II received contributions of 31% from zone I and 3% from zone III, while complete segregation existed between zone I and III. The same authors founding differences in core region markers indicated spatial segregation between three environmentally distinct nursery areas, probably related to Subtropical Water (Zone I), Subantarctic Water (Zone III) and the Subtropical Convergence (Zone II). Comparison of core and marginal regions, from juvenile and/or adult fish, suggested that adult fish remained close to their nursery areas.

The result of demographic units, obtained from the morphometry of the otolith of the present study and by Garces et al. (2019) using trace elements and stable isotopes of the otolith, are consistent with the differences in the growth pattern of juveniles found in our study (Chapter 3), where the results did not show significant difference in mean IW of otolith's daily rings between northern and north-central areas (Zone I and II). Although, they significantly varied with respect the southern area (zone III), where the lower values of IW were found.

Other finding from our analysis of otolith's shape indices (form factor, circularity, rectangularity, roundness, ellipticity), was the was how shape indices decreased with fish length, leading to a relative stability in morphological differences among sampling zones after fish reached sexual maturity (~14 cm TL). Similarly, the fish length effect has been reported for the clupeoid *Strangomera bentincki* by Curin-Osorio et al. (2012), as well as for other species (e.g. Tuset et al., 2003). Consequently, evaluation of otolith shape stability is important to determine when the otolith outline is not affected by fish ontogeny, but rather by genetic and environmental variability. Furthermore, it would be very interesting to evaluate whether the pattern of otolith shape stability found in *E. ringens* in the present study is also applicable to other engraulid species. For example, Zengin *et al*. (2015) and Jemaa et al. (2015) identified population units of *Engraulis encrasicolus* in the Black and Mediterranean seas respectively, although in both these studies only immature fish (11–12 cm TL) were used, when the shape or otolith outline has not been completely developed. In this sense, it is reasonable to infer that results derived from otolith shape analysis of adult anchoveta in the present study are reliable.

The similar classification accuracies found in 2013 and 2016, from Fourier analysis, provide the first evidence that otolith morphological differences among zones were not sensitive to the El Niño event, which dominated the oceanographic conditions during 2014–16 in the Southern Hemisphere (Vera and Osman, 2018). This finding suggests the absence of a generalized movement and mixing of schools in a southward direction, which could be expected as a result of movement of warm water masses from north to south. In fact, latitudinal stability in fish distribution under El Niño events has been observed in recruitment acoustic cruises (Castillo et al., 1998; Leiva et al., 2016), where a change in bathymetric distribution seems to be the dominant response to warm water conditions due to the El Niño event, where anchoveta move to deeper waters and are less vulnerable to purse seine fishing gear.

Growth pattern

Somatic growth is one of the key life history parameters in aquatic ectothermic organisms because is a concomitant variable (i.e., an output product) that can reflect the condition of a given population. In case of small pelagic fishes, the somatic growth is highly variable, particularly during their early life stages, likely linked to the variability of the main growth controllers, i.e., food availability and water temperature (Shepherd and Cushing, 1980; Kendall and Miller, 2009).

In the present Thesis, the Laird-Gompertz growth model was fitted to the relationship among anchoveta length, daily age, and increment width (IW), determined from otolith microstructure analysis of YOY. Also, a generalized additive model (GAM) was developed including as explanatory variables the zone of origin, the cohort, the sea surface temperature (SST), and concentration of chlorophyll-a, for three years (2015, 2016 and 2019).

The anchovy juveniles showed significant latitudinal differences in the otolith growth off Chilean coast. Such a consistent pattern was evidenced by the growth parameters derived from age-length relationships fitted to Laird-Gompertz models and when daily increment widths were analyzed using a GAM model. The growth parameters varied significantly between the three zones for all the cohorts (years). However, the chronological analysis of the IW only showed differences between the most septentrional zones (I and II) compared to the most meridional zone (III). These differences can be explained because the chronological analysis removes the effect of ontogeny and is independent of seasonality; unlike the approach of length-at-age models that require similar birth cohorts to be compared to reach reliable conclusions. This requirement was overlooked in part in the present study, because most juveniles come from winter and spring hatching seasons. The three years studied showed that temperature is an important environmental driver for the early growth of juveniles of this species, where higher temperatures were associated with high daily growth. Previous studies have reported that temperature was the main driver of somatic growth in larvae and juveniles of some other teleost fishes (Schismenou et al., 2014; 2016; Zenitani et al., 2009; Wenger et al., 2016; Gallagher *et al*., 2005). The positive relationship between growth and temperature has also been found for *Engraulis mordax* larvae from southern California (Methot and Kramer, 1979). Similarly, Namiki et al. (2010), in an experimental analysis, found that a low water temperature (<13–14∘C) limited the growth of the *Engraulis japonicus* otolith.

In the present study, the difference in SST between zones showed a north-south gradient, which could be enough to trigger significant changes in otolith growth and consequently in its somatic growth of this species. On the other hand, we found an inverse effect of chlorophyll-*a* on the chronology of anchovy growth, where the high productivity was not associated with higher growth in the HCS. i.e., Zone I and II have less Chlorophyll-a productivity and high growth, conversely, the zone III has high productivity and slower growth than the other zones. These findings appear somewhat contradictory, because an increase in Chlorophile-a should be associated with an increase in food availability; as has been shown in several studies in marine ecosystems and in the HCS as well (e. g., Thiel et al., 2007; Huot et al., 2007; Wernand et al., 2013). It is reasonable to infer that increased production of food does not imply that it can be available for consumption. Growth and survival depend on the match of more than one factor in the environment, not only productivity (enrichment), but also that food, supply and type, are available to larvae and pre-recruits at the right time (i.e., Lasker events, Lasker, 1981). For this,

water stability is decisive because low turbulence increases the concentration of food particles (Bakun and Parrish, 1980; Cury and Roy, 1989). This match occurs in Zone I and II, where moderate and permanent upwelling occurs during winter and spring, with low turbulence and Ekman transport, for a prolonged period (Thiel et al., 2007). This not only facilitates the retention of larvae, phytoplankton and zooplankton, but also provides a permanent stability, which increases the probability of encounters between larvae/juveniles and their prey. So, although Zones I and II on average has less primary production than zone III, the physical conditions of their habitat facilitate the feeding of anchoveta, and this may explain the higher growth recorded. Conversely, the physical conditions in zone III, where there is strong upwelling, lead to high primary production, followed by calm events that favor retention and feeding, which is continued in a new upwelling event in an intermittent cycle (e.g., Arcos et al., 1996). Such an oceanographic feature probably generates a discontinuity in the availability of food for the early stages of anchovy triggering a lower somatic growth.

There are different evolutionary explanations for the different growth trajectories of the same species, but of populations located at different latitudes or different thermal regimes, i.e.: 1) Genotype differ not in the maxima growth rate that can be achieved, but only in the temperature at which maximal and lesser growth is possible; 2) Growth rate may be maximized in each local environment, but no genotype is generally superior in all environments, or 3) it is possible that both of the above two models contribute to the overall pattern of the latitudinal compensation, Since temperature and length of growing season covary across latitudinal gradients, organisms may adopt a mixed strategy by adapting to differences in both temperature and seasonality (Conover and Present 1990; Yamahira and Conover 2002).

The latitudinal variations in anchovy growth from the HCS off Chile could be explained evolutionarily by the third alternative, that is: higher temperatures that are associated in the southern hemisphere with lower latitudes, modulated phenotypic adaptations of anchovy with higher growth. higher, than those anchovies that inhabit environments with lower temperature ranges. Furthermore, anchovies from the coast of Chile adapted to higher temperatures (Northern Zone) reach the maximum growth rate at a younger age than those from lower temperature environments (Southern Zone).

Growth-survival testing

To understand what triggers such a large difference in recruitment, it is essential to understand the role that environmental drivers play in the different stages of the life cycle of this species. The HCS is one of the most productive systems in the world, although it is not homogeneous in terms of abundance of pelagic fishes such as anchoveta. It is reasonable to infer that these differences could be associated to variable survivorship during the early stages where the higher mortalities are expected to occur (Anderson, 1988). The Chapter 2 showed that the growth of the survivors (YOY) was mainly influenced by temperature, and the highest growth was found for juveniles from the northern zone (Zone I), that coincidentally has the higher fisheries landings of Chile.

According to the previous argument the growth-mortality hypothesis was tested in spring cohorts of anchoveta (*Engraulis ringens*) in four different years (2014, 2019, 2020 and 2021) in Northern Chile (18-24°S) in the Humboldt Current system (HCS) using mixed effects models

(Chapter 4). The result showed that the anchovy survivors were always larger and grew faster when they were larvae in the first three weeks of life in the cohorts tested. These results match well with previous field studies, carried out using a comparable methodology with otolith analysis for small pelagic fishes and in other teleost as well, where a positive relationship between growth and survival has been reported (Table 4.5).

In the present study, the evidence in favor of the B-I-B and GR mechanisms were consistent for seasonal hatched cohorts over the 4-year study period, despite the fact that early growth patterns of anchoveta varied, given the significance of the random effect of year in the LMMs. To date, a few previous studies have evaluated these mechanisms on an interannual scale with old juveniles as survivors (e.g., Cotano and Alvarez, 2003; Robert et al. 2007; Takahashi et al., 2012). Robert et al. (2007), reported significant selection for fast growth when 1 year old juveniles were compared with late-stage larvae for a 1997 cohort in *Scomber scombrus* from southern Gulf of St. Lawrence; although growth did not differ in other two annual cohorts. However, our results should be taken with caution since the plankton sampling corresponds to a particular month in the spring season (October), so, although they coincide with the survivors in the birth season, they may not correspond in all cases to the same month of birth, or to the same week within the month. In an environment with highly variable conditions, such as the pelagic system off the coast of Chile, it is likely that growth can vary between months and even weeks within the same season of the year. This situation could suggest that the differences found are the result of comparing cohorts with a very wide time interval, that is, a grouping of cohorts from different months or weeks with their own characteristics such as growth. Even so, our results show very significant differences in growth between the larval stage and pre-recruits and recruits ($p<2e-16$), thereby highlighting the power of our results.

 A distinctive finding for *E. ringens* in the present study was that the older juveniles were larger and grew faster than the pre-recruits (young juveniles), up to about the second month of life. This was evident when comparing both radii and increment width-at age for the spring cohort of 2020 and 2021, where recruits that survived up to the first 150 days were larger and grew faster for the first 50 days than the pre-recruits. This was strong evidence that such an advantage persists into later life stages driving fish selection, likely related to predation (Leopold et al., 1998; Scharf et al., 2002; López et al., 2012; Takahashi et al., 2022). Therefore, it seems reasonable to infer that this selective advantage could be more critical for small pelagic fishes, because they are forage species that sustain trophic chains in many marine ecosystems.

It is important to underline that the definitive survivors are those that have successfully transited through the larval phase, the early and late juvenile phase, and have subsequently recruited into the adult population. In this context, the present study showed that indeed the oldest survivors analyzed were always the larger and faster growing individuals when they were in the previous stages.

As corollary, this thesis made a contribution to the knowledge of the population structure, the growth of juveniles and its importance for the survival of anchovy in HCS off Chile. However, several questions remain unaddressed. To what extent are these findings the rule for anchovies in these ecosystems? What about the influence of the spatial heterogeneity for a species with an extended latitudinal distribution? Or could such a fast growth only be linked to high cycles of productivity of these anchovy species? If there is a relationship between growth and survival, could this indicator be able to predict the strength of the year classes? Answering these

questions is not yet possible for anchovies, because early growth data are not available on a sequential monitoring basis. Hence, it is very important to continue accumulating information on early growth at different spatial and temporal scales in anchovies, because early growth could be used as health index in these important fisheries resources.

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Chapter 6: Conclusions

1. The fish length effects upon shape indices of anchoveta otoliths decreased with size, leading to a relative stability in morphological differences among geographical zones after fish reached their sexual maturity (~14 cm TL).

2. The otolith basic shape indices and EFDs, identified at least two completely independent demographic units (I and III) of anchoveta (*Engraulis ringens*) off the Chilean coast, lower discriminating power between zone I and II, suggests some level of mixing between these zones.

3. The growth parameters of anchoveta juvenile, derived from age-length relationships fitted to Laird-Gompertz models, showed significant latitudinal differences in the somatic growth off Chilean coast.

4. The chronological analysis of daily increment widths showed differences between the most septentrional zones (I and II) compared to the most meridional zone (III).

5. The three years studied showed that temperature is an important environmental driver for the early growth of juveniles of this species, where higher temperatures were associated with high daily growth. While, there is an inverse effect of chlorophyll-a on the chronology of anchovy growth, where high levels of chlorophyll-a are associated with poor growth.

6. The growth-mortality hypothesis tested in spring cohorts of anchoveta (*Engraulis ringens*) show evidence that survivors were bigger and grew faster than members of the original population.

7. The older juveniles were larger and grew faster than the pre-recruits (young juveniles), even up to about the second month of life. i.e., the oldest survivors analyzed were always the larger and faster growing individuals when they were in the previous stages, what we call "Carry-over effect on survivals selection".

8. In summary, this thesis has contributed to defining the anchovy population units of the HCS off Chile, improving knowledge of important traits of life history and ecological aspects, which will allow a best compression of the dynamics of anchovy populations in perspective of improving the management of the fishery and conservation of this important species of the pelagic ecosystem of Chile.