

Long-term trends in length and age at sexual maturity of hoki *Macruronus magellanicus* and southern hake *Merluccius australis* from Chilean Patagonia

Tendencias a largo plazo en la longitud y edad de madurez sexual de la merluza de cola *Macruronus magellanicus* y merluza del sur *Merluccius australis* de la Patagonia chilena

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Resumen.- Se analizó la tendencia a largo plazo de la longitud y edad media de madurez sexual (MLSM y MASM) de hembras de merluza de cola *Macruronus magellanicus* (2001 a 2019) y de merluza del sur *Merluccius australis* (1989 a 2019), en stocks sobreexplotados del Mar Exterior (OS) y Mar Interior (IS) de la Patagonia chilena. El estudio de las muestras se realizó mediante el análisis microscópico de las gónadas y parte de ellas se examinó histológicamente, junto con la lectura de otolitos. La MLSM y MASM se calcularon con un modelo lineal generalizado. La MLSM de la merluza de cola en el OS experimentó grandes fluctuaciones interanuales, con una diferencia de 16,2 cm entre los valores extremos, desde 55,3 cm longitud total (TL) en 2001 hasta 44,1 cm en 2019. El rango de la MASM fue entre 2,9 y 4,4 años. En el único año muestreado en el IS, se determinó maduración precoz en merluza de cola entre 32 y 35 cm TL. La MLSM en la merluza del sur mostró estabilidad dentro de un rango 5,1 cm, desde 73,5 cm TL en 1989 hasta 73,9 cm en 2019. La MASM presentó un rango desde 10,4 a 11,6 años. En los tres años muestreados en el IS, la MLSM fue menor en 4,5 cm TL al comparar con el OS. La información disponible sobre la historia de vida de estas especies sugiere que las favorables condiciones ambientales en el IS les permitirían alcanzar más tempranamente la MLSM que en el OS.

Palabras clave: Longitud y edad media de maduración sexual, estadios de madurez sexual, maduración precoz, Chile, Pacífico suroriental

Abstract.- Long-term trends of length and mean age at sexual maturity (MLSM and MASM) of female Patagonian hoki *Macruronus magellanicus* (2001 to 2019) and southern hake *Merluccius australis* (1989 to 2019) were analyzed in overexploited stocks from the Outer Sea (OS) and Inner Sea (IS) of Chilean Patagonia. The study of the samples was performed by microscopic analysis of the gonads and part of them were analyzed histologically, together with fish otoliths readings. MLSM and MASM were calculated using a generalized linear model. MLSM of hoki in the OS experienced large annual fluctuations, with a difference of 16.2 cm between extreme values. MLSM was 55.3 cm in 2001 and 44.1 cm in 2019. MASM ranged between 2.9 and 4.4 years. In the IS (only one year sampled) precocious maturation was found in females between 32 cm and 35 cm total length (TL). MLSM of southern hake was rather stable within a range of 5.5 cm, from 73.5 cm TL in 1989 to 73.9 cm in 2019. MASM ranged between 10.4 and 11.6 years. MLSM in the IS was 4.5 cm TL lower than in the OS in the three years of sampling. Life history information of these species suggests that favorable environmental conditions in the IS allow them to reach MLSM earlier than in the OS.

Key words: Mean length and age at sexual maturity, sexual maturity stages, precocious maturation, Chile, Southeastern Pacific

INTRODUCTION

Hoki *Macruronus magellanicus* Lönnberg, 1907 and southern hake *Merluccius australis* (Hutton, 1872) have a wide geographical distribution along the Pacific and Atlantic coasts, Patagonian shelf, and around the Falkland/Malvinas Islands (Niklitschek *et al.* 2014). Along the coast of Chile hoki is found from the tip of Patagonia to 30°S (Chong *et al.* 2007), and southern hake is found up to 38°22'S (Rubilar *et al.* 2002). Both species also inhabit the Patagonian channels, fjords

and Inner Sea (IS). This region is one of the largest estuarine areas of the world, extending from 41°20' to 55°58'S (Palma & Silva 2004), where different water masses enter and mix with freshwater from various sources, resulting in a unique environment which differs drastically with the Outer Sea (OS), according to Palma & Silva (2004). Both regions are important spawning grounds for many fish species, including hoki and southern hake (Balbontín & Bernal 1997, Landaeta & Castro 2006, Osorio-Zúñiga *et al.* 2018).



Hoki and southern hake have been heavily fished in both OS and IS. When they began to be exploited commercially in Chilean Patagonian waters in 1978, hoki was the most abundant fish resource in the southern region (Tascheri *et al.* 2010). This fishery concentrates primarily on the adults of the stock. As a result of the increased fishing effort, hoki became overfished in the OS by 1986 and in the IS by 1991 (Aguayo 1996). Subsecretaría de Pesca y Acuicultura (SUBPESCA) declared hoki stock as depleted and southern hake as overexploited (SUBPESCA 2022). Both species are being managed by establishing minimum catch lengths, bans during the spawning season, and catch quotas (SUBPESCA 2022).

The main spawning season of hoki in the OS according to sexual maturity stages and gonadal indices, starts in July, with a peak in August (Lillo *et al.* 2013), followed by a rapid decrease in September (Young *et al.* 1998, Chong 2000). The spawning season of southern hake in the OS is in winter (July to mid-September), with a peak also occurring in August (Balbontín & Bravo 1993, Aguayo *et al.* 2001, Lillo *et al.* 2013). However, eggs and larvae in ichthyoplankton samples indicate that in the IS southern hake spawns in spring (Flores *et al.* 2019).

Studies have shown that the length and age at which fish reach sexual maturity have important implications for their life history. For instance, size at sexual maturity is one of the variables for quantifying reproductive capacity of fish (Murua *et al.* 2003). Moreover, the length and age at which fish reach sexual maturity determine to a large extent the population's response to environmental stressors (Domínguez-Petit *et al.* 2017). As a consequence of commercial exploitation, various fish stocks have exhibited a decrease in the length and age at which 50% of individuals achieve sexual maturity (Barot *et al.* 2004). However, this trend may also be the expression of phenotypic changes resulting from long-term variations in temperature, population density or some other changes in the environment (Barot *et al.* 2004). The objective of this study was to examine long-term trends in the mean length and age at sexual maturity (MLSM-MASM) for hoki and southern hake inhabiting the Chilean Patagonia and to relate those changes to life history and environmental information, that could explain differences in the size at which they reach sexual maturity in IS and OS.

MATERIALS AND METHODS

SAMPLING

Most samples were obtained from hydroacoustic stock assessment cruises for hoki and southern hake in the southern Chilean region (Fig. 1). The average duration of the cruises was 17 days. Fishing hauls were performed using “Gloria 1408” or “Barracuda 608” trawls with 50 mm mesh size in the cover cod end. The cruises covered from 43°30' to 47°00'S in latitude, following 100 and 600 m isobaths offshore. Fishing hauls (*ca.* 28) in 38–48 transects were performed annually (not depicted in the map). Main sampling period was August and occasionally extending from July to September each year (Table 1). The historical data set on reproductive condition ranged from 2001 to 2019 in hoki and from 1989 to 2019 in southern hake, although samples were not available for some years. Additional sampling of southern hake was conducted offshore aboard commercial fishing ships from 45° to 46°S, and in some nearshore areas and inland waters (Anna Pink Bay, Darwin and El Morro Channels) in 1989 and 1991. In 2003 samples of both hoki and southern hake were collected in the IS, covering from Reloncaví Sound to Gulf of Aysén and Boca del Guafo (Fig. 1).

Gonads for reproductive studies and sagittal otoliths for age determination was based on length-stratified sampling on board. Total length (TL) in cm and body weight (BW) in g were recorded. Gonads were extracted, preserved in 10% formalin, and subsequently weighed in the laboratory. For a better fixation, formalin was buffered with Sorensen phosphate (Hunter 1985) from 2011 onward. Age for each female was included in the data base since 2002 for hoki and since 2013 for southern hake (Table 1). Age determination of females is lacking in the IS region since samples were not available.

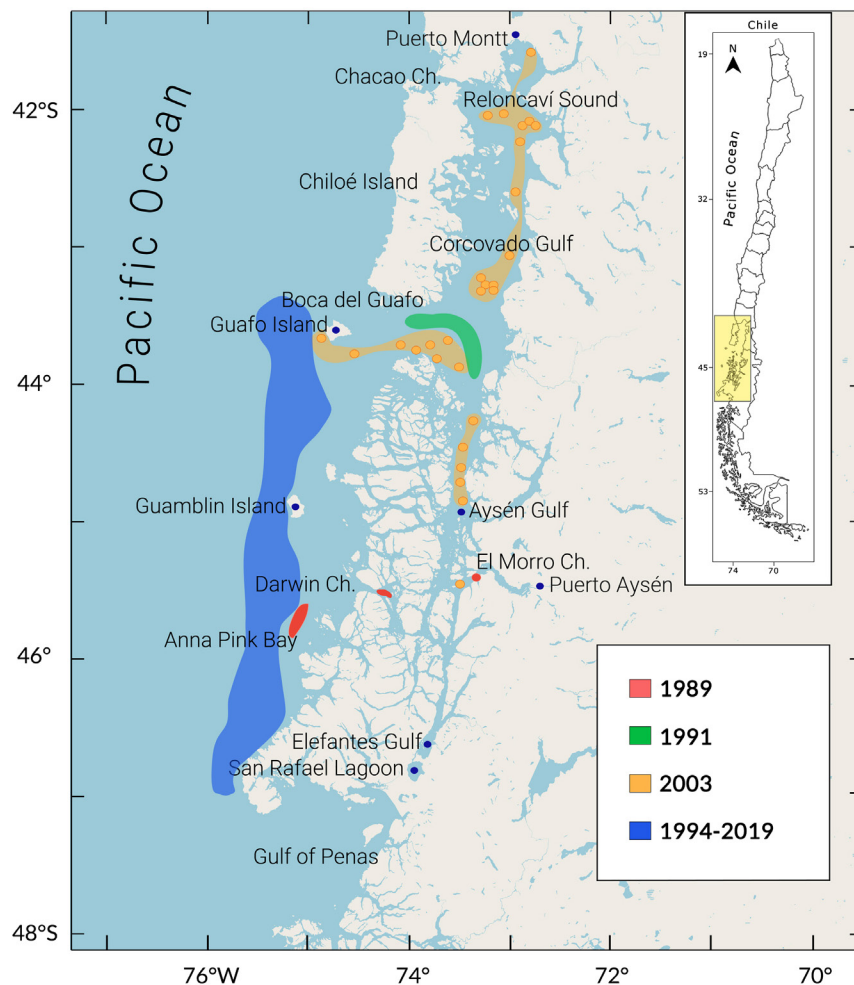


Figure 1. Localities in the Inner and Outer Seas of the Chilean Patagonia, with indication of sampling areas from 1989 to 2019. Fishing hauls were performed using Gloria 1408 or Barracuda 608 trawls, following 100 and 600 m isobaths offshore / Localidades en el Mar Interior y Exterior de la Patagonia chilena, con indicación de las áreas de muestreo desde 1989 a 2019. Los lances de pesca se realizaron con redes Gloria 1408 o Barracuda 608, siguiendo las isóbatas de 100 y 600 m mar afuera

LABORATORY ANALYSIS

The analysis of samples was conducted by the same team of researchers, ensuring the application of the same methodology. The sexual maturity stage (SMS) was determined through microscopic examination of gonad pieces, that were teased apart and the diameter of the most developed oocytes were measured (Balbontín & Bravo 1993). This method has been used to validate the degree of maturation of ovaries (West 1990, Kjesbu 1991). In addition, presence of hydrated oocytes with an oil globule and existence of some degree of oocyte reabsorption was verified. Macroscopic features of the ovaries were also recorded. Part of the terminology used to characterize SMS (ICES 2007, Brown-Peterson *et al.* 2011) was taken into consideration to modify the scale designed for southern hake (Balbontín & Bravo 1993) and apply it to hoki as well (Table 2). The scale includes six SMS, but only five

for hoki since in this species there is no stage corresponding to partial spawning (Chong 2000, Lillo *et al.* 2012). Females with gonads that conformed to the description of the *Immature* stage, particularly due to the presence of oocytes with a diameter less than 0.25 mm, were classified as immature. The rest of the stages represented mature females.

Gonads which presented some difficulty in defining the microscopic stage of maturity were analyzed histologically. The technique consisted in the post fixation of subsamples of ovarian tissue in 10% buffered formalin, followed by staining with hematoxylin and eosin. Histological stages of sexual maturity (Table 3) were identified using the criteria described for teleost fish (Wallace & Selman 1981, Brown-Peterson *et al.* 2011). The degenerative degree of postovulatory follicles (POF) was based on Alday *et al.* (2008).

Table 1. General sample data, length range (cm), number of ovaries and otoliths of *Macruronus magellanicus* and *Merluccius australis* analyzed each year / Datos generales de las muestras, rango de longitudes (cm), número de ovarios y otolitos de *Macruronus magellanicus* y *Merluccius australis* analizados cada año

Sampling period	Geographic area	Length range	<i>M. magellanicus</i>		Length range	<i>M. australis</i>		Reference
			Micro / Macro ovary samples	Otolith readings		Micro / Macro ovary samples	Otolith readings	
June - August 1989	Inner Sea	-	-	-	60-102	84 / -	-	Balbontín & Andrade (1990)
June - Sept 1989	Outer Sea	-	-	-	63-99	70 / -	-	Balbontín & Andrade (1990)
August 1991	Inner Sea	-	-	-	47-100	259 / -	-	Balbontín & Bravo (1993)
August - Sept 1994	Outer Sea	-	-	-	41-100	195 / 195	-	Lillo <i>et al.</i> (1995)
August - Sept 1996	Outer Sea	-	-	-	42-103	280 / 280	-	Lillo <i>et al.</i> (1997)
September 1999	Outer Sea	-	-	-	48-106	200 / 186	-	Aguayo <i>et al.</i> (2001)
August 2000	Outer Sea	-	-	-	43-110	1002 / 1042	-	Aguayo <i>et al.</i> (2001)
August 2001	Outer Sea	33-104	900 / 898	-	40-107	773 / 992	-	Lillo <i>et al.</i> (2002)
July - August 2002	Outer Sea	36-119	643 / 945	316	37-110	516 / 910	-	Lillo <i>et al.</i> (2003)
August 2003	Outer Sea	51-101	677 / 893	662	70-102	406 / 441	-	Lillo <i>et al.</i> (2004a)
September 2003	Inner Sea	48-76	118 / 118	-	48-101	105 / 106	-	Lillo <i>et al.</i> (2004b)
August - Sept. 2004	Outer Sea	39-118	749 / 1229	492	52-102	652 / 669	-	Lillo <i>et al.</i> (2005)
August 2005	Outer Sea	37-113	507 / 887	-	37-107	698 / 882	-	Córdova <i>et al.</i> (2006)
August - Sept. 2007	Outer Sea	-	-	-	35-107	505 / 957	-	Lillo <i>et al.</i> (2008a)
August - Sept. 2007	Outer Sea	37-103	253 / 370	180	-	-	-	Lillo <i>et al.</i> (2008b)
August 2008	Outer Sea	39-110	638 / 1258	180	33-108	500 / 734	-	Lillo <i>et al.</i> (2009)
August 2009	Outer Sea	23-113	504 / 889	237	32-108	628 / 842	-	Lillo <i>et al.</i> (2010)
August 2010	Outer Sea	23-108	594 / 909	-	33-106	602 / 1007	-	Lillo <i>et al.</i> (2011)
August 2011	Outer Sea	26-100	590 / 624	315	36-109	700 / 901	-	Lillo <i>et al.</i> (2012)
August 2012	Outer Sea	23-97	808 / 1012	410	60-106	609 / 623	-	Lillo <i>et al.</i> (2013)
August 2013	Outer Sea	23-108	851 / 898	410	33-105	790 / 829	739	Lillo <i>et al.</i> (2015a, b)
August 2014	Outer Sea	25-98	939 / 1058	407	38-107	885 / 949	865	Lillo <i>et al.</i> (2015c)
August 2015	Outer Sea	25-102	745 / 790	399	29-110	750 / 753	721	Lillo <i>et al.</i> (2016)
August 2016	Outer Sea	23-94	967 / 1067	411	35-105	858 / 961	832	Lillo <i>et al.</i> (2017)
August 2017	Outer Sea	29-104	506 / 507	430	31-108	762 / 767	726	Legua <i>et al.</i> (2018)
August 2018	Outer Sea	26-118	588 / 615	431	28-105	763 / 765	381	Legua <i>et al.</i> (2019)
August 2019	Outer Sea	32-105	607 / 607	423	31-110	782 / 782	431	Legua <i>et al.</i> (2020a, b)

Micro, microscopic analysis; Macro, macroscopic analysis; (-), no sample taken

Agreement between histological and microscopic gonadal scales was estimated. Considering that the two scales are made up of a different number of stages, these were grouped into immature and mature stages. In hoki, the agreement was 97.2% (n= 74) in 2011 and 93.4% (n= 59) in 2012 (Lillo *et al.* 2012, 2013), while in southern hake was 88.2% (n= 46) in 2012 and 100% (n= 76) in 2013 (Lillo *et al.* 2013, 2015a). In the scales described herewith, the use of the term maturity stage is maintained (Dominguez-Petit *et al.* 2017). The old database of hoki and southern hake SMS had to be updated according to the new design of the maturity scale, and in most cases, it was possible to match the stages with some phase or subphase of Brown-Peterson *et al.* (2011).

The technique applied in preparing otoliths of hoki, consisted in the hydration of both otoliths, which allowed the sharpness of the annuli, and some of them were dyed with indelible ink to create contrast. In southern hake, the left otolith was hydrated so as to observe the entire otolith, while the right otolith was cross sectioned, polished, and seared to enhance the annuli (Ojeda & Muñoz 2008).

Table 2. Sexual maturity stages of hoki *Macruronus magellanicus* and southern hake *Merluccius australis* females based on gonads microscopic and macroscopic characteristics. Terminology modified from Brown-Peterson *et al.* (2011) / Estadios de madurez sexual de hembras de merluza de cola *Macruronus magellanicus* y merluza del sur *Merluccius australis* basados en características microscópicas y macroscópicas de las gónadas. Terminología modificada de Brown-Peterson *et al.* (2011)

Stage	Description
<i>Immature</i>	Small, filiform ovaries. In a more advanced stage they are light pink, with thin walls, tubular section and with a maximum diameter of oocytes less than 0.25 mm
<i>Developing</i>	Pale yellow ovaries with little vascularity. Initial diameter of oocytes equal to or greater than 0.25 mm. As the vitellogenesis process progresses, the oocytes are visible to the naked eye. At the end of the stage the ovaries reach a large volume. Maximum oocyte diameter less than 0.85 mm
<i>Spawning Capable</i>	Turgent ovaries, often with translucent areas; with abundant vascularity, yellow or pale orange; at an advanced stage, oocytes flow from the ovary when pressing on the abdomen. Oocytes in the process of hydration or already hydrated, with or without oil globule. Diameter of the most advanced size mode of oocytes equal to or greater than 0.85 mm
<i>Spawning Capable with evidence of previous spawning*</i>	Ovary similar to <i>Spawning Capable</i> stage, with the same diameter of oocytes. Pale yellow, or orange. In addition, a small number of hydrated oocytes with oil globule that correspond to the remnant of a recent partial spawning are distinguished. These ovaries pass back to the <i>Spawning Capable</i> stage
<i>Regressing</i>	Ovary flaccid, with large lumen in hoki; ovary initially firm, later becoming flaccid and with large lumen in southern hake. In some cases, blood vessels stand out. Dark reddish color. Reduced number of oocytes in the process of reabsorption, some of them with an oil globule
<i>Regenerating</i>	Ovary with thick walls and small lumen. Pale yellow to pinkish color; no oocytes visible to the naked eye. With few unidentifiable oocyte debris or oil droplet in advanced reabsorption

* Partial spawning does not occur in *M. magellanicus*; therefore, this stage does not apply to the species

Table 3. Cytological and histological criteria to define sexual maturity stages in hoki *Macruronus magellanicus* with total spawning females, and southern hake *Merluccius australis* with partial spawning females. Terminology modified from Brown-Peterson *et al.* (2011) / Criterios citológicos e histológicos para definir los estadios de madurez sexual en merluza de cola *Macruronus magellanicus*, con hembras desovantes totales y merluza del sur *Merluccius australis* con hembras desovantes parciales. Terminología modificada de Brown-Peterson *et al.* (2011)

Stage	Histological characterization of the gonadal parenchyma
<i>Immature</i>	Immature ovary that has never spawned. Parenchyma represented by oogonia and oocytes in primary growth (premeiotic primary oocytes in leptotene). Sparse basophilic cytoplasm, chromatin nucleus. Large lumen and small broad-based lamellae
<i>Early developing</i>	Ovary with early signs of reproductive activity. Predominance of oocytes with vesicles in the cortical cytoplasm of varying diameter whose content is a product of oocyte synthesis. It is possible to detect oocytes with small yolk granules. Slightly basophilic cytoplasm. Externally, a thin zona radiata and flat to cubic follicular cells are visible. The presence of cortical vesicles indicates the beginning of the process of oocyte growth and development
<i>Late developing</i>	Ovarian parenchyma in full process of vitellogenesis, from the increase of small yolk granules in the peripheral cytoplasm of the oocyte to the final deposit of yolk globules throughout the cytoplasm, giving it a strong eosinophil appearance. Spherical nucleus (GV) central, multinucleolar, surrounded by oil droplets. Zona radiata developed. Low, cubic follicular cells with clear cytoplasm and spherical central nucleus
<i>Hydrated</i>	Migrant oocytes in the initial phase of fluid entry into the oocyte cytoplasm and oocytes in final hydration. Considerable increase in diameter of the oocyte. Dilution of the yolk due to fluid accumulation, taking on the appearance of plates in the cytoplasm. The follicular layer loses thickness. Spawning is imminent
<i>Spawning</i>	Ovaries in spawning activity. In species with partial spawning such as <i>Merluccius australis</i> , vitellated oocytes coexist with residual oocytes with a migrant nucleus and hydrated. The presence of new POFs is noteworthy as another element of the ovarian parenchyma. In total spawning species such as <i>Macruronus magellanicus</i> , the ovary does not have new batches of vitellated oocytes
<i>Regressing</i>	Lamellae with a predominance of non-vitellated reserve oocytes and few residual vitellogenic oocytes. Alpha and beta follicular atresia. Eventually POFs in advanced degenerative process
<i>Resting</i>	Resting ovary that has spawned at least once. Long, thin, interdigitated lamellae invade the lumen. Non-vitellated reserve oocytes surrounded by a thin layer of follicular cells. Central GV with numerous spherical and peripheral nucleolus. Finely granular, highly basophilic cytoplasm

POF, postovulatory follicle; GV, germinal vesicle. *Hydrated* and *Spawning* stages fall under *Spawning Capable* phase designation of Brown-Peterson *et al.* (2011)

STATISTICAL ANALYSIS

The length and mean age of maturity at which 50% of females were sexually mature and the maturity ogive were estimated using a generalized linear model (GLM), according to McCullagh & Nelder (1989). It was assumed that the number of mature females (response) follows a binomial distribution and a logistic link function between the linear predictor and the proportion of mature females. Parameters were estimated by applying the maximum likelihood method (Welch & Foucher 1988), using R version 3.6.3 program (R Core Team 2020). For the GLM, the linear predictor was expressed as $\eta_i = \beta_0 + \beta_1 \times X_i$, with $(\beta_0, \beta_1)^T$ being the coefficients of the model and X the i^{th} length or age of the specimen. The logistic link function:

$$g(P(X_i)) = \log\left(\frac{P(X_i)}{1 - P(X_i)}\right) = \eta_i$$

was applied. This relates the linear predictor to the expected number of mature females as follows: $\mu_i = g^{-1}(\eta_i) \times N_i = P(X_i) \times N_i$; where μ_i corresponds to the expected number of mature females at the " i " length or age and N_i is the total number of females in the i^{th} length or age. The expected length or age at which 50% of females are mature ($L_{50\%}$ or $A_{50\%}$) is reached when:

$$g(\eta_i) = \log(P(X_i) / (1 - P(X_i)))$$

resulting in:

$$X_{50\%} = -\frac{\beta_0}{\beta_1}$$

The analytical estimate was used to calculate uncertainty and confidence intervals in $L_{50\%}$ or $A_{50\%}$ (Roa *et al.* 1999).

All yearly data was used to determine an historical average of MLSM and MASM for the study period. An estimate, with its respective confidence intervals (CI), was calculated for each species.

RELATIVE INCREMENT OF THE GONADOSOMATIC INDEX (RI_{GSI})

SMS-based estimation of MLSM in hoki yielded great yearly variability. Therefore, RI_{GSI} was used as an alternative approximation. This method does not rely on SMS. It represents the length at which females start to accumulate yolk in their gonads and it was calculated as the largest increase of GSI in successive female total length classes (Finucane & Collins 1984, Flores & Smith 2010). Since there were no problems in calculating MLSM values for each year in southern hake, RI_{GSI} was not applied to this species.

RESULTS

HOKI

Some stages were selected from the gonad maturation cycle in order to characterize hoki ovaries histologically (Fig. 2). To determine the MLSM, histological examination of the ovaries evidenced the presence or absence of cortical alveoli oocytes as the main criterium to differentiate *Immature* (Fig. 2A) from *Early Developing* ovaries (Fig. 2B). Advanced stages of ovarian maturation are also shown (Fig. 2C and D).

MLSM in the OS was 55.4 cm TL (CI 55.0 and 55.7 cm), and 44.1 cm TL (CI 43.9 and 44.4 cm) for the first and last year of the data series (2001 and 2019). The minimum was 40.4 cm TL (CI 40.0 and 40.7 cm) in 2009 and the maximum was 56.6 cm TL (CI 56.4 and 56.9 cm) in 2005 (Fig. 3). The mean for the whole series was 50.7 cm TL (CI 50.6 and 50.8 cm), $n = 10,166$.

In 2003, 2008 and 2018, the MLSM of hoki was not calculated in the OS because the ratio of the length of immature to mature females did not allow the model a consistent fit to the data, where the intercept was not significant. In this area of the ocean, it is striking that in 2003, contrary to expectations, hoki females up to 63 cm TL were immature, despite their lengths being above the MLSM determined from the annual data (55.4 cm TL). In contrast, in the same year samples from the IS, the smallest female measured 32 cm TL and was sexually mature as were 96% of the rest of the females. Histological analysis of the gonads has shown that they were in a normal *Early Maturing* stage (Fig. 4). Three specimens of hoki from the IS between 33 and 35 cm TL already had hydrated oocytes.

The length distribution of small hoki females in the OS revealed the frequent occurrence of precocious females in the microscopic *Developing* stage, measuring between 31 and 38 cm TL (*i.e.*, lengths that coincide with those of females of the local IS population, with a high mean $GSI \pm SD$ equal to 7.0 ± 5.1). On the contrary, the rest of the females in the OS in the same length range had gonads in the *Immature* stage, with a maximum oocyte diameter of 0.20 mm and $GSI \pm SD$ of 0.35 ± 0.12 , *i.e.*, a GSI value typical of immature females and 20 times smaller than mature females. The presence of precocious females in the OS was detected in 2001, 2004 and from 2012 to 2019 (Fig. 5).

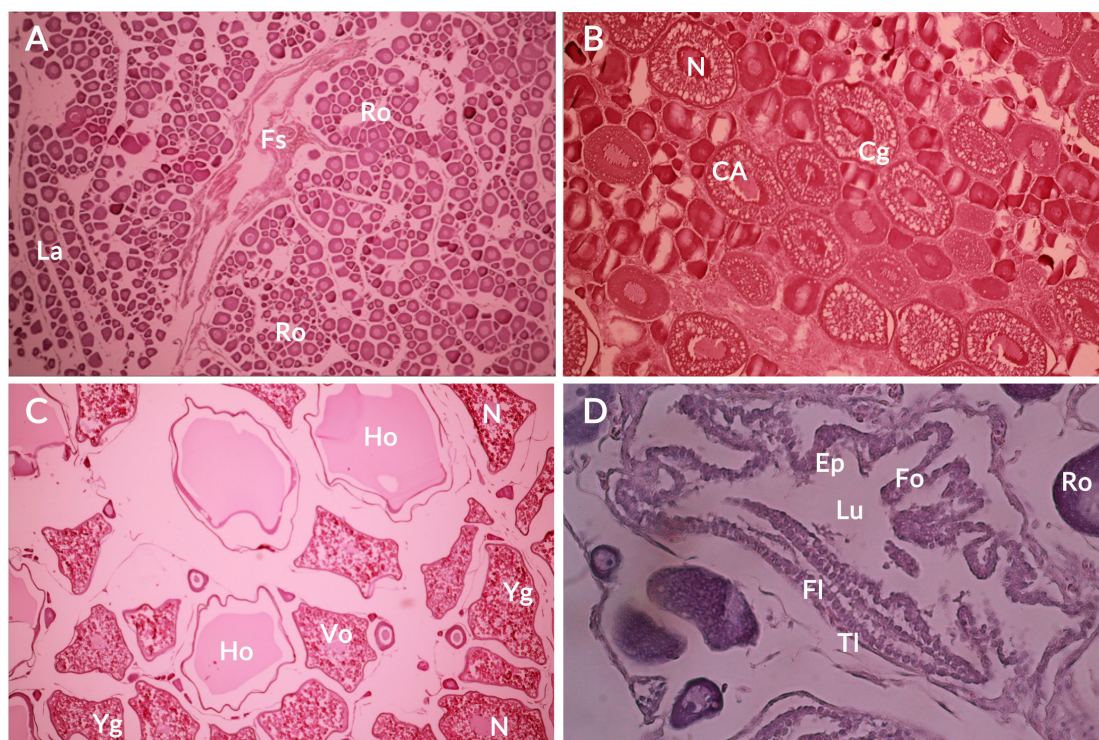


Figure 2. Ovarian histology of hoki *Macruronus magellanicus* in different stages of maturity. **A) Immature**, oocytes in primary growth stage (5x). **B) Early Developing**, yolk granules appear in the oocyte (10x). **C) Hydrated**, swelling of the oocyte due to hydration (5x). **D) Regressing**, recent follicular atresia. Postovulatory follicle 0 to 4 h of age (40x). CA: cortical alveoli; Cg: cortical yolk granules; Ep: eosinophilic pigment; Fl: follicular layer; Fo: fold; Fs: fibromuscular septum; Ho: hydrated oocyte; La: lamella; Lu: lumen; N: nucleus; Ro: reserve oocyte in chromatin nucleolus stage; Ti: theca layer; Vo: vitellated oocyte; Yg: yolk globules surrounding the nucleus / Histología ovárica de merluza de cola *Macruronus magellanicus* en diferentes estadios de madurez. **A) Inmaduro**, ovocitos en estadio de crecimiento primario (5x). **B) Desarrollo Temprano**, gránulos de vitelo aparecen en el ovocito (10x). **C) Hidratado**, aumento del volumen del ovocito debido a la hidratación (5x). **D) Regresión**, con atresia folicular reciente. Folículo postovulatorio de 0 a 4 h de edad (40x). CA: alvéolo cortical; Cg: gránulos corticales de vitelo; Ep: pigmento eosinofílico; Fl: lámina folicular; Fo: pliegue; Fs: septo fibromuscular; Ho: ovocito hidratado; La: lamela; Lu: lumen; N: núcleo; Ro: ovocito de reserva en estadio de cromatina nucleolar; Ti: Lámina tecal; Vo: ovocito vitelado; Yg: glóbulos de vitelo rodeando el núcleo

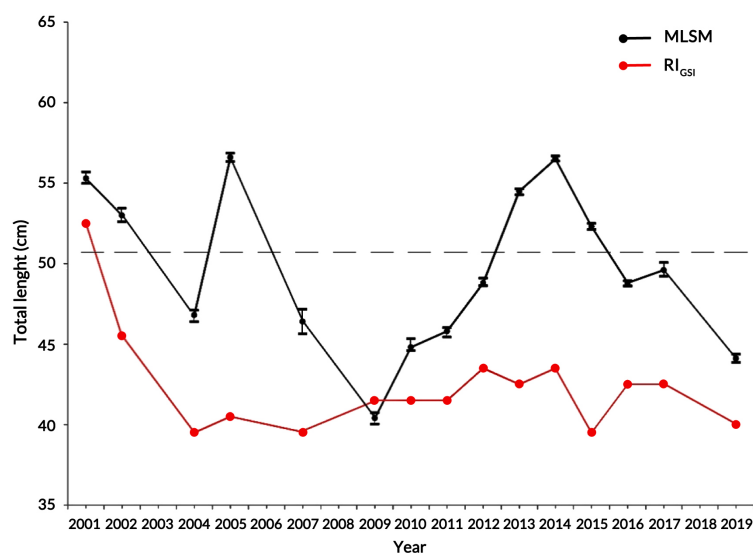


Figure 3. Mean length at sexual maturity (MLSM) and Relative Increment of the Gonadosomatic Index (RI_{GSI}) of hoki *Macruronus magellanicus* in the Outer Sea of Chilean Patagonia. MLSM: black line; RI_{GSI} : red line; vertical bars: 95% confidence intervals; dashed line: mean value of the entire data set / Longitud media de madurez sexual (LMMS) e Incremento Relativo del Índice Gonadosomático (IR_{IGS}) de la merluza de cola *Macruronus magellanicus* en el Mar Exterior de la Patagonia chilena. LMMS: línea negra; IR_{IGS} : línea roja; barras verticales: intervalos de confianza del 95%; línea de trazos: valor medio de toda la serie de datos

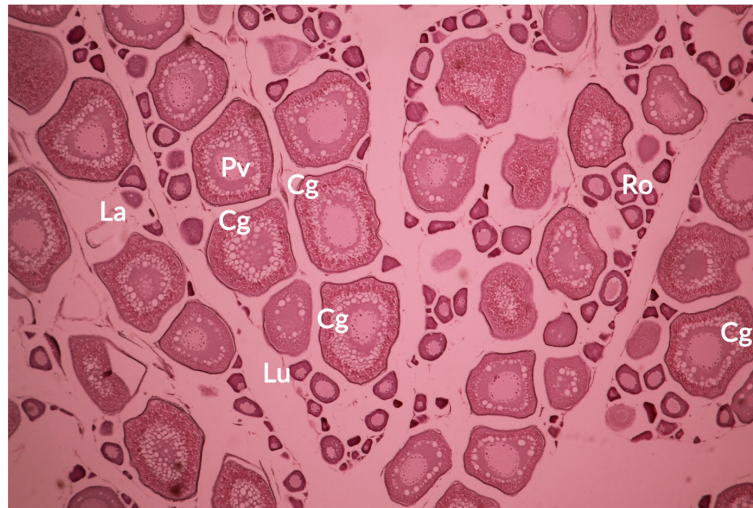


Figure 4. Ovarian histology of hoki *Macruronus magellanicus* in *Early Developing* stage. Female of 38 cm TL with precocious maturation (5x). Cg: cortical yolk granules; La: lamella; Lu: lumen; Pv: partially vitellated oocyte; Ro: reserve oocyte in chromatin nucleolar stage / Histología ovárica de merluza de cola *Macruronus magellanicus* en estadio *Desarrollo Temprano*. Hembra de 38 cm LT con maduración precoz (5x). Cg: gránulos corticales de vitelo; La: lamela; Lu: lumen; Pv: ovocito parcialmente vitelado; Ro: ovocito de reserva en estado de cromatina nucleolar

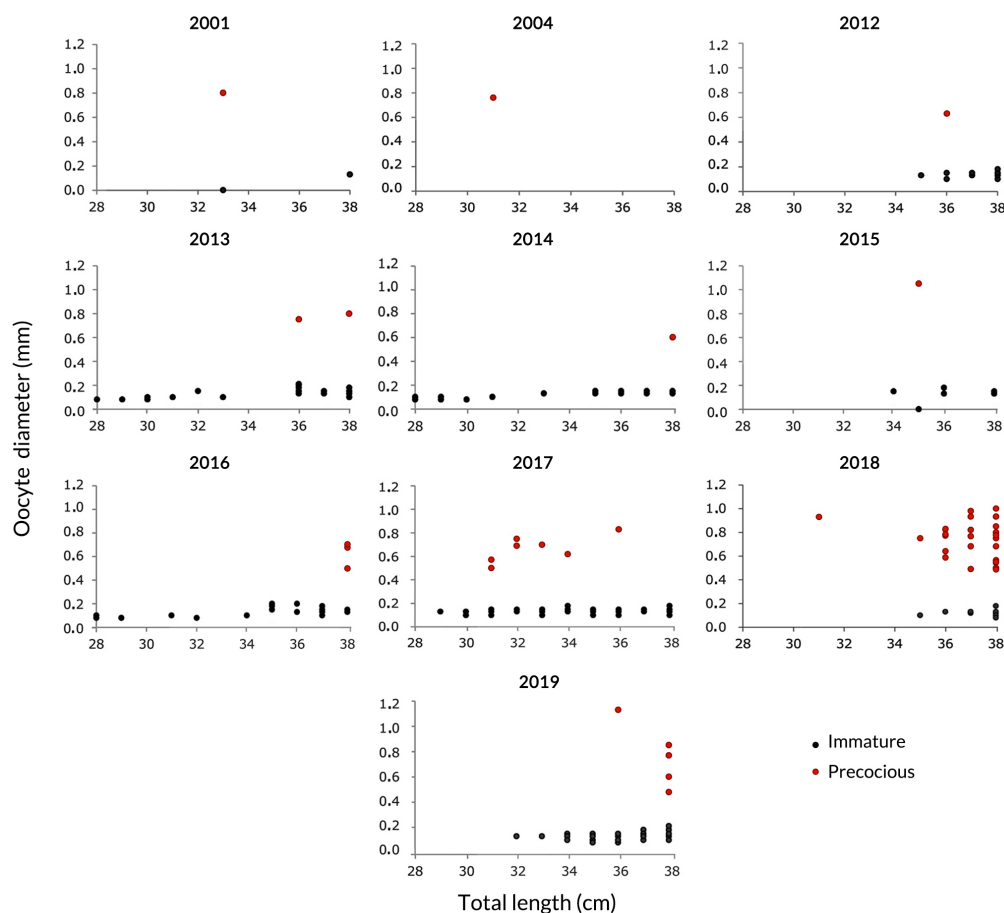


Figure 5. Distinction between immature and precocious hoki *Macruronus magellanicus* females of the Outer Sea, Chilean Patagonia, according to oocyte diameter. Years in which precocious females were present are shown / Distinción entre hembras inmaduras y precoces de la merluza de cola *Macruronus magellanicus* en el Mar Exterior de la Patagonia chilena de acuerdo con el diámetro ovocitario. Se muestran los años en que las hembras precoces estaban presentes

In 2001 data on immature and mature fish fit adequately around the maturity ogive, but from 2004 onwards there was greater heterogeneity in the length at maturity. In 2011, 86 to 90% of females of 43 to 45 cm TL were mature, while in 2016, this percentage was 69% at 44 cm TL (Fig. 6).

The data of RI_{GSI} results from 2003 was not included in the analysis because the index could not be calculated for the same reasons that MLSM could not be established. In 2001 the length was 52.5 cm TL, with a subsequent sharp decrease of the index to 39.5 cm TL in 2004. In the following years, the length estimated with this index stabilized until the end of the study period, with mean \pm SD of 42.4 ± 3.3 cm TL (Fig. 3).

MASM in hoki was 4.0 years (CI 3.5 and 4.4) at the beginning of the data series (2002) and 3.4 years (CI 3.2 and 3.7) in 2019. Mean age of maturity for the entire study period was 3.6 years (CI 3.5 and 3.7); $n=4,731$. There was a sustained decrease in age of maturity from 2002 to 2011, with a lowest value of 2.7 years (CI 2.3 and 3.0) in 2011 for this period. Then there was an increase until 2014, reaching 4.4 years (CI 4.1 and 4.6). Afterwards there was a slight decrease in the age of maturity (Fig. 7).

SOUTHERN HAKE

As in hoki, some stages of the southern hake gonadal maturation cycle were selected to characterize the ovaries histologically (Fig. 8). The presence of only primary growth oocytes in *Immature* ovaries (Fig. 8A) contrasts with the formation of yolk granules in ovaries during *Early Developing* stage (Fig. 8B). Ovarian parenchyma in full process of vitellogenesis is observed in Fig. 8C, and the presence of postovulatory follicles in *Regressing* gonads are depicted in Fig. 8D.

In southern hake, the MLSM in the OS was estimated at 73.5 cm TL (CI 72.7 and 74.4) in 1989 and 73.9 cm TL (CI 73.6 and 74.7) in 2019. MLSM ranged from 71.0 cm TL (CI 70.6 and 71.4) in 2011 and 76.1 cm TL (CI 75.8 and 76.3) in 2005. A consistent statistical result of MLSM, or any other type of analysis, could not be determined from the 2003 sample given that the smallest female measured 70 cm TL and was already sexually mature. Relatively low MLSMs were recorded from 1989 to 1999, followed by an increase from 2000 to 2005, a decrease over the following seven years, and

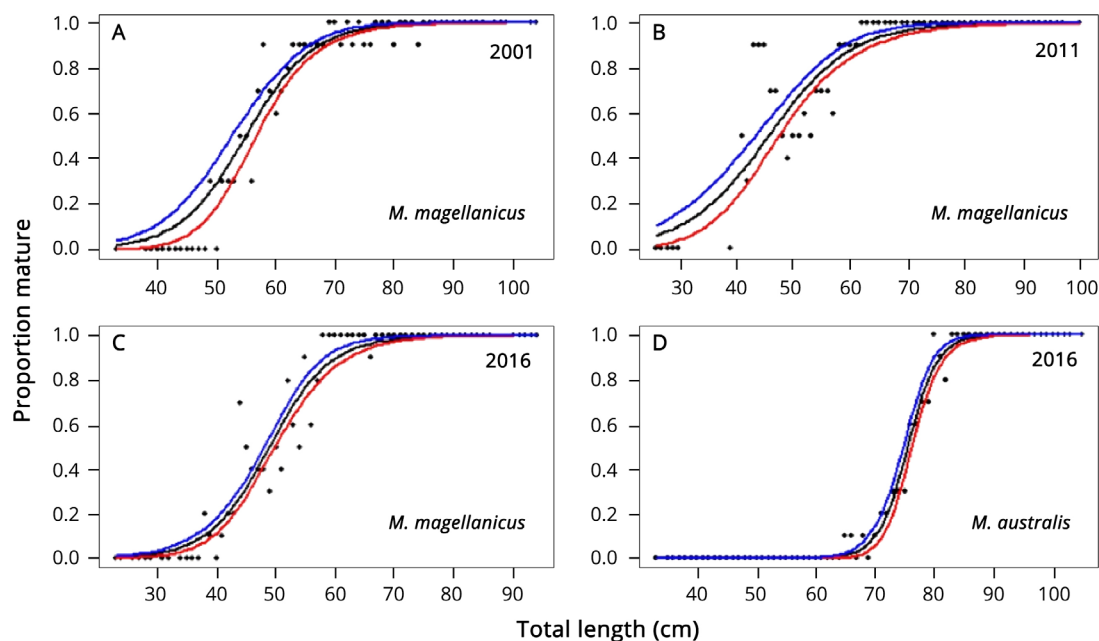


Figure 6. Examples of data fit to sexual maturity ogives in hoki *Macruronus magellanicus* (A-C) and southern hake *Merluccius australis* (D). A) data with weak scatter around the logistics curve in 2001. B-C) data with strong scatter in 2011 and 2016. D) data with weak scatter in 2016. Black dots: observed values; black line: proportion of mature females; blue and red lines: upper and lower 95% confidence intervals / Ejemplos del ajuste de datos a ojivas de madurez sexual en merluza de cola *Macruronus magellanicus* (A-C) y merluza del sur *Merluccius australis* (D). A) escasa dispersión de los datos alrededor de la curva logística en 2001. B-C) gran dispersión de los datos en 2011 y 2016. D) escasa dispersión de datos en 2016. Puntos negros: valores observados; línea negra: proporción de hembras maduras; líneas azul y roja: intervalos de confianza del 95% superior e inferior

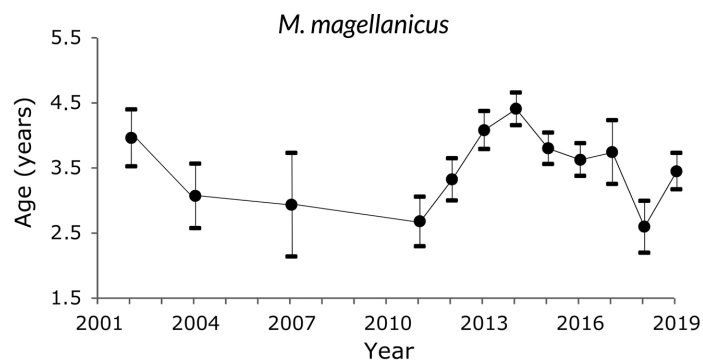


Figure 7. Mean age at sexual maturity of hoki *Macruronus magellanicus* from 2001 to 2019 in the Outer Sea of Chilean Patagonia. Vertical bars: 95% confidence intervals / Edad promedio de madurez sexual de la merluza de cola *Macruronus magellanicus* desde 2001 a 2019 en el Mar Exterior de la Patagonia chilena. Barras verticales: intervalos de confianza del 95%

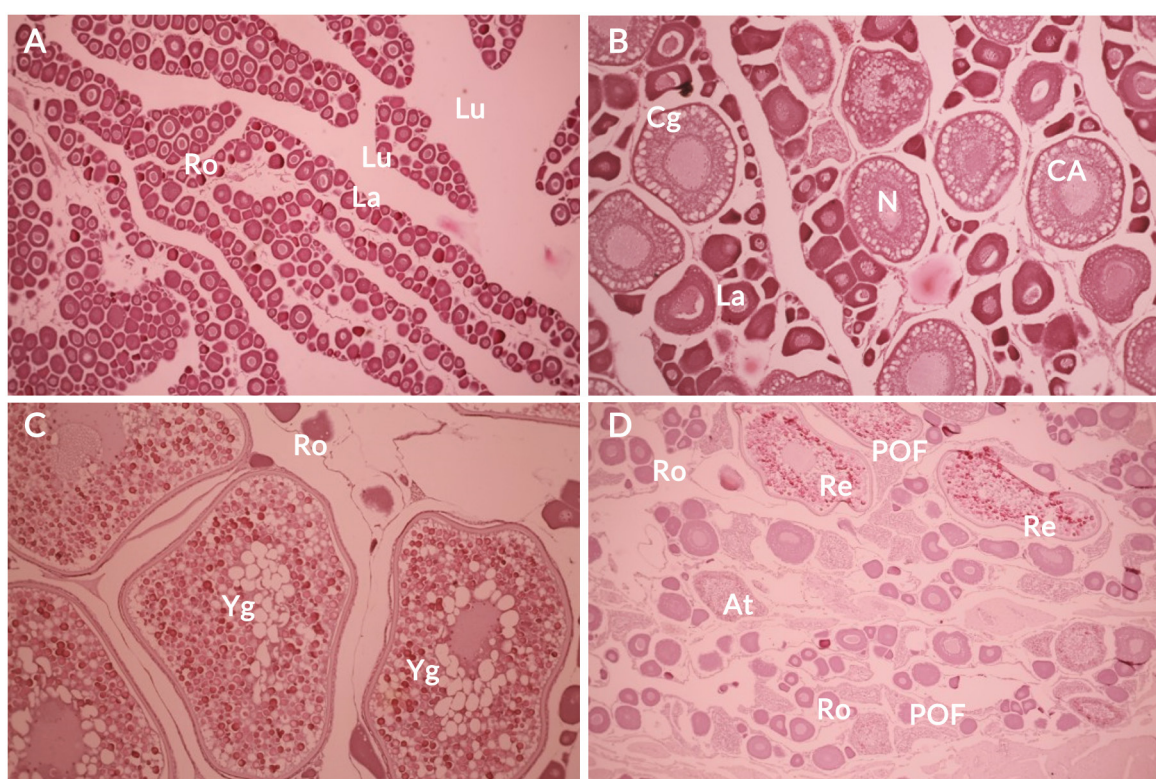


Figure 8. Ovarian histology of southern hake *Merluccius australis* in different stages of maturity. A) *Immature*, primary growth oocytes (5x). B) *Early Developing*, yolk granules appear in the oocyte (10x). C) *Late Developing*, oocytes in nuclear migration process (10x). D) *Regressing*, vitellated and non-vitellated oocytes present, as well as oocyte and follicular atresia (5x). At: atretic oocyte; CA: cortical alveoli; Cg: cortical yolk granules; La: lamella; Lu: lumen; N: nucleus; POF: postovulatory follicle; Re: residual vitellated oocyte; Ro: reserve oocyte in chromatin nucleolar stage; Yg: yolk globules surrounding the nucleus / Histología de ovarios de merluza del sur *Merluccius australis* en diferentes estadios de madurez. A) *Inmaduro*, ovocitos en crecimiento primario (5x). B) *Desarrollo Temprano*, gránulos de vitelo aparecen en el ovocito (10x). C) *Desarrollo Avanzado*, ovocitos en proceso de migración nuclear (10x). D) *Regresión*, ovocitos vitelados y no vitelados presentes, junto con atresia ovocitaria y folicular (5x). At: Ovocito atrésico; CA: alvéolo cortical; Cg: gránulos corticales de vitelo; La: lamela; Lu: lumen; N: núcleo; POF: folículo postovulatorio; Re: ovocito vitelado residual; Ro: ovocito de reserva en estadio de cromatina nucleolar; Yg: glóbulos de vitelo rodeando el núcleo

another increase in recent years. There was a 5.1 cm difference in MLSM between the extreme values (Fig. 9), with a mean in the total data set of 73.7 cm TL (CI 73.6 and 73.8); $n = 13,551$. In the three years for which data was available for the IS, MLSM ranged from 68.1 cm TL (CI 67.3 and 68.9) to 69.6 cm TL (CI 69.1 and 70.2) (Fig. 9), with a total data set mean of 69.2 cm TL (CI 68.8 and 69.6); $n = 434$.

In small females of southern hake from the OS similar in length to IS females (≤ 66 cm TL), the analysis of the degree of gonadal maturation according to oocytes diameter showed that most had gonads in microscopic *Immature* or *Developing* stages. In fact, in a 6-year data set, only seven small females were found to be in a *Developing* stage having oocytes from 0.50 to 0.73 mm in diameter.

From 2013 to 2019, MASM of southern hake females in the OS fluctuated within a narrow age range, from 10.4 years (CI 10.1 and 10.7) in 2014 to 11.6 years (CI 11.4 and 11.8) in 2016, with a mean for the total data series of 11.0 years (CI 10.9 and 11.1); $n = 2,753$ (Fig. 10).

DISCUSSION

There is agreement in the histological characterization of gonadal development of hoki, in particular distinction between immature and mature females on the basis of the presence of previtellogenic oocytes (Chong 2000). However, this study does not include photomicrographs of histological sections. For southern hake, the ovarian development agrees with previous photographic depictions of *Hydrated* and *Spawning* stages (Macchi & Pájaro 2003).

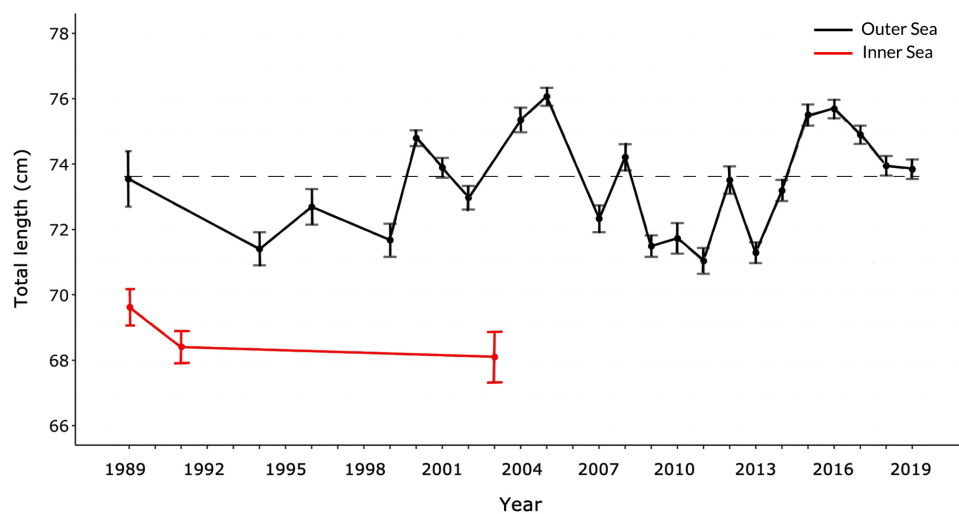


Figure 9. Mean length at sexual maturity of southern hake *Merluccius australis* in the Outer Sea (OS) and Inner Sea (IS) of Chilean Patagonia. Vertical bars: 95% confidence intervals; dashed line: mean value of the entire OS data set / Longitud promedio de madurez sexual de la merluza del sur *Merluccius australis* en el Mar Exterior (OS) y Mar Interior (IS) de la Patagonia chilena. Barras verticales: intervalos de confianza del 95%; línea de trazos: valor medio de toda la serie del OS

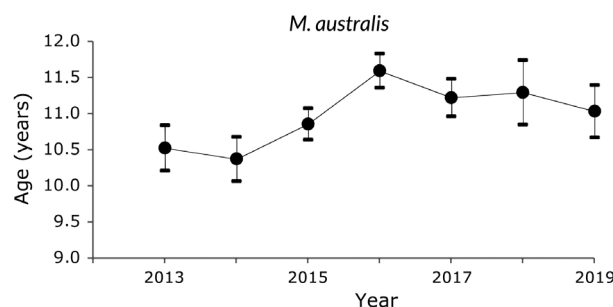


Figure 10. Mean age at sexual maturity of southern hake *Merluccius australis* in the Outer Sea of Chilean Patagonia. Vertical bars: 95% confidence intervals / Edad promedio de madurez sexual de la merluza del sur *Merluccius australis* en el Mar Exterior de la Patagonia. Barras verticales: intervalos de confianza del 95%

MLSM in hoki varied greatly from year to year, decreasing 11.2 cm from 2001 to 2019. The MASM trend was quite consistent with the TL-based estimate. The assumed heterogeneity in the geographic origin of hoki females in the spawning area can be explained through the analysis of stable isotopes in otoliths of preadults. Most recruits (79%) came from breeding areas of the shelf break of Patagonia, while 22% of recruits originated from breeding areas of the IS (Niklitschek *et al.* 2014). In fact, females migrating to the OS spawning areas were 24 cm smaller than the MLSM of hoki in that sector of the Patagonia (Fig. 5) and could be associated to those of the IS. Therefore, they may be considered as dwarf females under precocious maturation. This is also confirmed when comparing histological characteristics of hoki females from the OS at the *Early Developing* stage (Fig. 2B) with those from the IS at the same developing stage but with early maturation (Fig. 4). No differences in the gonadal structure were detected.

Dwarf females were identified as a subpopulation of *Merluccius productus* in Baja California Peninsula, together with a coastal subpopulation, which was explained as a result of exposure to different environmental conditions instead of a genetic differentiation (Vrooman & Paloma 1977, Bailey *et al.* 1982, Funes-Rodríguez *et al.* 2009). The importance of intrapopulation migratory groups has been thoroughly analyzed (Secor 1999) and could be applied to hoki. More information is required to evaluate the relevance of hoki females with differentiated gonadal development in inland waters. No references to its length at sexual maturity have been found in adult hoki population of the IS, which could be compared with the present results.

The analysis of isotopic and elemental composition of otoliths showed that adult southern hake coming from estuarine breeding areas dominated both estuarine and oceanic habitats (Toledo *et al.* 2019). There was a consistent tendency for estuarine fishes to be smaller during demersal recruitment compared to fish with different life cycle characteristics (Toledo *et al.* 2019). This smaller length at recruitment may be related to the smaller maturation size of IS females. It can be speculated that immature females from the IS who migrate to oceanic waters complete their sexual development at a length equal to the rest of the females of the OS. This finding is consistent with the oocyte diameter analysis of small-sized females from the OS, in which only seven mature females were similar in size to the MLSM of the IS females. A proper understanding of the gonadal maturation process in both hoki and southern hake caught in the OS should incorporate the study of IS females.

It should be noted that the RI_{GSI} is not equivalent to the SMS-based MLSM, since, when considering successive length classes into the calculation, small females that are first undergoing a build-up yolk, which is reflected by a sharp gain in ovarian weight, are responsible for the RI_{GSI} increase.

In the data base, hoki sexual maturity length estimated by RI_{GSI} remained relatively constant, except for the 2002 to 2004 period when the downward trend was similar to that in MLSM, resulting in a good concordance between both methods.

Hoki maturity ogives showed a good fit to the logistic curve, with an almost complete absence of early maturing females in 2001 (Fig. 6). In contrast, mature females of small size (38–45 cm LT) were observed in 2011 and 2016. While the MLSM estimate has been adequately validated by SMS, the RI_{GSI} has provided a better understanding of the large annual variations in the sexual behavior of hoki. By contrast, in the graph of southern hake in 2016, the data on immature and mature females fit adequately to the maturity ogive, a situation that was generally repeated in this species every year (Fig. 6). Consistent with these results, the size distribution of hoki between 2008 to 2019 has been characterized by a greater presence of individuals of 30–49 cm TL, while between 2001 and 2005 size distributions were dominated by adults (Legua *et al.* 2020b). It can be assumed that the low or moderately low MLSM observed in 2009, 2010, 2011 and 2019 was the result of the preponderance of early maturing females in the samples.

In Chile, the joint application of SMS to determine MLSM and the RI_{GSI} has been used in the common sardine *Strangomera bentincki* (Norman, 1936) and in the sea chub *Graus nigra* (Philippi, 1887), by Arancibia *et al.* (1994a) and Flores & Smith (2010), respectively.

The size structure of southern hake has been different from that of hoki, in that most of the southern hake population in the study area is made up of individuals between 70 and 99 cm TL (68%) and some juveniles (32%) smaller than 70 cm TL throughout the 2001–2018 period (Legua *et al.* 2018). This population structure is not expected in an overfished population which implies that such condition is consistent with the temporal stability of the MLSM.

Short-term temporal variations in MLSM have been observed in *Merluccius merluccius* of the coast of Galicia as well. After a nine-year period of decline in the MLSM there was a rapid increase over the following 10 years to enter a period of stabilization afterward (Domínguez-Petit *et al.* 2008). It may be argued that fishing mortality may be the cause of these variations, but contrary to what was expected, spawning stock biomass decreased in the period when MLSM increased (Domínguez-Petit *et al.* 2008). A change in the mean length at maturity is expected in fish stocks subject to intense exploitation, with sexual maturation occurring at a smaller length and at a younger age (Dieckmann & Heino 2007).

Changes in the MLSM of common hake *Merluccius gayi* (Guichenot, 1848), whose fishery off the coast of Chile extends south to 41° (Martínez 1976), can be compared to hoki and southern hake as a result of fishing exploitation. The MLSM of common hake was 36.0 cm LT in 1965 (Balbontín & Fischer 1981) while from 2002 onwards a decline in stock

size was observed in the face of overexploitation (Molina *et al.* 2020). As a consequence, the MLSM decreased to 31.4 cm TL in 2005 (Alarcón *et al.* 2008). This trend, with no clear signs of recovery, coincides with that observed in hoki, but not in the case of southern hake, where its MLSM in 2019 was the same as at the beginning of the study period.

Southern hake's MLSM in the OS showed small annual variations. IS mature females with a MLSM of 4.5 cm TL less than that of OS females are reported for the first time. This difference is noted not only in the length at maturation of southern hake but it is also observed in the fatty acid composition of some internal organs, suggesting that their food sources were different and that these groups of fish remained separated in each area during winter (Medina *et al.* 2014). This implies that there are alternative breeding areas of southern hake in the Chilean Patagonia (Medina *et al.* 2014) which seems consistent with our results of a likely local population in the IS.

The reproductive behavior of hoki and southern hake may be directly related to their early life stages in the fjords, channels and on the shelf break of the Patagonia. There is much evidence showing spawning in fjords and channels (Bustos *et al.* 2007, Balbontín *et al.* 2009). The average number of southern hake eggs in the OS was 30 times greater than in the IS in winter and four times greater in spring (Flores *et al.* 2019). There is no data regarding survival rate of the early stages of development of this species. However, the microstructure of southern hake larval otoliths and the oceanographic conditions in fjords were different to those of the Gulf of Penas larvae (Landaeta *et al.* 2018). Southern hake larvae of the same age were larger and grew faster in the IS compared to the larvae of the OS (Landaeta *et al.* 2018), suggesting the existence of favorable environmental conditions for larval survival in the IS.

Applying histological techniques, in 1997 the MLSM of hoki from the OS of the Patagonia was 54.4 cm TL (Chong 2000). Four degrees latitude north of Patagonia, the MLSM was 55.4 cm TL (Arancibia *et al.* 1994b). These estimates do not significantly differ from our estimates of 55.3 cm TL in 2001, made prior to the reduction of recruitment and overexploitation of hoki (Cubillos *et al.* 2014). Using a macroscopic sexual maturity scale, the MLSM of hoki females in the southwest Atlantic was 56.0 cm TL (Gorini & Pájaro 2014); at 57.3 cm TL corresponding to an age of 3.1 years (Zavatteri *et al.* 2016); at 58.0 cm TL and 3.6 years (males and females; Giussi *et al.* 2016). It should be pointed out that although data was consistent, macroscopic scales are less accurate than microscopic scales (Murua *et al.* 2003).

For southern hake off Pacific Patagonia, MLSM was 72 cm TL when applying a macroscopic maturity scale (Aguayo *et al.* 1985), which was similar to our microscopic estimate, and suggests great stability over time. Instead, macroscopic MLSM estimate of southern hake from the South Atlantic was as low as 61 cm TL (Giussi *et al.* 2001) and this might be the result of sampling both sexes together.

The current state of knowledge of life history stages of hoki and southern hake, and their relation to reproduction underscores the importance of the IS in maintaining the adult stock as suggested by (1) the smaller maturation length of southern hake in the IS compared to the OS; (2) the favorable environment of IS for the development and survival of the early larval stages due to high rates of phytoplankton growth, particularly in the spring (Antezana 1999, Pizarro *et al.* 2000).

The shift towards maturation at a smaller length with increasing fishing intensity has been observed in many commercial fish stocks. This rate has decreased when a fishing moratorium was implemented (Sharpe & Hendry 2009). However, the fishery management measures in southern Chile do not appear to have the same effect, where hoki spawning biomass continues to decline (SUBPESCA 2022). The length and age of sexual maturity of hoki did not recover its MLSM from early 2001, despite strong fluctuations. Instead, the MLSM of southern hake in the IS varied slightly in the 1989-2003 period (1.5 cm) and in the OS, despite small annual variation in the 1989-2019 period (23 years), the MLSM remained unchanged.

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