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Proximate composition and energy density in relation to Argentine hake females (*Merluccius hubbsi*) morphometrics and condition indices

Ezequiel Leonarduzzi[a,*], Karina A. Rodrigues[a,b], Gustavo J. Macchi[a,b]

[a] Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Paseo Victoria Ocampo N° 1, CC. 175, Mar del Plata 7600, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rivadavia 1917, Buenos Aires 1033, Argentina

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ABSTRACT

Argentine hake is the most important economic demersal resource for the fisheries of the South-West Atlantic Ocean. Nevertheless, not many studies on its bioenergetic dynamic have been reported. Proximate composition and energy density were analyzed in muscle, liver and gonads and they were related to maternal morphometrics (length and gutted weight) and condition factor (K), gonadosomatic index (GSI) and hepatosomatic index (HSI). Argentine hake females were collected during the beginning of their reproductive season in the main spawning ground reported for this species. The results support the idea that muscle represents the main deposit of proteins and the liver is the main deposit of lipids. Furthermore, it was observed that females with higher content of lipid in their liver had also greater lipid reserves in muscle. All hake females reached the same proportion of lipid and protein reserves in their gonads independently of lipid and protein stored in their liver. Water content reflected the lipid content on muscle, liver and gonad. Lipid content in the three tissues was positively related to maternal morphometrics. Being a good indicator of the physiological condition of hake female the HSI was the only variable that reflected changes in lipid content and energy density.

1. Introduction

The term condition has been commonly used to describe the overall health condition or the energy reserves of an animal (Green, 2001; Schulte-Hostedde et al., 2005). In fishes the proximate composition is an important ecological measure of condition that integrates both feeding and habitat quality (Jobling, 1995; Wicker and Johnson, 1987). Moreover, the biochemical composition of an individual describes the nutritional condition precisely, making possible the subsequent determination of energy available in each tissue. This assessment is important from the ecological, nutritional and food technology point of view (Connell, 1975; Huss, 1995; Zaboukas et al., 2006). In general, the biochemical composition and, especially, the fat content vary among marine species. At the same time, there are fluctuations of these components within the same species that could be associated to different factors: age, sex, gonadal development, migration stage, nutritional condition, area and other factors (Exler et al., 1975; Shearer and Swanson, 2000; Rueda et al., 1997; Akpinar et al., 2009). It has been observed that biochemical composition changes related to the individual condition affect the fertility and egg production of many species (Kjesbu et al., 1991; Kurita et al., 2003; Lambert and Dutil, 2000; Marteinsdottir and Begg, 2002; Korta et al., 2010). Hence, the knowledge about parental stock condition could help to understand the recruitment variations observed on the natural populations (Marteinsdottir and Thorarinsson, 1998; Scott et al., 1999). In many fish species it has been observed that the size or the age of the female usually affects the reproductive capacity. For example, the maternal conditions can affect the start date, the duration and/or the frequency of the spawning as well as the fertility and the quality of the eggs produced (Marteinsdottir and Steinarsson, 1998; Vallin and Nissling, 2000; Marteinsdottir and Begg, 2002; Macchi et al., 2004; Claramunt et al., 2007; Mehault et al., 2010).

Generally, morphometric changes may occur due to the mobilization or utilization of endogenous energy (proteins and lipids as energy sources) so, indices that relate the size to the individual weight or to a particular organ has been used to estimate the animal condition. K (condition factor) and HSI (hepatosomatic index) are the indices generally used (Lloret et al., 2002). For example, in the

* Corresponding author. Tel.: +54 223 4862586; fax: +54 223 4861830.
E-mail addresses: eleonard@inidep.edu.ar (E. Leonarduzzi), krodrigues@inidep.edu.ar (K.A. Rodrigues), gmacchi@inidep.edu.ar (G.J. Macchi).

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case of the Gadidae family, the hepatosomatic index is considered an useful indicator of energy storage that describes the condition of fish in a general way (Lambert and Dutil, 1997; Yaragina and Marshall, 2000).

Argentine hake (*Merluccius hubbsi*) is a demersal species distributed from 22° to 55° S at depths between 50 and 500 m (Cousseau and Perrotta, 1998). In the Argentine sea, the Patagonian stock, located at the south of the 41° S, is economically the most important one and it is currently being overexploited (Santos and Villarino, 2013). A strong decrease of the reproductive biomass of this species has been recorded since 1997; and changes on the structure of the parental stock (Santos and Villarino, 2013) and on the location of reproductive shoals (Macchi et al., 2005) have been observed. This species is a batch spawner with indeterminate annual fecundity (Macchi et al., 2004) showing a protracted spawning season during the southern spring and summer time, having its main peak from December to January (Macchi et al., 2004; Pájaro et al., 2005).

During the last few years different aspects of the reproductive biology of this population have been analyzed; such as the size composition and spatial variation of the breeders (Macchi et al., 2004; Pájaro et al., 2005; Macchi et al., 2010), its vertical migrations (Ehrlich et al., 2013), the reproductive potential (Macchi et al., 2004, 2006), and the variations of proximate composition (in gonad, liver and muscle) for each ovary developmental stage during the spawning season (Leonarduzzi et al., 2012). In this regard, it was observed that the egg production is influenced by age and size composition of the population (Macchi et al., 2004); that bigger females have a longer spawning season (Macchi et al., 2004); that the oocytes dry weight increases depending on the female size (Macchi et al., 2006); and that the oil droplets diameter in hydrated oocytes also increases depending on the females size (Macchi et al., 2013). Nevertheless, the influence of the maternal morphometrics and condition indices on the biochemical composition of different tissues of the Argentine hake spawning females has not been studied yet.

Thus, the goals of this research were to analyze the proximate composition and energy content of different tissues (liver, muscle and gonads) of hake females in spawning capable phase, and relate them to the maternal attributes (total length and gutted weight). Also, the relationships between the proximal composition of the tissues and the condition indices (HSI, K and GSI) were studied.

2. Materials and methods

Samples of Argentine hake females (*N* = 47) were collected in the main spawning ground of the Patagonian hake stock during a demersal trawl survey conducted by the National Institute for Fisheries Research and Development (INIDEP) during December 2008 in the early reproductive peak (see Macchi et al., 2004 and Macchi et al., 2007 for more details) (Fig. 1). Since *M. hubbsi* is an asynchronous batch spawner this sampling period was chosen to maximize the probability to find females at the beginning of the spawning season.

Total length (TL) to the nearest cm and total weight (TW), gutted weight (GuW), gonad weight (GW), and liver weight (LW) to the nearest gram, were recorded (Table 1). All female hakes sampled were at the spawning capable phase (fish are developmentally and physiologically able to spawn in this cycle) according to the macroscopic maturity key proposed by Brown-Peterson et al. (2011). Ovaries collected were characterized by the presence of yolked oocytes but without hydrated oocytes.

Three general condition indices were estimated for all sampled females: hepatosomatic index (HSI), gonadosomatic index (GSI) and condition factor (K). These indices were defined by the following equations:

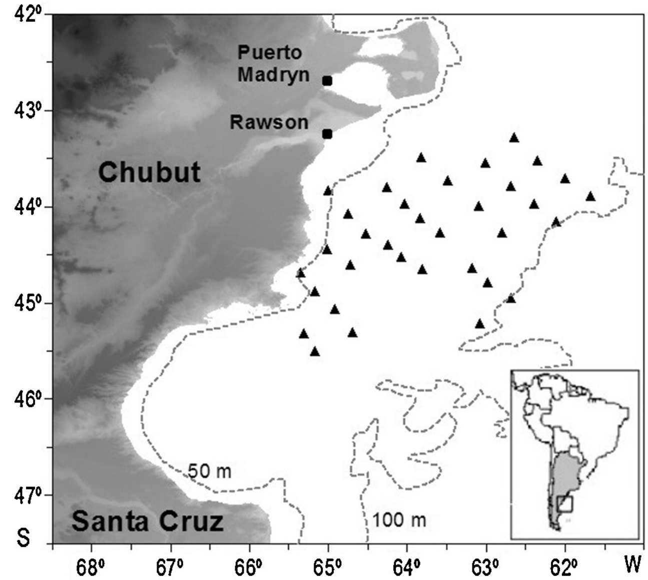

Fig. 1. Samples location of *Merluccius hubbsi* (triangles) collected during the early peak spawning in December 2008 in waters of the north Patagonian area.

$$HSI = \left(\frac{\text{Liver weight}}{\text{Gutted weight}}\right) * 100$$

$$GSI = \left(\frac{\text{Gonad weight}}{\text{Gutted weight}}\right) * 100$$

$$K = \left(\frac{\text{Gutted weight}}{\text{Total length}^3}\right) * 100$$

Muscle (fillet without skin), gonads and liver were dissected from each specimen sampled and preserved frozen at −22 °C in plastic bags vacuum sealed until their analysis in laboratory. These tissues were analyzed for lipids, protein, water and ash content (content refers to percentage of wet weight of tissue). To determine lipid content, sub-samples from frozen samples of different weight were taken according each tissue: muscle (10–20 g), gonads (5–10 g) and liver (3–5 g). This component was extracted by Bligh and Dyer method modified by Undeland et al. (1998), and gravimetrically quantified by Herbes and Allen (1983) method. Protein content was determined from frozen tissue (1 g) using bovine serum albumin (BSA) concentrated at 1 mg/ml as standard and following the protocol of Lowry et al. (1951).

In order to obtain water content (moisture) and inorganic matter (ash) subsamples up to 10 g were taken, as allowed the size of each tissue. The water content was estimated by drying the samples for 24 h at 105 °C, and weighed at room temperature. Later, for ash estimation, the dried samples were placed in a muffle furnace for 8 h at 550 °C (AOAC, 1995). After this period the ashes obtained were weighed at room temperature.

Table 1
Maximum and minimum values of the morphometric variables analyzed.

Morphometric variables	Minimum	Maximum	*n*
Total length (cm)	36	86	47
Gutted weight (g)	303	3300	47
Liver weight (g)	7	379	47
Gonad weight (g)	14	493	47

n, number of samples.

Table 2
Range and mean values of proximate composition (% of tissue wet weight) and energy density (ED, in kJ g^{-1}) for gonad, liver and muscle of *Merluccius hubbsi*.

	Muscle				Gonad				Liver			
	n	Range	Mean	*SD*	*n*	Range	Mean	*SD*	*n*	Range	Mean	*SD*
Lipid	39	0.71–4.95	2.38	0.99	45	8.06–13.19	10.92	1.07	43	4.25–50.10	29.71	11.95
Protein	38	8.49–23.25	15.84	3.69	42	16.86–26.03	21.95	1.75	39	9.82–23.97	16.38	3.22
Water	46	74.11–82.10	79.25	1.53	43	64.75–75.11	68.72	2.22	44	35.11–72.88	55.79	10.38
Ash	46	0.84–1.55	1.21	0.12	44	1.31–2.06	1.61	0.14	44	0.76–2.23	1.61	0.32
ED	32	3.54–6.43	4.72	0.79	42	7.19–10.58	9.49	0.71	36	5.82–24.36	15.93	4.68

n, number of samples; *SD*, standard deviation.

Finally, energy density in each female (ED, kJ g^{-1}) was estimated for each tissue (muscle, ovary, liver) by multiplying lipid and protein content (mg/g of wet mass) by the appropriate energy equivalents (lipid = 39.5 kJ g^{-1}, protein = 23.6 kJ g^{-1}; Kleiber, 1975). Lipid and protein energy were then summed within each tissue to determine the combined mass-specific energy. The contribution of each component (lipid and protein) to the energy density of each tissue was calculated and plotted. The term density refers to KJ per gram of tissue and avoids female size effects, because larger females have, in general, larger organs and body mass. Carbohydrate content was not measured because that component is generally low in marine species and its contribution to total energy content is near zero (Anthony et al., 2000; Eder and Lewis, 2005; Spitz et al., 2010). All determinations were performed in triplicate.

Simple linear regressions were used to analyze: (a) ED in each tissue as function of proximate composition (lipid or protein); (b) proximate composition (lipid, protein, water content and ash) as function of morphometric variables and general condition index. A correlation analysis was performed to study the biochemical composition between tissues. Normality and homocedasticity assumptions of residuals were checked graphically (Q–Q plot and plot of studentized residuals vs predicted values). All of the analyses were performed with R® software package (R Development Core Team, 2012).

3. Results

3.1. Proximate composition

In muscle and gonad the lipids average were 2.4% and 11%, respectively (Table 2), representing on average 19% and 45% of the energy content, respectively (Fig. 2a). On the other hand, lipids were the most abundant constituent of liver proximate composition, between 4.2% and 50.1% of tissue composition (Table 2), representing on average ca 29% and reaching up to 87% of the total energy depending of lipid content (Fig. 2a).

Regarding the proteins, these were the main constituent of proximate composition of muscle (8.5–23.2%) and gonad (16.9–26.0%) (Table 2), representing on average 81% and 55% of the energy content, respectively (Fig. 2a). The liver protein constitutes on average 16.4% of liver proximate composition (Table 2), representing between 13% and 71% of energy content depending of protein content (Fig. 2a).

Most of the wet weight of the muscle corresponded to water, representing ca. 79.2%, which showed the lowest standard deviation among the three tissues. During the spawning capable phase, the gonad water content represented ca 68.7% of the total wet weight, showing low variability. The liver was the tissue with the lowest water content levels (55.8%) as compared to gonad and muscle, and presented the highest fluctuation of the three tissues analyzed.

The mean ash content was lower and similar in the three tissues analyzed, being on average 1.2%, 1.6% and 1.6% for muscle, gonad and liver, respectively (Table 2).

ED mean value for muscle, gonad and liver was 4.7 kJ g^{-1}, 9.5 kJ g^{-1} and 15.9 kJ g^{-1}, respectively (Table 2). The increase of energy content in muscle was principally due to protein content increases, while the lipid remained relatively constant (Fig. 2b). In contrast, in liver the increase of ED was related to increases in lipid contents with no changes in protein quantity (Fig. 2b). However, the relative importance of protein in liver ED increased at low lipid levels (Fig. 2a). In gonad, the increase in ED was due to an increase in

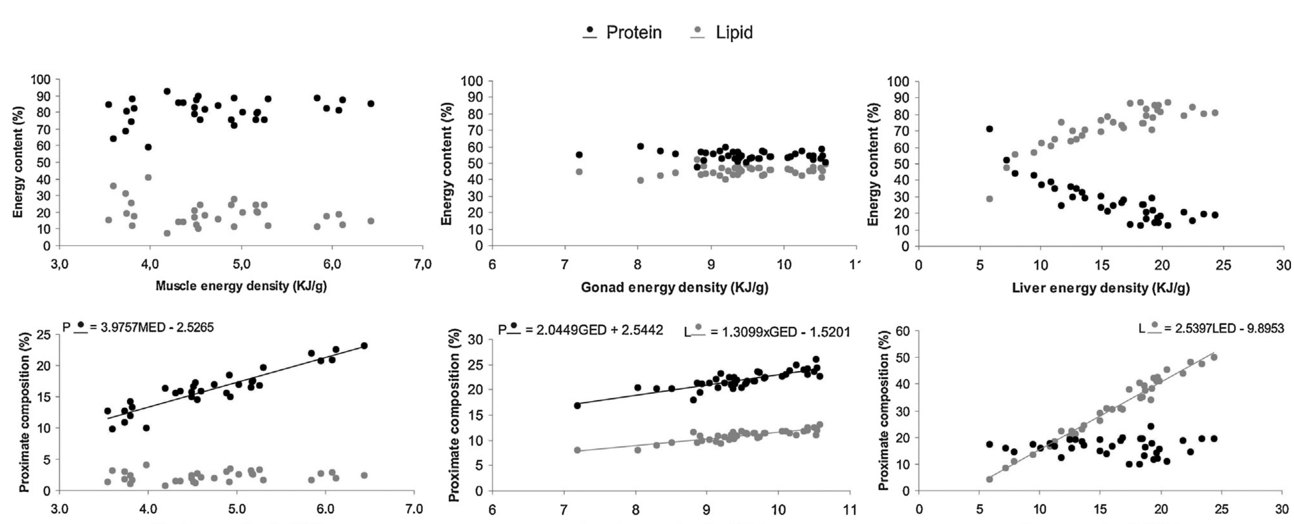

Fig. 2. (a) Energy relative contribution (%) of each component (protein and lipid) to energy density (sum of lipid and protein energy) in muscle, gonad and liver. (b) Relationships between proximate composition (% of wet weight of each component) and energy density for the different tissues analyzed in *Merluccius hubbsi*.

Table 3
Results of linear correlation of proximate composition (lipid, protein, water and ash) among tissues.

	Muscle vs liver		Muscle vs gonad		Liver vs gonad	
	r	*p*	*r*	*p*	*r*	*p*
Lipid	0.60	***	0.40	*	0.19	0.22
Protein	0.21	0.24	−0.08	0.62	0.02	0.88
Water	0.35	*	0.29	0.07	0.07	0.65
Ash	−0.49	***	−0.35	*	0.52	***

r, Pearson Product Moment Correlation; *p*, *p*-values.
* $p<0.05$.
*** $p<0.001$.

both lipid and protein content (Fig. 2b). In fact, the relative energy contribution of lipid and protein was similar (Fig. 2a).

3.2. Analysis of biochemical composition between tissues

Significant correlations were observed between some tissue components, but in general the Pearson's coefficients were low (Table 3). Lipids in muscle were positively correlated to the quantity of lipids in liver and gonad, but no significant relationship was observed between lipid content of liver and gonads. Otherwise, the protein content was independent among tissues. Water content in gonads did not show a significant relationship with the water content in muscle and liver. In contrast, the water content in muscle was positively related to liver water content. There was a significantly negative relationship between the muscle ash content and the gonad and liver ash content. On the other hand, a positive relationship between ash content in liver and gonads was observed.

3.3. Analysis of biochemical composition within each tissue

Significant negative relationships between lipid and water content were observed for the three tissues analyzed, showing the relationship between liver and water content the best fit (Fig. 3 and Table 4). In muscle, ash content was also significant and negatively correlated to water content, while lipids were positively correlated to ash contents. In gonad, a significant and positive relationship between lipid and protein content was observed, while a negative relationship was obtained between protein and water content (Table 4).

3.4. Proximate composition vs maternal morphometrics and condition indices

Lipids were the biochemical component that showed strongest correlation with maternal morphometrics and condition indices in Argentine hake. In muscle, positive significant relationships between lipid content and TL and GuW were observed (Fig. 4 and Table 5). With regard to condition indices, only HSI reflected

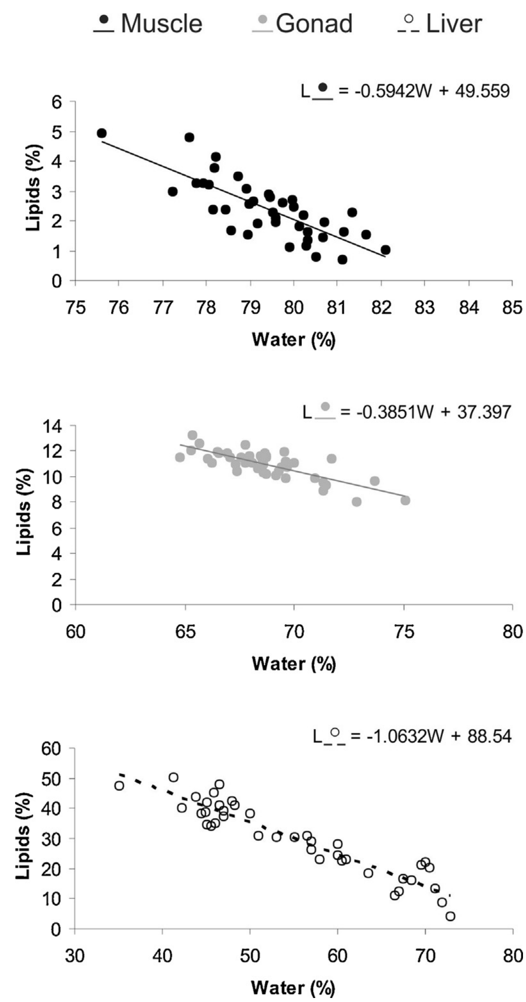

Fig. 3. Muscle, gonad and liver lipid content (g/100 g) in relation to water content (g/100 g) in each tissue sampled for *Merluccius hubbsi*.

positively lipid content in both muscle and liver (Fig. 4 and Table 5). GSI and K showed no significant relationships with lipid content for the three tissues analyzed (Table 5).

Protein content was not significantly related to maternal morphometrics (TL and GuW), HSI nor GSI in any of the tissues analyzed (Table 5). Otherwise, K was positively correlated to muscle protein, but the determination coefficient was very low (Table 5).

Muscle water content was significant negatively related with the female morphometrics (Fig. 5). In the case of liver, the water quantity showed a significant negative relationship with GuW, although the variance explained was low (Fig. 5 and Table 5). In relation to condition indices, HSI was negatively related to water content in muscle and liver, and GSI only was significant negatively related with gonad water content (Fig. 5 and Table 5). No significant relationships were observed between K and water quantity, for all tissues analyzed (Table 5).

Ash content in muscle was not statistical related neither to maternal morphometrics nor to condition indices (Table 5). In liver and gonad the relationships between ash content and maternal morphometrics (TL, GuW) and HSI were significant, showing a negative trend, but variability explained were low (Table 5).

In muscle and gonads no significant relationships between ED and the maternal morphometrics were obtained but there was a positive relationship between ED in gonads and GSI. In contrast, ED in liver was positively correlated to TL and GuW, but in all models the variability explained was very low (Fig. 6 and Table 5). Liver

Table 4
Determination coefficient (r^2) and *p*-values of linear regression among biochemical components in each tissue.

	Muscle		Gonad		Liver	
	r^2	*p*	r^2	*p*	r^2	*p*
Lipid vs protein	0.05	0.18	0.17	**	0.03	0.30
Lipid vs water	0.62	***	0.61	***	0.88	***
Lipid vs ash	0.25	***	0.03	0.21	0.01	0.52
Protein vs water	0.10	0.06	0.27	***	<0.01	0.82
Protein vs ash	<0.05	0.64	0.03	0.27	<0.01	0.65
Water vs ash	0.24	***	0.06	0.09	<0.01	0.52

** $p<0.01$.
*** $p<0.001$.

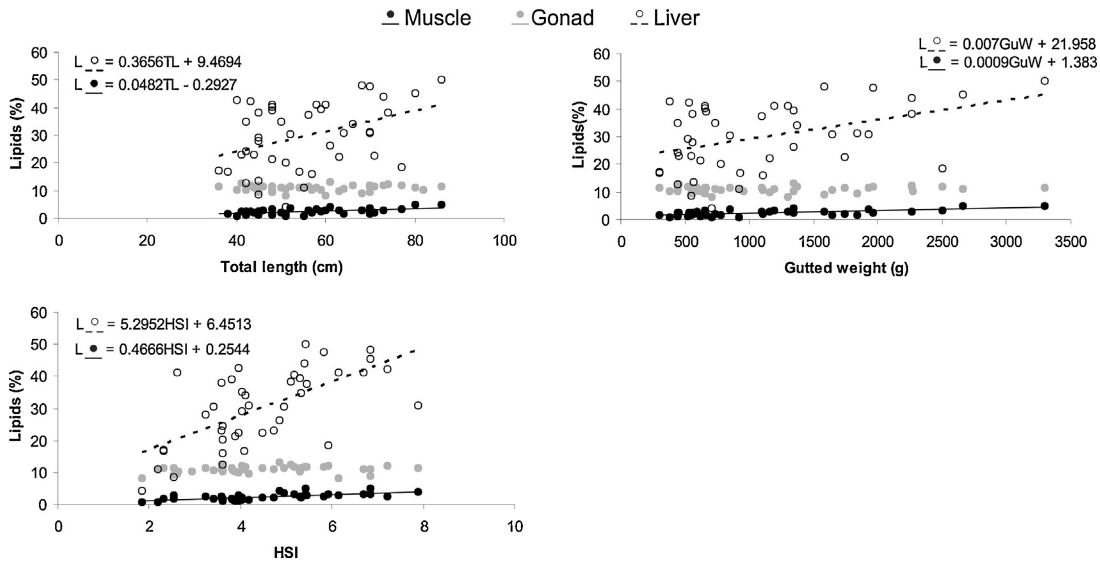

Fig. 4. Muscle, liver and gonad lipid content (g/100 g) in relation to female morphometrics (total weight and gutted weight) and somatic condition indices (HSI) obtained for *Merluccius hubbsi*.

ED was positively correlated to HSI (Fig. 6 and Table 5) and no significant relationship was observed between K and ED for the three tissues analyzed (Table 5).

4. Discussion

Biochemical parameters used to evaluate the energy reserves of fishes are important attributes to investigate because they have a large influence on growth, reproduction and survival (Shulman and Love, 1999). Particularly, the knowledge of the structure and energy reserves, especially in the liver of fish species, is important to understand metabolic processes and to assess the potential impact of physical and chemical environmental stressors in both wild and cultured stocks (Faahraeus-Van Ree and Spurrell, 2003). Lipid content of Argentine hake studies in this work ranged from 0.7% to 4.9% in muscle, from 8.1% to 13.2% in gonad, and from 4.2% to 50.1% in liver. Although in each analyzed tissue lipid concentration showed a great variability among individuals, the results revealed that in Argentine hake females the lipids are stored mainly in the liver. As hake is a batch spawner species with a protracted spawning season, the differences observed in the liver lipids content in females of similar size could be explained by the different individual reproductive histories during the spawning season (e.g. different number of batches already done or different timing within the spawning season). The lipid values estimated in liver were similar to those found in other Gadidae, 2–67% (Lloret et al., 2008) and 8.5–56% (Domínguez-Petit, 2007) in *Merluccius merluccius*, 16.2–59.5% in *Merluccius bilinearis* and 35.7–53.2% in *Merluccius senegalensis* (VNIRO, 2000), which confirms the important role of the liver as an energy reserve in this family (Lambert and Dutil, 1997; Alonso-Fernández and Saborido-Rey, 2012).

This study indicated that the proteins were the main constituent in both muscle and gonads tissues in female hake at the spawning capable phase. Also, the energy density in both tissues was related to the protein content showing the importance of proteins in these tissues. Protein content of Argentine hake ranged from 8.5% to 23.2%

Table 5
Determination coefficient (r^2) and p-values of linear regression between female morphometrics and somatic condition indices vs proximate composition.

		TL		GuW		HSI		GSI		K	
		r^2	p	r^2	p	r^2	p	r^2	p	r^2	p
Lipid	Muscle	0.36	***	0.41	***	0.45	***	0.05	0.22	0.01	0.44
	Gonad	<0.01	0.56	0.02	0.29	0.05	0.13	0.07	0.07	0.03	0.21
	Liver	0.15	**	0.17	**	0.41	***	<0.01	0.93	<0.01	0.92
Protein	Muscle	0.04	0.19	0.02	0.34	<0.01	0.88	<0.01	0.57	0.11	*
	Gonad	0.01	0.48	0.01	0.39	0.03	0.22	0.07	0.08	0.02	0.34
	Liver	0.01	0.46	0.01	0.46	0.05	0.17	<0.01	0.70	<0.01	0.75
Water	Muscle	0.22	**	0.28	***	0.26	**	<0.01	0.98	<0.01	0.86
	Gonad	0.02	0.32	0.03	0.23	0.01	0.51	0.26	***	<0.01	0.91
	Liver	0.07	0.07	0.11	*	0.43	***	<0.01	0.99	0.01	0.36
Ash	Muscle	0.04	0.16	0.06	0.09	0.04	0.19	0.03	0.24	0.02	0.30
	Gonad	0.10	*	0.09	*	0.16	**	<0.01	0.54	0.06	0.10
	Liver	0.18	**	0.17	**	0.16	**	<0.01	0.62	0.03	0.21
Energy density	Muscle	<0.01	0.81	<0.01	0.76	0.01	0.54	0.04	0.27	0.01	0.48
	Gonad	<0.01	0.58	0.02	0.34	<0.01	0.90	0.10	*	<0.01	0.78
	Liver	0.12	*	0.16	*	0.35	***	<0.01	0.57	<0.01	0.96

TL, total length; GuW, gutted weight; HSI, hepatosomatic index; GSI, gonadosomatic index; K, condition factor.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

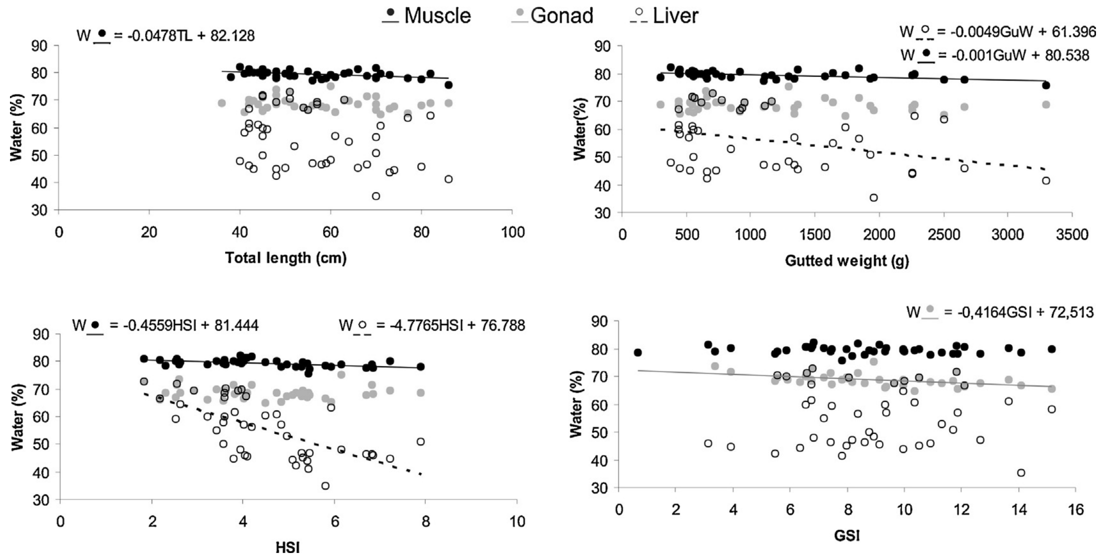

Fig. 5. Muscle, liver and gonad water content (g/100 g) in relation to female morphometrics (total weight and gutted weight) and somatic condition indices (HSI and GSI) obtained for the *Merluccius hubbsi*.

in muscle, from 16.9% to 26.0% in gonad, and from 9.8% to 24.0% in liver. These values were similar to that obtained by Leonarduzzi et al. (2012) for the same species and by Domínguez-Petit (2007) for European hake in Galician shelf waters.

The water content estimated here was on average 79.2% in muscle, 68.7% in gonad and 55.8% in liver, and were similar to the one obtained for European hake (Domínguez-Petit, 2007; Lloret et al., 2008). Generally, water tissue content follows the opposite trend with organic components since water proportion increases when energy is consumed and, thus, it is often considered as an indicator of individual condition (Lambert and Dutil, 2000; Dutil et al., 2003; Alonso-Fernández and Saborido-Rey, 2011). In this study, it was observed that the water content measurement allows a reasonable forecast of the lipid content present in the three tissues analyzed (muscle, gonads and liver). Thus, a simple condition index based on the estimation of moisture may be used as a fast indicator of lipid reserves present in tissues of Argentine hake as suggested in *M. merluccius* (Domínguez-Petit, 2007; Lloret et al., 2008) and in another species (Hartman and Margraf, 2008).

Proximate composition allows calculation of the energy available, and understanding of how energy stores are partitioned among different tissues and chemical constituents (Domínguez-Petit and Saborido-Rey, 2010). Energy density mean value for muscle, gonad and liver was 4.7 kJ g^{-1}, 9.5 kJ g^{-1} and 15.9 kJ g^{-1}, respectively. The muscle energy content was principally related to protein content representing on average 81% of its total energy. Liver was the tissue with the highest energetic level in comparison to gonad and muscle. The liver ED was related to lipid content reaching up to 87% of its total energy. In gonad, the increase in ED was due to an increase in both lipid and protein content. Although proteins are the main component in female gonadal tissue, changes in ovarian energy density are also closely associated to lipid content variations, like was mentioned by Alonso-Fernández and Saborido-Rey (2012) for *Trisopterus luscus*.

The low coefficient of determination between the lipids in muscle and gonads and the fact that there was not relationship between liver and gonad lipids content, does not allow a precise estimation of the relationship between maternal condition and gonadal

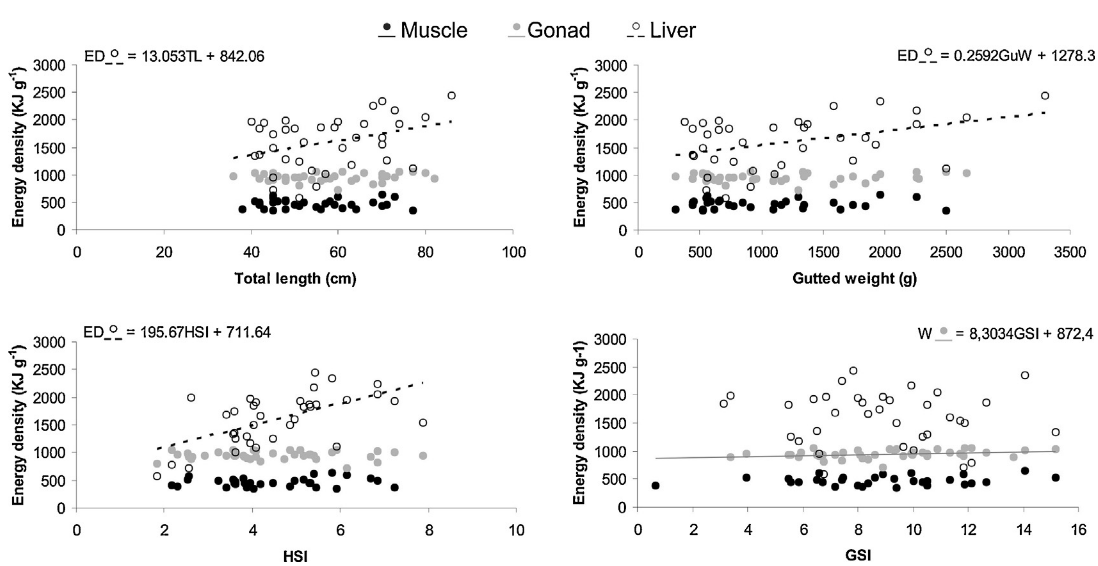

Fig. 6. Muscle, liver and gonad energy density (kJ g^{-1}) in relation to female morphometrics (total weight and gutted weight) and somatic condition indices (HSI and GSI) obtained for *Merluccius hubbsi*.

development. These are unexpected but interesting results, although there were differences in nutritional status (mainly due to lipids stored in the liver), all hake females reached the same proportion of lipid and protein reserves in their gonads. This agrees with the suggestion that the liver acts as a buffer between the soma and the ovaries, in order to ensure ovarian maturation even at low rates of energy intake (Allen and Wootton, 1982). Lipids and their constituent fatty acid have a particularly important role in the reproductive parameters of fish such as egg quality, spawning, hatching rate and survival of larvae (Sargent et al., 2002; Rainuzzo et al., 1997; Yanes-Roca et al., 2009). In this sense, studies of lipids class composition in gonads are needed to obtain a better overview of the link between the maternal condition and gonad development. On the other hand, the lack of a relationship between liver and muscle proximate composition and gonads may be due to the fact that these tissues, or their reserves, contribute to gonad composition for only short periods, and are thus not critical for ovary maturation (Domínguez-Petit, 2007; Domínguez-Petit et al., 2010).

It was observed that chemical composition, especially fat contents, change among individuals of the same species, being a common characteristic of free-living species (Shulman and Love, 1999). In many fish species it was also pointed out that the capacity of storing energy depends on body size and that lipid accumulation is directly related to the diet (Schultz and Conover, 1999, Lloret et al., 2008). In liver and muscle of *M. hubbsi* larger individuals showed a higher amount of energy stored in the form of lipids than smaller ones. This size effect could be explained as an ontogenetic variation in the hake diet; previous studies have recorded a change in the diet related to the length of the fish (Angelescu and Prenski, 1987; Sánchez, 2009). From 50 cm LT onwards, there is a transition from a diet based mainly on planktonic crustaceans to other based mainly on fish such as anchovy and squid. Even though there are no data on the proximal composition of planktonic crustaceans from the study area, in general squid and anchovy are characterized by high concentrations of fatty acids (Watanabe et al., 1984; Massa et al., 2007). On the other hand, Macchi et al. (2013) showed a decrease in frequency of empty stomachs with the size of the Argentine hake females, evidencing that feeding activity is higher in larger individuals. In fact, it was observed that Argentine hake females larger than 55 cm TL showed a change in oocyte dry mass vs TL relationship (Macchi et al., 2006), which may be associated to the variation in diet composition (Macchi et al., 2013). It was also observed that lipid contents in the liver of European hake (*M. merluccius*) females during the maturity stage depend on size (Lloret et al., 2008). This effect of size on the storage of lipid reserves was explained through an ontogenetic variation in the diet of the *M. Merluccius as*, similar to *M. hubbsi*, there was a transition from a diet based on crustaceans to another based on fish (Bozzano et al., 1997; Carpentieri et al., 2005). Our analyses also revealed that females having higher lipid reserves in their livers had high muscle reserves, which suggest that bigger breeding females have a better energy condition during the reproductive season. Similar results were found in the Brazilian flathead (*Percophis brasiliensis*) by Rodrigues (2012). The difference in condition of hake females associated to their size could eventually affect the reproductive potential of this species, affecting variables like the time and extension of the breeding season, spawning frequency and fecundity. However, the results also showed that there was not relationship between gonad lipids content and the female size; which suggest, on the contrary, that there is not difference in reproductive effort depending on female size (larger females do not have better gonad condition).

In fishery science and ecological studies, condition indices are widely used to express the energy reserves stored within individual fish. For this reason, it is important to validate the link between condition indices and the lipids and proteins content. The present study revealed that the condition factor K is not a good index for Argentine hake females, since it was not related to the lipids or the energy present in the three tissues analyzed. This index was only positively correlated to muscle proteins, although model prediction was very low. Similar results have been found in *T. luscus* another Bach spawner member of the family Gadidae (Alonso-Fernández and Saborido-Rey, 2012). Macchi et al. (2006), analyzed the influence of size, age and K on oocyte weight of *M. hubbsi*, concluding that this index is not the most appropriate to describe the female condition. Similar results were also observed in *M. merluccius* (Domínguez-Petit, 2007). On the other hand, different to what reported for European hake (Domínguez-Petit, 2007), the HSI in *M. hubbsi* was the index that best reflected the concentration of lipids within the liver and muscle. Similar results were found in other species with indeterminate annual fecundity such as yellowfin tuna, *Thunnus albacares* (Zudaire, et al.) and in the gadide *T. luscus* (Alonso-Fernández and Saborido-Rey, 2012). For this reason, would be important that futures studies on variations of the reproductive potential in relation to parental stock composition of *M. hubbsi* include HSI estimates, because it can be considered a better indicator of female condition.

5. Conclusions

Despite the handicap imposed by the asynchrony of the reproductive process of hake, not only at the individual, but also at the population level; in general, most of the results obtained demonstrates that (i) lipids are the best biochemical components to show female Argentine hake condition; (ii) energy reserves are mainly associated with the lipids stored in liver; (iii) HSI was the only index showing changes on lipid and energy content which can be considered a good estimator of the nutritional condition; and (iv) water content on different tissues (an easy variable to estimate) can be considered a good index to show female condition reflecting the lipid content on muscle, liver and gonad.

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References

Akpinar, M.A., Görgün, S., Akpinar, A., 2009. A comparative analysis of the fatty acid profiles in the liver and muscles of male and female *Salmo trutta macrostigma*. Food Chem. 112, 6–8.

Allen, J.R.M., Wootton, R.J., 1982. The effect of ration and temperature on the growth of the three-spined stickleback, *Gasterosteus aculeatus* L. J. Fish Biol. 20 (4), 409–422.

Alonso-Fernández, A., Saborido-Rey, F., 2011. Maternal influence on the variation of the reproductive cycle of *Trisopterus luscus* (Gadidae). Cienc. Mar. 37, 619–632.

Alonso-Fernández, A., Saborido-Rey, F., 2012. Relationship between energy allocation and reproductive strategy in *Trisopterus luscus*. J. Exp. Mar. Biol. Ecol. 416/417, 8–16.

Angelescu, V., Prenski, L.B., 1987. Ecología trófica de la merluza común del mar argentino (Merluccidae, *Merluccius hubbsi*): II. Dinámica de la alimentación analizada sobre la base de las condiciones ambientales, la estructura y las evaluaciones de los efectivos en su área de distribución. Contrib. Inst. Nac. Invest. Des. Pesq. Argentina 561, 205.

Anthony, J.A., Roby, D.D., Turco, K.R., 2000. Lipid content and energy density of forage fishes from the northern Gulf of Alaska. J. Exp. Mar. Biol. Ecol. 248, 53–78.

AOAC, 1995. Official Methods of Analysis, 16th ed. AOAC, Arlington, VA.

Bozzano, A., Recasens, L., Sartor, P., 1997. Diet of the European hake *Merluccius merluccius* (Pisces: Merluccidae) in the Western Mediterranean (Gulf of Lions). Sci. Mar. 61, 1–8.

Brown-Peterson, N.J., Wyanski, D.M., Saborido-Rey, F., Macewicz, B.J., Lowerre-Barbieri, S.K., 2011. A standardized terminology for describing reproductive development in fishes. Mar. Coast. Fish. 3, 52–70.

Carpentieri, P., Colloca, F., Cardinale, M., Belluscio, A., Ardizzone, G.D., 2005. Feeding habits of European hake (*Merluccius merluccius*) in the central Mediterranean Sea. Fish. Bull. 103, 411–416.

Claramunt, G., Serra, R., Castro, L.R., Cubillos, L., 2007. Is he spawning frequency dependent on female size? Empirical evidence in *Sardinops sagax* and *Engraulis ringens* off northern Chile. Fish. Res. 85, 248–257.

Connell, J.J., 1975. Control of Fish Quality. Fishing News Books Ltd., Farnham.

Cousseau, M.B., Perrotta, R.G., 1998. Peces Marinos de Argentina: Biología, distribución, pesca. INIDEP, Mar del Plata.

Domínguez-Petit, R., (Doctoral thesis) 2007. Study of reproductive potential of *Merluccius merluccius* in the Galician Shelf. University of Vigo, Spain, doi:10261/4377.

Domínguez-Petit, R., Saborido-Rey, F., 2010. New bioenergetic perspective of European hake (*Merluccius merluccius* L.) reproductive ecology. Fish. Res. 104, 64–72.

Domínguez-Petit, R., Saborido-Rey, F., Medina, I., 2010. Changes of proximate composition, energy storage and condition of European hake (*Merluccius merluccius*, L. 1758) through the spawning season. Fish. Res. 104, 73–82.

Dutil, J.D., Lambert, Y., Chabot, D., 2003. Winter and spring changes in condition factor and energy reserves of wild cod compared with changes observed during food deprivation in the laboratory. ICES J. Mar. Sci. 60, 780–786.

Eder, E.B., Lewis, M.N., 2005. Proximate composition and energetic value of demersal and pelagic prey species from the South-West Atlantic Ocean. Mar. Ecol. Prog. Ser. 291, 43–52.

Ehrlich, M., Macchi, G.J., Madirolas, A., Machinandiarena, L., 2013. Vertical distribution of hake *Merluccius hubbsi* in spawning aggregations in north Patagonian waters of the Southwest Atlantic. Fish. Res. 138, 89–98.

Exler, J., Kinsella, J.E., Watt, B.K., 1975. Lipids and fatty acids of important finfish: new data for nutrient tables. J. Am. Oil Chem. Soc. 52, 154–159.

Faahraeus-Van Ree, G.E., Spurrell, D.R., 2003. Structure of and energy reserves in the liver of wild and cultured yellowtail flounder, Limanda ferruginea. Mar. Biol. 143, 257–265.

Green, A.J., 2001. Mass/length residuals: measures of body condition or generators of spurious results? Ecology 82, 1473–1483.

Hartman, K.J., Margraf, F.J., 2008. Common relationships among proximate composition components in fishes. J. Fish Biol. 73, 2352–2360.

Herbes, S., Allen, C., 1983. Lipid quantification of freshwater invertebrates: method modification for microquantitation. Can. J. Fish. Aquat. Sci 40, 1315–1317.

Huss, H.H., 1995. Quality and quality changes in fresh fish. FAO Fish. Tech. Paper. 348.

Jobling, M., 1995. Environmental Biology of Fishes. Fish and Fisheries Series 16. Chapman & Hall, London.

Kjesbu, O.S., Klungsoyr, J., Kryvi, H., Witthames, P.R., Greer Walker, M., 1991. Fecundity, atresia and egg size of captive Atlantic cod (*Gadus morhua*) in relation to proximate body composition. Can. J. Fish. Aquat. Sci. 48, 2333–2343.

Kleiber, M., 1975. The Fire of Life: An Introduction to Animal Energetics. Robert E. Krieger Publishing Company, New York, pp. 453.

Korta, M., Domínguez-Petit, R., Murua, H., Saborido-Rey, F., 2010. Regional variability in reproductive traits of European hake *Merluccius merluccius* L. populations. Fish. Res. 104, 64–72.

Kurita, Y., Meier, S., Kjesbu, O.S., 2003. Oocyte growth and fecundity regulation by atresia of Atlantic herring (*Clupea harengus*) in relation to body condition throughout the maturation cycle. J. Sea Res. 49, 203–219.

Lambert, Y., Dutil, J.D., 1997. Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of Atlantic cod (*Gadus morhua*)? Can. J. Fish. Aquat. Sci. 54, 104–112.

Lambert, Y., Dutil, J.D., 2000. Energetic consequences of reproduction in Atlantic cod (*Gadus morhua*) in relation to spawning level of somatic energy reserves. Can. J. Fish. Aquat. Sci 57, 815–825.

Leonarduzzi, E., Massa, A., Manca, E., 2012. Variación de la composición bioquímica en hembras de merluza común (*Merluccius hubbsi*) durante su ciclo reproductivo. Rev. Invest. Desarr. Pesq. 21, 5–19.

Lloret, J., Gil de Sola, L., Souplet, A., Galzin, R., 2002. Large-scale habitat variability in condition of demersal fishery species in the north-western Mediterranean. ICES J. Mar. Sci. 59, 1215–1227.

Lloret, J., Demestre, M., Sanchez-Pardo, J., 2008. Lipid (energy) reserves of European hake (*Merluccius merluccius*) in the north-western Mediterranean. Vie Milieu 58, 75–85.

Lowry, O.H., Rosbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Macchi, G.J., Pájaro, M., Ehrlich, M., 2004. Seasonal egg production pattern of the Patagonian stock of Argentine hake (*Merluccius hubbsi*). Fish. Res. 67, 25–38.

Macchi, G.J., Pájaro, M., Madirolas, A., 2005. Can a change in the spawning pattern of Argentine hake (*Merluccius hubbsi*) affect its recruitment? Fish. Bull. 103, 445–452.

Macchi, G.J., Pájaro, M., Militelli, M.I., Radovani, N., Rivas, L., 2006. Influence of size, age and maternal condition on the oocyte dry weight of Argentine hake (*Merluccius hubbsi*). Fish. Res. 80, 345–349.

Macchi, G.J., Pájaro, M., Dato, C., 2007. Spatial variations of the Argentine hake (*Merluccius hubbsi*) spawning shoals in the Patagonian area during a reproductive season. Rev. Biol. Mar. Oceanogr. 42, 345–356.

Macchi, G.J., Martos, P., Reta, R., Dato, C., 2010. Offshore spawning of the Argentine hake (*Merluccius hubbsi*) Patagonian stock. Panam. J. Aquat. Sci. 5, 22–35.

Macchi, G.J., Leonarduzzi, E., Diaz, M.V., Renzi, M., Rodrigues, K., 2013. Maternal effects on the fecundity and egg quality of the Argentine hake *Merluccius hubbsi* patagonian stock. Fish. Bull. 111, 325–336.

Marteinsdottir, G., Begg, G.A., 2002. Essential relationships incorporating the influence of age, size and condition on variables required for estimation of reproductive potential in Atlantic cod *Gadus morhua*. Mar. Ecol. Prog. Ser. 235, 235–256.

Marteinsdottir, G., Steinarsson, A., 1998. Maternal influence on the size and viability of Iceland cod *Gadus morhua* eggs and larvae. J. Fish Biol. 52, 1241–1258.

Marteinsdottir, G., Thorarinsson, K., 1998. Improving the stock–recruitment relationship in Icelandic cod (*Gadus morhua* L.) by including age diversity of spawners. Can. J. Fish. Aquat. Sci. 55, 1372–1377.

Massa, A.E., Yeannes, M.I., Manca, E.A., 2007. Ácidos grasos poliinsaturados de la serie Omega – 3 en ejemplares bonaerenses y patagónicos de anchoíta argentina. Rev. grasas y aceites 66, 568–572.

Mehault, S., Domínguez-Petit, R., Cerviño, S., Saborido-Rey, F., 2010. Variability in total egg production and implications for management of the southern stock of European hake. Fish. Res. 104, 11–122.

Pájaro, M., Macchi, G.J., Martos, P., 2005. Reproductive pattern of the Patagonian stock of Argentine hake (*Merluccius hubbsi*). Fish. Res. 72, 97–108.

R Development Core Team, R., 2012. In: Computing, R.F.f.S. (Ed.), R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture 155, 103–115.

Rodrigues, K.A., (Doctoral thesis) 2012. Estrategia reproductiva del pez palo, Percophis brasiliensis (Quoy et Gaimard, 1824) en aguas costeras de la provincial de Buenos Aires. National University of Mar del Plata, Argentine, pp. 207.

Rueda, F.M., Lopez, J.A., Martinez, F.J., Zamora, S., Divanach, P., Kentouri, M., 1997. Fatty acids in muscle of wild and farmed red porgy, *Pagrus pagrus*. Aquacult. Nutr. 3, 161–165.

Sánchez, F., 2009. Alimentación de la merluza (*Merluccius hubbsi*) en el golfo San Jorge y aguas adyacentes. Technical Report INIDEP, Argentina 75/09, 21 pp.

Santos, B.A.Y, Villarino, M.F., 2013. Evaluación del estado de explotación del efectivo sur de 41° S de la merluza (*Merluccius hubbsi*) y estimación de las capturas biológicamente aceptables correspondiente al año 2014. Technical Report INIDEP, Argentina 29/13, 30 pp.

Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. , 3rd ed. Academic Press, San Diego, pp. 181–257.

Schulte-Hostedde, A.I., Zinner, B., Millar, J.S., Hickling, G.J., 2005. Restitution of mass-size residuals: validating body condition indices. Ecology 86, 155–163.

Schultz, E.T., Conover, D.O., 1999. The allometry of energy reserve depletion: test of mechanism for size-dependent winter mortality. Oecologia 119, 474–483.

Scott, B., Marteinsdottir, G., Wright, P., 1999. Potential effects of maternal factors on spawning stock–recruitment relationships under varying fishing pressure. Can. J. Fish. Aquat. Sci. 56, 1882–1890.

Shearer, K.D., Swanson, P., 2000. The effect of whole body lipid on early sexual maturation of 1+ age male Chinook salmon (*Onchorynchus tshawytscha*). Aquaculture 190, 343–367.

Shulman, G.E., Love, R.M., 1999. The biochemical ecology of marine fishes. In: Southward, A.J., Tayler, P.A., Young, C.M. (Eds.), Advances in Marine Ecology, vol. 36. Academic Press, London, 351 pp.

Spitz, J., Mourocq, E., Schoen, V., Ridoux, V., 2010. Proximate composition and energy content of forage species from the Bay of Biscay: high- or low-quality food? ICES J. Mar. Sci. 67, 900–915.

Undeland, I., Harrod, M., Lingnert, H., 1998. Comparison between methods using low-toxicity solvents for the extraction of lipids from herring (*Clupea harengus*). Food Chem. 61, 355–365.

Vallin, L., Nissling, A., 2000. Maternal effects on egg size and egg buoyancy of Baltic cod, *Gadus morhua*: implications for stock structure effects on recruitment. Fish. Res. 49, 21–37.

VNIRO, 2000. Chemical Composition and Processing Properties of Marine and Ocean Fishes: Handbook. VNIRO, Moscu.

Watanabe, T., Arakawa, T., Kitajima, C., Fujita, S., 1984. Effect of nutritional quality of broodstock diets on reproduction of Red Sea bream. Bull. Jpn. Soc. Sci. Fish. 50, 495–501.

Wicker, A.M., Johnson, W.F., 1987. Relationships among fat content, condition factor, and first-year survival of Florida largemouth bass. Trans. Am. Fish. Assoc. 116, 264–271.

Yanes-Roca., C., Rhody, N., Nystrom, M., Main, K.L., 2009. Effects of fatty acid composition and spawning season patterns on egg quality and larval survival in common snook (*Centropomus undecimalis*). Aquaculture 287, 335–340.

Yaragina, N.A., Marshall, C.T., 2000. Trophic influences on interannual and seasonal variation in the liver condition index of Northeast Arctic cod (*Gadus morhua*). ICES J. Mar. Sci., 42–55.

Zaboukas, N., Miliou, H., Megalofonou, P., Moraitou-Apostolopoulou, M., 2006. Biochemical composition of the Atlantic bonito *Sarda sarda* from the Aegean Sea (eastern Mediterranean Sea) in different stages of sexual maturity. J. Fish Biol. 69, 347–362.