

# LIPID (ENERGY) RESERVES OF EUROPEAN HAKE (*MERLUCCIIUS MERLUCCIIUS*) IN THE NORTH-WESTERN MEDITERRANEAN

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FISHERIES  
FISH ECOPHYSIOLOGY  
ENERGY RESERVES  
REPRODUCTION  
CONDITION INDICES

**ABSTRACT.** – This study analyses for the first time the lipid (energy) reserves of European hake (*Merluccius merluccius*) in the north-western Mediterranean from an ecophysiological perspective. Results show that there is a progressive accumulation of lipids in the liver of maturing hake -where the bulk of the fat is stored- as individuals grow. Results also indicate that female pre-spawners expend much energy on reproductive activities since they present lower liver lipid reserves than juveniles and maturing individuals. Furthermore, results show that female pre-spawners with higher lipid reserves in their livers had a higher amount of lipids in their ovaries, suggesting that maternal condition (spawner quality) may affect the reproductive potential of hake. Overall, the results of this study suggest that the analysis of liver lipid reserves during pre-spawning, along with the evaluation of the gonadosomatic index and the consideration of the reproductive stage, can contribute to improve the estimation of the reproductive potential of gadoid species such as hake.

## INTRODUCTION

The European hake (*Merluccius merluccius* Linnaeus, 1758) is an economically important species in the north-western Mediterranean and one of the main demersal target species of fisheries in this area (reviewed by Oliver & Massutí 1995). Although this species is widely distributed in the Mediterranean from 30 to 800 m depth, its distribution pattern changes spatially and seasonally in relation to the reproductive cycle, with juveniles and maturing fish mainly concentrated on the continental shelf (50-200 m) and adult females living deeper on the slope and in submarine canyons (Demestre & Sánchez 1998). Long-liners tend to catch large adult female hake in the slope and canyons as opposed to trawlers and gill-netters that take small and medium sizes respectively on the continental shelf (Aldebert *et al.* 1993).

Although several aspects of hake's biology in the Mediterranean have been studied such as larval distribution (Olivar *et al.* 2003), growth (Aldebert & Morales-Nin 1992, Morales-Nin & Moranta 2004), recruitment (Lloret *et al.* 2001, Lloret & Lleonart 2002, Maynou *et al.* 2003, Hidalgo *et al.* 2008), diet (Papaconstantinou & Caragitsou 1987, Bozzano *et al.* 1997, Carpentieri *et al.* 2005, Ferraton *et al.* 2007), maturity (Recasens 1992), population structure and dynamics (Aldebert *et al.* 1993, Recasens *et al.* 1998, Hidalgo *et al.* 2008) and bathymetric and spatial distribution (Demestre & Sánchez 1998, Orsi-Relini *et al.* 2002), information on the energy reserves of this species is still lacking. Fat content has been evaluated in the muscle of hake for nutritional purposes (Perez-Villarreal & Howgate 1987, Tornaritis *et al.* 1993, Soriguier *et al.* 1997). For ecological purposes, simple morphomet-

ric condition factors based on body and liver sizes have been considered (Lloret *et al.* 2002, Ferraton *et al.* 2007, Hidalgo *et al.* 2008). Whilst these condition indices provide a rough measurement of the well-being of fishes (Bolger & Connolly 1989), more accurate measurements of energy reserves based on biochemical factors, such as lipid content in different organs are needed (Shulman & Love 1999).

Energy reserves are a particularly important attribute of fishes because they have a large influence on growth, reproduction and survival (reviewed by Shulman & Love 1999). Low energy reserves may lower the chances of survival, leading to an increase of natural mortality (Cunjak 1988, Griffiths & Kirkwood 1995, Sogard & Olla 2000). Starvation due to exhaustion of energy reserves, particularly during the non-feeding and reproductive periods, weakens fishes and renders them more susceptible to predation and fishery, diseases and to a variety of environmental stressors (Shulman & Love 1999). Inadequate reserves have been implicated in the reduced reproductive potential of several fish species through reduced fecundity and quality of eggs and larvae or delayed maturation (reviewed by Lambert *et al.* 2003, Koops *et al.* 2004). A reduction in fish energy reserves does not only affect the own population success but may impact as well on the reproductive output of top predators (Wanless *et al.* 2005). Similar to this, condition of invertebrate species such as red shrimp (*Aristeus antennatus*) has been found to be an important aspect for the reproductive and recruitment success of these species (Carbonell *et al.* 2008).

From the available biochemical parameters for evaluating the energy reserves of fishes, determination of fat content has been the most widely used (Shulman 1974,

Shulman & Love 1999, Adams 1999, Shulman *et al.* 2005, Lloret *et al.* 2003, 2005, 2007). Lipids, together with proteins and carbohydrates, are principal energy stores in teleosts and are often mobilized during non-feeding and reproductive periods (Shulman & Love 1999). Lipids are also known to play a role as regulators of body density, cellular metabolism, detoxification, behaviour and reproduction, and a deficiency can negatively affect gonad development, fecundity, fertilization and hatching rates (Shulman & Love 1999, Adams 1999, Morris & Culkin 2000). Thus, lipid storage and dynamics within the organism are a particularly important aspect of fish health and population success. Fat content is mainly affected by habitat characteristics and the food supply, and thus possesses great potential as a 'marker' of habitat quality and feeding conditions (Shulman *et al.* 2005, Levi *et al.* 2005, Lloret *et al.* 2003, 2005, 2007).

The aim of the present study is to analyse for the first time, from an ecophysiological perspective, the lipid reserves in the liver (as a measure of somatic energy reserves) and ovary (as a measure of energy reserves invested by females into reproduction) of European hake. In particular, the work evaluates the liver and ovary lipid content in relation to fish size, sex and maturity stage, analyzes the relationship between the lipid reserves in the liver and the ovary and explores the use of simple condition indices based on liver weights and water content. The paper provides arguments on the need of evaluating the lipid reserves of exploited species in order to better understand their reproductive potential.

## MATERIALS AND METHODS

**Sample collection:** 57 juvenile and 35 maturing European hake (*Merluccius merluccius*) were captured in June 2004 on the continental shelf of the north-western Mediterranean (Fig. 1), between 40°24.36 and 43°13.06N (16 stations; Table I). These individuals were gathered by trawling during the annual Spanish and French MEDITS groundfish surveys and from commercial trawlers. Groundfish surveys followed the technical specifications given in Bertrand *et al.* (2002). Because there are significant bathymetric differences in condition of juvenile and maturing hake on the continental shelf of the north-western Mediterranean (Lloret *et al.* 2002, Ferraton *et al.* 2007), we restricted our sampling to depths comprised between 60 and 145 m. Total lengths of juveniles ranged between 19.0 and 30.0 cm (mean = 22.18 cm; Fig. 2a), whereas total length of maturing individuals ranged between 19.5 and 44.5 cm (mean = 30.20 cm; Fig. 2a). Maturing fishes were classified according to sex by visual inspection of the gonads (total: 25 males and 10 females) whilst the juveniles remained unsexed since it was difficult to distinguish their sex. Furthermore, 74 female pre-spawners measuring between 38.0 and 73.0 cm (mean = 51.38 cm; Fig. 2b) were sampled in two areas near the slope and the submarine canyons (100-258 m depth): Gulf of Lions (5 stations) and

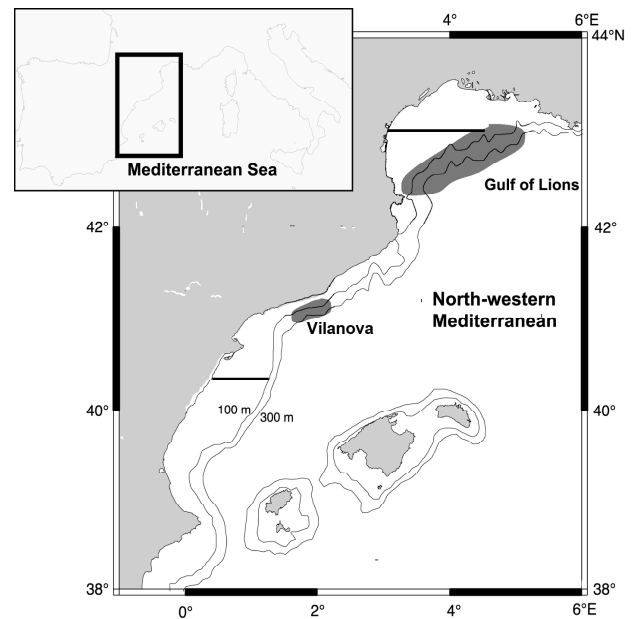


Fig.1. – Map of the north-western Mediterranean showing the area where hake were collected. The straight lines indicate the latitudinal limits of the continental shelf where juveniles and maturing individuals were collected with commercial trawls and groundfish surveys. The shadowed parts represent the two areas (Vilanova and Gulf of Lions) where female pre-spawners were sampled with commercial longlines.

Vilanova (3 stations; Table I, Fig. 1), from longliners during the peak of the reproductive season (between October and December 2004, Recasens 1992).

Individuals were eviscerated, and livers and ovaries were removed. All ovaries included in the lipid analysis were classified macroscopically to be in the late pre-spawning maturity stage: ovaries were large, with blood capillaries, yellow/orange coloured and with visible but still non-hydrated oocytes. Although initially more than 74 females were collected, some of them were in the early pre-spawning phase (their eggs and ovaries were small) or were already spawning (hydrated eggs), and were therefore disregarded for the analyses. Total length was measured to the nearest 0.5 cm, and eviscerated body, liver and ovary weights were measured to the nearest 0.01 g.

**Evaluation of lipid reserves:** Although it is already known that lipid concentration in the muscle of European hake is very low (less than ca 2 % wet weight or ca 6 % dry weight, Tornatiris *et al.* 1993, Soriguer *et al.* 1997) and that in other gadoid species, such as cod, lipids are stored primarily in the liver (Marshall *et al.* 1999), we checked the hake's lipid compartmentalization by analyzing the ratios of the lipid content in the liver and muscle of four female pre-spawners. Determination of total lipid was carried out in these tissues since mesenteric and subcutaneous fat depots were lacking. Given that in these four female pre-spawners lipids only constituted on average 3.10 % of the dry muscle compared to 69.06 % of the dry liver, and that the absolute fat content in the muscle of these four individuals averaged 4.07 g compared to 7.20 g in the liver, we assumed that muscle constitute a minor deposit of lipids in hake, and thus we proceed

Table I. – Number of individuals collected by reproductive stage at the different stations. MEDITS ES and MEDITS FR: Spanish and French groundfish surveys, respectively. V: Vilanova's area. GL: Gulf of Lions' area.

Fishing method	Area	Latitude	Longitude	Depth (m)	Juveniles	Maturing	Pre-spawning
Trawlers		40°24.36N	1°02.69E	75	22	10	0
MEDITS ES		41°01.41N	1°22.58E	143	4	0	0
		41°32.93N	2°47.93E	140	6	1	0
		41°08.77N	1°47.47E	56	11	0	0
		41°18.28N	2°15.68E	140	3	1	0
		41°23.95N	2°18.00E	61	3	5	0
		41°25.75N	2°30.49E	92	1	1	0
		41°58.77N	3°16.48E	128	0	1	0
		41°49.17N	3°09.37E	84	2	0	0
MEDITS FR		41°43.55N	3°15.03E	130	2	1	0
		43°09.89N	4°18.10E	86	1	1	0
		43°13.06N	4°10.72E	87	0	7	0
		43°01.41N	4°33.34E	113	0	3	0
		43°14.50N	4°24.46E	62	0	1	0
		43°01.88N	4°32.27E	116	1	3	0
		43°01.23N	4°33.41E	114	1	0	0
Long-liners	V	41°01.40N	1°32.19E	250	0	0	22
	V	41°07.80N	1°51.45E	237	0	0	13
	V	41°04.20N	1°48.90E	236	0	0	3
	GL	43°03.78N	5°01.54E	258	0	0	5
	GL	42°21.00N	3°12.00E	200	0	0	12
	GL	42°51.00N	3°42.00E	120	0	0	15
	GL	42°15.00N	3°12.00E	100	0	0	2
	GL	42°51.60N	3°58.44E	238	0	0	2

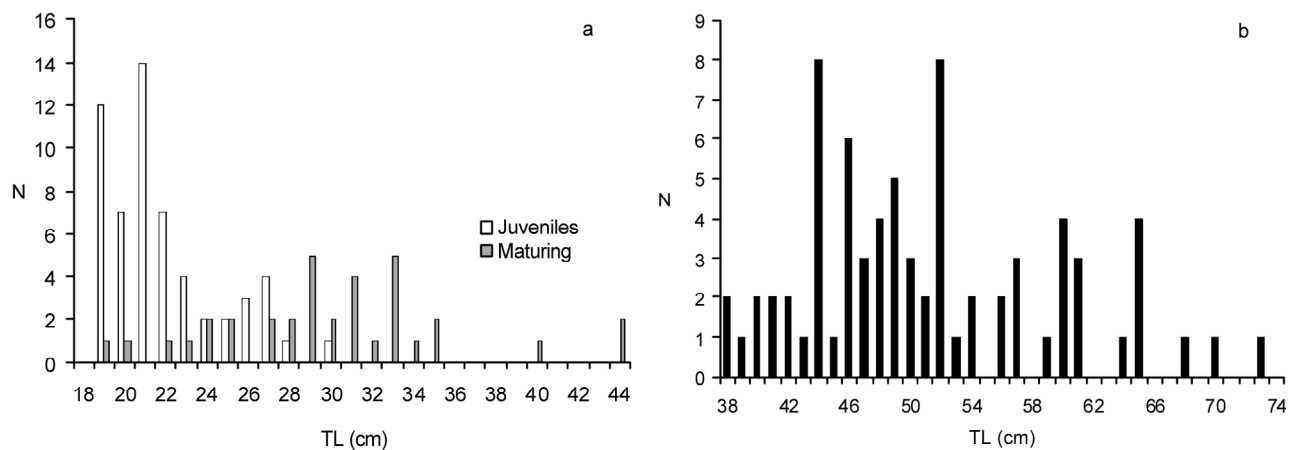


Fig. 2. – Length frequencies of juveniles and maturing individuals (a) and female pre-spawners (b) considered for lipid analyses.

to analyze only the total lipid content (% dry weight) in the liver of the 57 juveniles, 35 maturing individuals and 74 female pre-spawners (as a measure of somatic energy reserves), as well as the total lipid content (% dry weight) in the ovary of the 74 female pre-spawners (as a measure of energy reserves invested in reproduction).

The whole ovary and liver were taken for lipid analysis, whilst for the muscle a portion of ca 5 g was taken dorsally directly under the anterior dorsal fin and well above the lateral

line. After tissues were cleaned of skin, scales and bones, they were rinsed with seawater and immediately frozen at  $-30^{\circ}\text{C}$  for fat content determination in the laboratory.

Livers, ovaries and samples of muscle were unfrozen while deposited on a desiccant paper and were minced on a Petri dish. They were then desiccated to determine dry weights, first at  $70^{\circ}\text{C}$  during 24 h in a stove and second under vacuum for 12 h at ambient temperature. The dried livers, ovaries and muscle samples were put in a mortar together with anhydrous sodium sul-

phate previously desiccated in a stove at 100°C during 24 h, and were then ground to a fine powder.

Total lipids were determined gravimetrically with the Soxhlet method according to Shahidi (2001). Soxhlet is the most commonly used semi-continuous method applied to extraction of lipids from foods (James 1998), including fishes (see e.g. Belk 1993, Tornaritis *et al.* 1993, Rodrigo *et al.* 1998, Lloret *et al.* 2007). Lipids were extracted by repeated washing (percolation) with a volume of 200 ml of Hexane/Acetone (1:1 v/v) for ca 0.5-1.0 g of tissue. This organic solvent mixture flowed during several cycles through the tissue into a glass vitrified capsule (thimble) by distillation using heating mantles. By using pure (100 % fat) cod liver oil (same procedure as for the tissues), we estimated that the method we used was able to extract as much as 99.22 % of total lipids in the tissues.

The ground livers, ovaries and muscles were placed in the porous thimbles of the Soxhlet apparatus together with a number of little glass balls (boiling chips) to avoid splash projections during extraction. The extraction time was between 5 and 8 hours depending on tissue aggregation and taking into account that the distillation rate must be about 2 to 6 drops per second. After the extraction was complete -measured with an internal standard in a similar matrix- the content of the flask was filtered and evaporated using a rotary evaporator and weighed in a previously calibrated flask. The lipid content in the tissues was then calculated from differences in weight. After the gravimetric determination of the total lipid content in the tissues with a balance (to the nearest 0.1 mg), values were corrected for a recovery ratio of 99.22 %.

The relative liver, ovary and muscle lipid content, expressed as a percentage of dry masses, were given, and a Lipid Hepatosomatic index (LHSI) and a Lipid Gonadosomatic index (LGSI) were computed with the formulas  $LHSI = ANSL / EX \times 100$  and  $LGSI = ABSG / EW \times 100$ , where EW is the eviscerated wet weight and ABSL and ABSG are the absolute lipid content in the liver and ovary, respectively, computed by multiplying the respective lipid contents (in % wet weight) by total liver and ovary wet weights.

*Statistical analyses:* Linear regressions were used for identifying relationships between the lipid reserves and fish size and between individuals caught along the latitudinal gradient of the continental shelf. To compare lipid reserves among sexes and maturity stages, as well as to evaluate differences in these reserves between individuals caught on the slope and submarine canyons of the two sampled areas, we used analyses of variance (ANOVA) or analyses of covariance (ANCOVA) with fish size as a covariate when the length effect was significant. LSD post-hoc test was used for comparisons of significant effects after ANOVA. A p-value of 0.01 or less was considered statistically significant. The assumptions of normality and homogeneity of variances were tested with the Kolmogorov-Smirnov and the Cochran's tests, respectively. Data were log-transformed to meet these assumptions. For ANCOVA models, we furthermore tested the homogeneity of regressions (or slopes) among groups with the test of parallelism.

## RESULTS

In the 57 juveniles captured in June 2004 on the continental shelf, lipids constituted between 34.18 % and 86.32 % (mean = 71.98 %, SE = 1.470) of the dry liver whereas LSHI ranged between 0.225 and 3.164 (mean = 1.570, SE = 0.096). In the 35 maturing individuals captured in June 2004 on the continental shelf, the percentage of lipids in the dry liver ranged between 50.98 % and 90.00 % (mean = 74.72 %, SE = 1.626) whereas the LHSI ranged between 0.566 and 4.795 (mean = 2.030, SE = 0.212). In the 74 female pre-spawners sampled near the slope and the submarine canyons during the peak of the reproductive season (between October and December) in 2004, lipids constituted between 11.29 % and 88.37 % (mean = 63.23 %, SE = 2.019) of the dry liver and between 6.54 % and 33.96 % (mean = 21.73 %, SE = 0.508) of the dry gonad. LGSI of these 74 female pre-spawners ranged between 0.012 and 1.137 (mean = 0.346, SE = 0.027).

The conversion of the liver and ovary lipids in dry weight (dw) to wet weight (ww) can be done with the following linear equations, respectively (all individuals from the different reproductive stages together): % lipid ww =  $0.80 \times \% \text{lipid dw} - 17.72$  ( $R = 0.89$ ,  $p < 0.01$ ,  $n = 164$ ) and % lipid ww =  $0.35 \times \% \text{lipid dw} - 1.45$  ( $R = 0.87$ ,  $p < 0.01$ ,  $n = 74$ ).

The relative liver and ovary lipid contents (as % of dry weight) are inversely and significantly related to the water content in these tissues (all individuals from the different reproductive stages together; Fig. 3), even though the relationship is better in the liver ( $R = 0.80$ ) than in the ovary ( $R = 0.40$ ). The LHSI and the LGSI of the 74 female pre-spawners are also inversely and significantly related to the water content in the liver and ovary, respectively (Fig. 3), even though the relationship is better for the liver ( $R = 0.89$ ) than for the ovary ( $R = 0.37$ ) too. Furthermore, the relative lipid content in the liver and the LHSI are positively related to the HSI (all individuals from the different reproductive stages together;  $R = 0.54$ ,  $p < 0.01$  and  $R = 0.95$ ,  $p < 0.01$ ). Similar to this, the relative lipid content in the ovary of the 74 female pre-spawners and the LGSI are positively related to the GSI ( $R = 0.30$ ,  $p < 0.01$  and  $R = 0.89$ ,  $p < 0.01$  respectively).

There are no sexual differences between the relative lipid content in the liver of maturing individuals (Table II). Furthermore, the relative lipid content in the liver of juveniles is not statistically different from that of maturing individuals (males and females together), but in both cases the relative liver lipid content is significantly higher than that of female pre-spawners (ANOVA,  $F = 8.75$ ,  $df = 2$ ,  $p < 0.001$ , post-hoc LSD test, Fig. 4). Similar results are found when considering the LHSI. There are no sexual differences between LHSI of maturing individuals (Table II). The mean LHSI of juveniles is not different from that of maturing fishes but in both cases the mean LHSI is higher than that of female pre-spawn-



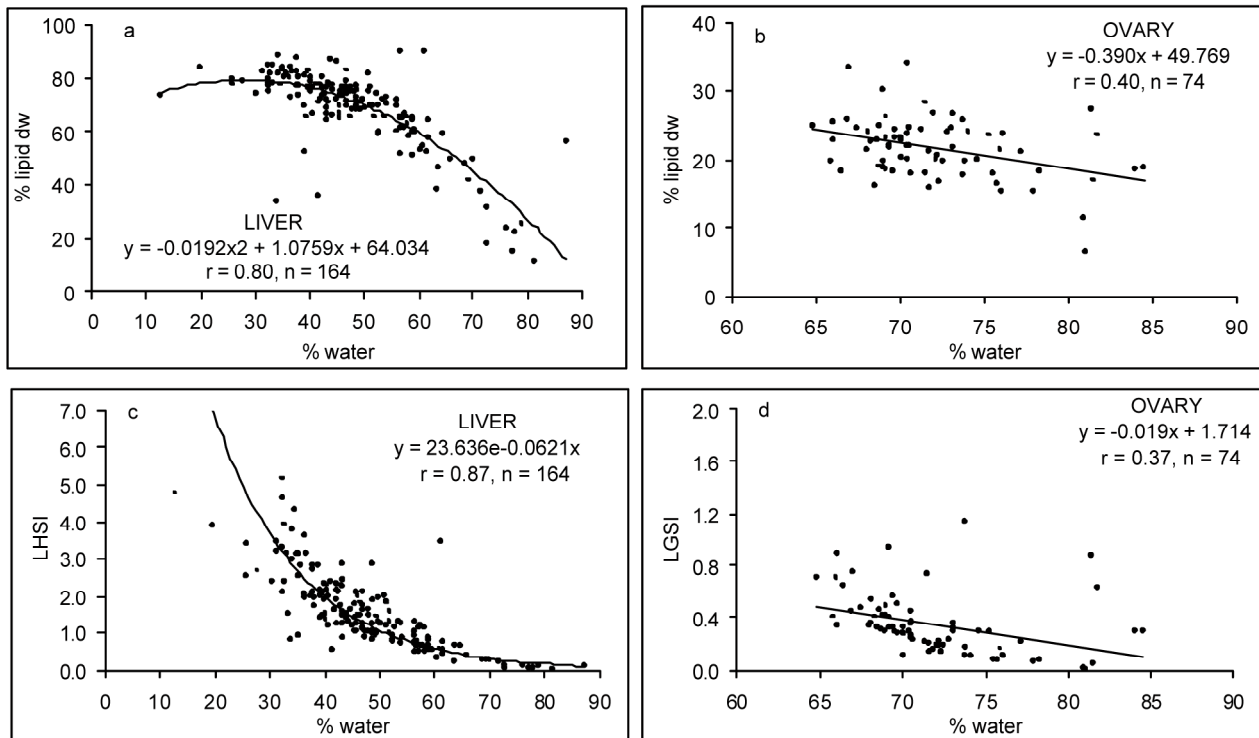


Fig. 3. – Relationships between the lipid content (% dry weight) and the water content in the liver (a, polynomial relation) and ovary (b, linear relation) of hake, between the LHSI and the lipid water content (c, exponential relation), and between the LGSI and the ovary water content (d, linear relation). Liver lipid content and LHSI consider all individuals whilst ovary lipid content and LGSI consider female pre-spawners only.

ers (ANOVA,  $F = 8.97$ ,  $df = 2$ ,  $p < 0.01$ , post-hoc LSD test, Fig. 4).

In maturing fish, the relative lipid content in the liver

and the LHSI were size-dependent, with small individuals having proportionally less lipids and lower LHSI values than larger individuals (Table II; Fig. 5). On the other

Table II. – Summary of results from statistical analyses evaluating the influence of different variables on the liver lipid content (% dry weight) and LHSI of juvenile and maturing hake collected in spring on the continental shelf, and on the liver and ovary lipid content (% dry weight), LHSI and LGSI of female pre-spawners collected in autumn on the slope and the submarine canyons. The symbol \* denotes significance at  $p < 0.01$ .

Variable	Test	Effect	Statistic	p
Juveniles (n = 57)				
% lipid liver	Linear regression	Length	$F = 0.949$ $R = 0.13$	0.334
LHSI	Linear regression	Length	$F = 4.332$ $R = 0.27$	0.052
Maturing individuals (n = 35)				
% lipid liver	ANCOVA	Sex	$F = 0.696$	0.696
		Length	$F = 11.623$	< 0.001*
		Sex x Length	$F = 0.154$	0.696
LHSI	ANCOVA	Sex	$F = 0.043$	0.836
		Length	$F = 11.135$	0.002*
		Sex x Length	$F = 0.517$	0.476
Pre-spawning females (n = 74)				
% lipid liver	Linear regression	Length	$F = 0.272$ $R = 0.006$	0.604
LHSI	Linear regression	Length	$F = 0.295$ $R = 0.006$	0.588
% lipid ovary	Linear regression	Length	$F = 0.030$ $R = 0.002$	0.864
LGSI	Linear regression	Length	$F = 1.208$ $R = 0.13$	0.275

hand, in juvenile fish, the relative lipid content in the liver as well as the LHSI, were size-independent (Table II). The liver and ovary lipid content, the LHSI and the LGSI of female pre-spawners were all size-independent within the length ranges of the fish analyzed (Table II).

There were no significant differences in the liver lipid content and the LHSI between juvenile and maturing individuals caught along the latitudinal gradient of the continental shelf (taking latitude as the explanatory variable) after considering the effect of fish size (linear regression,  $p > 0.01$ ,  $n = 92$ ). Furthermore, there were no significant differences between the liver and ovary lipid content (% dry weight), the LHSI and the LGSI of female pre-spawners caught on the slope and submarine canyons of the two sampled areas (ANOVA,  $p > .01$ ,  $n = 74$ ).

There are significant, positive relationships between the relative lipid content in the liver and the ovary of the 74 female pre-spawners (Fig. 6), as well as between their

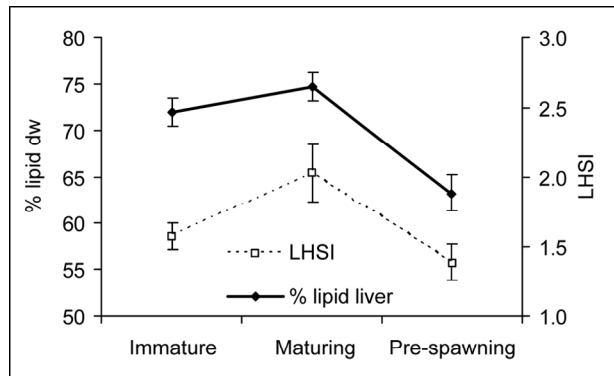


Fig. 4. – Differences in liver lipid content and LHSI (mean  $\pm$  SE) between immature, maturing fish and female pre-spawners.

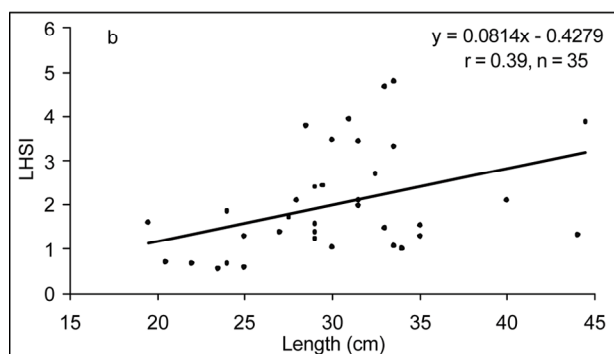
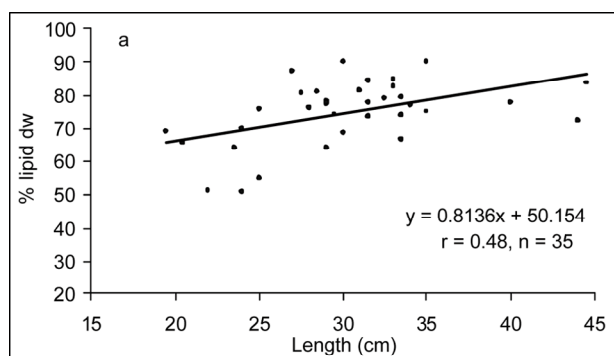


Fig. 5. – Linear relationship between the relative lipid content (a) and the LHSI (b) and the length of maturing hake.

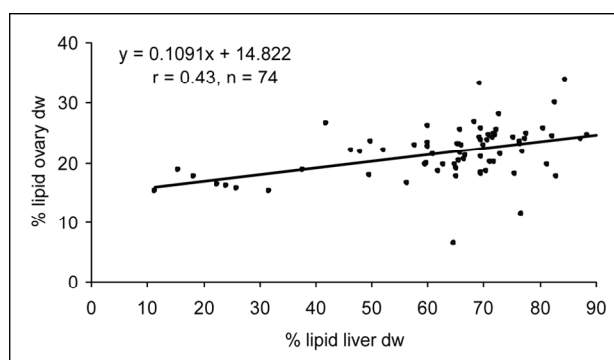


Fig. 6. – Linear relationship between the relative lipid content in the ovary and the relative lipid content in the liver of hake female pre-spawners.

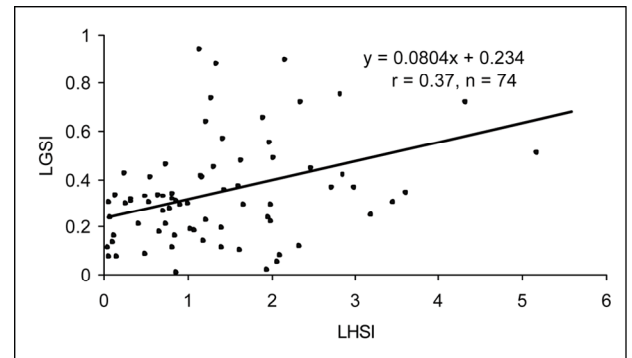


Fig. 7. – Linear relationship between the LGSI and the LHSI of hake female pre-spawners

LGSI and LHSI (Fig. 7). Thus, good condition female pre-spawners, i.e. those with higher relative lipid reserves in their livers and higher LHSI values, had higher relative lipid percentage in their ovaries and higher LGSI values than poor condition ones.

## DISCUSSION

Despite lipid concentrations varied greatly among individuals, results show that in the European hake lipids are stored mainly in the liver, which contains between 12 and 90 % total lipids dry weight (2 and 67 % in wet weight). These values are similar to those reported in the livers of other gadoids, e.g. cod (between 30 and 78 % wet weight, Falch *et al.* 2006), confirming the important role of the liver for energy storage in this family. During pre-spawning, lipids represented between 6 and 34 % of the dry ovary weights of hake. These values fall approximately within the range that has been reported for ovaries or eggs of other fish species, i.e. 8 to 35 % dry weight (reviewed by Riis-Vestergaard 2002). The high variation in lipid reserves between individuals of the same species is a common feature in wild fishes (Shulman & Love 1999) and may reflect the large differences in nutritional status and reproductive potential of individuals within a given population. This strong inter-population variability of lipid reserves probably explains the low correlation values found between the different variables studied.

The increase of the relative lipid content in the liver of maturing hake with size indicates a progressive accumulation of energy in the liver as fish grows till sexual maturation. This size effect may be explained by the ontogenetic variation in the feeding of hake since several studies (Bozzano *et al.* 1997, Cartes *et al.* 2004, Carpentieri *et al.* 2005, Ferraton *et al.* 2007) have indicated a transition from a crustacean-based to a fish-based diet as hake grows, with fish being more energetic as food supply than small crustaceans such as *Natantia* decapods (Ferraton *et al.* 2007).

Female pre-spawners seem to expend much energy on reproductive activities since they presented lower liver

lipid reserves than those of juveniles and maturing individuals. Thus, results suggest that lipids stored in the liver of hake during the maturing stage are mobilized towards the gonad during pre-spawning for reproductive purposes. In general, lipid reserves stored in the body of fishes are greatly diminished during breeding in order to supply energy to the ovary for intensive generative synthesis and to supply essential nutrients such as fatty acids and lipid soluble nutrients (Chelappa *et al.* 1989, Wiegand 1996, Brooks *et al.* 1997, Adams 1999, Morris & Culkin 2000, Okuda 2001). Although this study has detected differences in the lipid content of hake tissues depending on the development and reproductive stage, it must be noticed that these could be due in part to seasonal and bathymetric effects since juveniles and maturing individuals were collected in spring on the shelf, whereas pre-spawning females were collected in autumn on the canyons and the slope. The complex distribution pattern of hake, which changes spatially and seasonally in relation to the reproductive cycle (Demestre & Sánchez 1998), impedes the realisation of a more detailed analysis.

Since pre-spawning females with higher liver lipid reserves had significantly larger lipid reserves in their ovaries, results indicate that maternal condition may affect the reproductive potential of hake, suggesting that the analysis of liver lipid reserves during pre-spawning in gadoid species such as hake can contribute to improve the estimation of their reproductive potential. However, the relatively low correlation between liver and ovary lipid reserves does not allow estimating precisely the linkage between maternal and reproductive potential and suggests that other factors such as the amount or ratio of certain lipid classes in the ovary or the ovary protein reserves, are also important. Recent research has shown that the condition of adult females influences the reproductive potential of several fish species. Inadequate reserves have been implicated in the reduced reproductive potential through lower fecundity, e.g. cod *Gadus morhua* (Kjesbu *et al.* 1991, Lambert & Dutil 2000, Marteinsdottir & Begg 2002), herring *Clupea harengus* (Oskarsson *et al.* 2002) and haddock *Melanogrammus aeglefinus* (Blanchard *et al.* 2003). Low maternal condition has been linked to a lower total egg dry weight in cod (Lambert & Dutil 2000, Ouellet *et al.* 2001), a high incidence of atresia in herring (Oskarsson *et al.* 2002) and a smaller egg diameter in cod (Ouellet *et al.* 2001) and haddock (Trippel & Neil 2004). Low energy reserves can also lead to an increase in age and size at maturity, e.g. cod (Marteinsdottir & Begg 2002) and American plaice *Hippoglossoides platessoides* (Morgan 2004), and to a decrease in larval survival, e.g. sardine *Sardina pilchardus* (Riveiro *et al.* 2000). Ovarian lipids are particularly important since they are a source of energy at the larval stage before first-feeding, and therefore they will influence the larval survival (reviewed by Rainuzzo *et al.* 1997). Special attention should be given to anomalous low adult condition values since they may precede a recruitment failure. Thus for

example, the extreme low energy reserves of Atlantic cod in the Gulf of St Lawrence may have contributed to the collapse of this stock (Lambert & Dutil 2000).

The negative correlation between the concentration of water and fat in the liver of hake makes possible to estimate fat content when water content is known. Thus, a simple condition index based on liver water content could be used regularly as a rapid indicator of liver lipid reserve of this species. In general, fat content and water content of fish are usually related (Shulman & Love 1999, Shulman *et al.* 2005).

It should be also emphasized that this study did not find significant geographic differences in lipid reserves of juvenile and maturing hake caught on the continental shelf. Similar results were found by Lloret *et al.* (2001) and Ferraton *et al.* (2007) using simple condition indices based on individual lengths and weights and liver weights.

Overall, results of this study suggest that the analysis of liver lipid reserves during pre-spawning, along with the evaluation of the gonadosomatic index and the consideration of the reproductive stage, can contribute to improve the estimation of the reproductive potential of gadoid species such as hake. Since the large adult females of hake mainly inhabit the continental slope and submarine canyons of the north-western Mediterranean, the protection of these deep water habitats is important to safeguard the reproductive potential of hake stocks that are suffering from overfishing (Oliver & Massutí 1995, Lleonart & Maynou 2003).

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## REFERENCES

- Adams SM 1999. Ecological role of lipids in the health and success of fish populations. *In* Arts Lipids in freshwater ecosystems, Arts MT, Wainman BC eds. Springer-Verlag, New York: 318-325.
- Aldebert Y, Morales-Nin B 1992. La croissance des juvéniles du merlu dans le Golfe du Lion : nouvelles méthodes d'approche. *Rapp Comm Int Mer Médit* 33: 281-282.
- Aldebert Y, Recasens L, Lleonart J 1993. Analysis of gear interactions in a hake fishery: the case of Gulf of Lions (NW Mediterranean). *Sci Mar* 57: 105-109.
- Belk MC 1993. Growth and mortality of juvenile sunfishes (*Lepomis* sp.) under heavy predation. *Ecol Fresh Fish* 2: 91-98.

- Bertrand J, Gil de Sola L, Papaconstantinou C, Relini G, Souplet A 2002. The general specifications of the MEDITS surveys. *Sci Mar* 66(suppl 2): 9-17.
- Blanchard JL, Frank KT, Simon JE 2003. Effects of condition on fecundity and total egg production of eastern Scotian Shelf haddock (*Melanogrammus aeglefinus*). *Can J Fish Aquat Sci* 60: 321-332.
- Bolger T, Connolly PL 1989. The selection of suitable indices for the measurement and analysis of fish condition. *J Fish Biol* 34: 171-182.
- Bozzano A, Recasens L, Sartor P 1997. Diet of the European hake *Merluccius merluccius* (Pisces: Merlucciidae) in the Western Mediterranean (Gulf of Lions). *Sci Mar* 61(1): 1-8.
- Brooks S, Tyler CR, Sumpter P 1997. Egg quality in fish: what makes a good egg? *Rev Fish Biol Fish*: 387-416.
- Carbonell A, Lloret J, Demestre M 2008. Relationship between condition and recruitment success of red shrimp (*Aristeus antennatus*) in the Balearic Sea (Northwestern Mediterranean). *J Mar Syst* (in press).
- Carpentieri P, Colloca F, Cardinale M, Belluscio A, Ardizzone GD 2005. Feeding habits of European hake (*Merluccius merluccius*) in the central Mediterranean Sea. *Fish Bull* 103: 411-416.
- Cartes JE, Rey J, Lloris D, Gil de Sola L 2004. Influence of environmental variables on the feeding and diet of European hake (*Merluccius merluccius*) on the Mediterranean coasts. *J Mar Biol Ass UK* 84: 831-835.
- Chelappa S, Huntingford FA, Strang RH, Thomson RY 1989. Annual variation in energy reserves in male three-spined stickleback, *Gasterosteus aculeatus* L (Pisces, Gasterosteidae). *J Fish Biol* 35: 275-286.
- Cunjak RA 1988. Physiological consequences of overwintering in streams: the cost of acclimatization? *Can J Fish Aquat Sci* 45: 443-453.
- Demestre M, Sánchez P 1998. Spatio-temporal distribution of the European hake *Merluccius merluccius* off Catalan coast (Northwestern Mediterranean). *Rapp Comm Int Mer Médit* 35: 420-421.
- Falch E, Øverby A, Rustad T 2006. Natural antioxidants in cod liver oil: pitfalls during oxidative stability assessment. In *Seafood research from fish to dish: quality, safety and processing of wild and farmed fish*, Lutén JB ed: 127-137.
- Ferraton F, Harmelin-Vivien M, Mellon-Duval C, Souplet A 2007. Does spatio-temporal variation in diet affect condition and abundance of European hake (*Merluccius merluccius*) juveniles in the Gulf of Lions (NW Mediterranean). *Mar Ecol Prog Ser* 337: 197-208.
- Griffiths D, Kirkwood RC 1995. Seasonal variation in growth, mortality and fat stores of roach and perch in Lough Neagh, Northern Ireland. *J Fish Biol* 47: 537-554.
- Hidalgo M, Massutí E, Moranta J, Cartes J, Lloret J, Oliver P, Morales-Nin B 2008. Seasonal and short spatial patterns in European hake (*Merluccius merluccius*, L) recruitment process at the Balearic Islands (NW Mediterranean): the role of environment on distribution and condition. *J Mar Syst* (in press).
- James CS 1998. *Analytical Chemistry of Foods*. Germany, Springer.
- Kjesbu OS, Klungsoyr J, Kryvi H, Witthames PR, Walker MG 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.
- Koops MA, Hutchings JA, McIntyre T 2004. Testing hypotheses about fecundity, body size and maternal condition in fishes. *Fish Fish* 5: 120-130.
- Lambert Y, Dutil JD 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.
- Lambert Y, Yaragina NA, Kraus G, Marteinsdottir G, Wright PJ 2003. Using environmental and biological indices as proxies for egg and larval production of marine fish. *J N Atl Fish Sci* 33: 115-159.
- Levi F, Boutoute M, Mayzaud P 2005. Lipid composition of *Symphodus ocellatus* (Perciforms: Labridae) in the North-Western Mediterranean: influence of two different biotopes. *Mar Biol* 146(4): 805-814.
- Lleonart J, Maynou F 2003. Fish stock assessments in the Mediterranean: State of the art. *Sci Mar* 67(suppl 1): 37-49.
- Lloret J, Lleonart J, Solé I, Fromentin JM 2001. Fluctuations of landings and environmental conditions in the NW Mediterranean Sea. *Fish Oceanogr* 10(1): 33-50.
- Lloret J, Lleonart J 2002. Recruitment dynamics of eight fishery species in the Northwestern Mediterranean Sea. *Sci Mar* 66(1): 77-82.
- 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, Planes S 2003. Condition, feeding and reproductive potential of white seabream (*Diplodus sargus*) as indicators of habitat quality and the effect of protection in the north-western Mediterranean. *Mar Ecol Prog Ser* 248: 197-208.
- Lloret J, Galzin R, Gil de Sola A, Souplet A, Demestre M 2005. Habitat related differences in lipid reserves of some exploited fish species in the north-western Mediterranean continental shelf. *J Fish Biol* 67: 51-67.
- Lloret J, Demestre M, Sánchez-Pardo J 2007. Lipid reserves of red mullet (*Mullus barbatus*) in the north-western Mediterranean. *Sci Mar* 71(2): 269-277.
- Maynou F, Lleonart J, Cartes JE 2003. Seasonal and spatial variability of hake (*Merluccius merluccius*) in the NW Mediterranean. *Fish Res* 60: 65-78.
- Marshall CT, Yaragina NA, Lambert Y, Kjesbu O 1999. Total lipid energy as a proxy for total egg production by fish stocks. *Nature* 402: 288-290.
- Marteinsdottir G, Begg GA 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.
- Morales-Nin B, Moranta J 2004. Recruitment and post-settlement growth of juvenile *Merluccius merluccius* on the western Mediterranean shelf. *Sci Mar* 68: 399-409.
- Morgan MJ 2004. The relationship between fish condition and the probability of being mature in American plaice (*Hippoglossoides platessoides*). *ICES J Mar Sci* 61: 64-70.
- Morris RJ, Culkin F 2000. *Fish. In Marine biogenic lipids, fats and oils*, Ackman RG ed. Boca Raton, CRC Press: 850-900.
- Okuda N 2001. The costs of reproduction to males and females of a paternal mouthbrooding cardinalfish *Apogon notatus*. *J Fish Biol* 58: 776-787.
- Olivar MP, Quílez G, Emelianov M 2003. Spatial and temporal distribution and abundance of European hake, *Merluccius merluccius*, eggs and larvae in the Catalan coast (NW Mediterranean). *Fish Res* 60: 321-331.



- Oliver P, Massutí E 1995. Biology and fisheries of western Mediterranean hake (*M. merluccius*). In Hake: Biology, fisheries and markets, Alheit J, Pitcher TJ eds. London, Chapman and Hall: 182-202.
- Orsi Relini LC, Papaconstantinou S, Jukic-Peladic S, Souplet A, Gil de Sola L, Piccinetti C, Kavadas S, Rossi M 2002. Distribution of the Mediterranean hake populations (*Merluccius merluccius smiridus* Rafinesque, 1810) (Osteichthyes: Gadiformes) based on six years monitoring by trawl-surveys: some implications for management. *Sci Mar* 66 (Suppl 2): 21-38.
- Oskarsson GJ, Kjesbu OS, Slotte A 2002. Predictions of realised fecundity and spawning time in Norwegian spring-spawning herring (*Clupea harengus*). *J Sea Res* 48: 59-79.
- Ouellet P, Lambert Y, Bérubé I 2001. Cod egg characteristics and viability in relation to low temperature and maternal nutritional condition. *ICES J Mar Sci* 58: 672-686.
- Papaconstantinou C, Caragitsou E 1987. The food of hake (*Merluccius merluccius*) in Greek Seas. *Vie Milieu* 37: 77-83.
- Perez-Villarreal B, Howgate P 1987. Composition of European hake, *Merluccius merluccius*. *J Sci Food Agricult* 40(4): 347-356.
- Rainuzzo JR, Reitan K, Olsen Y 1997. The significance of lipids at early stages of marine fish: a review. *Aquaculture* 155: 103-115.
- Recasens L 1992. Dinàmica de població i pesqueria del lluç (*Merluccius merluccius*) al Golf de Lleó i la Mar Catalana. PhD Thesis, Univ Barcelona (in Catalan).
- Recasens L, Lombarte A, Morales-Nin B, Torres DJ 1998. Spatial-temporal variation in the population structure of the European hake in the NW Mediterranean. *J Fish Biol* 53: 387-401.
- Riis-Vestergaard J 2002. Energy density of marine pelagic eggs. *J Fish Biol* 60: 1511-1528.
- Riveiro I, Guisande C, Lloves M, Maneiro I, Cabanas JM 2000. Importance of parental effects on larval survival in *Sardina pilchardus*. *Mar Ecol Prog Ser* 205: 249-258.
- Rodrigo J, Ros G, Periago MJ, López C, Ortuño J 1998. Proximate and mineral composition of dried salted roes of hake (*Merluccius merluccius* L) and ling (*Molva molva* L). *Food Chem* 63(2): 221-225.
- Shahidi F 2001. Extraction and Measurement of Total Lipids. Current Protocols in Food Analytical Chemistry D1.1.-D1.1.11.
- Shulman GE 1974. Life cycles of fish. Physiology and biochemistry. New York, Hulsted Press, John Wiley and sons.
- Shulman GE, Love RM 1999. The biochemical ecology of marine fishes. *Advances in Marine Ecology*, 36. London, Academic Press.
- Shulman GE, Nikolsky VN, Yuneva TV, Minyuk GS, Shchepkin VY, Alla M Shchepkina AM, Ivleva EV, Dobrovlov IS, Ferit B, Kideys AE 2005. Fat content in Black Sea sprat as an indicator of fish food supply and ecosystem condition. *Mar Ecol Prog Ser* 293: 201-212.
- Sogard SM, Olla BL 2000. Endurance of simulated winter conditions by age-0 walleye pollock: effects of body size, water temperature and energy stores. *J Fish Biol* 56: 1-21.
- Soriguer F, Serna S, Valverde E, Hernando J, Martín-Reyes A, Soriguer M, Pareja A, Tinahones F, Esteva I 1997. Lipid, protein and caloric content of different Atlantic and Mediterranean fish, shellfish, and molluscs commonly eaten in the south of Spain. *Eur J Epidemiol* 13: 451-463.
- Tornaritis M, Peraki E, Georgulli M, Kafatos A, Charalambakis G, Divanack P, Kentouri M, Yiannopoulos S, Frenaritou H, Argyrides R 1993. Fatty acid composition and total fat content of eight species of Mediterranean fish. *Int J Food Sci Nutr* 45: 135-139.
- Trippel EA, Neil SRE 2004. Maternal and seasonal differences in egg sizes and spawning activity of northwest Atlantic haddock (*Melanogrammus aeglefinus*) in relation to body size and condition. *Can J Fish Aquat Sci* 61: 2097-2110.
- Wanless S, Harris MP, Redman P, Speakman JR 2005. Low energy values of fish as a probable cause of a major seabird breeding failure in the North Sea. *Mar Ecol Prog Ser* 294: 1-8.
- Wiegand MD 1996. Composition, accumulation and utilization of yolk lipids in teleost fish. *Rev Fish Biol Fish* 6: 259-286.

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