

Running title: Changes of proximate composition, energy storage and condition of European hake (*Merluccius merluccius*, L. 1758) through spawning season.

**Changes of proximate composition, energy storage and condition of
European hake (*Merluccius merluccius*, L. 1758) through the spawning
season.**

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ABSTRACT

European hake is one of the most important economic resources for South European fleets; however, not many studies on its reproductive biology have been carried out. Gonadosomatic and hepatosomatic indices and condition factor were estimated. Proximate composition was analyzed in gonad, liver and muscle as indicators of female condition status; these results were converted to energy values. Variations of these parameters during the spawning season and through the year were studied. The hake population in Galician waters has a protracted spawning season with peak spawning from February to March. The proximate composition of tissues changes considerably throughout the spawning season although population spawning asynchrony masks temporal patterns. This work corroborates that energy dynamics associated with egg production in European hake are different from those observed in species from temperate waters (5°-10°C), depending more on environmental conditions and food availability during the spawning season than on body energy reserves.

Key words: *Merluccius merluccius*, proximate composition, condition, reproduction, reproductive potential.

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INTRODUCTION

European hake (*Merluccius merluccius*) is one of the most important economic resources for South European fleets. For assessment purposes, the International Council for the Exploration of the Sea (ICES) considers the existence of two assessment areas for European hake in the North-east Atlantic, the so-called northern and southern stocks, the southern stock distributed from the Bay of Biscay to Morocco waters, excluding the Mediterranean Sea where it is assessed as an independent stock. Total landings of southern stock have decreased drastically in recent decades, from 22,300 t in 1983 to the lowest recorded value of 5,600 t in 2003. In recent years, however, landings increased considerably reaching 10,000 t in 2006. The stock is considered to be outside safe biological limits, or overexploited, and a recovery plan has been developed to be implemented in the near future.

One of the basic goals of fisheries management is to conserve sufficient reproductive potential in a stock to allow for sustainable exploitation. To achieve this, most stocks are managed based on maintaining certain levels or limit thresholds of Spawning Stock Biomass (SSB) on the assumption this is an indicator of the ‘viability’ of the stock. However, there is growing evidence indicating that SSB may not be directly proportional to reproductive potential (Marshall *et al.*, 1998, 2003; Marteinsdottir and Begg, 2002;). Fish in poor condition and first time spawners can have reduced fecundity and/or reproductive success or they can fail to spawn at all, affecting future recruitment (Marteinsdottir and Steinarsson, 1998; Burton, 1999; Wigley, 1999; Marteinsdottir and Begg, 2002; Kurita and Kjesbu, 2003; Saborido-Rey *et al.*, 2004; Morgan and Bratney, 2005; Jørgensen *et al.*, 2006). Trippel (1999) emphasized the importance of integrating such basic reproductive biology as spawners’ ages and sizes, maturation, condition and reproductive history into stock assessment, and

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introduced the new term of Stock Reproductive Potential (SRP) that “represents the annual variation in a stock’s ability to produce viable eggs and larvae that may eventually recruit to adult population or fishery”. According to Tomkiewicz *et al.* (2003) it is necessary to encourage efforts to improve SRP indices for potential application in assessment and management in order to establish reference points, which are basic to a precautionary approach to fisheries management and sustainable fisheries. Several indices based on reproductive potential have been studied (Marteinsdottir and Begg, 2002; Marshall *et al.*, 2003); among them, those indices based on female physiological condition have improved the understanding of SRP (Lambert and Dutil, 1997a; Marshall *et al.*, 1999; Lambert *et al.*, 2000; Yaragina and Marshall, 2000). Individual fecundity and egg size, among other reproductive features, are influenced basically by the availability and quality of energetic reserves, or by food assimilation (Tyler and Colow, 1985), which directly affect spawners’ condition, and at the same time determine maturation of individuals (Saborido-Rey and Kjesbu, in press). Fish condition can be assessed using a variety of criteria, ranging from simple morphometric measures (length-weight relationship or K) to physiological (liver or hepatosomatic index, gonadosomatic index) and biochemical measures (body proximate composition as lipid, protein and other components in fish tissues).

Lipids play an important role as energy reserves and as regulators of body density, cellular metabolism and reproduction (Love, 1980; Chellappa *et al.*, 1989; Jonsson *et al.*, 1997; Blanchard *et al.*, 2005); and they strongly affect ovary development, fecundity, fertilization, egg quality and hatching rates (Shearer and Swanson, 2000; Hendry *et al.*, 2001; Kurita *et al.*, 2003; Lambert *et al.*, 2003). Proteins are the main component of muscular tissue and are not only the principal energy source of active metabolism of fish, but also ovary growth takes place at the expense of body proteins (Tyler and Colow, 1985; Black and Love, 1986). Traditionally, glycogen has been

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considered an insignificant biochemical component in fish despite the indisputable function that this component carries out in other marine organism like molluscs (Wilbur and Hochachka, 1983). Nevertheless, Love (1970) already highlighted the importance of glycogen in general metabolism, including reproduction, especially under stress situations when a high proportion of muscle glycogen is mobilized. Regarding tissue water content, it normally follows the opposite trend than organic components, thus sometimes is considered to be a proxy of condition (Lambert and Dutil, 2000; Dutil *et al.*, 2003a).

In spite of the economic and ecological importance of hake and the depletion of the European stocks, studies on hake reproductive potential are scarce (Murua *et al.*, 1998). The objective of the present study is:

- To analyze variations of condition, proximate composition and energy storage during the spawning season.
- To explain how energy stores and chemical constituents are distributed among different tissues.
- To determine how these stores are mobilized to fuel reproductive development.
- To improve the understanding about reproductive ecology of European hake on the Galician Shelf, and the factors that determine its reproductive potential.

MATERIAL AND METHODS

Sampling.- A total of 2,012 female hake were collected from January 2003 to November 2004 (Table I). Sampling was conducted weekly during the peak of spawning (January-May), and twice a month the rest of the year, except September 2003. The sampling area covered the entire Galician

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shelf, although highest sampling intensity was in the Western area (Figure 1). Samples were collected onboard from commercial fleets both for adults (gillnet) and juveniles (bottom trawl). Sampling was stratified by length class (Table I), and total length (cm), total and gutted weight (g), sex, macroscopic maturity stage and ovary and liver weight (g) were recorded for each individual. All the ovaries were preserved in 4% buffered formaldehyde and histologically processed using standard paraffin embedding and Haematoxylin-Eosin staining techniques. Ovary development was staged microscopically (Table II). During sampling, one ovary, the liver and a one centimetre thick muscle slice from the posterior part of the body were taken and preserved frozen at -22°C in plastic bags until their analysis in the laboratory. In the case of immature and spent females, only a small number of individuals were analyzed because ovary size was generally too small to estimate tissue proximate composition.

Condition analyses.- Three general condition indices were calculated for all sampled females: ovariosomatic index (GSI), hepatosomatic index (HSI) and condition factor (K). These indices are defined by the following equations:

$$(i) \ GSI = \frac{gonadW}{guttedW} \cdot 100$$

$$(ii) \ HSI = \frac{liverW}{guttedW} \cdot 100$$

$$(iii) \ K = \frac{guttedW}{length^3}$$

Where W refers to weight.

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Proximate composition was determined in three different tissues, liver, muscle and ovary for a total of 50 females well-distributed by size and month (Table III). From each tissue, two replicates of 3-5g samples were taken, skin, bones and scales were removed from muscle, and parasites were also removed from ovary and liver. Then, samples were manually homogenised. Each sample was divided in two subsamples, one of them was dried for 24 hours at 100°C, and weighed at ambient temperature (0.001g) to determine water content (wet mass-dry mass). The other one was used for biochemical analysis. Lipid was measured in two replicates of 1.5 ± 0.4 g wet. Lipid extraction followed the method developed by Bligh and Dyer (1959). For lipid quantification, the gravimetric method of Herbes and Hallen (1983) was applied. Protein content was determined in two replicates of frozen tissue (100 mg) using Bovine seroalbumin (BSA) concentrated at $0.33 \text{ mg}\cdot\text{ml}^{-1}$ as standard and following the protocol of Lowry *et al.* (1951). Glycogen content was determined according to the method of Strickland and Parsons (1968) in previously lyophilized subsamples (22.27 ± 3.99 mg). The base solution to elaborate the glucose standard was obtained from D(+)-anhydrous glucose $180.16 \text{ g}\cdot\text{mol}^{-1}$. Due to differences in glycogen concentration between tissues, it was necessary to create two different standards, one for muscle, with values of D(+)-anhydrous glucose from $0.003 \text{ mg}\cdot\text{ml}^{-1}$ to $0.1 \text{ mg}\cdot\text{ml}^{-1}$ and another for ovary and liver with values that cover from $0.01 \text{ mg}\cdot\text{ml}^{-1}$ to $0.2 \text{ mg}\cdot\text{ml}^{-1}$, so that tissue glycogen concentration values were between detection limits of the standard. Glycogen concentrations were then measured in a spectrophotometer (Beckman Coulter DV 640) at 490nm. Proximate composition values are presented throughout the text as milligrams per gram of tissues' dry mass. Energy density ($\text{kJ}\cdot\text{g}^{-1}$) was estimated for each tissue (ovary, liver, muscle) by multiplying lipid, protein and glycogen content (mg/g of dry mass) by the appropriate energy equivalents (lipid= $39.5 \text{ kJ}\cdot\text{g}^{-1}$, protein= $23.6 \text{ kJ}\cdot\text{g}^{-1}$; glycogen= $17.1 \text{ kJ}\cdot\text{g}^{-1}$; Kleiber, 1975). Lipid, protein and glycogen energy were then summed within each tissue to determine the combined mass-specific energy. Values of all

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variables are presented throughout the text as mean values \pm standard deviation and in figures as mean values \pm standard error.

Statistical analyses.- Simple regression analysis was used to study existing relationship between general condition index, as well to analyze the relationships of proximate composition between tissues. Analysis of variance was used to study the variation of condition indexes between months and between ovary developmental stages. In the case of proximate composition and energy density, analysis of variance was only used to study variation between ovary developmental stages, but for monthly variation, Kruskal-Wallis' non-parametric test was carried out, because variances were not homogeneous in this case. Relationships between energy density in each tissue during the spawning season were also studied. Immature females were not considered for the statistical analyses because of the low number of specimens sampled, but they were included in the graphs as a reference value.

RESULTS

Seasonal variations

In 2003, GSI varied between 0.11 and 29.47 showing significant differences between months ($F(10, 792)=13.28$, $p<0.001$; Figure 2a) and a clear and steadily decreasing trend from the maximum average values in January (9.24 ± 4.12) to the minimum average value in October (3.72 ± 2.68). A secondary peak of GSI was observed in May-June (5.66 ± 3.76). In 2004 significant differences between months were also detected ($F(10, 652)=14.64$, $p<0.001$; Figure 2a). GSI followed a similar pattern as in the previous year, but the main peak of GSI was observed in February (8.83 ± 5.25) and

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the secondary one in July (7 ± 5.42). The large variation within each month for both years indicates an important asynchrony at the population level of ovarian development and spawning activity.

HSI also showed significant monthly variations in both 2003 ($F(10, 588)=17.98$, $p<0.001$) and 2004 ($F(10, 277)=9.36$, $p<0.001$; Figure 2b). Thus, in 2003, HSI was significantly high towards the end of the year, from August (5.61 ± 1.24) to December (5.79 ± 1.68). However, between January and July, it fluctuated between 4.0 and 4.9. The maximum value of HSI was recorded in October (6.92 ± 3.19). A similar pattern was observed in 2004 when HSI fluctuated around 4 between January and June (Figure 2b), but the maximum mean value was reached in July (7.91 ± 3.16), decreasing in subsequent months. Seasonal variation of K also showed significant differences between months in 2003 ($F(10, 792)=4.72$, $p<0.001$) and in 2004 ($F(10, 654)=5.17$, $p<0.001$). As well as GSI and HSI, condition factor K showed large variation within each month. Nevertheless, in general there is an increasing trend of K from spring to autumn in both years (Figure 2c). In 2003, K values were between 0.40 and 1.16 whereas in 2004, in general, they were lower, ranging between 0.26 and 0.84. HSI and K mean values remained constant or increased towards the last quarter of the year in comparison to the first quarter whereas GSI tended to decrease. When correlations between GSI and the other two condition indices were carried out, results showed a significantly negative relationship between them, as expected, but the correlation coefficient was very low in both cases ($r = -0.07$, $p<0.01$ for HSI and $r = -0.21$, $p<0.001$ for K).

Variations in the prevalence of different ovarian development stages showed that the highest proportions of ovulating-hydrated females (h) occurred from January to August (Figure 3); i.e. main spawning activity takes place during this period; in consequence, proximate composition and energy density analyses were focused on those months. None of the chemical components analyzed

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(lipids, protein, and glycogen content and energy density) showed significant monthly variation during the spawning season in any tissue ($p>0.05$; Figure 4). Although monthly differences were not significant some trends were identified. Gonad lipid content ranged between 194 and 597 $\text{mg}\cdot\text{g}^{-1}$. It showed two peaks (Figure 4a), coinciding with maximum mean values of GSI in January ($353\pm150 \text{ mg}\cdot\text{g}^{-1}$) and June ($377\pm89\text{mg}\cdot\text{g}^{-1}$). Its minimum mean value was recorded in February ($281\pm51 \text{ mg}\cdot\text{g}^{-1}$). On the contrary, gonad protein content that ranged between 219 and 667 $\text{mg}\cdot\text{g}^{-1}$, followed the opposite trend than lipids (Figure 4b); minimum mean values were observed in January ($487\pm195 \text{ mg}\cdot\text{g}^{-1}$) and June ($482\pm100 \text{ mg}\cdot\text{g}^{-1}$) and the maximum in February ($590\pm44 \text{ mg}\cdot\text{g}^{-1}$). Peaks of glycogen coincided with GSI peaks (Figure 4c). The primary one in January ($86\pm33 \text{ mg}\cdot\text{g}^{-1}$), but the secondary one was observed slightly earlier than in gonad lipids, in May ($96\pm50 \text{ mg}\cdot\text{g}^{-1}$). In liver, lipid content followed the same trends as in gonad except in April and May when it was the opposite (Figure 4a). It ranged between 319 and 866 $\text{mg}\cdot\text{g}^{-1}$. The main peak was detected in June ($778\pm88 \text{ mg}\cdot\text{g}^{-1}$) and the secondary one in January ($697\pm126 \text{ mg}\cdot\text{g}^{-1}$). For liver protein content, the observed pattern was exactly the same as gonad protein content (Figure 4b), fluctuating from 39 to 294 $\text{mg}\cdot\text{g}^{-1}$. Liver glycogen content varied without any clear trend between 3 and 280 $\text{mg}\cdot\text{g}^{-1}$ (Figure 4c). Muscle presented the lowest values of lipid content of the three tissues (23-118 $\text{mg}\cdot\text{g}^{-1}$) and followed the same trend as lipids in gonad (Figure 4a) and gonad proteins that were the main muscle component (286-946 $\text{mg}\cdot\text{g}^{-1}$), and varied in a similar way as gonad proteins except in July when they were lower (Figure 4b). Glycogen content did not show any clear pattern in muscle either (Figure 4c).

Energy density in the three tissues followed the same pattern with two peaks that coincided with GSI peaks, one in January and the other in June (Figure 4d). In gonad, energy density fluctuated between 24 and 31 $\text{kJ}\cdot\text{g}^{-1}$, in liver between 27 and 37 $\text{kJ}\cdot\text{g}^{-1}$ and in muscle between 8 and 24 $\text{kJ}\cdot\text{g}^{-1}$.

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In summary, although some similarities could be detected between GSI and the tissues' proximate composition trends, variability was so high in all of them that monthly variations were not significant, and thus could not be associated clearly with reproductive process. This could be due to population asynchrony during the reproductive period. To elucidate if the liver and muscle energy store fluctuations were mobilized in relation to egg production, a second set of analyses were performed based on ovary developmental stages instead of months.

Variations between ovary developmental stages

Figure 5 shows the variation of condition indices in relation to ovary developmental stages. Significant differences were observed between stages in all of them (Figure 5), GSI ($F(4, 1908)=325.40$, $p<0.001$), HSI ($F(4, 1053)=6.44$, $p<0.001$) and K ($F(4, 1911)=22.40$, $p<0.001$). GSI reflects changes in ovary mass and results were as expected; the lowest mean GSI values appeared in immature individuals (0.33 ± 0.19) and maximum (7.82 ± 4.97) in ovulating-hydrated females. HSI increased from immature ovaries (4.23 ± 1.39) to ripening ones (4.71 ± 1.58), and remained constant around 4.60 during the rest of maturity stages, increasing slightly in inactive mature ovaries. K followed exactly the opposite pattern from GSI with the highest values for immature females (0.66 ± 0.07) and the lowest for ovulating-hydrated ones (0.63 ± 0.06). Nevertheless, if linear regression analysis between condition indices is carried out, a direct relationship between them is not so evident as in analysis of variance, due to high data dispersion; so the regression between GSI and HSI although significant, showed very low Pearson r coefficient ($r=-0.07$, $p<0.05$) and something similar was observed between GSI and K ($r=-0.20$, $p<0.001$).

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Figure 6 shows the mean concentration of lipid, protein and glycogen and energy density in ovary, liver and muscle for each ovarian developmental stage. In none of the three tissues observed were significant differences among developmental stages ($p>0.05$). But as with the monthly variations of proximate composition, certain trends could be identified. In all three tissues studied, lipid content decreased while the reproductive process advanced (from ripening to late spawning females), but rising in inactive mature females (Figure 6a). Proteins (Figure 6b) content varied in the same way following the contrary trend to lipids. Glycogen content varied without any trend in muscle, but in gonad showed a peak in ovulating-hydrated females, and decreased progressively in inactive mature ones. In the case of liver, glycogen content was maximum in late spawning females, decreasing also in inactive mature ones. Energy density decreased progressively from ripening to late spawning females in gonad, liver and muscle, while for inactive mature females it increased in gonad and liver but not in muscle; this could, however, be due to an anomalously low energy density value observed in one female from the inactive mature group.

Relationship between tissues proximate composition

Linear regression analysis between gonad biochemical components and liver and muscle biochemical components was carried out in order to elucidate relationships between gonad development and energy depletion in the other two tissues, liver and muscle, to clarify dependence of ovary maturation on body energy reserves. Results are shown in Table IV. Significant relationships were observed between some tissue components, but in general showed low Pearson coefficients that only reached values higher than 0.45 in a few cases; these will be described now. Liver protein content was negatively related to gonad lipid and positively to protein content ($r = -0.4987$, $p<0.001$ and $r = 0.5445$, $p < 0.001$ respectively). Muscle lipid content was positively related

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to gonad lipid and negatively to gonad protein content ($r = 0.4999$, $p < 0.001$ and $r = -0.6223$, $p < 0.001$ respectively). Gonad energy density increased significantly with liver energy density ($r = 0.4198$, $p < 0.01$), but did not show any relationship with muscle energy content.

DISCUSSION

The main difficulty with this study was the asynchrony of the reproductive cycle of hake, not only at the individual, but also at the population level. Asynchronous development masks temporal variations of the factors analysed, and led us to analyse the changes of condition between microscopically determined ovarian developmental stages. The particular reproductive characteristics of the hake and inability to maintain it in captivity make its study difficult.

Condition indices have been shown to be important factors to refine the estimates of stock reproductive potential in a number of species (Marshall *et al.*, 1999; Lambert *et al.*, 2000; Yagarina and Marshall 2000). It is assumed that liver and ovary indices measure the energy reserves of fish more accurately (Shulman and Love, 1999), but proximate composition describes condition more precisely, as it allows calculation of the energy available, and understanding of how energy stores are partitioned among different tissues and chemical constituents. However, condition is a good index of reproductive potential when it varies at seasonal scales, due to seasonality in feeding rate and energy allocation within individual fish. Many of the studies of fish condition variations have been conducted in temperate water species, where clear seasonal changes in food supply, temperature and photoperiod occur, and yolk accumulation and spawning normally occur in periods of food deprivation. As a consequence, condition indices are often strongly coupled to feeding, growth and maturation of individual fish and the allocation of energy between somatic and ovary

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production (ICES, 2003). Often, condition indices reflect geographical and temporal variations in environmental conditions, prey availability and composition, spawning and feeding behaviour or genetic factors (Rätz and Lloret, 2003; Hidalgo *et al.*, 2008). Thus in cod from Greenland waters, HSI has shown a wider range of values (Lloret and Rätz, 2000) compared with Northwest Atlantic cod (Dutil *et al.*, 2003b), in which values were lower than those observed in cod from Newfoundland and Iceland (Marteinsdottir and Begg, 2002; Mello and Rose, 2005). Similar variations have been observed in K in cod (Krohn *et al.*, 1997; Lambert and Dutil, 1997b, 2000; Dutil *et al.*, 2003b; Mello and Rose, 2005). However, for subtropical waters species (10°-20°C), seasonality, especially in food availability, is not always so apparent. In many of those species, like hake, spawning seasons are protracted, and females in spawning condition are found throughout the year (Murua, 2006; Domínguez-Petit, 2007). In this study, female hake HSI and K fluctuate during the year, but there were no clear seasonal patterns in their variation. K varied between 0.26 and 1.16, but the average monthly values hardly fluctuate (0.60-0.67), in accordance with other hake species, such as *M. hubbsi* (Montecchia *et al.*, 1990; Méndez and González, 1997). These values were well below those reported for cod in a number of stocks (Krohn *et al.*, 1997; Lloret and Rätz, 2000; Koops *et al.*, 2004). In contrast, hake HSI varied notably (1.76-14.52), with average values between 4.02 and 6.92; slightly above values for other European hake stocks, such as that of the Northern Tyrrhenian Sea, (1.5-5; Biagi *et al.*, 1995), but similar to *M. australis* (Balbontín and Bravo, 1993) where HSI has been reported to average 4.2 ± 5 . Surprisingly, in the Gulf of St Lawrence cod, a temperate water species whose gamete production depends completely on energy reserves, HSI varied seasonally, but monthly values were lower than in hake (Lambert and Dutil, 1997a). In any case, both indices, K and HSI, tend to increase towards the last quarter of the year. It can be hypothesized that energy stored in liver or muscle (female condition) in hake may plays an

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important role in reproduction, but the asynchronous spawning activity and the protracted spawning season in hake mask the dynamics of energy allocation and mobilization.

To avoid asynchrony effects, HSI and K were analysed independently of temporal variations, i.e. based on ovary developmental stages. In these analyses significant variations were recognized through the spawning season. On one hand, K followed the opposite pattern to GSI, decreasing from immature to ovulating-hydrated females and recovering slightly in late spawning and inactive mature stages. On the other hand, HSI increased progressively from immature females to inactive mature ones. These ANOVA results suggest that gonad development depends completely on muscle energy reserves, whereas liver condition seems to be independent of reproductive processes. Nevertheless, when direct relationships between these indices and GSI were analyzed, correlation between variables was weak, so the dependence of gonad development on muscle energy reserves is either weak or nonexistent. Knowledge of the structures and energy reserves is important in understanding metabolic processes, and in assessing the impact of potential environmental physical and chemical stressors on fish stocks (Faahraeus-Van Ree and Spurrell, 2003). In hake, this study indicates that conditions indices (HSI and K) do not seem to be good proxies of stock reproductive potential. In other species it has been reported that HSI and K are representative of energy storage, direct mobilization of that energy from liver and muscle to gonad for gamete production has been observed (Love, 1970; Kjesbu *et al.*, 1991; Lambert and Dutil, 1997a; Komova, 2002; Blanchard *et al.*, 2003), and they can be used as proxies of stock reproductive potential. According to our results, this criterion is not applicable to all fish species. HSI and K represent the sizes of liver and carcass in relation to the whole body, but liver and carcass size do not depend necessarily on energy accumulation. Their sizes may be related with accumulation of non-energetic compounds such as

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water that affect their size and weight. Because of this, a more detailed approach, i.e. proximate composition, was taken here.

There are few studies of hake proximate composition, and most are focused on muscle (edible portion) because of its importance in human nutrition (Dill, 1925; Gordon and Roberts, 1977; Pérez-Villareal and Howgate, 1987; Montecchia *et al.*, 1990; Méndez and González, 1997; Soriguer *et al.*, 1997; Pagano *et al.*, 2001; Roldán *et al.*, 2005). Few previous studies of liver and ovary proximate composition in hake have been published (Lloret *et al.*, 2008), so comparative analyses are made here with proximate composition values of similar species from other genera (Montecchia *et al.* 1990).

In our study, ovary proximate composition did not show any significant pattern of variation during the year, although decreases of gonad lipid, glycogen, water and energy content were recorded during the first quarter. Lack of temporal variations in proximate composition was previously observed in *M. hubbsi* and *M. australis* (Eder and Lewis, 2005). Variations of ovary proximate composition through ovary developmental stages were more evident in these species but not statistically significant. Nor were these variations reflected in liver and muscle proximate composition. Relationships between biochemical compounds of gonad, liver and muscle were not consistent with ovary development based on body energy reserve theories, since linear regressions are significant for only a few of these compounds, and those in the gonad are not always negatively related to those in liver and muscle, as would be expected if energetic dependence exists. This suggests either that a direct relationship between ovary development and somatic energy reserves in hake is not as strong as in temperate water species, or that stored energy can be mobilized to the gonad during vitellogenesis and then might be recovered rapidly by compensatory growth

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mechanisms (Ali *et al.*, 2003). In stable environments, large energy reserves are less essential, in contrast with environments where food resources vary seasonally (Stickney and Torres, 1989). At high latitudes, ecosystem productivity is restricted to the spring-summer period when conditions of turbulence and light intensity allow phytoplankton blooms, the basis of the ocean food web. Consequently, during late summer-early autumn, food is abundant for top predators like large gadoids. However, during winter when breeding takes place, the availability of food is lower. This situation forces females to accumulate energy in liver and muscle during late summer-early autumn period in order to provide enough energy for gamete production during winter. Energy spent during reproduction is recovered in next months, after spawning season ends. In contrast, in habitats where environmental fluctuations are not so marked (deep-sea demersal habitats, subtropical- and tropical-waters habitats), homogeneous proximate composition has been observed through the whole year (Koslow *et al.*, 2000) probably because energy reserves are recovered during the spawning season because food availability is relatively constant the whole year.

Unlike other fish species in which energy reserves tend to be maximum just prior to the spawning season, and decrease progressively as it advance (Lloret and Rätz, 2000; Richoux *et al.*, 2004), in this study no significant differences of energy density were observed either between months or between ovary developmental stages. The fact that energy density remains constant through the spawning season supports the idea that European hake does not stop feeding during reproduction. This suggests that hake reproduction does not completely depend on energy reserves. Trade-offs in the energy budget distribution must exist which affect growth and reproductive dynamics (Saborido-Rey and Kjesbu, in press). As mentioned previously, the primary production cycle and environmental conditions in temperate waters force fish to spawn in late winter-spring and develop ovaries during autumn-winter, experiencing periods of food depletion. The strategy in these cases is

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to store parts of the assimilated energy for later use when the food supply is limited (Bagenal, 1967). In subtropical waters, on the contrary, environmental conditions allow the existence of protracted spawning seasons and less marked periods of food depletion. Oocyte development is done at expenses of food intake (i.e. energy surplus) during spawning season rather than from reserves, which allows modulate egg production in response to food surplus (indeterminate fecundity). The present results support rather convincingly the idea that hake reproduction follows this pattern, although energetic components of the ovary are created in the liver (vitellogenin), and muscle may provide temporally some energy for ovary development. Nevertheless, the energy content of organs and quality of energetic compounds (fatty acids, amino acids and protein classes) may vary depending on environmental conditions (Dutil *et al.*, 2003b), and both maternal energy reserves and biochemical composition might affect fertilization rates, catabolism, and the energy reserves of eggs (Buckley *et al.*, 1990; Tamaru *et al.*, 1992; Finn *et al.*, 1995); this is true even in species with the strategy assumed here for hake, with the subsequent effect on reproductive potential of stock.

Assuming that hake egg production does not depend directly on body energy reserves (liver and muscle) determined some months before spawning season beginning, then necessarily it has to depend on energy consumed during the spawning season (energy surplus). Subsequently, energy reserves may indicate female capacity to obtain food, to mate, and to produce more eggs. Thus, stock reproductive potential is indirectly affected by female condition (Dominguez-Petit and Saborido-Rey, in press). On the other hand, environmental conditions determine food availability and female metabolic rates, and also influence stock reproductive potential. To study changes in proximate composition of gonad, liver and muscle through ovary development in European hake from Northern areas would allow corroboration of the effects of environmental conditions

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(seasonality of energetic resource availability) on body energy reserve distribution. All these aspects highlight the need to review management criteria for subtropical water species which have been assessed according to temperate water species models.

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FIGURE CAPTIONS

Figure 1.- Sampled areas of Galician Shelf in 2003 and 2004. Red line: European hake distribution area.

Figure 2.- Monthly variations (mean \pm standard error) of a) GSI, b) HSI and c) K for 2003 (solid line) and 2004 (dashed line).

Figure 3: Prevalence of different ovary developmental stages during the year.

Figure 4.- Monthly variations (mean \pm standard error) of lipids, proteins and glycogen content and energy density in gonad (solid line), liver (dashed line) and muscle (dotted line).

Figure 5.- Mean values \pm standard error of a) GSI (solid line), b) HSI (dashed line) and c) K (dotted line) in relation to ovary developmental stages.

Figure 6.- Mean concentrations \pm standard error of lipid, protein and glycogen content and energy density in gonad (solid line), liver (dashed line) and muscle (dotted line) in relation to ovary developmental stages.

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629 **TABLES**

630

631 **Table I.-** Length class distribution of females sampled each month, 2003 and 2004

	2003												2004												Total
Length (cm)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov			
<45	18	16	13	30	26	33	25	14	21	25	24	15	4	25	22	44	37	30		1	17	23	463		
45-50	1	16	20	11	9		2	1	5	1	12	8	7	10	14	35	8	9	6	10	3	1	189		
50-55	13	63	49	45	51	13	11	5	11	4	8	28	39	37	36	40	16	15	7	12	13		516		
55-60	18	27	35	40	73	37	35	24	20	6	9	41	37	47	29	24	10	10	8	12	6		548		
>60	7	3	27	21	24	32	21	12	11	4	10	18	30	26	15	18	3	4	4	2	4		296		
Total	57	125	144	147	183	115	94	56	68	40	63	110	117	145	116	161	74	68	25	37	43	24	2012		
Total immature	17	16	7	23	29	34	26	15	25	26	29	15	5	18	20	39	36	31		2	18	23	454		
Total mature	40	109	137	124	154	81	68	41	43	14	34	95	112	127	96	122	38	37	25	35	25	1	1558		

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634 **Table II.-** Descriptions of ovary developmental stages determined microscopically

STAGE	DESCRIPTION
Immature (i)	All the oocytes in the ovary are in primary growth stage
Ripening (r)	Occurrence of cortical alveoli and/or vitellogenic oocytes is observed, but post-ovulatory follicles are not present, and no signs of advanced spawning process such as thick ovary wall, high vascularization of gonad and/or disorganization of lamellae, are observed either.
Ovulating-Hydrated (h)	There is a high percentage of hydrated oocytes at the beginning of the hydration process, or post-ovulatory follicles younger than 72 hours are observed throughout the ovary, together with vitellogenic oocytes in different stages. Signs of advanced spawning process are not necessarily observed.
Late spawning (ls)	Ovary with vitellogenic oocytes and without post-ovulatory follicles younger than 72 hours, but with signs of advanced spawning process such as high number of blood vessels, swollen ovary wall, atresia, disorganization of ovary structures, etc.
Inactive mature (im)	Females at this stage will no longer produce oocytes to be released during the current breeding season. The cessation of egg production may be due to the end of the spawning season (spent females), or an earlier interruption of it (skip spawners), or ovary is without mature oocytes, with wide ovary wall, lamellae are not so compact as in immature ovaries, and blood vessels use to be more visible too. These structures indicate that this ovary has produced eggs in the previous spawning season, and that it is recovering for the next one.

635

636

637 **Table III.-** Number of females with bionergetic analyses from each length class by month.

Length (cm)	2003							Total
	Jan	Feb	Mar	Apr	May	Jun	Jul	
<50		4	1	1	1		1	8
50-55	2	2	2	3	1	2		12
55-60	2	1	1	2	3	3	3	15
>60	2	1	2	2	3	3	2	15
Total	6	8	6	8	8	8	6	50

638

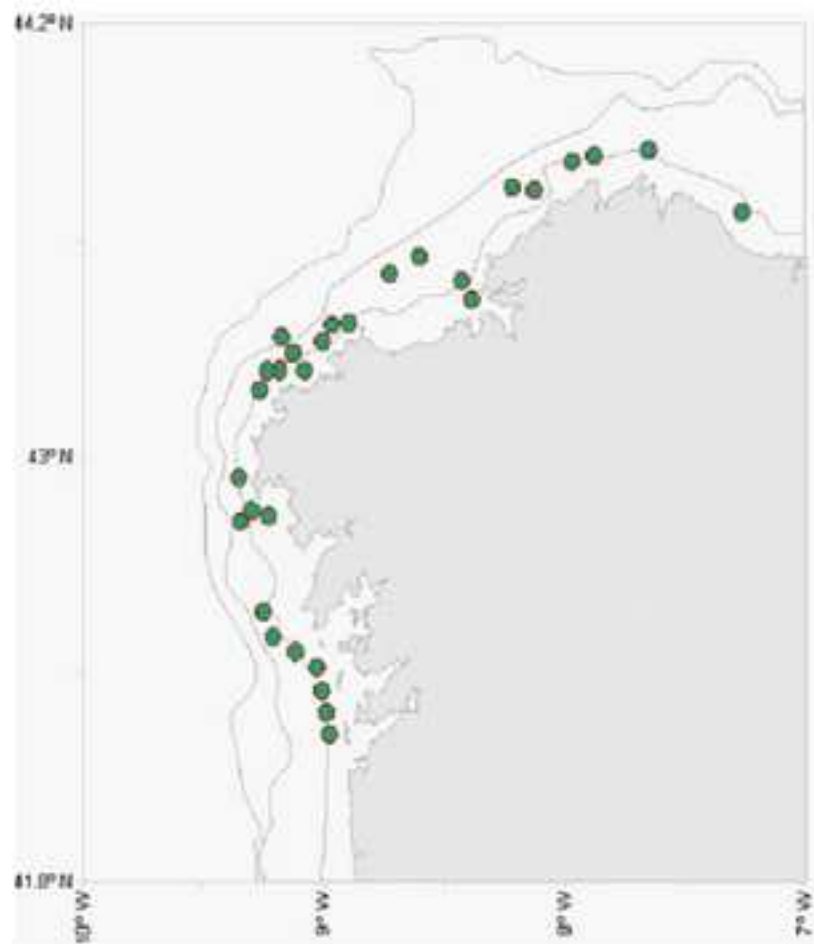
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Table IV.- Results of linear correlation between gonad and liver and muscle biochemical compounds (N=50). * = $p < 0.05$; ** = $p < 0.01$ and *** = $p < 0.001$

Gonad compound	Liver/Muscle compound	Pearson r	p	Gonad compound	Liver/Muscle compound	Pearson r	p
Gonad lipid	Liver lipid	0.3139	*	Gonad water	Liver lipid	0.1429	0.32
	Liver protein	-0.4987	***		Liver protein	-0.3030	*
	Liver glycogen	-0.2197	0.13		Liver glycogen	-0.0398	0.78
	Liver water	-0.2253	0.12		Liver water	-0.1748	0.22
	Liver energy	0.4077	**		Liver energy	0.1681	0.24
	Muscle lipid	0.4999	***		Muscle lipid	0.2773	0.05
	Muscle protein	-0.2510	0.08		Muscle protein	-0.1355	0.35
	Muscle glycogen	0.2804	*		Muscle glycogen	0.2203	0.12
	Muscle water	0.2235	0.12		Muscle water	0.3008	*
	Muscle energy	-0.1021	0.48		Muscle energy	-0.0464	0.75
Gonad protein	Liver lipid	-0.3043	*	Gonad energy	Liver lipid	0.2473	0.08
	Liver protein	0.5445	***		Liver protein	-0.4080	**
	Liver glycogen	0.2547	0.07		Liver glycogen	-0.2344	0.10
	Liver water	0.1324	0.36		Liver water	-0.2041	0.16
	Liver energy	-0.3970	**		Liver energy	0.4198	**
	Muscle lipid	-0.6223	***		Muscle lipid	0.4093	**
	Muscle protein	0.2289	0.11		Muscle protein	-0.2198	0.13
	Muscle glycogen	-0.2496	0.08		Muscle glycogen	0.2368	0.10
	Muscle water	-0.3592	*		Muscle water	0.1653	0.25
	Muscle energy	0.0348	0.81		Muscle energy	-0.0910	0.53
Gonad glycogen	Liver lipid	0.1989	0.17				
	Liver protein	-0.1753	0.22				
	Liver glycogen	0.0938	0.52				
	Liver water	-0.1316	0.36				
	Liver energy	0.0734	0.61				
	Muscle lipid	0.1671	0.25				
	Muscle protein	-0.0254	0.86				
	Muscle glycogen	0.1770	0.22				
	Muscle water	-0.0391	0.79				
	Muscle energy	0.0645	0.66				

Figure

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Figure

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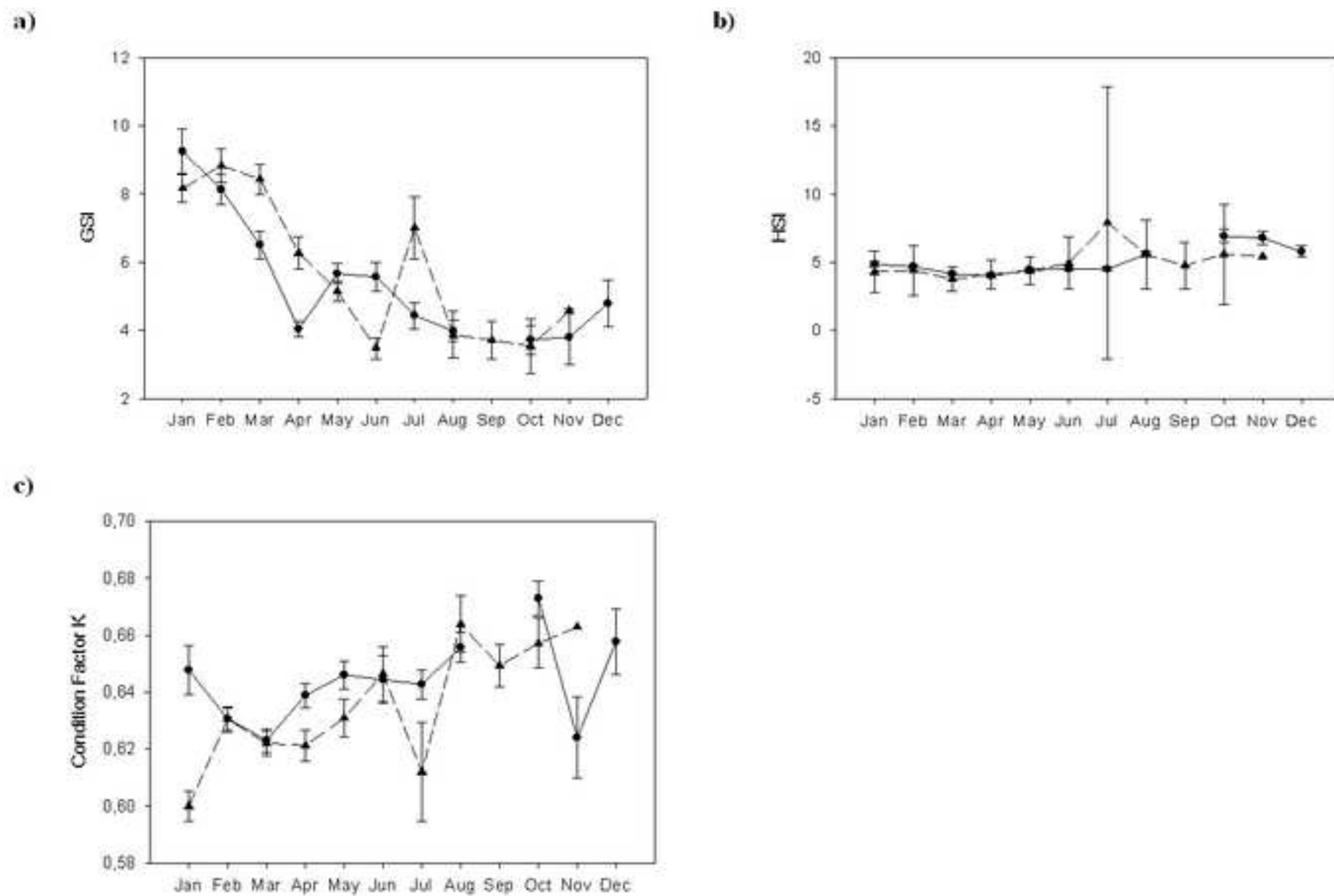
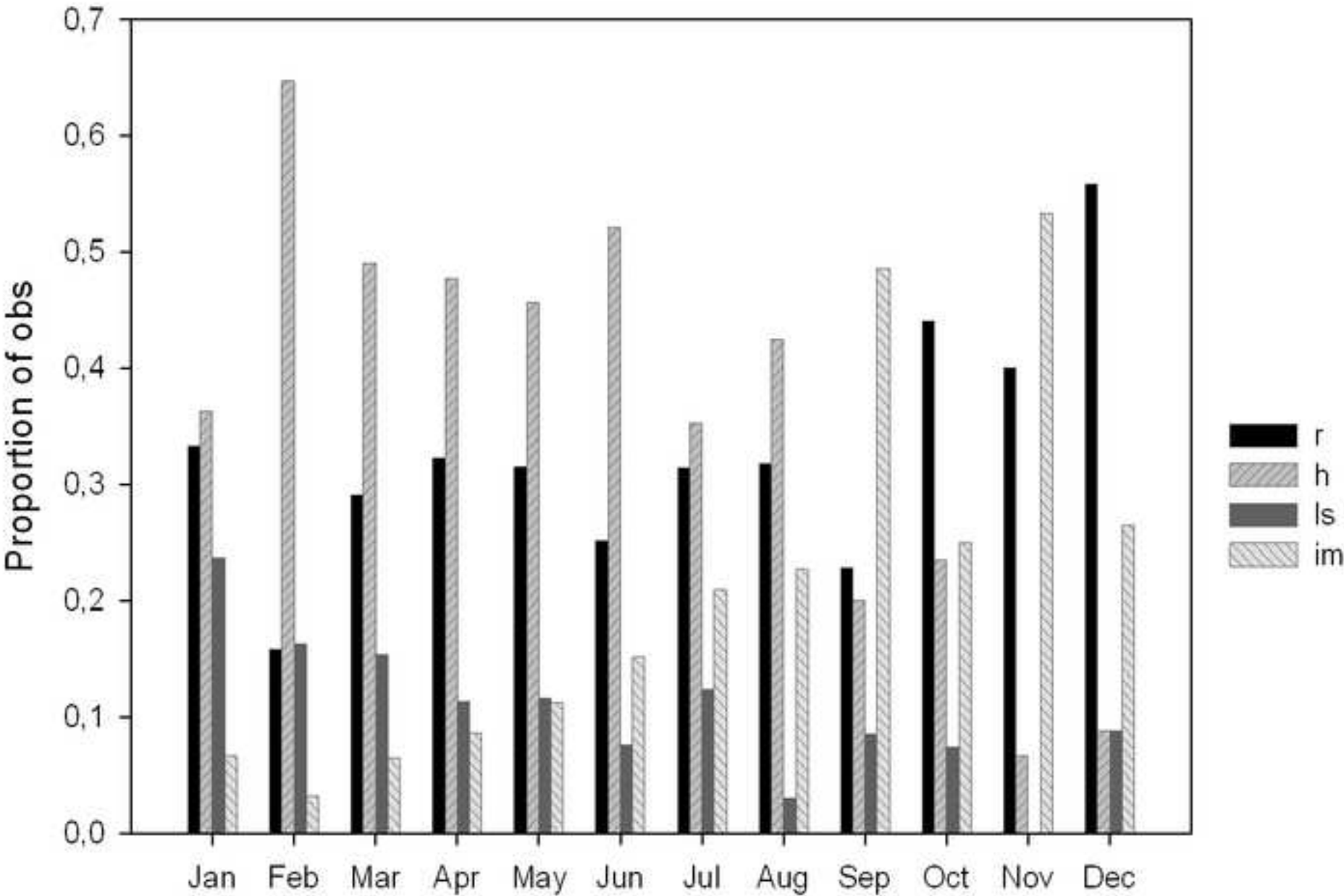


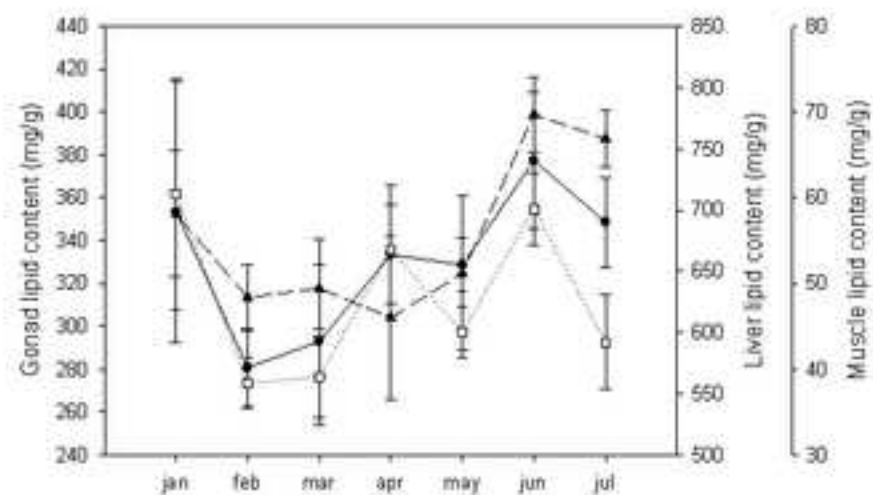
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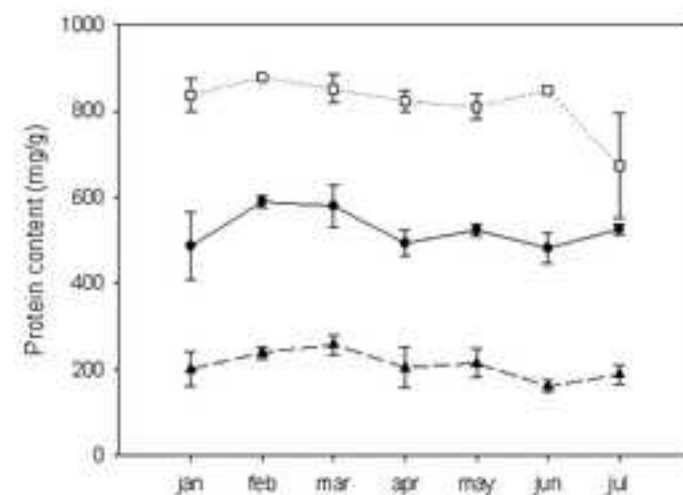
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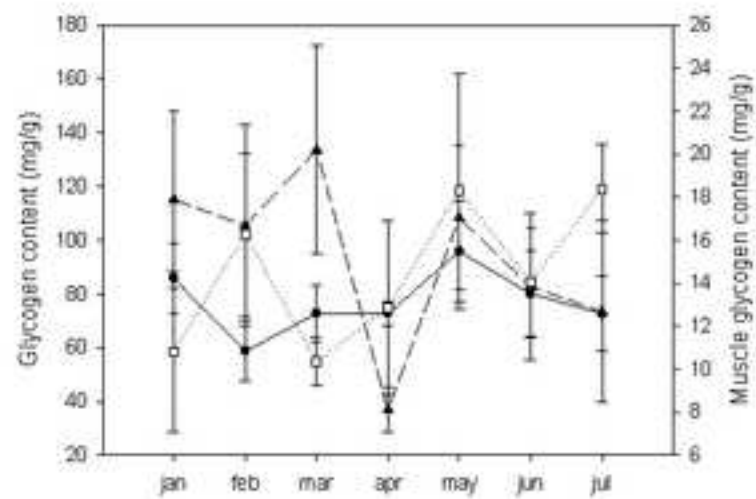
a)



b)



c)



d)

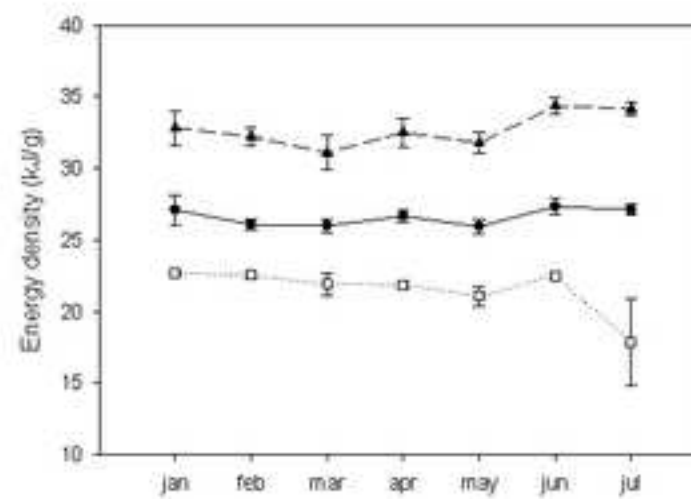
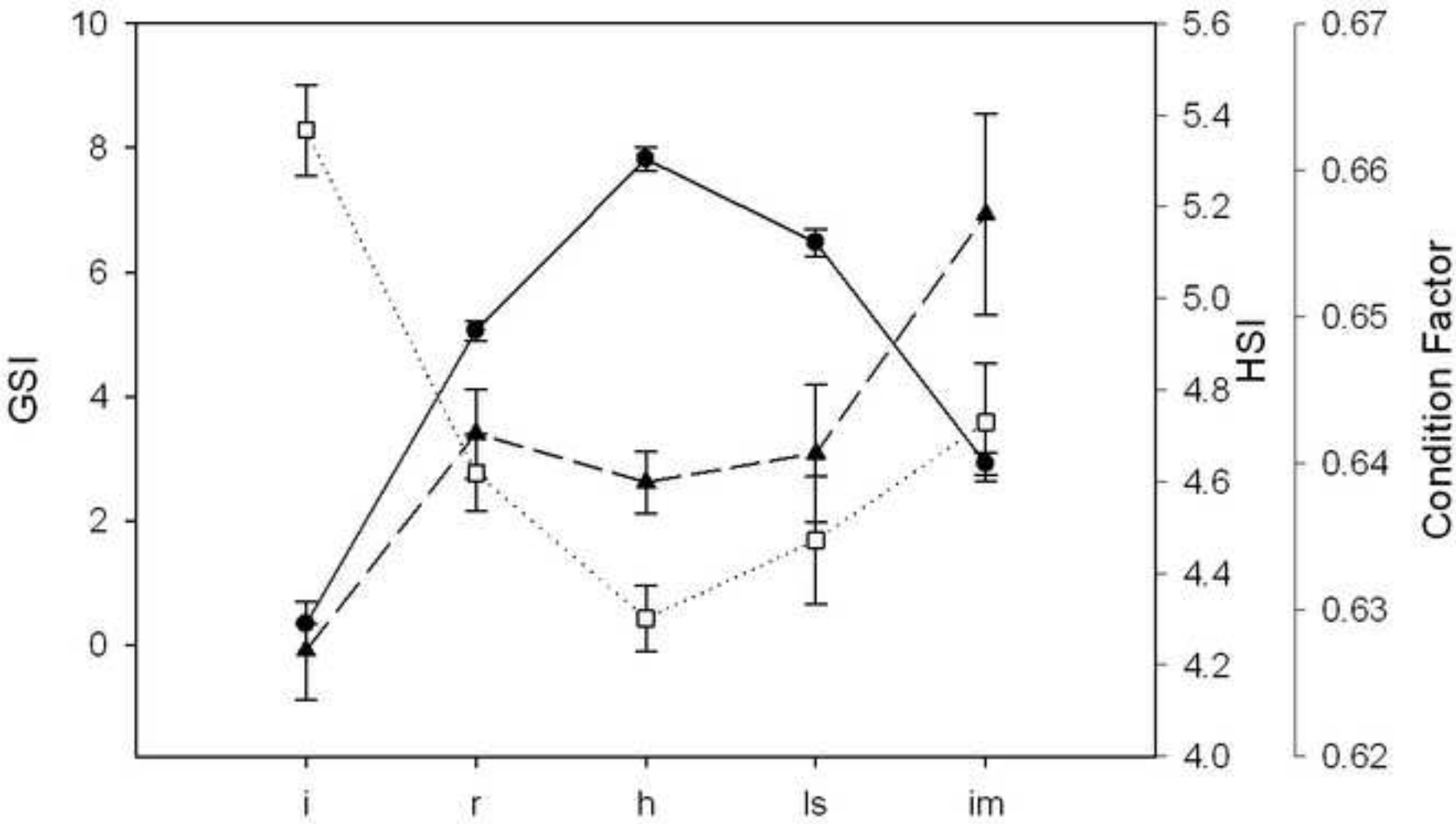


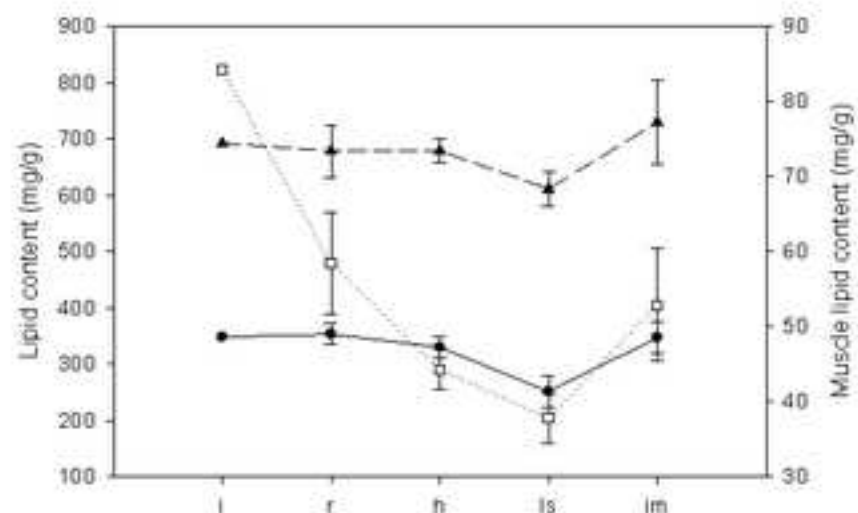
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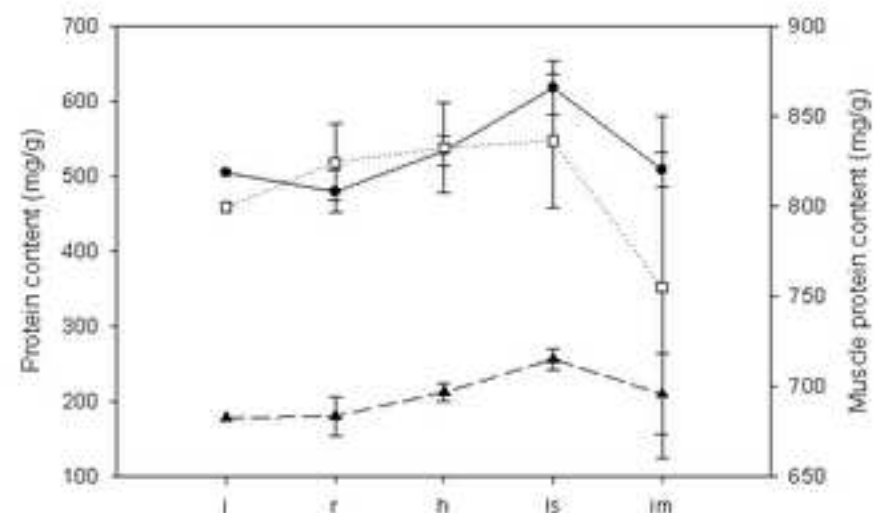
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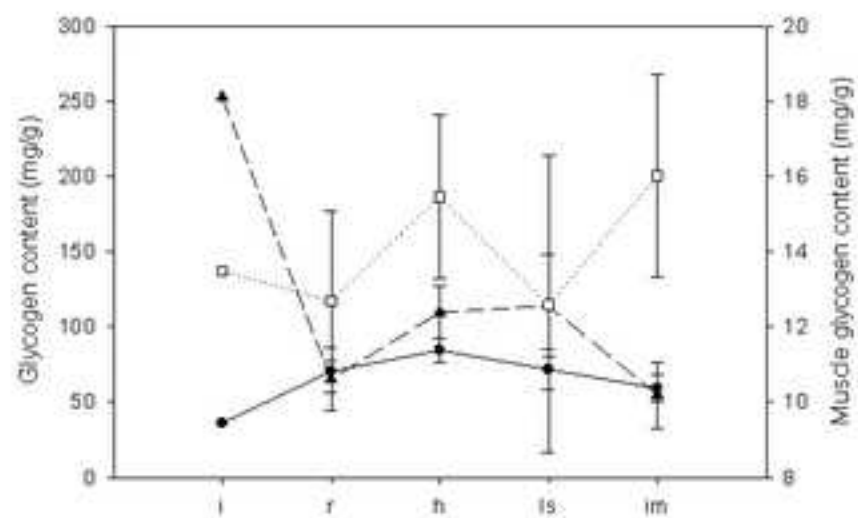
a)



b)



c)



d)

