

1 Relationship between energy allocation and reproductive strategy
2 in *Trisopterus luscus*

3 Runnig Head: Energy allocation and reproduction in *Trisopterus luscus*

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9 ABSTRACT

10 Proximate composition and energy content in *Trisopterus luscus* have been studied to
11 address energy allocation strategy. Energy reserves in pouting females are mainly
12 associated with lipids stored in the liver. Water content appeared to be a useful index in
13 order to obtain very accurate predictions of energy density, particularly for ovaries and
14 liver. Condition indices, HSI and K, are suitable to analyze nutritional status of fish,
15 since they reflect changes in energy content and proximate composition in the different
16 tissues studied. The seasonality of energy reserves was detected and is closely related to
17 the sexual cycle, indicating that females pouting display a period of energy storage,
18 mainly through lipid deposition in the liver. Therefore, *T. luscus* should be considered a
19 capital rather than an income breeder, as the energy for reproduction comes essentially
20 from stored energy.

21

22 KEY WORDS: fish reproduction, energy allocation, proximate composition, condition
23 index, *Trisopterus luscus*

24

25 INTRODUCTION

26 A central assumption of life-history theory is the existence in iteroparous organisms
27 of a trade-off between present reproduction and future reproductive output, this being
28 the consequence of an ultimate trade-off among survival, growth and reproduction
29 (Roff, 1992; Stearns, 1992). The energy required for survival varies as fish grow and
30 changes after each reproductive episode. Species that reproduce only once
31 (semelparous) invest most of their energy in reproduction before dying. Those with
32 repeated reproduction (iteroparous) save some energy for survival and possible further
33 growth. Reproduction has energetic and physiological costs; therefore, if reproductive
34 investment is excessive, it may reduce life-span, increasing parent mortality risks. This
35 is especially evident under stress conditions, for instance limited resources for
36 metabolic use (Callow, 1985). If priority is given to growth, increased body size will
37 likely reduce the risk of being predated upon and also improve feeding opportunities.
38 This is reflected in size-dependent survival (Beverton and Holt, 1959; Pauly, 1980;
39 Pepin, 1991). This leads to an unavoidable trade-off between growth and reproduction,
40 i.e. maturation and gamete production, which has an important effect on different
41 reproductive strategies.

42 Consequently, the energetic costs of reproduction and the faculty to restore energy
43 after each reproductive event influence both mortality and the ability to spawn in future
44 years. Therefore the offspring could be partially dependent on energy acquisition by the
45 fish stock prior to spawning. This includes the replenishment of energy from the
46 previous spawning (Henderson et al., 1996; Lambert and Dutil, 2000).

47 Marine environments show important seasonal and annual fluctuations in energy
48 availability (food resources), and organisms have developed several strategies for
49 energy acquisition and allocation to reproduction. The classical division of these types

50 of strategies is made between capital and income breeders (Drent and Daan, 1980;
51 Stearns, 1989). Capital breeders store energy to be used later on for reproduction, and
52 thus storage constitutes the primary energy source for reproduction. In pure income
53 breeders, reproduction is fully financed by means of current energetic income, i.e.
54 current feeding activity, and there is no need (or mechanisms) for storing energy. These
55 are the extremes of a continuum; certain species may compensate for inadequate energy
56 deposits with income derived from feeding (Stearns, 1989; Henderson et al., 1996).

57 As a consequence, and as it stems from energy allocation rules, seasonal growth and
58 energy storage cycles are common among cold and temperate fish and are related to
59 environmental production cycles, usually reflecting the impact of reproduction on
60 physiological condition (Callow, 1985; Stearns, 1992; Aristizabal, 2007).

61 Pouting, *Trisopterus luscus*, is a member of the Gadoid family and is of major
62 commercial importance for the artisanal fleet of a number of European countries,
63 primarily France, Portugal, and Spain. *T. luscus* is distributed, from the Skagerrak and
64 the British Isles to southern Morocco, and into the western Mediterranean. *T.luscus*,
65 considered a batch spawner (Merayo, 1996a), shows a protracted spawning season
66 during winter and spring time (Gherbi-Barre, 1983; Desmarchelier, 1985; Merayo,
67 1996a). Pouting presents asynchronous ovarian development organization and females
68 reach maturation at about 15 cm, based on histology (Alonso-Fernández et al., 2008;
69 Alonso-Fernández, 2011). Despite the asynchronous ovarian organization, several
70 authors consider it as a determinate fecundity species making it possible to estimate
71 potential fecundity (Merayo, 1996a; Alonso-Fernández et al., 2008).

72 Our study makes use of chemical and morphometric data to properly address the
73 bioenergetics dynamics of reproduction. To analyse seasonal variation in energy
74 allocation to reproduction, it is crucial to determine first the reproductive modality of

75 the species regarding energy allocation. Our hypothesis is that pouting females appear
76 to be capital breeders, rather than income ones, based on the trends in condition indices
77 throughout the year as stated in preceding studies (Merayo, 1996b). In order to test this
78 hypothesis, three main objectives are addressed in this section: i) study of proximate
79 composition and levels of energy stored in different fish tissues, ii) suitability of simple
80 condition indices to study changes in energy reserves and iii) quantification of seasonal
81 changes and energy dynamics related to reproductive annual cycle. Therefore, it should
82 be possible to clarify when energy is acquired and how energy is allocated to
83 reproduction in female pouting.

84

85 MATERIAL AND METHODS

86 Samples were taken on a monthly basis over a three-year period, from December 2003
87 to December 2006. The sampling strategy was devised to achieve proper sampling
88 distribution across years, seasons and sizes, always subject to availability and providing
89 good seasonal and population coverage (Table 1).

90 Pouting samples were collected from landings in fish markets of Ribeira and Vigo.
91 Sampling locations were distributed throughout the main fishing grounds along the
92 coastal waters of the Galician shelf (Figure 1). Therefore, this research covers
93 significant spatial range in terms of the pouting population on the Galician shelf. A total
94 of 2094 females were sampled and histologically analysed (Table 1).

95 Ovaries were removed from all specimens and one lobe was fixed immediately in 10%
96 formalin, buffered with $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (0.046 M, molar concentration) and
97 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (0.029 M) for subsequent histology.

98 Central portions of the fixed ovaries were extracted, dehydrated, embedded in paraffin,
99 sectioned at 3 μm and stained for microscopic analysis. The Haematoxylin-Eosin
100 staining protocol was used for preparation of histological slides. Reproductive phases
101 are defined as Immature (Imm), Developing (Dev), Spawning Capable (SC) and
102 correspondent sub-phase Actively Spawning (AS), Regressing (Rgs) and Regenerating
103 (Rgn) (Brown-Peterson et al., 2011).

104 The following information was collected from each fish: total length (TL $\pm 0.1\text{cm}$); total
105 weight and gutted weight (TW and W $\pm 0.01\text{g}$); gender; reproductive phase; and ovary
106 weight and liver weight (GW and LW, $\pm 0.01\text{g}$). Several condition indices were
107 estimated for each mature female: the gonadosomatic index (GSI), the hepatosomatic
108 index (HSI) and condition factor (K).

109 $\text{GSI} = 100 \cdot \text{GW} / \text{W}$

110 $HSI = 100 * LW / W$

111 $K = 100 * W / L^3$

112 The temporal variation of GSI was analyzed by comparing monthly mean values to
113 describe sexual cycle seasonality.

114 One ovary lobule, a liver portion and one muscle slice from the middle part of the body
115 per specimen were frozen at -80°C for proximate composition analysis. In order to cover
116 as much as possible proximate composition variation in *T. luscus*, sample selection
117 included all maturity phases, and therefore, also temporal variation was reflected in this
118 subset. In order to estimate proximate composition, the correspondent portion of each
119 tissue was lyophilized, homogenized in a grinder and stored at -20°C until analysis of
120 lipids, proteins, glycogen and water.

121 Analyses were made on two replicates, and samples with coefficient of variation above
122 10% were repeated. If the coefficient of variation of a sample remained higher than
123 10%, the sample was excluded from the analysis. Ultimately, sample sums were 27, 43
124 and 76 for ovary, liver and muscle tissue, respectively. Due to the reduction in sample
125 tissue during lyophilisation, it was necessary to dilute the sample to increase the
126 quantity of substrate, in order to carry out all biochemical quantifications. To confirm
127 concentration of tissue component it was used a subsample of this solution. A
128 subsample of known volume from the original sample solution was kept in an oven for
129 24 hours at 100°C and then weighed at ambient temperature. This difference was used
130 to obtain the correct values of the biochemical analyses. Lipids were extracted
131 following a modification of (Bligh and Dyer, 1959). Proteins were determined
132 following the methodology described by (Lowry et al., 1951). Total carbohydrates
133 (glycogen) were quantified as glucose, as it is the standard for the phenol/sulphuric acid
134 colorimetric assay (Strickland and Parsons, 1968). For estimation of water content the

135 portions of each tissue were re-weight after lyophilisation to estimate water loss. Once
136 the tissue was lyophilized a subsample was weighted in a precision weight scale
137 (+0.0001mg) and processed in a furnace muffle for approximately 12 hours for
138 estimation of ash content.

139 Energy density in each tissue was calculated by applying energy equivalents of 39.5,
140 23.6 and 17.1 kJ/g for lipid, protein and glycogen, respectively (Kleiber, 1975). Total
141 tissue energy in each fish was calculated multiplying the energy density by the total
142 weight (ovary, liver and muscle respectively). Muscle weight was calculated extracting
143 the amount of fish skin and bones to the gutted weight using the following relationship
144 (Kjesbu et al., 1998):

$$145 \text{ Fish skin and bones (g)} = 0.0026 * (\text{Female length, cm})^{3.2142}; n=32, p<0.001, R^2=0.98$$

146 The term content refers to percentage of tissue wet weight; and the term density refers
147 to kJ per gram of tissue through the text.

148 Differences in average biochemical composition and energy density in tissues were
149 assessed using Generalized Least Squares, GLS, to properly modelate variance structure
150 (Pinheiro et al., 2010).

151 The contribution of each component (lipid, protein and glycogen) to the energy density
152 of each tissue was calculated, so, multiple linear regression models were fitted to the
153 energy density and component content relationships. ANOVA model comparisons were
154 used for model selection (Zuur et al., 2007; Zuur et al., 2010).

155 Simple linear regressions were used to explore the existing relationships of energy
156 density (ED) and proximate composition (PC: protein, lipid or glycogen %) as a
157 function of water content (WC). :

$$158 \text{ PC or ED} = \alpha + \beta * \text{WC} + \varepsilon$$

159 Therefore, following successful establishment of tight relationships (see below), water content
160 of tissues from all the specimens were used to increase the number of data (sample size)
161 for further analysis (n=559, 1172 and 661 for ovary, liver and muscle tissue,
162 respectively). In this way, the biochemical database notably improved in quantity,
163 providing better information on proximate and energy variation of *T. luscus* females.

164 Several aspects of condition indices were explored i) independence of energy reserves
165 of body size and ii) direct correlation with the energy stores.

166 Seasonality of total energy was evaluated by using Generalized Additive Models, GAM
167 (Wood, 2006) to properly depict monthly variation in fish condition. Length was
168 included in the model to remove fish size effect. Immature individuals were excluded
169 from the analysis:

$$170 \log(\text{Total energy}) = \alpha + \log(\text{length}) + \varphi(\text{Month}) + \varepsilon$$

171 Total energy and length were natural log transformed to properly meet normality
172 assumptions. The variable month has only 12 values, and to avoid over-smoothing we
173 decided to use 4 degrees of freedom for the smoothing term. Additionally, variation in
174 nutritional status in the different reproductive phases was studied through total energy
175 using GLS. Length was also included in this analysis as a covariate to remove fish size
176 effect.

177 Statistical analyses were conducted using the R software package (R Development Core
178 Team 2011). For all the data sets an initial data exploration was conducted following the
179 protocol proposed by (Zuur et al., 2010).

180

181 RESULTS

182 Proximate composition and energy density were analysed in ovary, muscle and liver of
183 female pouting. The average biochemical composition and energy density for the whole
184 period studied was significantly different across tissues for all the components analysed
185 (Table 2).

186 Variation in ovary energy content is significantly associated with lipids and proteins
187 (Table 3, Figure 2). Proteins are the major constituent of ovary proximate composition
188 (Table 2), representing ca. 18% of tissue composition, which is around 80% of energy
189 content in ovaries. Although lipids amount to less than 5% of ovary tissue composition,
190 they average ca. 20% of energy content (Figure 2). In fact, the amount of variation in
191 energy density purely related to lipids is not so far from the variation related to proteins.
192 Both components, proteins and lipids, shared almost 50% of the explained variation in
193 ovary energy density (Table 3). Results of glycogen content showed negligible
194 concentrations, less than 0.2%, and energy variations in ovaries due to changes in
195 glycogen content are not appreciable and did not significantly contribute to explain
196 energy density variation (Table 3). Thus, the resultant final model includes only lipids
197 and proteins and it is expressed as:

198 $OED = 0.3331 + 0.5197*OLC + 0.1956*OPC$

199 Where OED is Ovary Energy Density (kJ/g), OLC is Ovary Lipid Content and OPC is
200 Ovary Protein Content (% of lipids and proteins on a wet-weight basis).

201 The relative energy contribution of proteins and lipids is not stable (Figure 2). Higher
202 ovary energy levels are found when the contribution of lipids increases. However,
203 proteins were still the main component and energetic constituent of ovaries, although
204 lipid and protein concentrations exhibited significant positive correlation in ovaries
205 (Pearson's p correlation, $r=0.52$, $n=27$, $p\text{-value}<0.01$).

206 Most of the wet weight corresponds to water, representing more than 70% of the total
207 weight of the ovary. Besides, there was a significant inverse relationship between water
208 content and concentration of lipids and proteins in ovaries (Table 4). Water content
209 accounted for a large portion of variation in ovary lipids, but especially for variation in
210 energy density and proteins (Table 4 and Figure 3).

211 The liver was the tissue with the highest energetic levels as compared to ovary and
212 muscle (Table 2). Lipids can reach over 65% of liver wet weight; however, lipid content
213 dropped even below 4% of wet weight in fish with low condition. Consequently, energy
214 density is strongly related to lipid concentration representing up to 80-90 % (Figure 4).
215 Most of the energy variation detected in the liver is generated by lipid fluctuation,
216 which accounts on its own for almost 95% of total energy, whilst proteins, although
217 significant, account for less than 1% of total energy variation (Table 3). Glycogen did
218 not significantly contribute. Lipids and proteins share only 1.91% of the total
219 information of the final model (Table 3), summarized in the following equation:

$$220 \text{ LED} = 0.0675 + 0.4216 * \text{LLC} + 0.2198 * \text{LPC}$$

221 Where LED is Liver Energy Density (kJ/g), LLC is Liver Lipid Content and LPC is
222 Liver Protein Content (% of lipids and proteins on a wet-weight basis).

223 Protein contribution to total liver energy ranged from 5.33% to 76.24% in an inverse
224 relationship (Figure 4). Minor variations in glycogen content were observed. Therefore,
225 lipid energy reserves are the capital source of energy in the liver; when lipids decrease,
226 the relative importance of protein increases (Figure 4).

227 Water content in liver demonstrated the highest fluctuations of all tissue analysed (Table
228 2). Water variation in the liver distinctly reflected changes in lipid concentration and
229 energy density. A significant negative linear relationship between lipid and water
230 content was observed (Table 4 and Figure 3).

231 The muscle had the smallest energy variation found among tissues analysed (Table 2).
232 Proteins are the capital components of this tissue, averaging over 20% of the wet
233 weight. In terms of energy, proteins represent on average almost 95% of total muscle
234 energy (Figure 5).

235 Lipid and glycogen levels remained relatively constant with muscle energy variation,
236 and only changes in protein content significantly explained variations in muscle energy
237 density (Table 3). The relationship is described by the following equation:

$$238 \text{ MED} = -0.4674 + 0.2687 * \text{MPC}$$

239 Where MED is Muscle Energy Density (kJ/g) and MPC is Muscle Protein Content (%
240 of proteins on a wet-weight basis).

241 Muscle water content is the least variable of all tissues analysed, only 5.51% percent
242 points. There was a strong inverse relationship between water and protein content in
243 muscle (Table 4). Water content accounted for a lower percentage of muscle energy
244 variability, (Table 4 and Figure 3), although it was still significant.

245 The following indices were examined as expression of physiological fish condition:
246 hepatosomatic index (HSI) and body condition factor (K). GSI was studied to search for
247 possible energetic patterns in ovaries during ovary development. No statistical
248 relationship was found between ED and fish total length for any of the tissues and
249 maturity phases analysed: ovary, liver and muscle ($p > 0.05$). HSI increased significantly
250 with liver energy density ($n=1172$, $df=2$, $p\text{-value} < 0.001$, $r^2=0.462$). Hepatosomatic
251 index is related to liver energy density, hence HSI act as a good proxy for energetic
252 status. Condition factor K was significantly and positively related to energy density in
253 muscle; but the prediction power of these models was considerably lower ($n=661$, $df=2$,
254 $p\text{-value}=0.029$, $r^2=0.007$).

255 Seasonal energy trends in total energy, in the three different tissues, were analysed by
256 pooling the data per month for all years sampled. Following removal of the female size
257 effect, all tissues analysed presented significant variation along the year on a monthly
258 basis (GAM, Table 5). Model predictions at two different female sizes, 20 and 30 cm,
259 revealed that liver is the tissue which mobilized the highest amount of energy through
260 the annual sexual cycle (Table 6). Throughout an annual cycle, total liver energy
261 underwent important variations: the maximum value occurred in December, just prior to
262 the peak of the spawning season, while the lowest value was observed in April, when
263 the spawning season has severely declined (Figure 6). Energy storage in the liver occurs
264 immediately after the end of spawning season, from May to July, as indicated by
265 monthly net energy variation (Figure 6). During the period ensuing reproduction
266 (August-October), low storage activity occurs in liver, and increased over again in
267 November and December. Liver energy consumption (negative net energy variation)
268 becomes apparent in January (Figure 6) and continues during the spawning season.

269 Total energy trends were also evaluated by reproductive phases (removing also the
270 females size effect in the model), and energy storage showed a clear fluctuation pattern
271 related to reproduction, as was expected regarding the above results (Figure 7).
272 Maximum values occurred in developing and recovering females. Significant
273 differences were found comparing those developing and spawning capable maturity
274 phases where it is expected the higher energy investment in ovary development (GLS:
275 $t=-5.517$, $p<0.001$ in liver; $t=-3.850$, $p<0.001$ in muscle; $t=16.724$, $p<0.001$ in ovary).

276

277 DISCUSSION

278 Ovaries of *T. luscus* are primarily composed of proteins and lipids. It is assumed that
279 proteins and lipids serve as as building blocks for ovary development, among other fish
280 requirements (Kamler, 1992). Bearing in mind our a priori hypothesis, i.e. that pouting
281 is a capital breeder, there must exist an endogenous source of energy for ovary
282 development, gained in advance of the reproductive period.

283 From a bioenergetics approach, the discrimination between lipid and protein reserves is
284 not a trivial issue when we want to understand the dynamics of energy allocation. It has
285 been demonstrated in many fishes that eggshell and egg yolk proteins use the liver as
286 their main major site of synthesis and these substances are then transported to the
287 oocyte for uptake (Arukwe and Goksoyr, 2003). Vitellogenin (precursor of yolk
288 proteins) is a complex calcium-binding phospholipoglycoprotein, and now, it is known
289 that the ability to transport fat, in the form of lipoprotein through the circulatory system
290 by eukaryotes is one of their most significant functions. Also, protein synthesis is a
291 major energy-demanding process (high energetic cost) and mobilization of fat reserves
292 results in the highest energetic yield as compared with other substrates, i.e., proteins
293 (Bonnet et al., 1998). On grounds of all these considerations, it is well-founded to think
294 of liver as the main source of energy reserves in pouting. However, the role of energy
295 stored in muscle, mostly as proteins, cannot be diminished, especially at the end of the
296 spawning season.

297 It is widely accepted that reproduction comprises several endocrine and metabolic
298 changes to mobilize and reallocate both materials and energy (Jobling, 1995). Energy
299 allocation can vary, depending on the life-history strategy of a fish species. It is possible
300 that the amount of food ingested during the spawning season may partially offset the
301 cost of reproduction, as it is the case of *Pagrus pagrus* (Aristizabal, 2007). However,

302 when energy intake is not enough to cover the costs of ovarian growth and somatic
303 maintenance, it is necessary to transfer resources from stored body energy (Adams et
304 al., 1982; Henderson and Morgan, 2002). To this respect, the evolutionary advancement
305 of storing energy in the form of fat has provided organisms with enormous advantage in
306 adapting to environmental and developmental changes.

307 One interesting finding is the strong inverse relationships found between energy density
308 and content of main biochemical components with water content for all the tissues
309 analysed: ovary, liver and muscle. These inverse relationships between
310 energy/proximate composition and water content are well known in fishes. Similar
311 results were found for liver and muscle tissue in pouting from the Cantabrian Sea
312 (Merayo, 1996b); however, no equations for later comparisons or predictive purposes
313 were provided. Different relationships between the dry mass proportion and energy
314 density of the fish were assessed for a variety of species from the North Sea and that
315 study showed that there were insignificant differences across species, indicating at some
316 point that the usefulness of generalized models can be established (Pedersen and Hislop,
317 2001). Contrary to this conclusion, some studies recommended the use of the lowest
318 taxon model possible, since they found species-specific differences, but also ecosystem-
319 specific, in the energy density relationship (Hartman and Brandt, 1995; Dubreuil and
320 Petitgas, 2009). Despite this controversy, the general trend is still there, i.e. water
321 percentage is inversely correlated to energy density in fish tissue. Therefore, water/dry-
322 matter relationships should be properly compared from a variety of tissues, taxa and
323 ecosystems, using normalized methodologies in an attempt to infer a general model for
324 fish -if there is one to be found.

325 Body condition indices are generally used in an attempt to determine the amount of
326 mass in the individual, correlated to energy reserves after removal of the structural body

327 size effect. However, this is not always completely true, as a certain extent of the
328 variation in condition may indicate variation in all the constituents of body composition,
329 not only in energy reserves (Schulte-Hostedde et al., 2005). In our study, only HSI
330 clearly reflects an increase in the stock of energy reserves. This is related to the role of
331 the liver as a deposit of lipids, already shown in pouting (Merayo, 1996b). In addition,
332 the importance of hepatosomatic index as indicator of reproductive success in Atlantic
333 cod (*Gadus morhua*) has been pointed out, linked to the role of lipids (Marshall et al.,
334 1999). This fact supports the idea that the liver represents the main source of energy for
335 female pouting. The incorporation of endogenous proteins as energy reserves entails the
336 mobilization of large amounts of water, as compared to lipids in the liver. Therefore;
337 energy storage through lipid accumulation seems to be more efficient than through
338 proteins (Bonnet et al., 1998).

339 It is well known that energy allocation strategies vary across organisms. A number of
340 studies on fish condition variations have been carried out in cold-water species, where
341 marked seasonal changes in food supply, temperature and photoperiod occur, and
342 spawning usually takes place in periods of limited food availability. Consequently,
343 vitellogenesis occurs during environmentally adverse periods and must rely on energy
344 stored over the months preceding follicle development. That situation is characteristic of
345 determinate fecundity species, like most gadoids, e.g. haddock, *Melanogrammus*
346 *aeglefinus*, and cod among others (Eliassen and Vahl, 1982; Kjesbu et al., 1991;
347 Lambert and Dutil, 1997; Pedersen and Hislop, 2001; Trippel and Neil, 2004).
348 However, in some temperate species condition indices fluctuate throughout the year
349 with no clear seasonal pattern, as is the case of *Merluccius hubbsi* and *M. merluccius*,
350 two indeterminate fecundity species (Méndez and González, 1997; Domínguez-Petit et
351 al., 2010). In contrast, tropical environments exhibit minimal seasonal variation of

352 temperature and photoperiod; in consequence, it has been suggested (Junk, 1985) that in
353 neotropical areas only migratory fish display cycles of energy accumulation/depletion,
354 whilst non-migratory species do not present this seasonality. However, recent studies
355 suggest that even non-migratory tropical species may exhibit seasonal patterns of
356 energy storage related to other environmental conditions (Arrington et al., 2006).

357 We hypothesised that *T. luscus* reproductive cost is afforded mainly at the expense of
358 energy reserves stored in advance of breeding, i.e. following a capital breeding strategy
359 (Drent and Daan, 1980; Callow, 1985; Stearns, 1989). It is known that energy storage
360 and expenditure is an important component of life-history strategies, directly affecting
361 the trade-off across current reproduction, survival and future reproduction (Roff, 1992;
362 Stearns, 1992). Assessing the cost of reproduction and determination of the energy
363 allocation strategy became an important issue, since reproductive potential may be
364 dependent upon available energetic resources, rather than on food resources during the
365 spawning season.

366 In the present study total liver energy exhibits seasonal variation as it was already
367 shown in pouting (Merayo, 1996b). Seasonal changes in fish energy are normally
368 associated with seasonal changes in food consumption, diet and the allocation of
369 assimilated energy to the growth-reproduction dynamic (Jobling, 1995; Pedersen and
370 Hislop, 2001). Our results are in agreement with previous studies in a variety of species
371 (Henderson et al., 1996; Merayo, 1996b; Xie et al., 1998), where the maximum values
372 of energy reserves are found immediately before the onset of ripening and are depleted
373 at the end of the spawning period. Hence, it seems that the energy to be allocated to
374 reproduction is stored in advance and mobilized later on, to supply the energy required
375 for physiological functions related to reproduction in female pouting. The drastic
376 variation in the lipids stored in the liver, provides evidence of the role of this source of

377 energy, which is likely linked to reproductive activity. Some authors suggest as well
378 that the liver acts as a buffer between the soma and the ovaries, in order to ensure
379 ovarian maturation even at low rates of energy intake (Allen and Wootton, 1982).

380 Seasonal changes in feeding activity have not been observed in pouting, even during the
381 spawning season (Merayo, 1991; Hamerlynck and Hostens, 1993). Seasonal changes in
382 energy levels also could correspond to shifts in prey preference. This aspect it is yet to
383 be investigated in pouting; however, in related species, like *Trisopterus minutus*, it was
384 not detected a clear change in the quality of food ingested along the year and
385 reproduction seems to have little effect on feeding intensity (Politou and
386 Papaconstantinou, 1994). Therefore, the observed seasonal variation in energy reserves
387 may respond exclusively to changes in the energy requirements associated to the sexual
388 cycle. This seems to support as well the hypothesis of *T. luscus* being a capital rather
389 than income breeder. Hence, energy reserves may have some impact on reproductive
390 potential; as is suggested in a recent study (Alonso-Fernández and Saborido-Rey, 2011).

391 In some species, a protracted spawning season and population asynchronicity in
392 spawning activity may impede the ecological study of the dynamic of energy allocation
393 and mobilization, as is the case of European hake (Domínguez-Petit et al., 2010). So, it
394 was deemed convenient to analyse energy reserves variation by female maturity phases,
395 rather than based on a temporal pattern. This new analysis corroborates the observed
396 seasonal pattern and suitably reflects the accumulation/depletion cycle of energy
397 reserves and its direct relation with ovary development and reproductive activity in
398 general.

399 Capital and income breeders represent the ends of a continuum, and some species are
400 able to compensate for inadequate energy deposits with concurrent food intake
401 (Henderson et al., 1996). It was suggested that in ectothermic organisms, including fish

402 species, the strategy of capital breeding is the most widespread, because ectothermic
403 features tend to pre-adapt organisms to store energy prior to its use (Bonnet et al.,
404 1998). Despite this apparent tendency to store energy, found in fish species under
405 changing environmental conditions, there are few studies that relate the presence of
406 income breeding species among teleostei (Aristizabal, 2007; Domínguez-Petit and
407 Saborido-Rey, 2010). These two examples, *Pagrus pagrus* and *Merluccius merluccius*,
408 exhibit a strategy based on lower energy allocation and a higher dependence on food
409 during the spawning time in order to ensure successful reproduction. Reproductive
410 strategy may influence at some point which approach characterizes a fish species
411 concerning energy allocation. In capital breeders, the stored energy allows individuals
412 “to predict” the potential number of eggs to be spawned (determinate fecundity), but
413 normally yolk accumulation in late vitellogenesis is done at the expense of stored reserves,
414 leading to a modality of group-synchronous oocyte development. In income breeders,
415 on the contrary, the lack of stored energy prevents the prediction of egg production and
416 both oocyte recruitment and vitellogenesis is done at the expense of surplus energy from
417 concurrent feeding, leading to asynchronous oocyte development (Saborido-Rey et al.,
418 2010). Interestingly, pouting exhibits determinate –or nigh to determinate (Alonso-
419 Fernández, 2011)— fecundity, but also asynchronous oocyte development (Alonso-
420 Fernández et al., 2008), indicating that it is a suitable candidate to possess an
421 intermediate strategy, combining capital and income breeding.

422 In between these end strategies, capital-income breeders, species that store large
423 amounts of energy but which show important feeding activity during the spawning
424 season are expected to be found. Ultimately, the females of *T. luscus* show a marked
425 seasonal pattern concerning energy reserves, apparently exhibiting determinate
426 fecundity, but also asynchronous oocyte development (Alonso-Fernández et al., 2008).

427 Although oocyte recruitment is initiated a few months before spawning, vitellogenesis
428 takes only a few weeks to be completed (Alonso-Fernández, 2011). Subsequently, it
429 could be hypothesized that energy intake from concurrent feeding influences egg
430 production, although it is clear that the liver acts as the main energy storage. It is still
431 ignored which of the energy available (as storage or recently assimilated) is diverted for
432 reproduction, as well the allocation norm, i.e. which type of energy and in what amount
433 is devoted to reproduction (gamete production and reproductive behaviour) and
434 maintenance. Nevertheless, the results presented here show an important seasonality in
435 energy reserves related to reproduction. Besides, it has been demonstrated that the liver
436 is the tissue that mobilize more energy during the reproductive season; although the
437 muscle energy is also contributing.

438 Summarizing, energy reserves in pouting females are mainly associated with the lipids
439 stored in liver. Water content appeared to be a useful index in order to obtain very
440 accurate predictions of energy density, particularly for ovaries and the liver. HSI has
441 been detected as the best condition index to analyse nutritional status of fish, since it
442 reflects the main changes in fish energy. The seasonality of energy reserves was clearly
443 detected and is closely related to the sexual cycle, indicating that pouting females
444 display a period of energy storage, mainly through lipid deposition in the liver.
445 Therefore, pouting should be considered a capital rather than an income breeder, as the
446 fuel for reproduction comes essentially from stored energy. However, regarding its
447 particular reproductive strategy (asynchronous/determinate), it is hypothesized for
448 pouting that a fraction of the direct intake of energy may also be allocated to
449 reproduction. The extent of this influence on the reproductive output is yet to be
450 assessed.

451

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458 discussions held within the NAFO Working Group on Reproductive Potential and the
459 COST Action FA0601 Fish Reproduction and Fisheries (FRESH).

460

461 TABLES

462 Table 1. Summary of sampling effort of female *T. luscus*, body length (cm) range and
 463 gutted weight (g) range, used in this study.

Month	Specimens (n)	Number of hauls	Length range (cm)	Gutted Weight range (g)
Jan	159	4	16.7 - 40	48.2 - 914.2
Feb	232	6	13.9 - 35.1	26.1 - 458.1
Mar	149	10	17.1 - 39.7	44.5 - 652.6
Apr	310	9	14.8 - 33.6	31.0 - 429.7
May	293	8	16.5 - 33.7	46.8 - 439.7
Jun	157	4	17 - 41.7	38.4 - 861.3
Jul	127	4	17.2 - 33.5	53.5 - 466.7
Aug	107	3	16.6 - 30.7	46.7 - 325.1
Sep	91	10	16.2 - 31.1	37 - 328.5
Oct	95	4	16.9 - 32.5	49.3 - 388.5
Nov	175	6	15.2 - 29.7	40.9 - 299.7
Dec	199	6	13.6 - 35.9	29.0 - 597.3
TOTAL	2094	75	11.9 - 41.7	26.1 - 914.2

464

465

466 Table 2. Summary of biochemical composition and energy density for ovaries, liver and
 467 muscle of female *T. luscus*, in 2003-2006 (values are given as % of tissue wet weight
 468 and energy density, ED, in kJ/g). p-value corresponds to the GLS comparing
 469 composition among tissues.

	Ovary			Liver			Muscle			GLS
	n	Range	Mean	n	Range	Mean	n	Range	Mean	p-value
Lipid	27	0.71-3.87	2.39	43	3.31-66.20	32.54	76	0.279-1.362	0.634	p<0.01
Protein	27	12.50-22.93	17.65	43	4.83-18.69	10.75	76	18.13-23.63	21.04	p<0.01
Glycogen	27	0.009-0.165	0.067	43	0.021-0.272	0.099	76	0.015-0.181	0.056	p<0.01
Ash	27	0.090-0.188	0.142	43	0.041-0.160	0.092	76	0.026-0.200	0.138	p<0.01
Water	27	73.27-86.08	79.76	43	26.3-85.76	56.52	76	75.55-81.06	78.13	p<0.01
ED	27	3.32-7.09	5.03	43	3.85-31.34	16.15	76	4.18-6.43	5.19	p<0.01

470

471

472 Table 3. Results of the multiple linear regression between tissue energy density (ED)
 473 and biochemical components as explanatory variable in *T. luscus*.

ED	n	df	r ²	p-value	Partial Explained Variation (%)		
					Lipid	Protein	Shared
Ovary	27	3	0.924	<0.001	18.47	26.39	47.49
Liver	43	3	0.973	<0.001	94.41	0.95	1.91
Muscle	76	2	0.450	<0.001	-	44.97	-

474

475 Table 4. Results of linear regression between lipid and protein concentrations and
 476 energy density (ED) versus water content for each tissue analysed in *T. luscus*.

477 Lipid/Protein/ED = $\alpha + \beta * \text{Water}$

		n	df	r ²	AIC	p-value	α	Std.Error	β	Std.Error
Ovary	Lipids	27	2	0.472	53.8	<0.001	16.693	3.030	-0.179	0.038
	Proteins	27	2	0.955	49.8	<0.001	82.281	2.815	-0.810	0.035
	ED	27	2	0.866	18.6	<0.001	25.094	1.578	-0.252	0.020
Liver	Lipids	43	2	0.963	236.8	<0.001	89.848	1.840	-1.014	0.031
	ED	43	2	0.964	161.0	<0.001	40.115	0.762	-0.424	0.013
Muscle	Proteins	76	2	0.973	-25.2	<0.001	96.253	1.466	-0.963	0.019
	ED	76	2	0.406	69.7	<0.001	24.662	2.737	-0.249	0.035

478

479 Table 5. Summary of GAMs fitted to total energy variation by month for each tissue
 480 (ovary, liver and muscle) for *T. luscus*. Gaussian distribution was adopted for all

481 response variables. Degrees of freedom (df) and estimated degrees of freedom (edf).

482 Total energy and length were natural log transformed.

Total energy	n	Length df	t	p-value	Month edf	F	p-value	Deviance explained
Ovary	556	1	25.98	<0.001	4	111.1	<0.001	75.5%
Liver	1138	1	37.33	<0.001	4	219.1	<0.001	65.5%
Muscle	635	1	74.78	<0.001	4	46.76	<0.001	90.1%

483

484 Table 6. Net monthly energy variation in the three tissue analysed (liver, muscle and
 485 ovary) in *T. luscus*. Estimations were obtained from predictions of corresponding GAM
 486 s (Table 5) using two standard fish size: 20 cm and 30 cm female size.

Month	20cm female			30cm femlae		
	Liver	Muscle	Ovary	Liver	Muscle	Ovary
January	-34.26	-19.22	5.74	-201.10	-65.15	33.64
February	-32.75	-29.89	1.32	-192.22	-101.28	7.71
March	-12.26	-16.31	0.21	-71.97	-55.28	1.23
April	-2.09	0.13	-1.75	-12.26	0.44	-10.28
May	4.51	13.52	-2.90	26.50	45.80	-16.99
June	10.34	18.77	-2.53	60.69	63.61	-14.85
July	13.03	13.71	-1.55	76.50	46.47	-9.08
August	9.62	3.26	-0.72	56.47	11.04	-4.20
September	4.64	-3.28	-0.15	27.26	-11.13	-0.89
October	5.12	-1.17	0.30	30.08	-3.97	1.74
November	12.08	6.71	0.76	70.88	22.75	4.45
December	22.01	13.78	1.28	129.18	46.71	7.51

487

488

489 FIGURE CAPTIONS

490

491 Figure 1. *Trisopterus luscus* collection locations (dots). Arrows point out sample fish
492 market of origin.

493

494 Figure 2. Relationships between gonad proximate composition (protein, lipid and
495 glycogen) and gonad energy density in *T. luscus*. Values given as: a) % of wet weight
496 and b) % of energy.

497

498 Figure 3. Linear relationships between energy density and water content for gonad, liver
499 and muscle tissue in *T. luscus*. Parameter estimations for each linear regression are
500 presented in Table 4.

501

502 Figure 4. Relationships between liver proximate composition (protein, lipid and
503 glycogen) and liver energy density in *T. luscus*. Values given as: a) % of wet weight and
504 b) % of energy.

505

506 Figure 5. Relationships between muscle proximate composition (protein, lipid and
507 glycogen) and muscle energy density in *T. luscus*. Values given as: a) % of wet weight
508 and b) % of energy.

509

510 Figure 6. Estimated seasonal variations (by month) in total energy in liver (black solid
511 line) and gonad (dashed line) and net monthly energy variation (kJ/g*day) for liver
512 (vertical bars) in a 30 cm female *T. luscus*. (estimated from corresponding GAM, Table
513 5). Dotted lines represent 95% confidence intervals.

514

515 Figure 7. Estimated total energy for gonad, liver and muscle (corresponding lines) and
516 net energy variations through maturity phases (bars) in a 30 cm female *T. luscus*.

517

518 REFERENCES

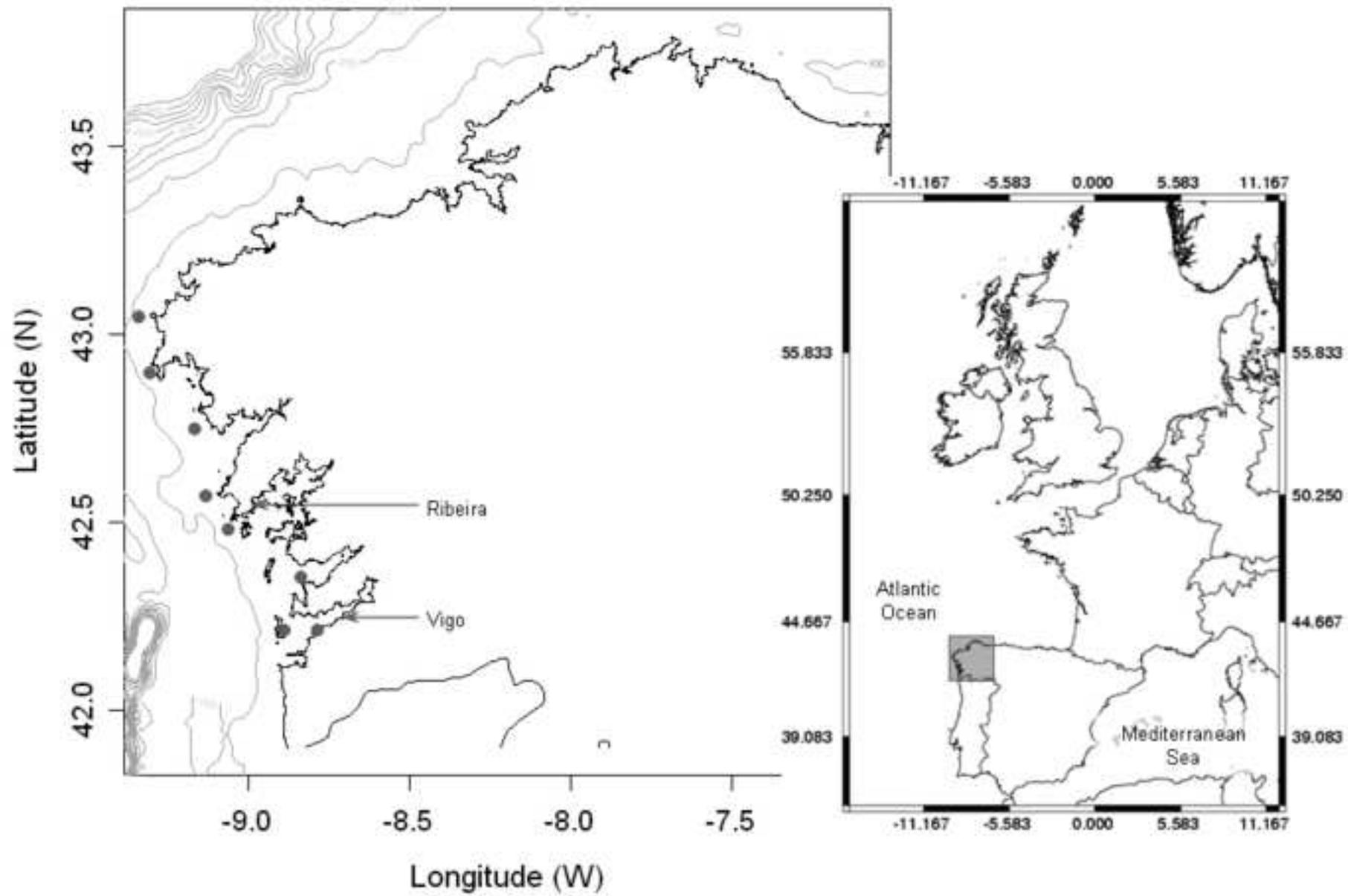
- 519 Adams, S.M., McLean, R.B., Parrotta, J.A., 1982. Energy Partitioning in Largemouth
520 Bass under Conditions of Seasonally Fluctuating Prey Availability. Transactions
521 of the American Fisheries Society 111(5), 549-558.
- 522 Alonso-Fernández, A., 2011. Bioenergetics approach to fish reproductive potential: case
523 of *Trisopterus luscus* (Teleostei) on the Galician Shelf (NW Iberian Peninsula).
524 University of Vigo, Vigo, pp. 350.
- 525 Alonso-Fernández, A., Domínguez-Petit, R., Bao, M., Rivas, C., Saborido-Rey, F.,
526 2008. Spawning pattern and reproductive strategy of female pouting *Trisopterus*
527 *luscus* (Gadidae) on the Galician shelf of north-western Spain. Aquatic Living
528 Resources 21(4), 383-393.
- 529 Alonso-Fernández, A., Saborido-Rey, F., 2011. Maternal influence on the variation of
530 the reproductive cycle of *Trisopterus luscus* (Gadidae). Ciencias Marinas 37(4B
531), 619–632.
- 532 Allen, J.R.M., Wootton, R.J., 1982. The effect of ration and temperature on the growth
533 of the three-spined stickleback, *Gasterosteus aculeatus* L. Journal of fish biology
534 20(4), 409-422.
- 535 Aristizabal, E.O., 2007. Energy investment in the annual reproduction cycle of female
536 red porgy, *Pagrus pagrus* (L.). Marine Biology 152(3), 713-724.
- 537 Arrington, D.A., Davidson, B.K., Winemiller, K.O., Layman, C.A., 2006. Influence of
538 life history and seasonal hydrology on lipid storage in three neotropical fish
539 species. Journal of fish biology 68(5), 1347-1361.
- 540 Arukwe, A., Goksoyr, A., 2003. Eggshell and egg yolk proteins in fish: hepatic proteins
541 for the next generation: oogenetic, population, and evolutionary implications of
542 endocrine disruption. Comparative Hepatology 2(1), 4.
- 543 Beverton, R.J.H., Holt, S.J., 1959. A Review of the Lifespans and Mortality Rates of
544 Fish in Nature, and Their Relation to Growth and Other Physiological
545 Characteristics, Ciba Foundation Symposium - The Lifespan of Animals
546 (Colloquia on Ageing). John Wiley & Sons, Ltd, pp. 142-180.
- 547 Bligh, E., Dyer, W.A., 1959. A rapid method of total lipid extraction and purification.
548 Journal of Biochemical Physiology 37(Journal Article), 911-917.
- 549 Bonnet, X., Bradshaw, D., Shine, R., 1998. Capital versus Income Breeding: An
550 Ectothermic Perspective. Oikos 83(2), 333-342.
- 551 Brown-Peterson, N.J., Wyanski, D.M., Saborido-Rey, F., Macewicz, B.J., Lowerre-
552 Barbieri, S.K., 2011. A standardized terminology for describing reproductive
553 development in fishes. Marine and Coastal Fisheries: Dynamics, Management,
554 and Ecosystem Science 3, 52-70.
- 555 Callow, P., 1985. Adaptative aspects of energy allocation In Fish Energetics: New
556 perspectives. In: Tyler, P., Callow, P. (Eds.). Croom Helm Ltd, Sydney, pp. 13-
557 31.
- 558 Desmarchelier, M., 1985. Growth and reproduction of the whiting-pout (*Trisopterus*
559 *luscus* L. 1758) in the English Channel and in the south of the North Sea, Counc.
560 Meet., 1985, of the Int. Counc. for the Exploration of the Sea, (London (UK)), (7
561 Oct 1985). ICES, COPENHAGEN (DENMARK).
- 562 Domínguez-Petit, R., Saborido-Rey, F., 2010. New bioenergetic perspective of
563 European hake (*Merluccius merluccius* L.) reproductive ecology. Fisheries
564 Research 104(1-3), 83-88.
- 565 Domínguez-Petit, R., Saborido-Rey, F., Medina, I., 2010. Changes of proximate
566 composition, energy storage and condition of European hake (*Merluccius*

- 567 merluccius, L. 1758) through the spawning season. *Fisheries Research* 104(1-3),
568 73-82.
- 569 Drent, R.H., Daan, S., 1980. The prudent parent: energetic adjustments in avian
570 breeding. *Ardea* 68(1-4), 225-252.
- 571 Dubreuil, J., Petitgas, P., 2009. Energy density of anchovy *Engraulis encrasicolus* in the
572 Bay of Biscay. *Journal of Fish Biology* 74(3), 521-534.
- 573 Eliassen, J.E., Vahl, O., 1982. Seasonal variations in biochemical composition and
574 energy content of liver, gonad and muscle of mature and immature cod, *Gadus*
575 *morhua* (L.) from Balsfjorden, northern Norway. *Journal of fish biology* 20(6),
576 707-716.
- 577 Gherbi-Barre, A., 1983. Biologie de *Trisopterus luscus* L. de la baie de Douarnenez
578 (Reproduction, Croissance, Régime alimentaire). University of Brest, pp. 1-92.
- 579 Hamerlynck, O., Hostens, K., 1993. Growth, feeding, production, and consumption in
580 0-group bib (*Trisopterus luscus* L.) and whiting (*Merlangius merlangus* L.) in a
581 shallow coastal area of the south-west Netherlands, *ICES Journal of Marine*
582 *Science*, pp. 81-91.
- 583 Hartman, K., Brandt, S.B., 1995. Estimating Energy Density of Fish. *Transactions of*
584 *the American Fisheries Society* 124(3), 347-355.
- 585 Henderson, B.A., Morgan, G.E., 2002. Maturation of walleye by age, size and surplus
586 energy. *Journal of Fish Biology* 61(4), 999-1011.
- 587 Henderson, B.A., Wong, J.L., Nepszy, S.J., 1996. Reproduction of walleye in Lake
588 Erie: allocation of energy. *Canadian Journal of Fisheries and Aquatic Sciences*
589 53(1), 127-133.
- 590 Jobling, M., 1995. *Environmental biology of fishes* Chapman & Hall, London, 455 pp.
- 591 Junk, W.J., 1985. Temporary Fat Storage, an Adaptation of Some Fish Species to the
592 Water Level Fluctuations and Related Environmental Changes of the Amazon
593 River. *Amazoniana* 9(3), 315-351.
- 594 Kamler, E., 1992. *Early life history of fish: An energetics approach* Chapman & Hall,
595 London (UK), 267 pp.
- 596 Kjesbu, O.S., Klungsoyr, J., Kryvi, H., Witthames, P.R., Greer Walker, M., 1991.
597 Fecundity, atresia, and egg size of captive Atlantic cod (*Gadus morhua*) in
598 relation to proximate body composition. *Canadian Journal of Fisheries and*
599 *Aquatic Sciences* 48(12), 2333-2343.
- 600 Kjesbu, O.S., Witthames, P.R., Solemdal, P., Greer Walker, M., 1998. Temporal
601 variations in the fecundity of Arcto-Norwegian cod (*Gadus morhua*) in response
602 to natural changes in food and temperature. *Journal of Sea Research* 40(3-4),
603 303-321.
- 604 Kleiber, M., 1975. *The fire of life: an introduction to animal energetics* Krieger
605 Publishing, New York.
- 606 Lambert, Y., Dutil, J.D., 1997. Can simple condition indices be used to monitor and
607 quantify seasonal changes in the energy reserves of Atlantic cod (*Gadus*
608 *morhua*)? In: Campbell, J.S., Schwinghamer, P., Symons, P.E.K. (Eds.), *Symp.*
609 *on the Biology and Ecology of Northwest Atlantic Cod*, St. John's, NF (Canada),
610 24-28 Oct 1994, pp. 104-112.
- 611 Lambert, Y., Dutil, J.D., 2000. Energetic consequences of reproduction in Atlantic cod
612 (*Gadus morhua*) in relation to spawning level of somatic energy reserves.
613 *Canadian Journal of Fisheries and Aquatic Sciences* 57(4), 815-825.
- 614 Lowry, O.H., Rosbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement
615 with the folin phenol reagent. *Journal of Biological Chemistry* 193(Journal
616 Article), 265-275.

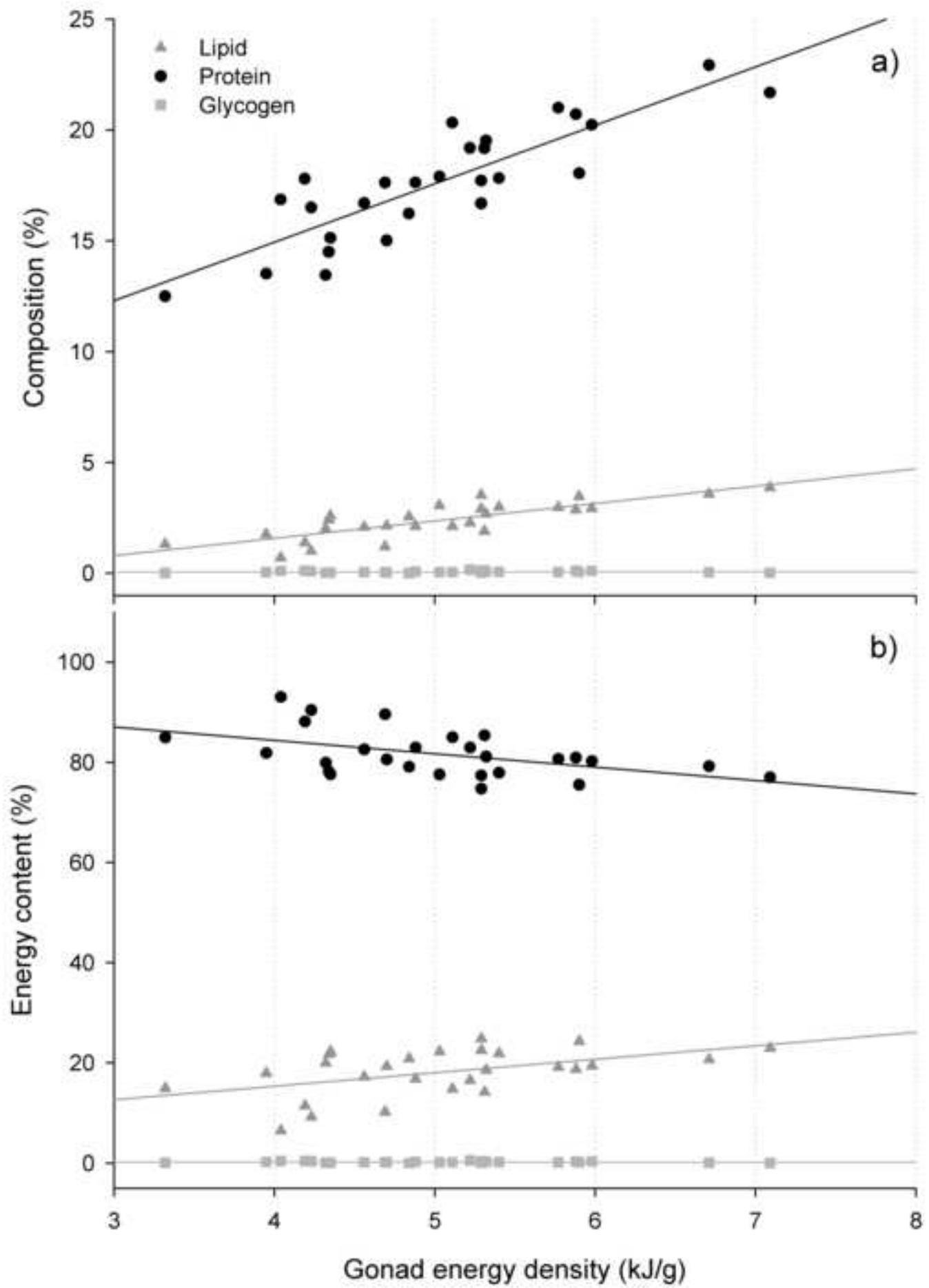
- 617 Marshall, C.T., Yaragina, N.A., Lambert, Y., Kjesbu, O.S., 1999. Total lipid energy as a
618 proxy for total egg production by fish stocks. *Nature* 402(6759), 288-290.
- 619 Méndez, E., González, R.M., 1997. Seasonal changes in the chemical and lipid
620 composition of fillets of the Southwest Atlantic hake (*Merluccius hubbsi*). *Food*
621 *Chemistry* 59(2), 213-217.
- 622 Merayo, C.R., 1991. Biología y pesca de la faneca *Trisopterus luscus* (Linnaeus, 1758),
623 en las aguas de Asturias.
- 624 Merayo, C.R., 1996a. Reproduction and fecundity of the bib *Trisopterus luscus*
625 (Linnaeus, 1758) (Pisces, Gadidae) in the central region of the Cantabrian Sea
626 (northern Spain). *Boletín del Instituto Español de Oceanografía* 12(1), 17-29.
- 627 Merayo, C.R., 1996b. Seasonal changes in the biochemical composition of the muscle
628 and liver of bib (*Trisopterus luscus* L.) (Pisces, Gadidae) from the Cantabrian
629 Sea (N Spain), *Scientia Marina*, pp. 489-495.
- 630 Pauly, D., 1980. On the interrelationships between natural mortality, growth parameters,
631 and mean environmental temperature in 175 fish stocks. *ICES Journal of Marine*
632 *Science: Journal du Conseil* 39(2), 175-192.
- 633 Pedersen, J., Hislop, J.R., 2001. Seasonal variations in the energy density of fishes in
634 the North Sea, *Journal of Fish Biology*. Academic Press, pp. 380-389.
- 635 Pepin, P., 1991. Effect of temperature and size on development, mortality, and survival
636 rates of the pelagic early life history stages of marine fish. *Canadian Journal of*
637 *Fisheries and Aquatic Sciences* 48(3), 503-518.
- 638 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2010. nlme: Linear and Nonlinear Mixed
639 Effects Models, R package version 3.1-97. R Development Core Team.
- 640 Politou, C.Y., Papaconstantinou, C., 1994. Feeding ecology of Mediterranean poor cod,
641 *Trisopterus minutus capelanus* (Lacepede), from the eastern coast of Greece.
642 *Fisheries Research* 19(3-4), 269-292.
- 643 Roff, D.A., 1992. *The Evolution of Life Histories: Theory and Analysis* Chapman and
644 Hall, New York, 535 pp.
- 645 Saborido-Rey, F., Murua, H., Tomkiewicz, J., Lowerre-Barbieri, S., 2010. Female
646 reproductive strategies: an energetic balance between maturation, growth and
647 egg production. In: Brown-Peterson, D.M.W.a.N.J. (Ed.), *Fourth Workshop on*
648 *Gonadal Histology of Fishes*, El Puerto de Santa Maria, Spain, pp. 15-17.
- 649 Schulte-Hostedde, A.I., Zinner, B., Millar, J.S., Hickling, G.J., 2005. Restitution of
650 mass-size residuals: validating body condition indices. *Ecology* 86(1), 155-163.
- 651 Stearns, S.C., 1989. The Evolutionary Significance of Phenotypic Plasticity. *BioScience*
652 39(7), 436-445.
- 653 Stearns, S.C., 1992. *The evolution of life histories* Oxford Univ. Press, New York, 249
654 pp.
- 655 Strickland, D.H., Parsons, T.R., 1968. A practical handbook of seawater analysis. *Bull.*
656 *Fish. Res. Bd. Can.* 167(Journal Article), 11-16.
- 657 Trippel, E.A., Neil, S.R.E., 2004. Maternal and seasonal differences in egg sizes and
658 spawning activity of northwest Atlantic haddock (*Melanogrammus aeglefinus*)
659 in relation to body size and condition. *Canadian Journal of Fisheries and Aquatic*
660 *Sciences* 61(11), 2097-2110.
- 661 Wood, S.N., 2006. *Generalized Additive Models: An Introduction with R* Chapman and
662 Hall/CRC.
- 663 Xie, X., Long, T., Zhang, Y., Cao, Z., 1998. Reproductive investment in the *Silurus*
664 *meridionalis*. *Journal of Fish Biology* 53(2), 259-271.
- 665 Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid
666 common statistical problems. *Methods in Ecology and Evolution* 1(1), 3-14.

667 Zuur, A.F., Leno, E.N., Smith, G.M., 2007. Analysing ecological data : Statistics for
668 biology and healthSpringer, New York.
669
670

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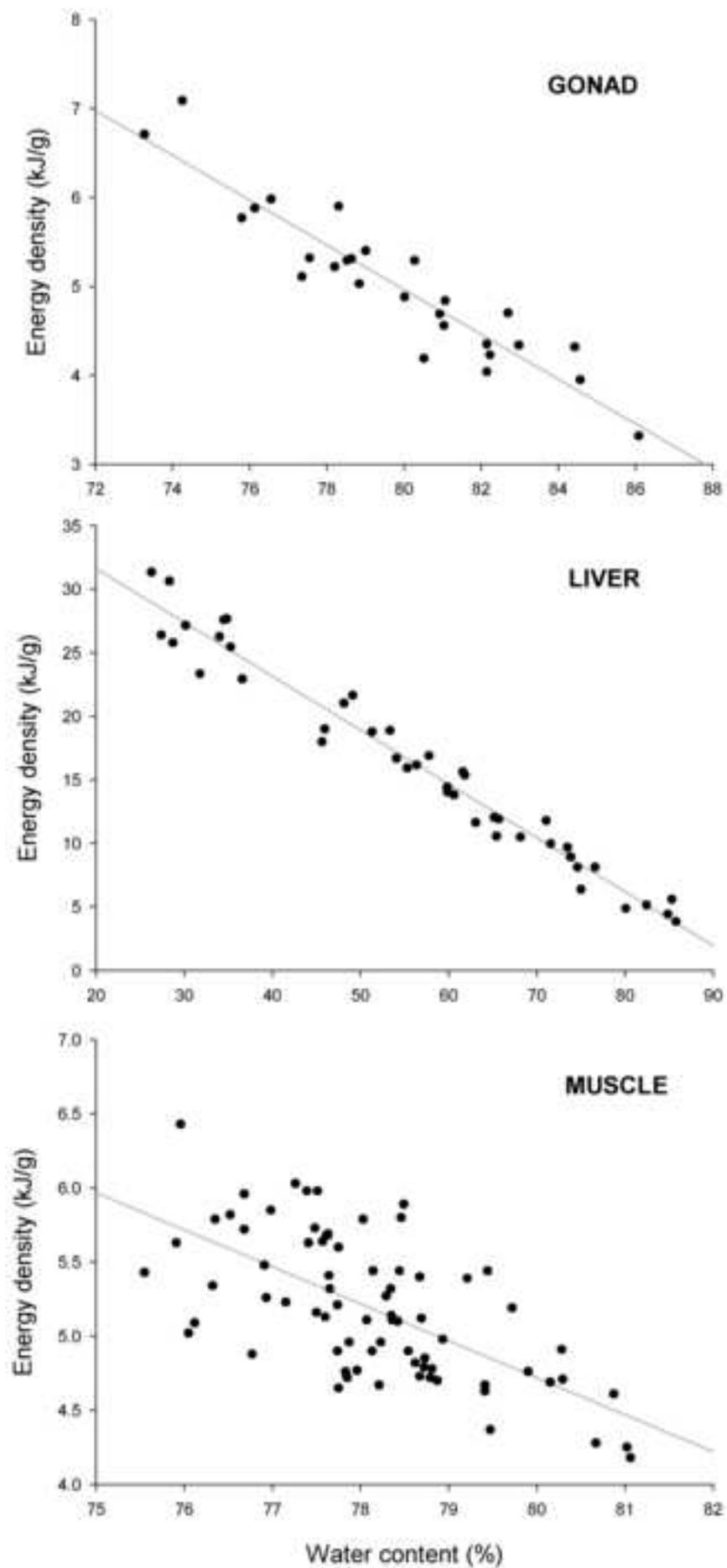


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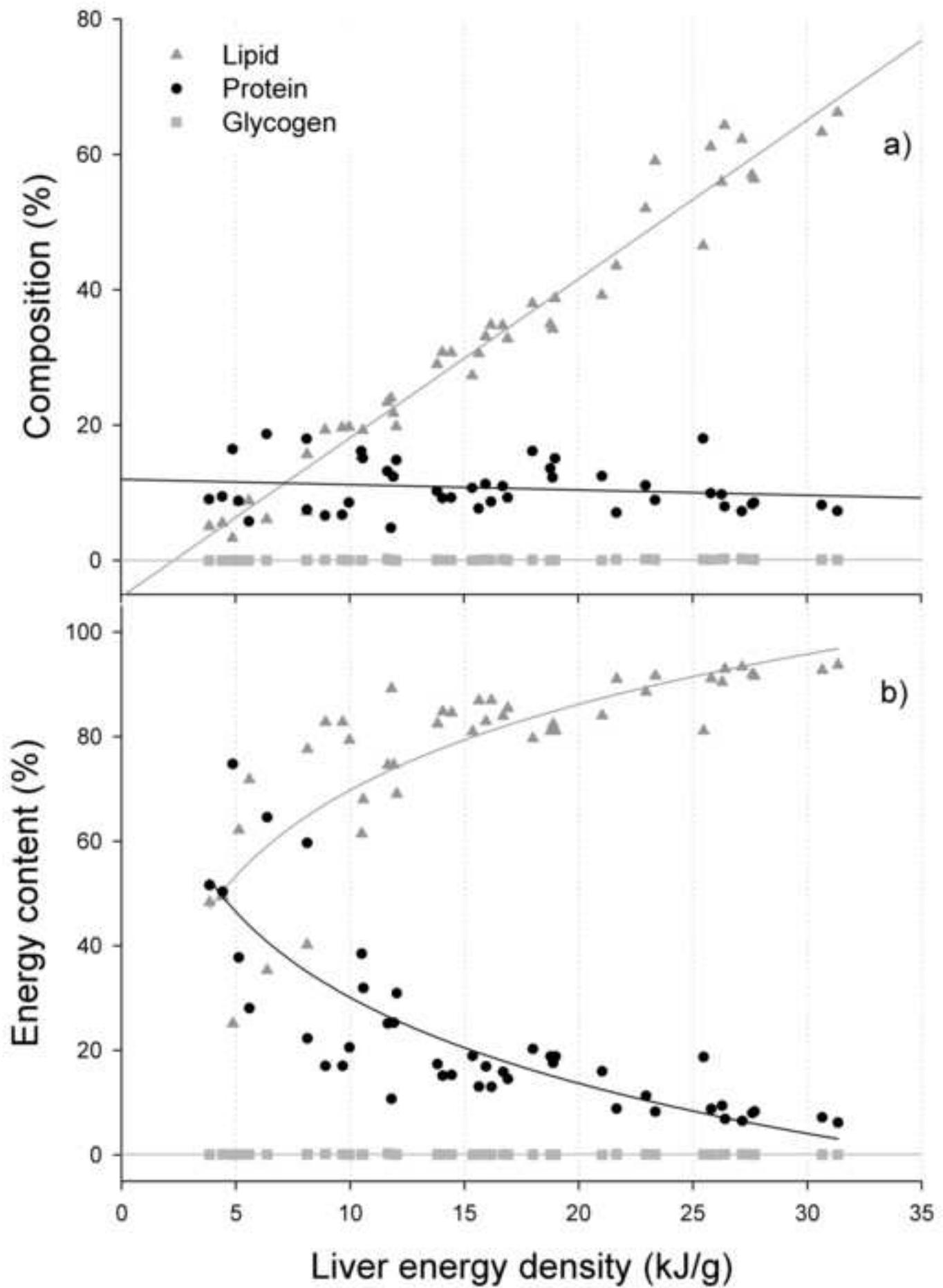


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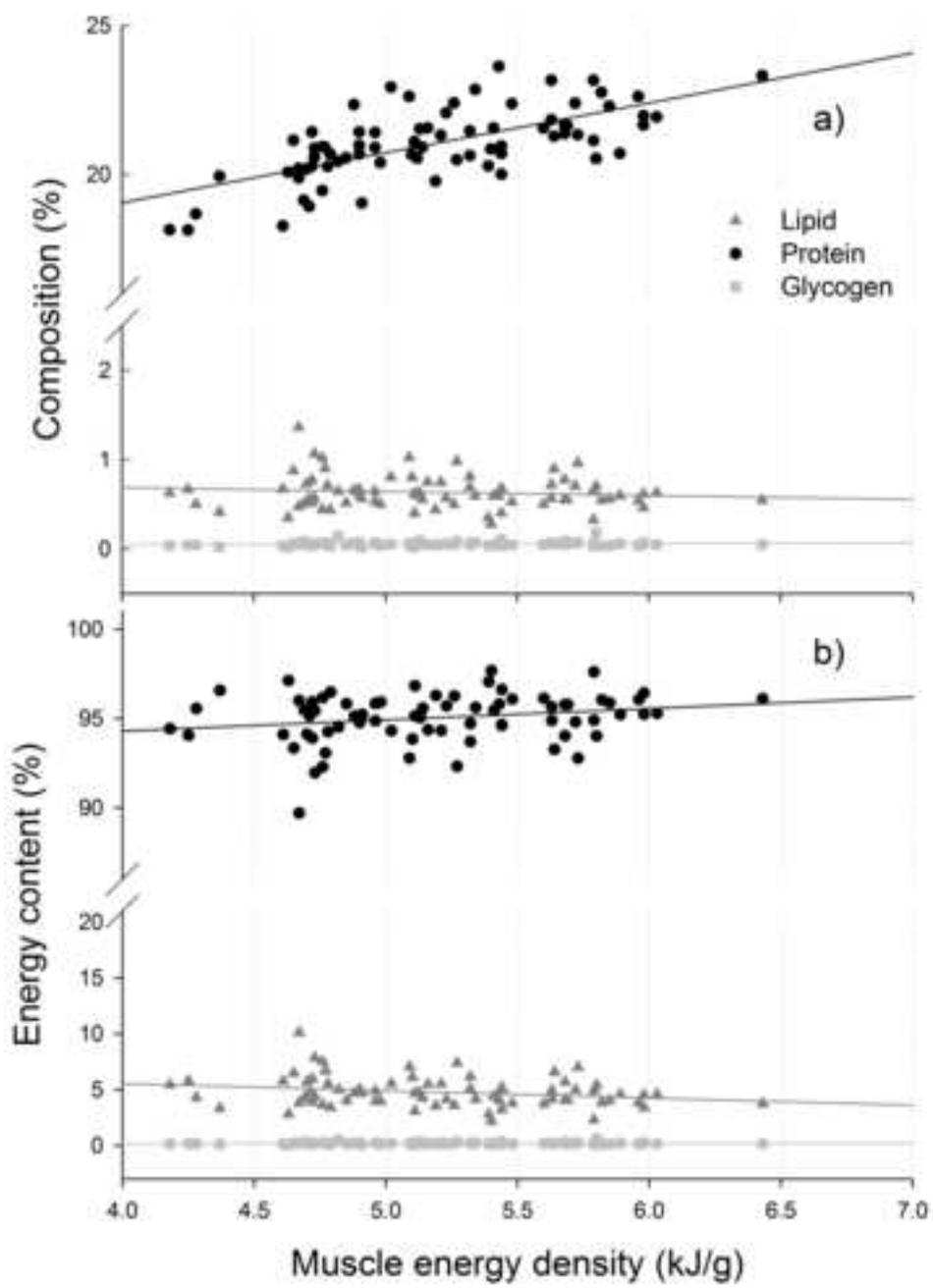


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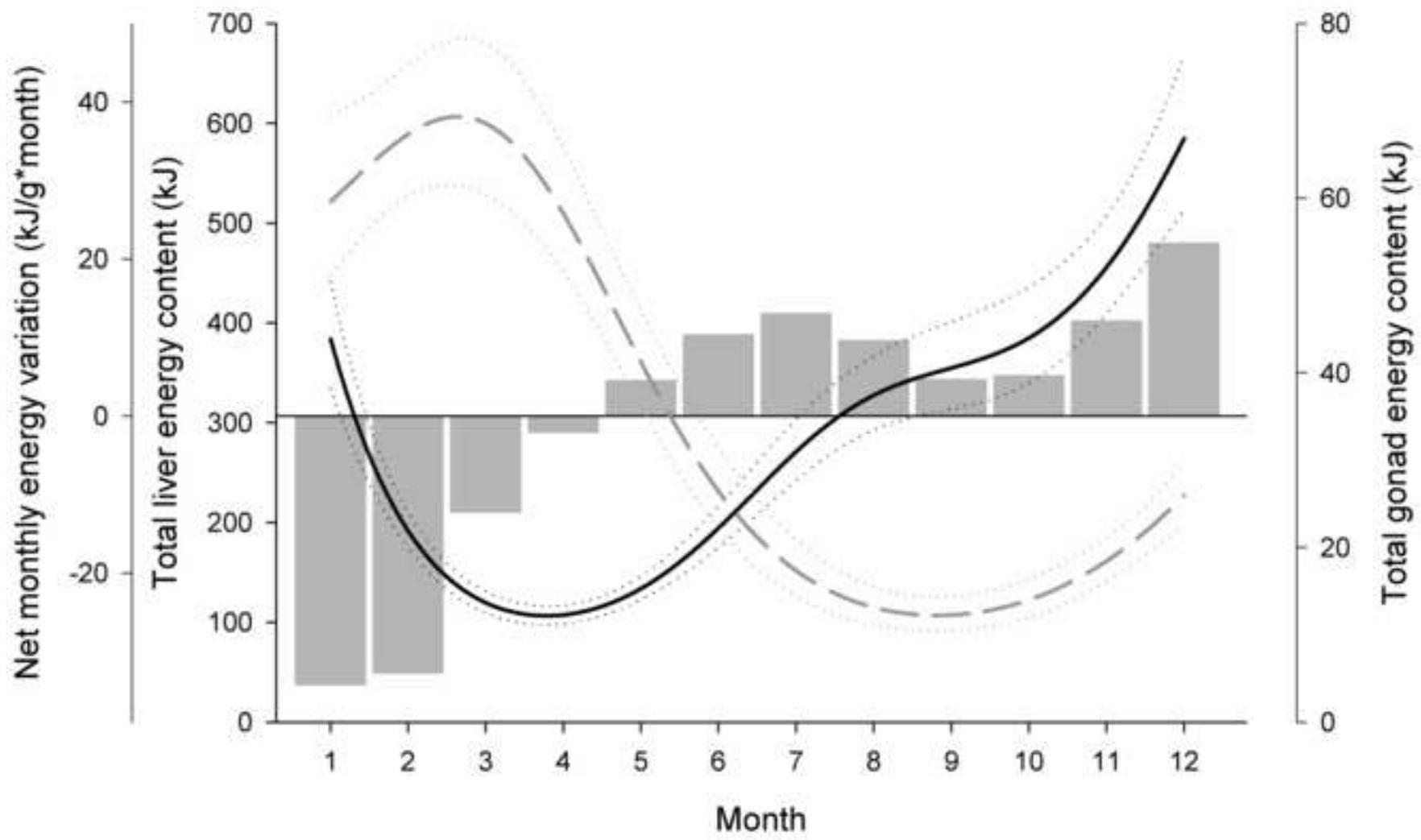


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