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Condition states in anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) revealed by energy and proximate composition relationships

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Abstract :

Energy content has long been proposed as a fundamental, integrated, and reliable indicator of the condition of individuals as it reflects past bioenergetics and influences future life-history traits. There is a direct biochemical link between energy density and body composition described by four main compounds in fish (protein, lipid, ash, and water), with proteins and lipids being the sources of energy. If relationships between water content, or lipid content, and energy density have been well described in relative terms, the absolute mass variations in the proximate composition have been overlooked and thus their interpretation is often equivocal. In our study, based on a large and unique dataset on the proximate composition and energy density of anchovy (Engraulis encrasicolus) and sardine (Sardina pilchardus) from sampling in the Bay of Biscay and the English Channel, we aimed to better explain the patterns between water content and other proximate components or energy density, based on the dynamics of proteins, lipids, and water absolute masses. For the first time, we defined good, intermediate, and poor condition states in wild fish, based on water content, corresponding to the different dynamics of lipids and proteins in the metabolism of individuals. Anchovy and sardine exhibited remarkably similar patterns of variation in the compounds and in the limits between the condition states with respect to water content. Those patterns revealed that water mass remained constant for a given fish size whatever its condition state, and that variability in water content only resulted from the variation in lipid and protein masses. Furthermore, the differential dynamics of proteins and lipids, with only lipids mobilized in the good condition state, only proteins in the poor condition state, and both proteins and lipids in the intermediate condition state, elucidates the nonlinear pattern observed in the relationship between energy density and water content. Overall, our results highlight the importance of monitoring the intraspecific variations in water content to predict the proximate composition and energy content in small pelagic fish and better assess individual and population conditions in changing ecosystems as well as to better parameterize bioenergetic models.

Keywords : Bay of Biscay, condition, energy, lipid, protein, small pelagic fish, water content

1. Introduction

Energy content has long been proposed as a fundamental, integrated and reliable indicator of the condition of individuals (Molnár *et al.*, 2009), populations (Pagano *et al.*, 2018), and ecosystems (Benoit-Bird, 2004; Spitz and Jouma'a, 2013; Pothoven and Fahnenstiel, 2014). Energy content is key to the understanding of the observed variability in animal life history traits such as growth, reproduction and mortality and is biochemically linked to body composition. As such, body composition has been studied for a long time in both farmed and wild mammals (Moulton, 1923; Noren et al., 2003), birds (Eits *et al.*, 2002; Rivera-Torres *et al.*, 2011; Guglielmo, 2018) and fish (Groves, 1970), for its large significance in various aspects of individual physiology, biology and ecology. Body composition mostly varies in response to feeding (Gerking, 1955; Bonvini et al., 2015) and ontogeny (Wuenschel *et al.*, 2006; Bakhtiarvandi *et al.*, 2014). It can give insights into the well-being or condition of the animal through the amount of lipid or protein reserves (McCue, 2010).

Lipids are the most variable component within an animal's body, serving as the primary energy reserve to be catabolised (McCue, 2010). Their quantity is regarded as a good indicator of an organism's condition (Lloret, 2014) and can vary significantly, not only between individuals, but also throughout an individual life cycle, due to both internal factors, reproductive status and ontogeny, and environmental variations, ranging from 2% to 20% in fish for example (Lloret, 2014). Proteins usually represent the dominant component of animals weight, after water, with their quantity being relatively stable (approximately 20% of wet mass in fish, Lloret, 2014), although they can be catabolised to provide energy when lipids are depleted (Savitz, 1971; Black and Love, 1986). While both proteins and lipids serve as sources of energy, proteins primarily contribute to structural components, whereas lipids represent mobilisable energy reserves. Ash is a highly stable component, as it constitutes inorganic molecules (scales, skeleton) and is strongly correlated with the organism’s length (McComish et al., 1974). In fish studies, four components are typically considered: water, ash, protein and lipid. Carbohydrates are generally not considered, as they constitute a very small proportion of wet mass (<0.14%; Brett and Groves, 1979). As water and ash are not energetic, energy content is intrinsically linked to protein and lipids content and can then be assessed using the energy density of these components (Breck, 2008, 2014; Schloesser and Fabrizio, 2015).

In nature, all animals can face periods of food deprivation, and some, such as fish, can cope with long periods of starvation (McCue, 2010). During these periods, proximate composition varies, eventually reaching energy depletion. For an animal with plenty of reserves, food deprivation is usually followed by three phases for birds (Le Maho *et al.*, 1981; Robin *et al.*, 1988; Groscolas and Robin, 2001), mammals (Cherel *et al.*, 1988, 1991, 1992; Caloin, 2004) and fish (Bar, 2014). Phase 1 is short and characterised by the mobilisation of protein and/or lipids to produce energy, phase 2 lasts longer and involves lipids catabolisation, until phase 3 where lipids are depleted and proteins become the main source of energy (Bar, 2014). Studying the effect of starvation on animals has been mainly assessed in controlled environments, following individuals in longitudinal experiments.

Identifying condition states and understanding the respective contributions of lipids and proteins when reserves are catabolised in wild organisms remains challenging. The scarcity of extensive in-situ datasets documenting fish proximate composition and energy density, over a wide range of fish condition, has limited our understanding of the variations in wild fish condition.

Water content (or dry mass content), which shows a strong relationship with energy density ($R^2 > 0.90$) for many taxa (Ciancio et al., 2007), provides a quick and reliable method for assessing energy density. This relationship is mainly related to the catabolism of lipids, which once mobilised lead to a relative increase of water content and a decrease in energy density. For fish, numerous studies have explored the correlation between water content (or dry mass content) and energy density (Hartman and Brandt, 1995; Tirelli et al., 2006; Dubreuil and Petitgas, 2009; Gatti et al., 2018). However, Hartman and Brandt, (1995) and Gatti *et al (*2018) have shown that this relationship was nonlinear. This non linearity was discussed with respect to size and ontogeny effects (Wuenschel et al., 2006). Although energy density and proximate composition have been extensively studied in fish, their interdependent variations still remain misunderstood. We here suggest that the usual representation of proximate composition, water content and energy density in relative terms, has maintained confusion in the interpretation of the absolute variation of these variables along a gradient of fish condition. Specifically, a change in the relative value of one component will inevitably affect the relative value of another component, even if the absolute term remains constant. Unraveling the patterns in their absolute variations would therefore benefit the community.

Small pelagic fish (SPF) are high-fat, high-energy forage species, that play a key role in pelagic ecosystems, transferring energy from plankton to higher trophic levels (Cury, 2000). In temperate shelf ecosystems, such as the Bay of Biscay or the English channel, SPF energy density displays large variations, with low energy density in winter and spring after overwintering and high energy density in summer and autumn during and after the feeding season (Spitz and Jouma'a, 2013; Gatti *et al.*, 2018). Because farming small pelagic fish remains challenging (but see Bandarra et al, 2018

Queiros *et al.*, 2019, 2021), no SPF proximate composition has been studied in vivo. These fish species are hence absent from existing reviews of experimental studies exploring strategies for dealing with starvation and biochemical processes at stake (McCue, 2010; Bar, 2014).

Our study was based on a large and unique dataset of proximate composition and energy density of European anchovy *Engraulis encrasicolus* (Linnaeus 1758), hereafter anchovy, and European sardine *Sardina pilchardus* (Walbaum 1792), hereafter sardine, sampled in the Bay of Biscay and the English Channel. We aimed to provide new insights on the link between proximate composition, state in fish condition and associated energy density. First, we characterised the patterns of variation of protein and lipid contents over the observed range of water content, to derive distinct condition states. Second, we tested the effect of internal (reproductive status and length) and external (season and region) factors on the individuals condition state. Third, we checked if the water mass in absolute terms was varying across the different condition states in response to the variations of proximate composition. Fourth, we assessed the respective contributions of proteins and lipids to energy supply in each condition state, when energy has to be mobilised. Finally, we further investigated the non linearity in the well-known relationship between energy density and water content, in light of lipid and protein content variations.

2. Material and methods

2.1. Study area and sampling

Juvenile and adult anchovy and sardine were collected from 2014 to 2017 in the Bay of Biscay and the English Channel (Figure 1). All the data used in this study were made available in Huret et al. (2024). Fish were sampled during the PELGAS (Doray et al., 2000; 2018b), EVHOE (Laffargue et al, 1987), CGFS (Giraldo et al, 1988) and CAMANOC (Travers-Trolet and Verin, 2014) scientific surveys conducted by IFREMER on the RV “Thalassa”, during the JUVENA survey conducted by AZTI (Boyra et al, 2003), and from commercial landings within the European Data Collection

Framework (DCF). The PELGAS integrated pelagic survey monitors small pelagic fish and their ecosystemin spring, while EVHOE bottom trawl survey evaluates demersal fish communities in autumn, both in the Bay of Biscay. The CGFS and CAMANOC bottom trawl surveys evaluate demersal fish communities and their environment in the English Channel in autumn. Despite their focus on demersal fish communities, EVHOE, CGFS and CAMANOC surveys catch small pelagic fish in sufficient numbers to get samples representative of the fish condition in autumn (Gatti et al, 2018). JUVENA survey monitors the abundance of juvenile anchovy in early autumn in the Bay of Biscay. Samples from commercial landings were obtained as part of the national CAPTAIN research project.

A total of 503 anchovies and 976 sardines sampled between 2014 and 2017 were used in this study (Table 1). Sampling locations were representative of the species core distribution areas in the Bay of Biscay and the English Channel (Figure 1). Each fish was individually measured to the nearest tenth of a centimeter and weighed to the nearest tenth of a gram. Maturity stages were determined following ICES guidelines (ICES, 2008) based on macroscopic gonads observation and using a six-stage key as follows: stages 1 & 2 indicate immature and developing individuals, stages 3–5 indicate three steps of increasing gonad development and the spawning period (stage 3: partial pre-spawning; stage 4: spawning (hydrated); stage 5: partial post-spawning), and stage 6 features the final post-spawning period. Fishes characterised by maturity stages 3, 4 or 5 were considered as being in an active reproductive period as opposed to fishes in stages 1, 2 or 6. These measurements were taken either at sea or later in the laboratory. A wide range of body lengths (4.7 – 19.1 cm for anchovy and 8 – 26.8 cm for sardine) and masses (0.5 – 50.2 g for anchovy and 3.4 – 162.2 g for sardine) was available, representing the size spectrum of the two species across juvenile and adult stages. The collected fish were frozen individually at -20°C before laboratory processing.

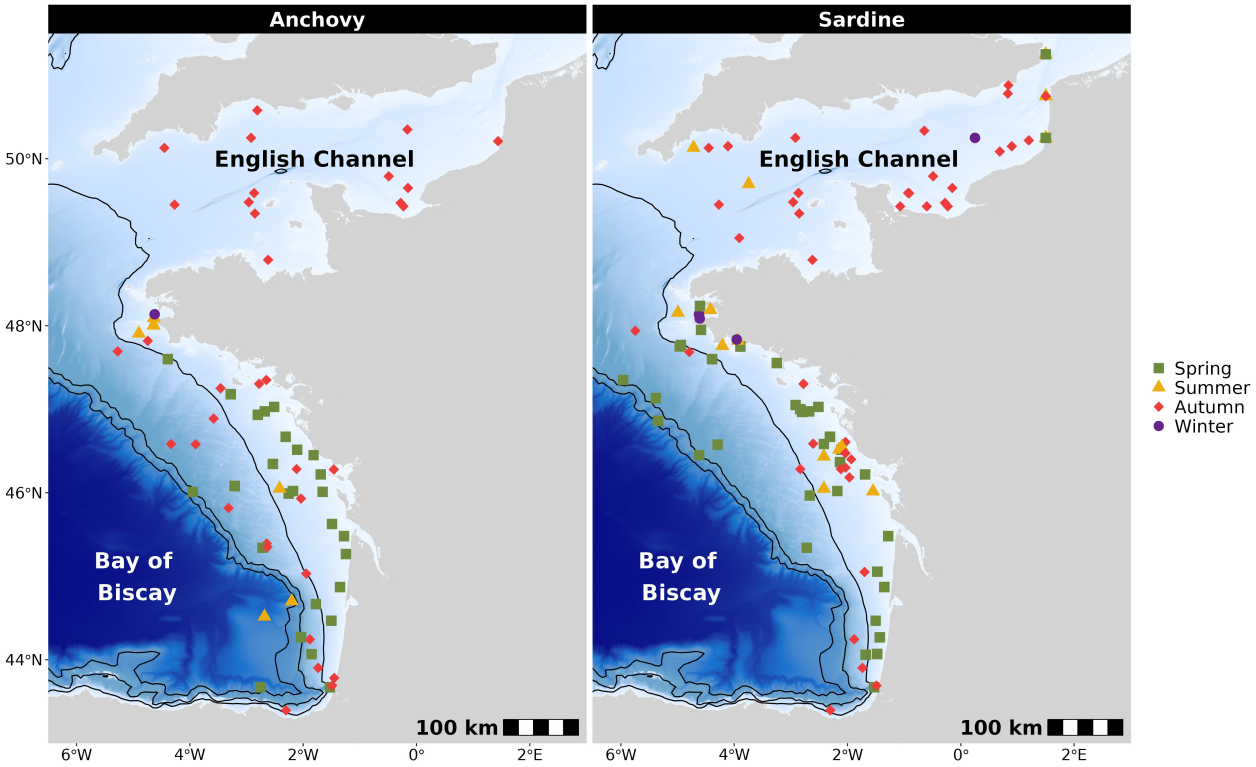

Figure 1: Sampling locations and associated seasons, for anchovy (left) and sardine *(right) from the six data sources considered: PELGAS (spring in the Bay of Biscay), JUVENA and EVHOE (autumn in the Bay of Biscay) and CGFS/CAMANOC (autumn in the English Channel) surveys, as well as commercial landings (CAPTAIN project, several seasons and both regions). Black lines represent isobaths of 100, 200 and 1000 meters.*

2.2. Water content

In the laboratory, fish were unfrozen, ground and freeze-dried for at least 48 hours. Water content of the entire fish was determined from dry mass (M_D) and wet mass (M_W) ratio. Then, fish were ground again to obtain fine homogeneous dry powder for subsequent analysis. In this study, the term “content” refers to the relative proportion of the component on the total fish mass. It will be used for ash, lipid, protein and water components.

2.3. Proximate composition analysis

Ash content was determined gravimetrically by combusting dried tissue in a muffle furnace at 550°C for six hours. Lipids and proteins were analysed by a certified laboratory (Labocea, Plouzané, France). Protein content was estimated using the Kjeldahl method (Sáez-Plaza *et al.*, 2013). It consists in first determining the quantity of nitrogen contained in the sample, and to convert it into protein content using a conversion factor (6.25 here, Sáez-Plaza *et al.*, 2013). Lipid content was determined by hydrolysis, using petroleum ether as an organic solvent. Carbohydrates represent less than 1% of fish mass and were thus neglected as in previous studies (Brett and Groves, 1979; Craig, 1977; Craig et al, 1978). Protein, lipid and ash content did not exactly sum to 1 in dry weight (DW, anchovy: mean = 0.91, sd = 0.04; sardine: mean = 0.90, sd = 0.04). This discrepancy may arise from residual water, measurement uncertainties, or to a lesser extent the exclusion of carbohydrates. Body component contents have been normalised by dividing each component by the sum of lipids, proteins and ash content, to sum to one, enabling comparisons between fishes, assuming proportional errors across the components (Breck, 2014). Values of water, protein, lipids and ash masses were calculated by multiplying the fish body mass by the normalised percentages of fish components. A total of 104 and 116 proximate composition analyses were performed for anchovy and sardine, respectively (Table 1).

2.4. Energy density

Energy density measurements were performed following the protocols of Dubreuil and Petitgas, (2009) and Spitz and Jouma'a, (2013). Two subsamples of fish powder were placed in an adiabatic bomb calorimeter (IKA C-4000 adiabatic bomb calorimeter, IKA-WerkeGmbh & co. KG) for energy measurements. The energy density (ED, $kJ.g^{-1}$ dry mass) was determined by measuring the heat released through the combustion of a small subsample, approximately 200 mg. If the coefficient of variation between the two measurements exceeded 3%, a third measurement was made. Finally, ED subsamples measurements were averaged and assigned to each individual fish.

Energy density analyses were conducted on 503 individuals for anchovy and 976 individuals for sardine (Table 1).

Table 1: Sampling design: data sources, region, season, number of samples per species for energy density and proximate composition analysis.

Data sources	**Region**	**Season**	**Energy density**		**Proximate composition**	
			Anchovy	**Sardine**	**Anchovy**	**Sardine**
PELGAS	Bay of Biscay	Spring (May)	186	204	17	17
EVHOE	Bay of Biscay	Autumn (Oct-Nov)	130	91	31	12
CGFS/ CAMANOC	English Channel	Autumn (Sept-Oct)	91	164	24	25
JUVENA	Bay of Biscay	Autumn (Sept)	22	0	0	0
CAPTAIN	Bay of Biscay, English Channel	All	74	517	32	62
Total			**503**	**976**	**104**	**116**

2.5. Statistical analysis

The contents of proximate components, namely lipids, proteins and ash, were used as response variables and water content as the explanatory variable (Breck, 2008, 2014) in segmented linear models (Muggeo, 2008). Segmented linear models (SLM) are linear regression models where the relationships between the response and one or more explanatory variables are piecewise linear, represented by two or more straight lines connected at unknown values, referred as breakpoints. SLM were used to identify breakpoints delimiting intervals in the relationship between proximate components and water content. These intervals were assumed to represent fish condition states, defined by thresholds in water content.

Multinomial logistic regression (Starkweather and Moske, 2011) analysis were used to examine the influence of both internal (length and reproductive status) and external factors (season and region) on the condition states, the response variable comprising three categories: poor, intermediate, and good. This regression method extends the binomial logistic regression to situations where the dependent variable is nominal with more than two categories. The intermediate state was chosen as the reference category, representing the state with the most available data, while good and poor states were the alternative categories. The optimal model for each species was determined through stepwise analysis based on the Akaike Information Criterion (AIC). Odds ratios (ORs) with 95% confidence intervals (CI) and associated p-values were used to display the results. The OR represents the likelihood of the independent variable to belong to a specific category relative to the reference category. The OR is therefore greater than 1 if the individual has a higher probability to be in the considered category than in the reference category, and less than 1 if it has a lower probability to be in the considered category.

Breck (2008) showed that proteins are closely linked to water content. The loss of protein during starvation was shown to be accompanied by water losses. Thus, we investigated the effect of the content in proximate components other than water on the water mass, independently of fish length, using the following multiple linear regression model:

$$W \sim L + p + l + a + \epsilon \quad (1)$$

With W the mass of water in gram, L the fish length in cm, p, l and a the protein, lipids and ash contents, respectively and ε the residuals term.

Log-log linear relationships were fitted with proximate components masses as response variables, and water mass as explanatory variable, for each condition state determined from the segmented regression, for anchovy and sardine. Additionally, a covariance analysis (ANCOVA) was applied to assess if the relationships between water mass and proximate component mass differed among condition states.

The respective contributions and their associated uncertainties of lipids and proteins to energy mobilisation were calculated using the variation of each component (i.e. regression slopes) within the different intervals (i.e. condition state). Considering the higher energy content of lipids compared to proteins, we assessed energy contributions in terms of both mass and energy. Usually, energy density of lipids ranges from 34.7 to 39.5 kJ.g^{-1} (Schloesser and Fabrizio, 2015), and energy density of proteins is taken as 23.6 kJ.g^{-1} (Paine, 1971; Beamish et al, 1975; Brett and Groves, 1979). Consequently, we used the value of 23.6 kJ.g^{-1} for protein and 38.5 kJ.g^{-1} for lipids, based on the measurement of mesenteric fat of sardine using bomb calorimetry (n=4, CV = 1.2%) which fits in the range of the literature. Calculations of the respective contributions based on the regressions are detailed in appendix A.

Finally, the relationship between energy density and water content was further investigated. The former was used as the response variable and the latter as the explanatory variable using two linear regression models. The standard one was fitted to all individual data together. An alternative model was a segmented linear model between water content and energy fitted over each condition state.

Linear models goodness of fit was assessed using classical diagnosis tools (coefficients of determination (R^2), residuals Q-Q plots, residuals versus fitted values plots, standard error and confidence interval at 95% of regression coefficients). Statistical tests were performed at a significance level of 0.05. Analyses were performed using the statistical R software (R Core Team, 2023). Segmented regression analysis was performed using the package *segmented* (Muggeo, 2008). Multinomial logistic regression models were performed using the function *multinom* in package *nnet*.

2.6. Ethical statement

Ethical review and approval were not required for this study because it did not involve animal experimentation or harm. No animals were involved in the conduct of this study as the analysis was done on previously collected data on wild-caught fish that were sampled within the European Data Collection Framework.

3. Results

3.1. Fish condition states from proximate composition

Strong relationships were found between contents of water and proximate components, namely proteins and lipids (Figure 2). The segmented regression analysis identified two breakpoints, characterising a change in the regression slopes between water content and contents in proximate components for both anchovy and sardine (Figure 2, Table 2 and Table A1). Variation in water content explained a great proportion of lipid content variation in the segmented regression (R^2 equal to 0.90 and 0.91 for anchovy and sardine, respectively). Water content variation explained a lower proportion of protein variation (R^2 equal to 0.52 and 0.50 for anchovy and sardine, respectively). Six among eight breakpoints were significant. Only the second lipid breakpoint of anchovy and the second protein breakpoint for sardine had p-values higher than 0.05 (0.32 and 0.12, respectively, Table 2). Despite this non significance, these breakpoints have been considered in the rest of the results as their complementary breakpoints (2nd protein breakpoint of anchovy and 2nd lipid breakpoints for sardine), as well as their counterparts in the other species, are significant, which suggests two changes in the pattern of proximate composition against water content that appeared interesting to further investigate in our study.

For anchovy and sardine, the first lipid breakpoint occurred respectively at 68.4% and 66.2% of water content, and the second at 75% and 74.5%. Similarly, the first protein breakpoint occurred respectively at 68.4% and 69.4% of water content, and the second at 75.5% and 75.6%. For each species, the two protein and lipid breakpoints were close, the maximum difference being 3.2% of water content between the first breakpoints for sardine. Considering these similarities, and in order to isolate three distinct composition intervals for each species, we used the average breakpoints calculated over the corresponding protein and lipid breakpoints as common breakpoints, for each species (Table 2). Based on these average breakpoints we identified three condition states, which exhibited remarkable similarities between the two species (Figure A1, Table 2). In the first state, fish water content was lower than 68.4% and 67.8% for anchovy and sardine, respectively, and lipid content was higher than 5% in wet mass for both species. Here, we assumed that the higher the lipid content, the better the condition state. Within this ‘good condition’ state, lipid content strongly decreased as water content increased, while protein content slightly increased. The second state extended from 68.4% to 75.2% and from 67.8% to 75% in water content for anchovy and sardine, respectively. Both lipid and protein contents decreased with increasing water content, albeit at different rates in this ‘intermediate condition’ state. In the third state, fish water contents were above 75.2% and 75% for anchovy and sardine, respectively. Lipid content reached a minimal value “threshold” of ca. 1% suggesting exhaustion of lipid reserves, while protein levels significantly decreased. Fish were considered in a ‘poor condition’ state.

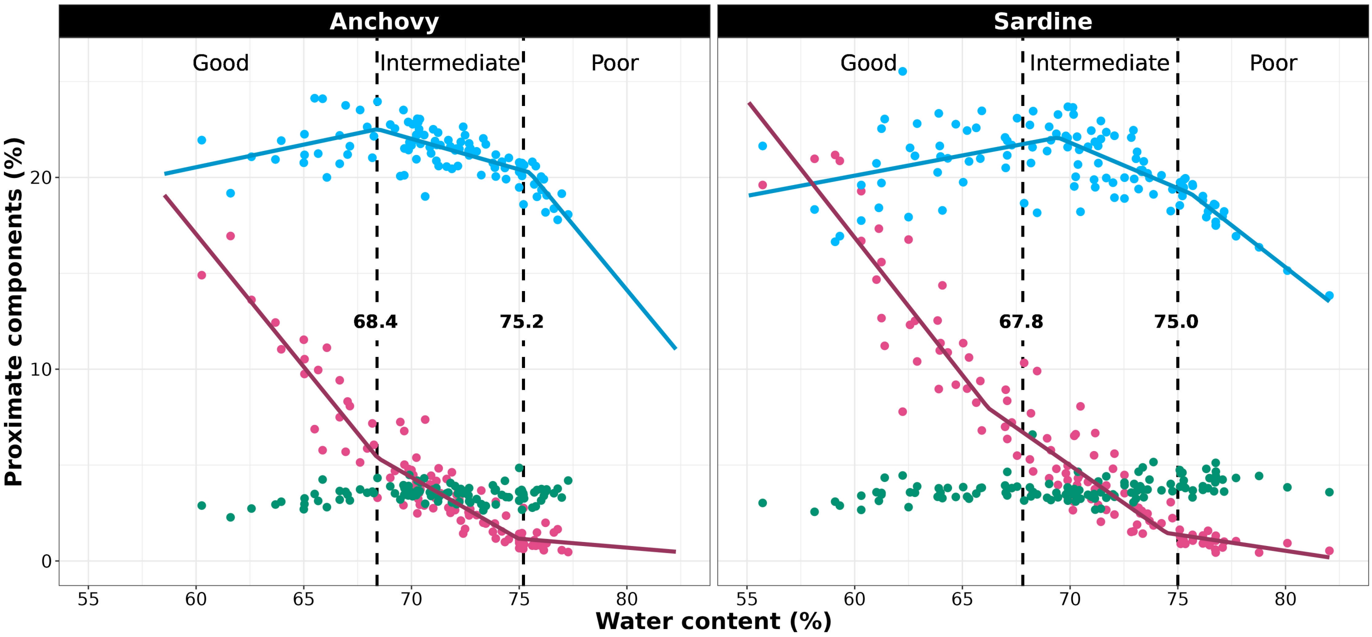

Figure 2: Observed contents of proteins (blue dots), lipids (purple dots) and ash (green dots) in anchovy and sardine body composition, as a function of water content. Solid lines: segmented regression models for proteins (blue lines), and lipids (purple lines). The dotted vertical lines represent the mean values of the breakpoints identified from proteins and lipids segmented regressions.

Table 2: Breakpoint values for lipids and proteins, defining condition states in anchovy and sardine. Slope coefficient, associated standard error and confidence intervals for each segment are presented in Table A1

	Anchovy							**Sardine**						
	Protein			*Lipid*			*Mean*	*Protein*			*Lipid*			*Mean*
	Value	P-value	R^2	Value	P-value	R^2	Value	Value	P-value	R^2	Value	P-value	R^2	Value
Breakpoint 1	0.684	$3.3.10^{-5}$	0.52	0.684	$1.6.10^{-4}$	0.90	0.684	0.694	$6.7.10^{-5}$	0.50	0.662	$3.4.10^{-3}$	0.91	0.678
Breakpoint 2	0.755	$2.3.10^{-2}$	0.52	0.750	$3.2.10^{-1}$	0.90	0.752	0.756	$1.2.10^{-1}$	0.50	0.745	$2.6.10^{-3}$	0.91	0.750

3.2. Effect of length, season, reproductive status and region on the condition state

Through multinomial logistic regression analyses, we explored the impact of season, region, length and reproductive status on the condition states of anchovy and sardine, with the intermediate condition state serving as the reference category (Figure 3, Table A2, Table A3). For anchovy, the optimal model, as determined by the AIC, included all variables (season, reproductive status, region, and length). In contrast, for sardine, the reproductive status variable was excluded from the best model, while season, region, and length variables were retained.

For anchovy, seasonal variations significantly influenced its condition state (Figure 3). During summer [OR = $6.91.10^{1}$, 95% CI = 2.27 - $2.10.10^{3}$] and autumn, they were more likely to be in good condition than in winter, whereas in spring, the likelihood decreased. Conversely, for all seasons, the likelihood of being in a poor condition was lower than in winter. Similar patterns are observed for sardine, despite non significant ORs for summer and autumn, for poor condition state. Regarding region, both species exhibited comparable patterns although significance was not always verified. Anchovy and sardine from the English Channel were more likely to be in good condition (p-value = 0.052 for anchovy and 0.061 for sardine), and less likely to be in poor condition compared to those from the Bay of Biscay (p-value < 0.001 for anchovy and p-value = 0.54 for sardine). Both species also displayed size-related patterns (Figure 3). Larger sardine were more likely to be in good (OR > 1 and p-value < 0.001) and poor (OR > 1 and p-value < 0.001) condition state than smaller sardine. Results are similar for anchovy with larger individuals more likely to be in good (OR > 1 and p-value < 0.05) and poor (OR > 1 but p-value > 0.05) condition state than smaller ones. Graphical representation supports these results (Figure A2). For anchovy, being in good condition was unlikely during reproduction, whereas being in poor condition was more likely (Figure 3). ORs, associated 95% CIs and p-values are available in Table A2 and Table A3. A graphical representation of individual condition states for anchovy and sardine considering each explanatory variable is provided in Figure A2.

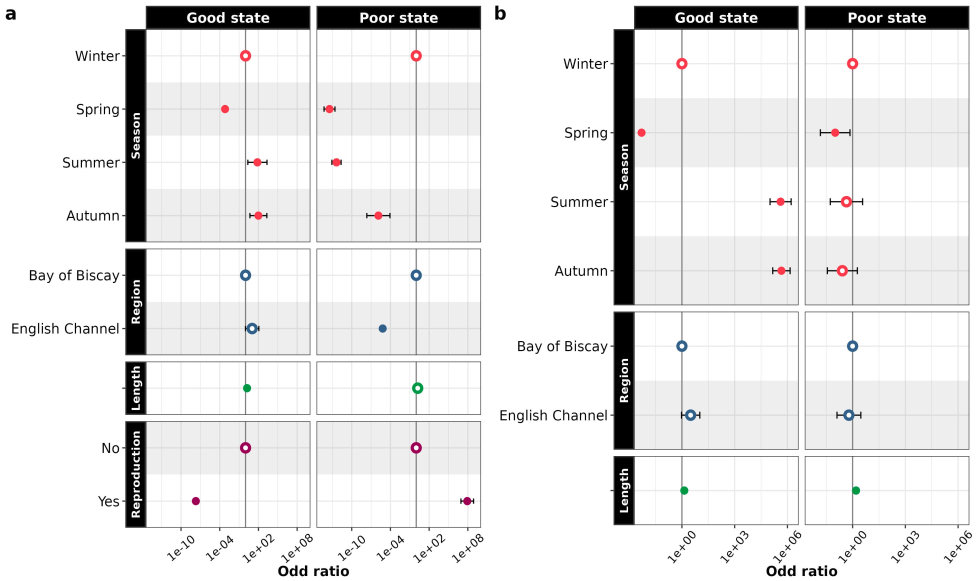

Figure 3: Summary of multinomial logistic regressions for anchovy (a) and sardine (b). Odds ratios and 95% confidence intervals for explanatory variables associated with condition states. The intermediate condition state served as the reference group. Filled dots indicate significance (p-value < 0.05) and empty dots indicate non-significance (p-value > 0.05).

3.3. Variation in water mass

The influence of proximate component content on water mass independently of fish length was explored using a multiple linear regression (Eq.1) (Table 3). First, length had a significant effect on water mass (p-value < 2.10^{-16}) for both species, explaining 88% of the variance for anchovy and 94% for sardine. Second, protein or lipid content had no significant effect on water mass (p-value > 0.05) for both anchovy and sardine. Third, ash content had a significant effect on water mass for anchovy (although explaining only 2.21% of the variance) but not for sardine.

Table 3: Results of the multiple regression of water mass as a function of proximate component content and length, for anchovy and sardine.

	Anchovy			**Sardine**		
	Coefficient	*p-value*	*Explained variance*	*Coefficient*	*p-value*	*Explained variance*
Intercept	-23.0 ± 3.5	$3.6.10^{-9}$		-56.0 ± 8.0	$2.2.10^{-10}$	
Length	3.0 ± 0.1	< 2.10^{-16}	88%	5.6 ± 0.2	< 2.10^{-16}	94%
Protein	3.6 ± 13.5	0.80	0.002%	-30.0 ± 28.3	0.29	0.06%

Lipids	9.8 ± 6.0	0.10	1.44%	-3.7 ± 11.4	0.74	0%
Ash	-234.1 ± 44.8	$9.9.10^{-7}$	2.21%	-67.3 ± 105.0	0.52	0.02%

3.4. Relationships between mass of water and proximate components

Strong log-log linear relationships were found between protein and water masses, as well as between ash and water masses, for anchovy and sardine (Table A4). R^2 were similar for protein (0.93 and 0.95 for anchovy and sardine, respectively) but differed for ash (0.89 and 0.96 for anchovy and sardine, respectively). Lipid mass exhibited higher variability for a given water mass, with a low associated R^2 (< 0.3) for both species.

The relationships between the mass of lipids, of proteins, of ash and that of water were investigated for each of the three condition states (Figure 4). Log-log linear model relating lipid to water masses, segmented by condition state, displayed higher explained variance ($R^2 > 0.7$) for individuals in good state for both anchovy and sardine (Table A4). Significant differences in intercepts were found between condition states in the segmented linear models of protein and water masses and lipids and water mass (all P-values < 0.001 for anchovy and sardine in ANCOVA results), but not in the model relating ashes to water (ANCOVA P-values = 0.21 for anchovy and 0.36 for sardine, Figure 4, Table 4). The interaction between the condition state and water mass had also a significant effect on proteins and lipids (all ANCOVA P-values < 0.001 for anchovy and sardine) but not on ashes (ANCOVA P-value = 0.16 for anchovy and 0.14 for sardine, Table 4).

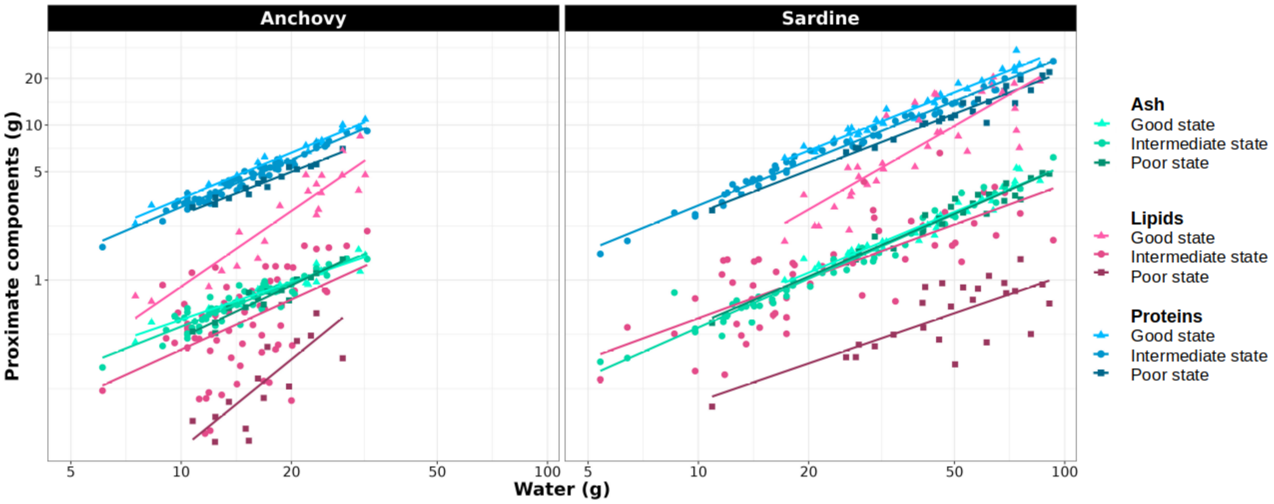

Figure 4: Observed mass (y-axis) of proteins (blue dots), lipids (purple dots) and ash (green dots) in anchovy and sardine body composition, as a function of water mass (x-axis). Solid lines: regression models for the three condition states for proteins (blue lines), lipids (purple lines) and ash (green lines).

Table 4: P-values of ANCOVA testing the effect of the condition state on the proximate components mass versus water mass relationship for anchovy and sardine.

	Anchovy			**Sardine**		
	Proteins	Lipids	Ashes	Proteins	Lipids	Ashes
Water mass	$< 2.10^{-16}$	$< 2.10^{-16}$	$< 2.10^{-16}$	$< 2.10^{-16}$	1.10^{-13}	$< 2.10^{-16}$
State	$< 2.10^{-16}$	$< 2.10^{-16}$	0.207	$< 2.10^{-16}$	$< 2.10^{-16}$	0.357
Water mass * State	$1.2.10^{-4}$	1.10^{-13}	0.163	$2.1.10^{-7}$	4.10^{-14}	0.143

3.5. Lipids and proteins contribution to energy mobilisation

Based on the slope coefficients estimated within the three intervals, we determined the contributions and associated uncertainties of lipids and proteins to energy mobilization in terms of mass and energy (Figure 5, Table A5, Table A6). Within the good condition state, lipids emerged as the primary source of energy mobilisation in both anchovy and sardine, representing nearly all energy mobilized (94% and 96% in mass and energy, for anchovy and 93% and 96% in mass and energy, for sardine). In the intermediate condition state, lipids and proteins contributed roughly equally to energy utilization in terms of mass for both species (53% lipids and 47% proteins for anchovies; 52% lipids and 48% proteins for sardines). However, given the higher energy density of lipids compared to proteins, lipids remained the dominant contributor to energy in terms of energy (65% lipids and 35% proteins for anchovies; 64% lipids and 36% proteins for sardines). In the poor condition state, proteins supplanted lipids as the primary source of energy for both species, due to lipid depletion (94% in mass and 90% in energy for anchovies; 86% in mass and 79% in energy for sardines).

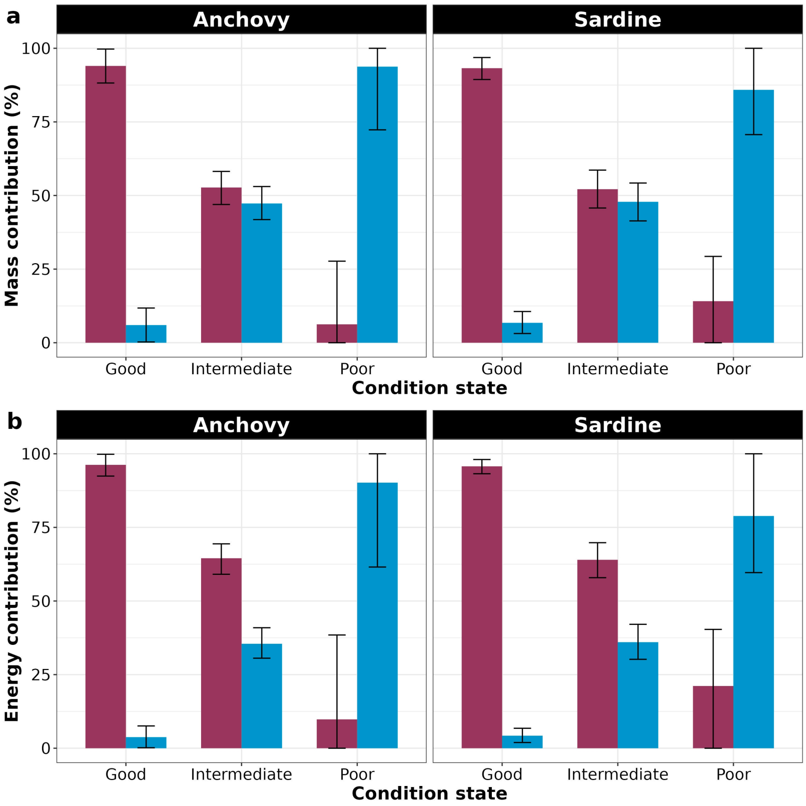

Figure 5: Relative contribution to energy mobilisation of lipids (purple area) and proteins (blue area) with associated uncertainty, expressed in terms of mass (a) and energy (b), for anchovy and sardine according to the condition states (good, intermediate or poor).

3.6 Relationship between fish water content and energy density

The linear models between energy density and water content, segmented or not by condition state, are presented in Figure 6a. Significant differences in regression slopes (Table A7) were observed between unsegmented and segmented linear regression, particularly for states of good and poor conditions. The regression slopes for good condition (-46.3±3.74 for anchovy and – 43.5±1.15 for sardine) were higher, while the slopes for poor condition (-18.8±1.14 for anchovy and -28.6±1.29 for sardine) were lower than the regression slopes of unsegmented models (-31.2±0.53 for anchovy and -39.8±0.26 for sardine). Using both segmented and unsegmented regression equations (for good, intermediate, and poor states), we predicted energy density based on water content for both anchovy and sardine. Energy density predictions from the unsegmented linear model were more accurate for sardine ($R^2 = 0.959$) than for anchovy ($R^2 = 0.874$), primarily due to poor predictions of extreme energy density values for anchovy (Figure 6b). Using a linear model segmented by each condition state (Figure 6c) improved the accuracy of energy density predictions for fish in good and poor condition, and also globally, particularly for anchovy ($R^2 = 0.913$).

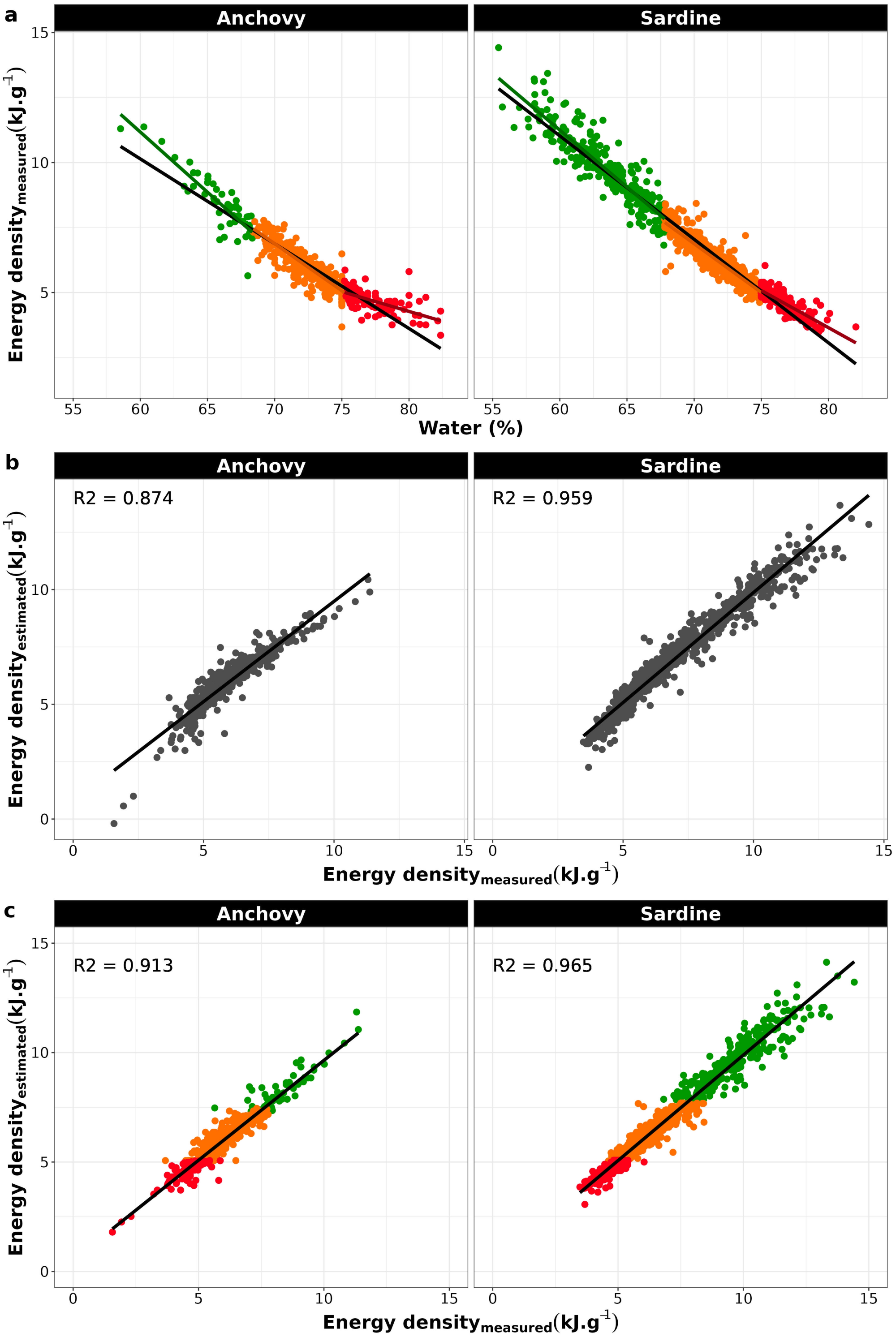

Figure 6: (a) Measured energy density as a function of water content (x-axis) and associated linear models based on all individuals (black line), individuals in good (green dots and line), intermediate (orange dots and line) and poor (red dots and line) condition states. Measured energy density (y-axis) as a function of predicted energy density (x-axis) from a linear models fitted to all individuals (b) and from segmented linear models fitted to individuals in good (green), intermediate (orange) and poor (red) condition state (c).

4. Discussion

Based on a unique dataset of energy density and proximate composition measured on almost 1500 anchovy and sardine sampled over a large region encompassing the Bay of Biscay and the English Channel, we derived original patterns of variation and dependency between energy, water, lipid and protein content. Working on wild individuals preclude any longitudinal studies in which individuals and their condition are followed through time. Instead, we developped a cross-sectional approach on a large number of individuals over a large range of size and from different seasons to uncover those interesting patterns.We defined for the first time different condition states in wild fishes, corresponding to different levels of lipid and protein contents, as well as to different contribution of those metabolites when the lack of direct energy from food requires body reserves to be mobilised. Following the identification of those condition states, we were able to i) assess that absolute water mass, unlike relative water content, does not vary with fish condition, and ii) improve the modelling of the non-linear relationship between energy density and water content.

4.1. Three condition states based on relative proximate composition

We identified three distinct condition states in wild anchovy and sardine, based on their lipid and protein composition, relative to water content. Our finding is consistent with previous studies describing three phases of starvation for mammals (Cherel *et al.*, 1988, 1991, 1992; Caloin, 2004), birds (Le Maho *et al.*, 1981; Robin *et al.*, 1988; Groscolas and Robin, 2001) and fish (Bar, 2014). We can not however ascertain that fish between good and intermediate states were starving, as we did not follow individuals in a controlled longitudinal experiment. Instead, we may consider that the three condition states identified in our study correspond to three different phases of energy mobilisation or reserve constitution. The patterns of variation in the proximate composition were strikingly similar between anchovy and sardine. Breck (2014) have also represented lipid and protein variations relative to water content for five fish species (no small pelagic fish) but without demonstrating different condtion states. Investigating these variations in other fish species, in particular small pelagics, would allow to investigate the genericity of these three condition states or phases of energy mobilisation.

Our results confirmed that fish in good condition have high lipid (> 5%) and protein (> 20%) and low water (< 68 %) contents. While some studies indicated that fish may use protein as primary energy source (Marqueze *et al.*, 2017) or a combination of lipid and protein (Van Dijk *et al.*, 2005; Gunasekera *et al.*, 2001.), we showed that anchovy and sardine primarily used lipids as source of energy which was also described for other fish species (Savitz, 1971; Hung *et al.*, 1997). In the intermediate condition state, fish exhibited lower lipid levels (< 5%), high yet decreasing protein levels (> 20%), and average water content ranging from 68 to 75%. Within this state, fish continued to utilise lipids as a source of energy but started to consume proteins, which is in agreement with observations from other studies (Bar, 2014). Within the poor condition state, fish had nearly depleted all lipids and only relied on protein mobilisation for energy production (Black and Love, 1986). We considered that the remaining ~1% lipids were structural (Bayse *et al.*, 2018; Biro *et al.*, 2021) and could not be mobilised. De Leonardis and Macciola (2004) showed that the difference of total lipid between lean and fat sardine were exclusively due to increase of reserve lipid and not affecting structural lipids. Fish in poor condition exhibited high water content in our study (>75%), consistent with Breck's (2008) model results. Because protein metabolism is energetically demanding (Schmidt-Nielsen., 1997; Anthony *et al.*, 2000), poor condition state impacts metabolic rates (Queiros *et al.*, 2021) and is likely brief and critical. However, Queiros *et al.* (2021) showed that some sardine were able to survive 57 days without food *in-vivo*. Survival time when starving should be even shorter *in-situ*, as poor condition can impact swimming capacity (Martínez *et al.*, 2003; Faria *et al.*, 2011), and reduce predator escapement capacity (Biro *et al.*, 2021). Reversibility from poor to intermediate condition state has been observed in controlled environments (Marqueze *et al.*, 2017), but has never been documented in the wild so far.

4.2. What determines the fish condition state?

We identified three condition states along the gradient of water content corresponding to different utilisation strategies (when water content increases), or creation (when water content decreases), of lipid reserves or proteins. Since we were not in a starvation experiment framework, we explored the internal and external drivers that could explain the position of the fish in a given state.

We found that season strongly influenced anchovy and sardine condition state. Our results are in agreement with other studies studying anchovy and sardine condition in the Bay of Biscay through energy density (Gatti et al, 2018; Rosa et al, 2010) or Le Cren body condition index (Véron et al, 2020). They found minimum energy density (or Le Cren index) in winter and spring when food is limiting and a peak in summer and autumn after energy storage during the feeding season. Same patterns have been shown for the Northwest Atlantic forage species (Wuenschel et al, 2024). Seasonality in energy density in small pelagic fish has also been demonstrated in Mediterranean sea, although the peak of energy density was in spring for anchovy and in autumn for sardine (Albo-Puigserver *et al.*, 2017; Campanini et al, 2021; Schismenou et al, 2024).The seasonal effect under environmental variability could also influence the lipid and fatty acid composition (Bandarra et al, 1997 ; Biton-Porsmoguer et al, 2020), affecting the fish physiology as some fatty acid are important components for growth, survival and egg quality (Almansa et al; 1999; Bruce et al; 1999; Garrido et al, 2007; Tocher, 2003). However, variations in energy density are mainly due to changes in total lipid content varying between 36.2 to 39.5 kJ.g-1 (Schloesser and Fabrizio, 2015). Despite this strong effect of season on the condition state, we found some fish in very poor condition in autumn, i.e. two large sardines in reproduction. Conversely, no fish in good condition were found in winter when food was limited, nor in spring when reserves did not have time to replenish.

Region has also an important impact on condition state with anchovy and sardine in good condition mostly sampled in the English Channel, in agreement with other studies (Gatti *et al.*, 2018; Menu *et al.*, 2023) which is most likely explained by more abundant food (Menu *et al.*, 2023), and can be generalised along a latitudinal gradient in temperate systems (Huret et al., 2019; Ljungström et al., 2023).

For both species, length significantly influenced the condition state, with larger individuals of both anchovy and sardine more likely to be in good condition, yet also more prone to being in poor condition (though not significant for anchovy). Larger individuals seemed capable of accumulating substantial reserves (Albo-Puigserver et al., 2017) but also mobilizing proteins when lipid reserves are depleted, in agreement with Gatti et al. (2018), who observed a decrease in energy density in larger size classes of sardine. Conversely, the prevalence of smaller individuals in the intermediate state suggested limited reserves and their absence in the poor condition state indicate an inability to utilize protein for energy mobilization. These may use protein to prioritize rapid growth, but if unsuccessful, they may succumb quickly when facing adverse conditions such as during winter (Dubreuil and Petitgas, 2009).

The influence of reproductive status on fish condition has been extensively discussed (Pethybridge et al., 2014; Brosset et al., 2015a; Albo-Puigserver et al., 2017; Campanini et al., 2021). In our study, reproductive status was only retained in the anchovy’s model, indicating that anchovies were less likely to be in good condition and more likely to be in poor condition during reproduction.

Working on two species with two different spawning strategies highlighted that the main pattern of variability in energy or proximate composition in our temperate study area was driven by external factors (i.e. season and region). The significant effect of internal factors was mostly driven by their interaction with external factors. Indeed, spawning sardines could be in poor or good condition, mostly depending on their reproduction timing in spring or autumn, but not because of their reproductive status. However some studies have demonstrated that reproduction has an effect on lipid content and composition in the Mediterranean Sea (Vila-Belmonte et al, 2024). We were not able to highlight this effect for sardine in the Bay of Biscay. We mention that our dataset, although extensive, is not fully representative of all variable combinations (season, region, length, and reproductive status). This limitation was mainly due to species phenology as well as migration patterns, e.g., anchovy being distributed in the North Sea and not in the English Channel during spring and summer (Huret et al., 2020).

4.3. Water mass does not vary with body proximate composition

We showed that length had a significant effect on water mass, unlike the proportions of lipid or protein. Therefore, water mass primarily relies on fish ontogeny rather than its lipid and protein content. Although a significant negative correlation exists between lipids and water, it is only valid in relative content. As lipid content decreases, water content increases but only in relative terms, not in mass. These results did not agree with previous fish studies, which stated that fish replaced the loss of lipids or protein with water to maintain osmotic pressure, cell size, and body shape (Love, 1970; Pangle and Sutton, 2005; McCue, 2010; Rosa *et al.*, 2010; Bar, 2014; Nemova *et al.*, 2021) or on the opposite found that water did not replace lipid (Savitz, 1971; Breck, 2008) but rather decreased together with lipids explaining the largest part of weight loss (Niimi, 1972; Breck, 2008 but see Collins and Anderson, 1995). Focusing on small pelagic fish, Dubreuil and Petitgas, (2009) stated that anchovy replaced water with organic matter during feeding season and replaced organic matter with water in winter. Breck (2008) argued that proteins are strongly associated with water, leading to a loss of water when proteins are consumed. Groves (1970), suggested that proteins are lost more rapidly than water and argued that a fish in starvation will have a lower protein:water ratio than a fish in good condition, for the same length. We also showed that fish in poor condition state exhibited a lower protein:water ratio, or lipid:water ratio, although we showed, thanks to an analysis based on absolute mass rather than relative content, that this discrepancy is only attributable to protein and lipid loss and not variation in water mass.

4.4. Differential use of lipids and proteins among condition states explain the non linear relationship between water content and energy density

The strength of the relationship between water content (or dry content) and energy density has been extensively documented (Hartman and Brandt, 1995; Tirelli *et al.*, 2006; Dubreuil and Petitgas, 2009; Gatti *et al.*, 2018). Non-linearity has been reported in this relationship (Hartman and Brandt, 1995; Gatti *et al.*, 2018), often attributed to ontogenetic and size-related effects (Wuenschel *et al.*, 2006) but without clear consensus on the mechanisms at stake. In our study, we showed that the well-established relationship between water content and energy density varies for both anchovy and sardine in response to fish condition state and body composition. Non-linearity in the energy to water relationship can be modelled by applying a piece-wise regression, over each condition state derived from proximate composition vs. water content models. The regression slopes for good condition were steeper while the slopes for poor condition were flatter as compared to the regression slopes of unsegmented models. A pronounced negative slope means a more pronounced reduction in energy as water content increases, consistent with the initial loss of lipids with higher energy density than proteins, followed by the loss of proteins within the poor state. Our findings increase the predictability of energy density based on a water content proxy. The absence of non-linearity in this relationship reported in some studies (Tirelli *et al.*, 2006; Dubreuil and Petitgas, 2009) may have stemmed from two main reasons. First, the relationship may not be nonlinear if most fish are in the same condition state, e.g. in the case of reduced sample size and/or space-time range. Second, differences in the contributions of lipids and proteins may be difficult to detect in the case of lean fish with low lipid content (Anthony *et al.*, 2000). However, the genericity of this non-linearity across species and beyond small pelagic species only (Hartman and Brandt, 1995) calls for consideration of segmented regressions in future studies before using it to predict energy density.

4.5. Conclusion

This study highlights the importance of monitoring and predicting the intraspecific variations in water content, proximate composition and energy content in small pelagic fish. We revealed striking similarities between anchovy and sardine, suggesting that similar studies should be conducted on other small pelagic fish species to verify the larger genericity of the observed patterns. However, before doing so, these relationships should be determined on a case by case basis (McPherson) to validate the use of proxy such as water content or fatmeter measurements, despite some limitations of this device (Brosset et al, 2015, Schismenou et al, 2024).In particular, lipid storage organs may vary from one species to another (e.g. muscle, liver, gonads), which should influence the generalisation of this relationship. Integrating water content measurements during surveys could provide new insight on individual and population conditions, especially in the context of the decline condition in the European Atlantic (Doray *et al.*, 2018a; Véron *et al.*, 2020; Taboada et al., 2024) and Mediterranean areas (Brosset *et al.*, 2017). Sardine spawners in better condition have higher reproduction outcomes, i.e. more batches and more viable eggs (Rosa *et al.*, 2010; Brosset *et al.*, 2016), potentially impacting recruitment. Monitoring changes in the proportion of fish in good and poor condition states could offer insights into the evolution of natural mortality rates, which may be increasing in the Bay of Biscay (Véron *et al.*, 2020) but considered as invariant in stock assessment models (ICES, 2023). Finally, our observation of individuals in very poor condition in the wild raises the following questions: Is this state reversible? If so, what proportion of individuals survives annually? Is it a common state in the fish life cycle? A better characterisation of the overwintering dynamics, especially the survival of individuals, and of the interplay with reproductive strategies would certainly bring important knowledge for a better understanding of the recruitment process. Furthermore, our research improved our understanding of the dynamics of reserves (lipids and proteins) that should be taken into account for improved realism of bioenergetic models (Breck, 2008; Gatti *et al.*, 2017).

Author contributions

A.F. and M.H. designed the methodology. A.F. ran the analysis. S.L.M acquired the data. M.H. raised fundings. A.F. wrote the original manuscript.. A.F., M.H., J.S., and M.D. contributed to revising and editing the manuscript.

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Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of this article.

References

Albo-Puigserver, M., Muñoz, A., Navarro, J., Coll, M., Pethybridge, H., Sánchez, S., Palomera, I., 2017. Ecological energetics of forage fish from the Mediterranean Sea: Seasonal dynamics and interspecific differences. Deep Sea Research Part II: Topical Studies in Oceanography 140, 74–82. https://doi.org/10.1016/j.dsr2.2017.03.002

Almansa, E., Perez, J., Cejas, J.R., Badıa, P., Villamandos, J.E., Lorenzo, A., 1999. Influence of broodstock gilthead seabream žSparus aurata L./ dietary fatty acids on egg quality and egg fatty acid composition throughout the spawning season.

Anthony, J.A., Roby, D.D., Turco, K.R., 2000. Lipid content and energy density of forage fishes from the northern Gulf of Alaska. Journal of Experimental Marine Biology and Ecology 248, 53–78. https://doi.org/10.1016/S0022-0981(00)00159-3

Bakhtiarvandi, K., Kenari, A., Nazari, M., 2014. Ontogenetic changes in lipids, fatty acid, and body composition during larval stages of Caspian Kutum. Iranian Journal of Fisheries Sciences, 13(2) 365-383.

Bandarra, N.M., Batista, I., Nunes, M.L., Empis, J.M., Christie, W.W., 1997. Seasonal Changes in Lipid Composition of Sardine (Sardina pilchardus). Journal of Food Science 62, 40–42. https://doi.org/10.1111/j.1365-2621.1997.tb04364.x

Bandarra, N.M., Marçalo, A., Cordeiro, A.R., Pousão-Ferreira, P., 2018. Sardine (Sardina pilchardus) lipid composition: Does it change after one year in captivity? Food Chemistry 244, 408–413. https://doi.org/10.1016/j.foodchem.2017.09.147

Bar, N., 2014. Physiological and hormonal changes during prolonged starvation in fish. Can. J. Fish. Aquat. Sci. 71, 1447–1458. https://doi.org/10.1139/cjfas-2013-0175

Bayse, S.M., Regish, A.M., McCormick, S.D., 2018. Proximate composition, lipid utilization and validation of a non-lethal method to determine lipid content in migrating American shad Alosa sapidissima. Journal of Fish Biology 92, 1832–1848. https://doi.org/10.1111/jfb.13624

Beamish, F.W.H., Niimi, A.J., Lett, P.F.K.P., 1975. Bioenergetics of teleost fishes: Environmental influences. In: Bolis, L., Maddrell, S.H.P., Schmidt-Nielsen, K. (Eds.), Compara- tive physiology: Functional aspects of structural materials. North-Holland Publishing Company, Amsterdam, pp. 187–209.

Benoit-Bird, K.J., 2004. Prey caloric value and predator energy needs: foraging predictions for wild spinner dolphins. Marine Biology 145: 435-444. https://doi.org/10.1007/s00227-004-1339-1

Biro, P.A., Post, J.R., Beckmann, C., 2021. Autumn lipid reserves, overwinter lipid depletion, and high winter mortality of rainbow trout in experimental lakes. Can. J. Fish. Aquat. Sci. 78, 738–743. https://doi.org/10.1139/cjfas-2020-0276

Biton-Porsmoguer, S., Bou, R., Lloret, E., Alcaide, M., Lloret, J., 2020. Fatty acid composition and parasitism of European sardine (Sardina pilchardus) and anchovy (Engraulis encrasicolus) populations in the northern Catalan Sea in the context of changing environmental conditions. Conservation Physiology 8, coaa121. https://doi.org/10.1093/conphys/coaa121

Black, D., Love, R.M., 1986. The sequential mobilisation and restoration of energy reserves in tissues of Atlantic cod during starvation and refeeding. J Comp Physiol B 156, 469–479. https://doi.org/10.1007/BF00691032

Bonvini, E., Parma, L., Mandrioli, L., Sirri, R., Brachelente, C., Mongile, F., Gatta, P.P., Bonaldo, A., 2015. Feeding common sole (Solea solea) juveniles with increasing dietary lipid levels affects growth, feed utilization and gut health. Aquaculture 449, 87–93. https://doi.org/10.1016/j.aquaculture.2015.01.013

Boyra, G., Nogueira, E., & Sobradillo, B. (2003). JUVENA survey. AZTI. doi.org/10.57762/A6T5-9D32

Breck, J.E., 2014. Body composition in fishes: body size matters. Aquaculture 433, 40–49. https://doi.org/10.1016/j.aquaculture.2014.05.049

Breck, J.E., 2008. Enhancing Bioenergetics Models to Account for Dynamic Changes in Fish Body Composition and Energy Density. Transactions of the American Fisheries Society 137, 340–356. https://doi.org/10.1577/T05-240.1

Brett, J. R., and T. D. D. Groves. 1979. Physiological energetics. Pages 279–352 in W. S. Hoar, D. J. Randall, and J. R. Brett, editors. Fish physiology, volume 8. Academic Press, New York.

Brosset, Pablo, Fromentin, J.-M., Ménard, F., Pernet, F., Bourdeix, J.-H., Bigot, J.-L., Van Beveren, E., Pérez Roda, M.A., Choy, S., Saraux, C., 2015a. Measurement and analysis of small pelagic fish condition: A suitable method for rapid evaluation in the field. Journal of Experimental Marine Biology and Ecology 462, 90–97. https://doi.org/10.1016/j.jembe.2014.10.016

Brosset, P, Ménard, F., Fromentin, J., Bonhommeau, S., Ulses, C., Bourdeix, J., Bigot, J., Van Beveren, E., Roos, D., Saraux, C., 2015b. Influence of environmental variability and age on the body condition of small pelagic fish in the Gulf of Lions. Mar. Ecol. Prog. Ser. 529, 219–231. https://doi.org/10.3354/meps11275

Brosset, P., Lloret, J., Muñoz, M., Fauvel, C., Van Beveren, E., Marques, V., Fromentin, J.-M., Ménard, F., Saraux, C., 2016. Body reserves mediate trade-offs between life-history traits: new insights from small pelagic fish reproduction. R. Soc. open sci. 3, 160202. https://doi.org/10.1098/rsos.160202

Brosset, P., Fromentin, J.-M., Van Beveren, E., Lloret, J., Marques, V., Basilone, G., Bonanno, A., Carpi, P., Donato, F., Čikeš Keč, V., De Felice, A., Ferreri, R., Gašparević, D., Giráldez, A., Gücü, A., Iglesias, M., Leonori, I., Palomera, I., Somarakis, S., Tičina, V., Torres, P., Ventero, A., Zorica, B., Ménard, F., Saraux, C., 2017. Spatio-temporal patterns and environmental controls of small pelagic fish body condition from contrasted Mediterranean areas. Progress in Oceanography 151, 149–162. https://doi.org/10.1016/j.pocean.2016.12.002

Bruce, M., Oyen, F., Bell, G., Asturiano, J.F., Farndale, B., Carrillo, M., Zanuy, S., Ramos, J., Bromage, N., 1999. Development of broodstock diets for the European Sea Bass žDicentrarchus labrax/ with special emphasis on the importance of n y 3 and n y 6 highly unsaturated fatty acid to reproductive performance.

Caloin, M., 2004. Modeling of lipid and protein depletion during total starvation. American Journal of Physiology-Endocrinology and Metabolism 287, E790–E798. https://doi.org/10.1152/ajpendo.00414.2003

Campanini, C., Albo-Puigserver, M., Gérez, S., Lloret-Lloret, E., Giménez, J., Pennino, M.G., Bellido, J.M., Colmenero, A.I., Coll, M., 2021. Energy content of anchovy and sardine using surrogate calorimetry methods. Marine Environmental Research 172, 105510. https://doi.org/10.1016/j.marenvres.2021.105510

Cherel, Y., Attaix, D., Rosolowska-Huszcz, D., Belkhou, R., Robin, J.-P., Arnal, M., Maho, Y.L., 1991. Whole-body and tissue protein synthesis during brief and prolonged fasting in the rat. Clinical Science 81, 611–619. https://doi.org/10.1042/cs0810611

Cherel, Y., Burnol, A.-F., Leturque, A., Le Maho, Y., 1988. In vivo glucose utilization in rat tissues during the three phases of starvation. Metabolism 37, 1033–1039. https://doi.org/10.1016/0026-0495(88)90063-7

Cherel, Y., Robin, J.-P., Heitz, A., Calgari, C., Le Maho, Y., 1992. Relationships between lipid availability and protein utilization during prolonged fasting. J Comp Physiol B 162, 305–313. https://doi.org/10.1007/BF00260757

Ciancio, J.E., Pascual, M.A., Beauchamp, D.A., 2007. Energy Density of Patagonian Aquatic Organisms and Empirical Predictions Based on Water Content. Transactions of the American Fisheries Society 136, 1415–1422. https://doi.org/10.1577/T06-173.1

Collins, A.L., Anderson, T.A., 1995. The regulation of endogeneous energy stores during starvation and refeeding in the somatic tissues of the golden perch. Journal of Fish Biology 47, 1004–1015. https://doi.org/10.1111/j.1095-8649.1995.tb06024.x

Craig, J. F. 1977. The body composition of adult perch, Perca fluviatilis, in Windermere, with reference to seasonal changes and reproduction. Journal of Animal Ecology 46:617–632.

Craig, J.F., Kenley, M.J., Talling, J.F., 1978. Comparative estimations of the energy content of fish tissue from bomb calorimetry, wet oxidation and proximate analysis. Freshwater Biology 8, 585–590. https://doi.org/10.1111/j.1365-2427.1978.tb01480.x

Cury, P., 2000. Small pelagics in upwelling systems: patterns of interaction and structural changes in "wasp-waist" ecosystems. ICES Journal of Marine Science 57, 603–618. https://doi.org/10.1006/jmsc.2000.0712

De Leonardis, A., Macciola, V., 2004. A study on the lipid fraction of Adriatic sardine filets (Sardina pilchardus). Nahrung 48, 209–212. https://doi.org/10.1002/food.200300408

Doray, M., Duhamel, E., Huret, M., Petigas., P, Masse, J. 2000. PELGAS, https://doi.org/10.18142/18

Doray, M., Petitgas, P., Huret, M., Duhamel, E., Romagnan, J.B., Authier, M., Dupuy, C., Spitz, J., 2018a. Monitoring small pelagic fish in the Bay of Biscay ecosystem, using indicators from an integrated survey. Progress in Oceanography 166, 168–188. https://doi.org/10.1016/j.pocean.2017.12.004

Doray, M., Petitgas, P., Romagnan, J.B., Huret, M., Duhamel, E., Dupuy, C., Spitz, J., Authier, M., Sanchez, F., Berger, L., Dorémus, G., Bourriau, P., Grellier, P., Massé, J., 2018b. The PELGAS survey: Ship-based integrated monitoring of the Bay of Biscay pelagic ecosystem. Progress in Oceanography 166, 15–29. https://doi.org/10.1016/j.pocean.2017.09.015

Dubreuil, J., Petitgas, P., 2009. Energy density of anchovy *Engraulis encrasicolus* in the Bay of Biscay. Journal of Fish Biology 74, 521–534. https://doi.org/10.1111/j.1095-8649.2008.02143.x

Eits, R.M., Kwakkel, R.P., Verstegen, M.W.A., 2002. Nutrition Affects Fat-Free Body Composition in Broiler Chickens. The Journal of Nutrition 132, 2222–2228. https://doi.org/10.1093/jn/132.8.2222

Faria, A., Chícharo, M., Gonçalves, E., 2011. Effects of starvation on swimming performance and body condition of pre-settlement Sparus aurata larvae. Aquat. Biol. 12, 281–289. https://doi.org/10.3354/ab00345

Garrido, S., Rosa, R., Ben-Hamadou, R., Cunha, M.E., Chícharo, M.A., Van Der Lingen, C.D., 2007. Effect of maternal fat reserves on the fatty acid composition of sardine (Sardina pilchardus) oocytes. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 148, 398–409. https://doi.org/10.1016/j.cbpb.2007.07.008

Gatti, P., Petitgas, P., Huret, M., 2017. Comparing biological traits of anchovy and sardine in the Bay of Biscay: A modelling approach with the Dynamic Energy Budget. Ecological Modelling 348, 93–109. https://doi.org/10.1016/j.ecolmodel.2016.12.018

Gatti, P., Cominassi, L., Duhamel, E., Grellier, P., Le Delliou, H., Le Mestre, S., Petitgas, P., Rabiller, M., Spitz, J., Huret, M., 2018. Bioenergetic condition of anchovy and sardine in the Bay of Biscay and English Channel. Progress in Oceanography 166, 129–138. https://doi.org/10.1016/j.pocean.2017.12.006

Gerking, S.D., 1955. Influence of Rate of Feeding on Body Composition and Protein Metabolism of Bluegill Sunfish. Physiological Zoology 28, 267–282. https://doi.org/10.1086/physzool.28.4.30152188

Giraldo, C., Martin-Baillet, V., Le Roy, D. 1988. CGFS: Channel Ground Fish Survey, https://doi.org/10.18142/11

Groscolas, R., Robin, J.-P., 2001. Long-term fasting and re-feeding in penguins. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 128, 643–653. https://doi.org/10.1016/S1095-6433(00)00341-X

Groves, T.D.D., 1970. Body Composition Changes During Growth in Young Sockeye (*Oncorhynchus nerka*) in Fresh Water. J. Fish. Res. Bd. Can. 27, 929–942. https://doi.org/10.1139/f70-100

Guglielmo, C.G., 2018. Obese super athletes: fat-fueled migration in birds and bats. Journal of Experimental Biology 221, jeb165753. https://doi.org/10.1242/jeb.165753

Gunasekera, R.M., Silva, S.S.D., Ingram, B.A., n.d. Chemical changes in fed and starved larval trout cod, Maccullochella macquarensis during early development.

Hartman, K., Brandt, S., 1995. Estimating Energy Density of Fish. Transactions of The American Fisheries Society - TRANS AMER FISH SOC 124, 347–355. https://doi.org/10.1577/1548-8659(1995)124

Hung, S.S.O., Liu, W., Li, H., Storebakken, T., Cui, Y., 1997. Effect of starvation on some morphological and biochemical parameters in white sturgeon, Acipenser transmontanus. Aquaculture 151, 357–363. https://doi.org/10.1016/S0044-8486(96)01506-2

Huret, M., Tsiaras, K., Daewel, U., Skogen, M., Gatti, P., Petitgas, P., Somarakis, S., 2019. Variation in life-history traits of European anchovy along a latitudinal gradient: a bioenergetics modelling approach. Mar. Ecol. Prog. Ser. 617–618, 95–112. https://doi.org/10.3354/meps12574

Huret, M., Lebigre, C., Iriondo, M., Montes, I., Estonba, A., 2020. Genetic population structure of anchovy (Engraulis encrasicolus) in North-western Europe and variability in the seasonal distribution of the stocks. Fisheries Research 229, 105619. https://doi.org/10.1016/j.fishres.2020.105619

Huret, M., Favreau, A., Gatti; P., Le Mestre S. 2024. Energy density and proximal composition of anchovy and sardine along the french Atlantic coast. SEANOE. https://doi.org/10.17882/101384

ICES. 2008. Report of the Workshop on Small Pelagics (Sardina pilchardus, Engraulis encrasicolus) maturity stages (WKSPMAT), 10–14 November 2008, Mazara del Vallo, Italy. ICES CM 2008/ACOM:40. 82 pp. https://doi.org/10.17895/ices.pub.19280426.

ICES, 2023. Working group on southern horse mackerel, anchovy and sardine (WGHANSA).

Laffargue, P., Salaun, M., Garren, F., Bellail, R., Mahe, JC., Poulard JC. 1987. EVHOE Evaluation Halieutique Ouest de l’Europe. https://doi.org/10.18142/8

Le Maho, Y., Vu Van Kha, H., Koubi, H., Dewasmes, G., Girard, J., Ferre, P., Cagnard, M., 1981. Body composition, energy expenditure, and plasma metabolites in long-term fasting geese. American Journal of Physiology-Endocrinology and Metabolism 241, E342–E354. https://doi.org/10.1152/ajpendo.1981.241.5.E342

Ljungström, G., Langbehn, T., Jørgensen, C., 2023. Bergmann patterns in planktivorous fishes: A light-size or zooplankton community-size rule is just as valid explanation as the temperature-size rule. Global Ecology and Biogeography n/a. https://doi.org/10.1111/geb.13782

Lloret, 2014. Condition and Health Indicators of Exploited Marine Fishes, 1st ed. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118752777

Love, R. M. 1970. The chemical biology of fishes. Academic Press, New York.

Marqueze, A., Garbino, C.F., Trapp, M., Kucharski, L.C., Fagundes, M., Ferreira, D., Koakoski, G., Rosa, J.G.S., 2017. Protein and lipid metabolism adjustments in silver catfish (Rhamdia quelen) during different periods of fasting and refeeding. Braz. J. Biol. 78, 464–471. https://doi.org/10.1590/1519-6984.169333

Martínez, M., Guderley, H., Dutil, J.-D., Winger, P.D., He, P., Walsh, S.J., 2003. Condition, prolonged swimming performance and muscle metabolic capacities of cod *Gadus morhua*. Journal of Experimental Biology 206, 503–511. https://doi.org/10.1242/jeb.00098

McComish, T.S., Anderson, R.O., Goff, F.G., 1974. Estimation of Bluegill (*Lepomis macrochirus*) Proximate Composition with Regression Models. J. Fish. Res. Bd. Can. 31, 1250–1254. https://doi.org/10.1139/f74-151

McCue, M.D., 2010. Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 156, 1–18. https://doi.org/10.1016/j.cbpa.2010.01.002

Menu, C., Pecquerie, L., Bacher, C., Doray, M., Hattab, T., van der Kooij, J., Huret, M., 2023. Testing the bottom-up hypothesis for the decline in size of anchovy and sardine across European waters through a bioenergetic modeling approach. Progress in Oceanography 210, 102943. https://doi.org/10.1016/j.pocean.2022.102943

Molnár, P.K., Klanjscek, T., Derocher, A.E., Obbard, M.E., Lewis, M.A., 2009. A body composition model to estimate mammalian energy stores and metabolic rates from body mass and body length, with application to polar bears. Journal of Experimental Biology 212, 2313–2323. https://doi.org/10.1242/jeb.026146

Moulton, C.R., 1923. AGE AND CHEMICAL DEVELOPMENT IN MAMMALS. Journal of Biological Chemistry 57, 79–97. https://doi.org/10.1016/S0021-9258(18)85527-2

Muggeo, V.M.R., 2008. segmented: An R Package to Fit Regression Models with Broken-Line Relationships 8.

Nemova, N.N., Kantserova, N.P., Lysenko, L.A., 2021. The Traits of Protein Metabolism inthe Skeletal Muscle of Teleost Fish. J Evol Biochem Phys 57, 626–645. https://doi.org/10.1134/S0022093021030121

Niimi, A.J., 1972. Changes in the proximate body composition of largemouth bass (*Micropterus salmoides*) with starvation. Can. J. Zool. 50, 815–819. https://doi.org/10.1139/z72-110

Noren, D.P., Crocker, D.E., Williams, T.M., Costa, D.P., 2003. Energy reserve utilization in northern elephant seal (Mirounga angustirostris) pups during the postweaning fast: size does matter. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 173, 443–454. https://doi.org/10.1007/s00360-003-0353-9

Pagano, Durner, Rode, 2018. High-energy, high-fat lifestyle challenges an Arctic apex predator, the polar bear. https://doi.org/10.1126/science.aan8677

Paine, R.T., 1971. The Measurement and Application of the Calorie to Ecological Problems. Annu. Rev. Ecol. Syst. 2, 145–164. https://doi.org/10.1146/annurev.es.02.110171.001045

Pangle, K.L., Sutton, T.M., 2005. Temporal changes in the relationship between condition indices and proximate composition of juvenile Coregonus artedi. Journal of Fish Biology 66, 1060–1072. https://doi.org/10.1111/j.0022-1112.2005.00660.x

Pethybridge, H., Bodin, N., Arsenault-Pernet, E., Bourdeix, J., Brisset, B., Bigot, J., Roos, D., Peter, M., 2014. Temporal and inter-specific variations in forage fish feeding conditions in the NW Mediterranean: lipid content and fatty acid compositional changes. Mar. Ecol. Prog. Ser. 512, 39–54. https://doi.org/10.3354/meps10864

Pothoven, S.A., Fahnenstiel, G.L., 2014. Declines in the energy content of yearling non-native alewife associated with lower food web changes in Lake Michigan. Fisheries Management and Ecology 21, 439–447. https://doi.org/10.1111/fme.12092

Queiros, Q., Fromentin, J.-M., Gasset, E., Dutto, G., Huiban, C., Metral, L., Leclerc, L., Schull, Q., McKenzie, D.J., Saraux, C., 2019. Food in the Sea: Size Also Matters for Pelagic Fish. Front. Mar. Sci. 6, 385. https://doi.org/10.3389/fmars.2019.00385

Queiros, Q., Saraux, C., Dutto, G., Gasset, E., Marguerite, A., Brosset, P., Fromentin, J.-M., McKenzie, D.J., 2021. Is starvation a cause of overmortality of the Mediterranean sardine? Marine Environmental Research 170, 105441. https://doi.org/10.1016/j.marenvres.2021.105441

Rivera-Torres, V., Noblet, J., Van Milgen, J., 2011. Changes in chemical composition in male turkeys during growth. Poultry Science 90, 68–74. https://doi.org/10.3382/ps.2010-00633

Robin, J.P., Frain, M., Sardet, C., Groscolas, R., Le Maho, Y., 1988. Protein and lipid utilization during long-term fasting in emperor penguins. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 254, R61–R68. https://doi.org/10.1152/ajpregu.1988.254.1.R61

Rosa, R., Gonzalez, L., Broitman, B., Garrido, S., Santos, A., Nunes, M., 2010. Bioenergetics of small pelagic fishes in upwelling systems: relationship between fish condition, coastal ecosystem dynamics and fisheries. Mar. Ecol. Prog. Ser. 410, 205–218. https://doi.org/10.3354/meps08635

Sáez-Plaza, P., Michałowski, T., Navas, M.J., Asuero, A.G., Wybraniec, S., 2013. An Overview of the Kjeldahl Method of Nitrogen Determination. Part I. Early History, Chemistry of the Procedure, and Titrimetric Finish. Critical Reviews in Analytical Chemistry 43, 178–223. https://doi.org/10.1080/10408347.2012.751786

Savitz, J., 1971. Effects of Starvation on Body Protein Utilization of Bluegill Sunfish (Lepomis macrochirus Rafinesque) with a Calculation of Caloric Requirements. Transactions of the American Fisheries Society 100, 18–21. https://doi.org/10.1577/1548-8659(1971)100<18:EOSOBP>2.0.CO;2

Schismenou, E., Chatzifotis, S., Tsiaras, K., Somarakis, S., 2024. Anchovy and sardine condition and energy content in the North Aegean Sea (eastern Mediterranean) in relation to their contrasting reproductive strategies. Journal of Fish Biology 2024; 1-11. https://doi.org/10.1111/jfb.15872

Schloesser, R.W., Fabrizio, M.C., 2015. Relationships among Proximate Components and Energy Density of Juvenile Atlantic Estuarine Fishes. Transactions of the American Fisheries Society 144, 942–955. https://doi.org/10.1080/00028487.2015.1052557

Schmidt-Nielsen, K. (Ed.), 1997. Animal Physiology: Adaptation and Environment, 5th Edition, Cambridge University Press, New York, p. 607.

Spitz, J., Jouma'a, J., 2013. Variability in energy density of forage fishes from the Bay of Biscay (north-east Atlantic Ocean): reliability of functional grouping based on prey quality. Journal of Fish Biology 82, 2147–2152. https://doi.org/10.1111/jfb.12142

Starkweather, J. and A.K. Moske, Multinomial logistic regression. 2011. University of North Texas.

Taboada, F.G., Chust, G., Santos Mocoroa, M., Aldanondo, N., Fontán, A., Cotano, U., Álvarez, P., Erauskin-Extramiana, M., Irigoien, X., Fernandes-Salvador, J.A., Boyra, G., Uriarte, A., Ibaibarriaga, L., 2024. Shrinking body size of European anchovy in the Bay of Biscay. Global Change Biology 30, e17047. https://doi.org/10.1111/gcb.17047

Tirelli, V., Borme, D., Tulli, F., Cigar, M., Fonda Umani, S., Brandt, S.B., 2006. Energy density of anchovy Engraulis encrasicolus L. in the Adriatic Sea. Journal of Fish Biology 68, 982–989. https://doi.org/10.1111/j.0022-1112.2006.00987.x

Tocher, D.R., 2003. Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish. Reviews in Fisheries Science 11, 107–184. https://doi.org/10.1080/713610925

Travers-Trolet Morgane, Verin Yves (2014) CAMANOC cruise, RV Thalassa, https://doi.org/10.17600/14001900

Van Dijk, P.L.M., Hardewig, I., HÖlker, F., 2005. Energy reserves during food deprivation and compensatory growth in juvenile roach: the importance of season and temperature. Journal of Fish Biology 66, 167–181. https://doi.org/10.1111/j.0022-1112.2005.00590.x

Véron, M., Duhamel, E., Bertignac, M., Pawlowski, L., Huret, M., 2020. Major changes in sardine growth and body condition in the Bay of Biscay between 2003 and 2016: Temporal trends and drivers. Progress in Oceanography 182, 102274. https://doi.org/10.1016/j.pocean.2020.102274

Vila-Belmonte, M., Bou, R., Lloret, E., Lloret, J., 2024. Fatty acid content and profile of round sardinella (Sardinella aurita), an expanding thermophilic species in the NW Mediterranean. Mediterr. Mar. Sci. 25, 300–310. https://doi.org/10.12681/mms.35917

Wuenschel, M.J., Bean, K.A., Rajaniemi, T., Oliveira, K., 2024. Variation in energy density of northwest Atlantic forage species: Ontogenetic, seasonal, annual, and spatial patterns. Marine and Coastal Fisheries 16, e10287. https://doi.org/10.1002/mcf2.10287

Wuenschel, M.J., Jugovich, A.R., Hare, J.A., 2006. Estimating the Energy Density of Fish: The Importance of Ontogeny. Transactions of the American Fisheries Society 135, 379–385. https://doi.org/10.1577/T04-233.1

Appendix A. *Calculation of lipid and protein contributions to the variation in fish mass*

The contribution of lipid and protein, in both mass and energy, to energy mobilisation has been estimated as the mass variation of each of the two components in relation to the variation in total body mass. As we did not follow individuals through time like in experimental studies, we had to assume that the patterns of variation between the body components explored in our study based on our field sampling is representative of the patterns of variation of these components for an individual through time.

The starting point is thus the segmented regressions between the lipid or protein content and water content and its associated slope (equation 1A), which we had to transform in order to obtain a contribution in absolute mass of lipid or protein to the total body mass variation (equation 8A).

$$\frac{d\left(\frac{W_C}{W_T}\right)}{d\left(\frac{W_W}{W_T}\right)}=a_C \qquad (1A)$$

With W_c the component (lipid or protein) mass, W_W the water mass, W_T the fish body mass and *a* the component slope of the segmented regression.

Using $\left(\frac{u}{v}\right)'=\frac{u'v-uv'}{v^2}$ we can transform equation (1A) into equation (2A)

$$\frac{\frac{dW_C * W_T - W_C * dW_T}{W_T^2}}{\frac{dW_W * W_T - W_W * dW_T}{W_T^2}}=a_C \qquad (2A)$$

This can be simplified as:

$$\frac{dW_C * W_T - W_C * dW_T}{dW_W * W_T - W_W * dW_T}=a_C \qquad (3A)$$

Then, as we demonstrated (see our result section) that water mass does not vary, $dW_w = 0$:

$$dW_C * W_T - W_C * dW_T = -a_C * W_W * dW_T \qquad (4A)$$

Dividing both terms by dW_T we obtain:

$$\frac{dW_C * W_T}{dW_T} - W_C = -a_C * W_W \qquad (5A)$$

Or:

$$\frac{dW_C * W_T}{dW_T} = -a_C * W_W + W_C \tag{6A}$$

Dividing both terms by W_T we obtain:

$$\frac{dW_C}{dW_T} = \frac{-a_C * W_W + W_C}{W_T} \tag{7A}$$

Finally, simplifying W_W/W_T and W_C/W_T we obtain:

$$Contrib_C = \frac{dW_C}{dW_T} = -a_C * \%W + \%C \tag{8A}$$

With $Contrib_C$ the mass contribution of the component C to energy mobilisation, $\%W$ the water content and $\%C$ the component (lipid or protein) content determined from the mean of all individual data on considered interval. Since the contributions are based on independent regressions, we do not obtain contributions somming to 1. Thus we normalised protein and lipid contribution.

Finally, to consider the energy contribution of each component, we had to weight the mass contributions by the energy density of both components (equation 10A).

$$Contrib_{LEnergy} = \frac{Contrib_{Lnorm} * 38.5}{Contrib_{Lnorm} * 38.5 + Contrib_{Pnorm} * 23.6} \tag{9A}$$

With $Contrib_{LEnergy}$ the lipid energy contribution to energy mobilisation, $Contrib_{Lnorm}$ and $Contrib_{Pnorm}$ the normalized lipid and protein, respectively, masses contribution to energy mobilisation. Protein energy contribution $Contrib_{PEnergy} = 1 Contrib_{LEnergy}$.

To calculate the uncertainties associated to both mass and energy contribution to energy mobilisation, we calculated mass and energy contribution (equations 8A to 10A) by taking the minimum and maximum value of slope $a_{Cmin} = a_C - standard\ error$ and $a_{Cmax} = a_C + standard\ error$ for each component.

Appendix B. *Segmented regression equations*

Table A1: Slope coefficients and associated standard error (SE) and confidence interval at 95% (IC 95%) of observed proportions of proteins and lipids in sardine and anchovy body composition, as a function of water content for each condition state determined by the segmented regression analysis.

		Anchovy			**Sardine**		
	State	*Coefficient*	*SE*	*IC 95 %*	*Coefficient*	*SE*	*IC 95 %*
Water ~ Lipids	Good	-1.38	±0.11	[-1.59 ; -1.18]	-1.43	±0.12	[-1.66 ; -1.20]
	Intermediate	-0.64	±0.09	[-0.82 ; -0.46]	-0.78	±0.10	[-0.97 ; -0.59]
	Poor	-0.09	±0.31	[-0.71 ; 0.53]	-0.16	±0.18	[-0.53 ; 0.20]
Water ~ Proteins	Good	0.23	±0.09	[0.05 ; 0.42]	0.21	±0.06	[0.09 ; 0.33]
	Intermediate	-0.32	±0.06	[-0.43 ; -0.21]	-0.47	±0.11	[-0.69 ; -0.26]
	Poor	-1.36	±0.51	[-2.36 ; -0.34]	-0.87	±0.21	[-1.29 ; -0.45]

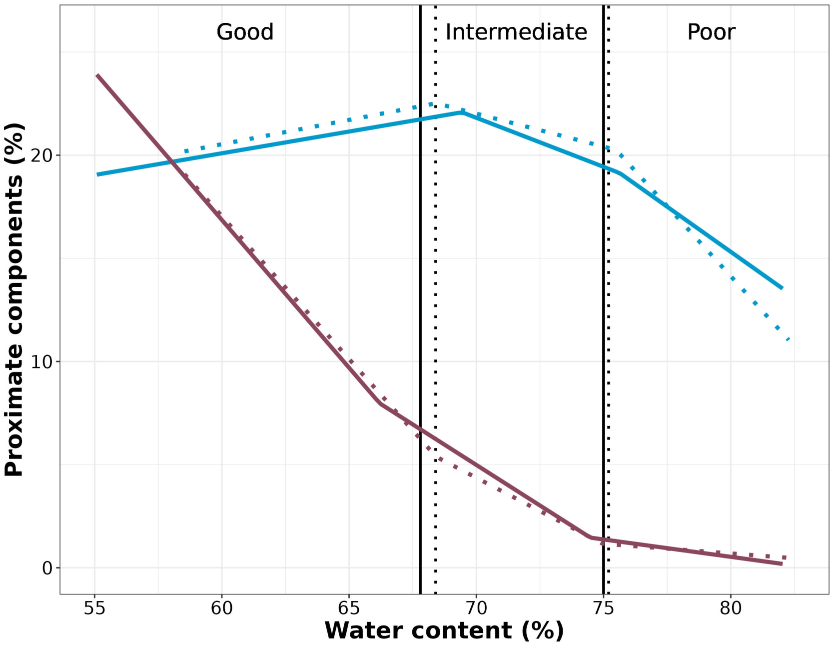

Figure A1: Comparison of segmented regressions of lipids (purple lines) and proteins (blue lines) versus water content for sardine (solid lines) and anchovy (dotted lines), across different condition states. The vertical lines represent the mean values of the breakpoints identified from proteins and lipids segmented regressions for sardine (solid lines) and anchovy (dotted lines).

Appendix C. *Length, season, reproductive status and region versus condition state*

Table A2. Multinomial logistic regression table showing the odd ratio and 95% confidence interval of explanatory variables associated with condition states for sardine (reference group = intermediate state).

SARDINE	**Good state**	**Poor state**
Season		
Winter	1	1
Spring	**5.09.10^{-3} [5.09.10^{-3} ; 5.10.10^{-3}] *****	**1.02.10^{-1} [1.47.10^{-2}; 7.10.10^{-2}] ***
Summer	**4.09.10^{5} [1.02.10^{5} ; 1 62.10^{6}]*****	0.45 [0.05 ; 3.70]
Autumn	**4.55.10^{5} [1.45.10^{5} ; 1 42.10^{6}]*****	0.26 [0.04 ; 1.86]
Region		
Bay of Biscay	1	1
English Channel	3.13 [0.95 ; 10.4]	0.62 [0.13 ; 2.93]
Length	**1.37 [1.15 ; 1.63]*****	**1.57 [1.25 ; 1.97]*****
P-value: * <0.05,** <0.01, *** =<0.001		

Table A3. Multinomial logistic regression table showing the odd ratio and 95% confidence interval of explanatory variables associated with condition states for anchovy (reference group = intermediate state).

ANCHOVY	**Good state**	**Poor state**
Season		
Winter	1	1
Spring	**6.68.10^{-4} [6.68.10^{-4} ; 7.00.10^{-4}]*****	**3.58.10^{-14} [5.08.10^{-15} ; 2.54.10^{-13}]*****
Summer	**6.91.10^{1} [2.27 ; 2.10.10^{3}]***	**4.33.10-^{13} [8.39.10^{-14} ; 1.88.10$^{-1é}$]*****
Autumn	**9.75.10^{1} [4.76 ; 2.00.10^{3}]****	**1.36.10^{-6} [2.18.10^{-8} ; 1.00.10^{-4}]*****
Region		
Bay of Biscay	1	1
English Channel	1.06.10^{1} [0.97 ; 1.15.10^{2}]	**6.33.10^{-6} [6.32.10^{-6} ; 6.34.10^{-6}]*****
Length	**1.72 [1.03 ; 2.87]***	1.70 [0.89 ; 3.22]
Reproductive status		
No	1	1
Yes	**1.98.10^{-8} [1.98.10^{-8} ; 1.98.10^{-8}]*****	**8.16.10^{7} [8.56.10^{6} ; 7.77.10^{8}]*****

P-value: * <0.05,** <0.01, *** =<0.001

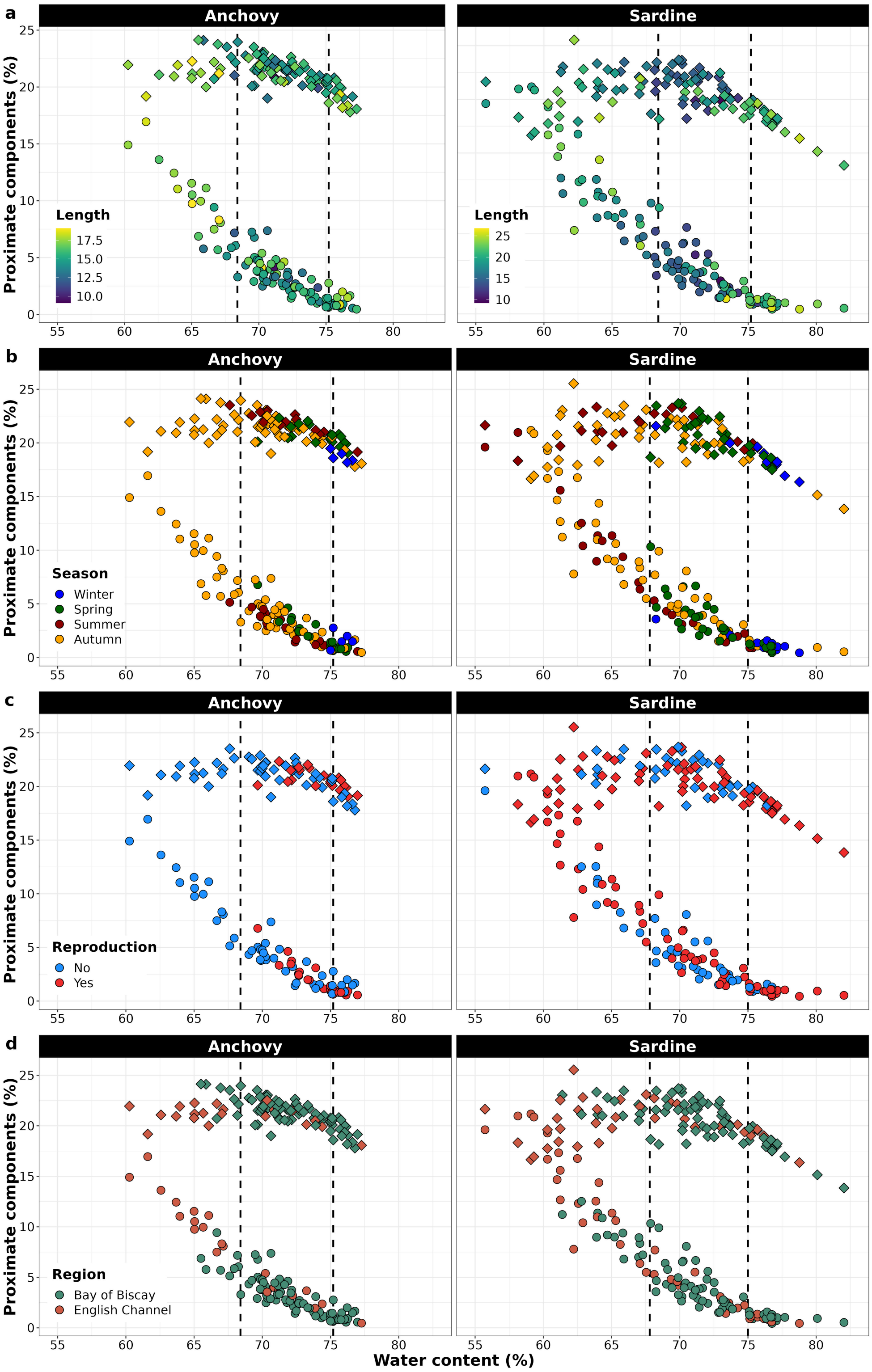

Figure A2: Observed content of proteins (diamond) and lipids (circle) in sardine and anchovy body composition, as a function of water content with colors representing (a) the length, (b) the season, (c) the reproductive status and (d) the region. The dotted vertical lines represent the mean values of the breakpoints identified from proteins and lipids segmented regressions, allowing to separate the three condition states.

Appendix D. *Regression equation characteristics of proximate components versus water for each condition state*

Table A4: Regression equation and associated standard error (±SE) and determination coefficient (R^2) of observed mass of proteins, lipids and ash in sardine and anchovy body composition, as a function of water mass for all individuals (all) and for each condition state (good, intermediate, poor)

		Anchovy	**Sardine**
log(Proteins) ~ log(Water)	All	-1.26 (±0.07) + 1.01(±0.03) x, R^2 = **0.93**	-1.01 (±0.07) + 0.93(±0.02) x, R^2 = **0.95**
	Good	-1.05 (±0.09) + 0.98(±0.03) x, R^2 = **0.98**	-0.90 (±0.12) + 0.94(±0.03) x, R^2 = **0.96**
	Intermediate	-1.24 (±0.06) + 1.01(±0.02) x, R^2 = **0.97**	-1.10 (±0.05) + 0.96(±0.02) x, R^2 = **0.98**
	Poor	-1.14 (±0.15) + 0.92(±0.05) x, R^2 = **0.95**	-1.11 (±0.17) + 0.91(±0.04) x, R^2 = **0.95**
log(Lipids) ~ log(Water)	All	-4.58 (±0.64) + 1.51(±0.23) x, R^2 = **0.29**	-2.39 (±0.53) + 0.85(±0.15) x, R^2 = **0.21**
	Good	-3.83 (±0.44) + 1.62(±0.15) x, R^2 = **0.85**	-3.00 (±0.54) + 1.35(±0.15) x, R^2 = **0.71**
	Intermediate	-3.25 (±0.56) + 0.98(±0.21) x, R^2 = **0.23**	-2.56 (±0.31) + 0.86(±0.10) x, R^2 = **0.59**
	Poor	-6.93 (±1.32) + 1.92(±0.46) x, R^2 = **0.53**	-3.67 (±0.59) + 0.81(±0.15) x, R^2 = **0.53**
log(Ash) ~ log(Water)	All	-2.80 (±0.09) + 0.92(±0.03) x, R^2 = **0.89**	-3.08 (±0.07) + 1.04(±0.02) x, R^2 = **0.96**
	Good	-2.44 (±0.15) + 0.81(±0.05) x, R^2 = **0.93**	-2.83 (±0.16) + 0.98(±0.04) x, R^2 = **0.94**
	Intermediate	-2.84 (±0.12) + 0.93(±0.04) x, R^2 = **0.87**	-3.11 (±0.09) + 1.05(±0.03) x, R^2 = **0.96**
	Poor	-3.42 (±0.30) + 1.12(±0.10) x, R^2 = **0.89**	-2.98 (±0.20) + 1.01(±0.05) x, R^2 = **0.94**

Appendix E. *Lipid and protein contributions to the variation in fish mass*

Table A5: Slope coefficients in segmented regression of protein and lipids and their relative contribution as energy source (calculated using equations 2 and 3) in anchovy across the condition states. a_L: lipid slope and associated standard error, a_P: protein slope and associated standard error C_L mass: lipid mass contribution, C_P mass: protein mass contribution, C_L energy: lipid energy contribution, C_P energy: protein energy contribution.

	Anchovy					
	Lipids			***Proteins***		
	a_L	C_L *mass*	C_L *energy*	a_P	C_P *mass*	C_L *energy*
Good	1.38 ± 0.11	94%	96%	0.23 ± 0.09	6%	4%
Intermediate	-0.64 ± 0.09	53%	65%	-0.32 ± 0.06	47%	35%
Poor	-0.09 ± 0.31	6%	10%	-1.36 ± 0.51	94%	90%

Table A6: Slope coefficients in segmented regression of protein and lipids and their relative contribution as energy source (calculated using equations 2 and 3) in sardine across the condition states. a_L: lipid slope and associated standard error, a_P: protein slope and associated standard error C_L mass: lipid mass contribution, C_P mass: protein mass contribution, C_L energy: lipid energy contribution, C_P energy: protein energy contribution.

	Sardine					
	Lipids			***Proteins***		
	a_L	C_L *mass*	C_L *energy*	a_P	C_p *mass*	C_L *energy*
Good	-1.43 ± 0.12	93%	96%	0.21 ± 0.06	7%	4%
Intermediate	-0.78 ± 0.10	52%	64%	-0.47 ± 0.11	48%	36%
Poor	-0.16 ± 0.18	14%	21%	-0.87 ± 0.21	86%	79%

Appendix F. *Difference between simple and segmented regression for water~energy density regression*

Table A7: Regression equation and associated standard error (±SE) and determination coefficient (R^2) of observed energy density as a function of percent water for all individuals (all) and for each condition state (good, intermediate, poor)

		Anchovy	**Sardine**
Energy density ~ water	Simple	28.7 (±0.39) – 31.2 (±0.53) x, R^2 = **0.87**	34.9 (±0.19) – 39.8 (±0.26) x, R^2 = **0.96**
	Strong	38.9 (±2.46) – 46.3 (±3.74) x, R^2 = **0.75**	37.3 (±0.73) – 43.5 (±1.15) x, R^2 = **0.83**
	Intermediate	32.2 (±0.78) – 36.2 (±1.07) x, R^2 = **0.77**	33.1 (±0.55) – 37.5 (±0.77) x, R^2 = **0.83**
	Poor	19.2 (±0.88) – 18.8 (±1.14) x, R^2 = **0.71**	26.5 (±0.99) – 28.6 (±1.29) x, R^2 = **0.71**