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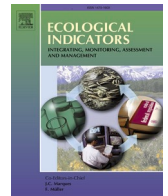
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## Fish morphometric body condition indices reflect energy reserves but other physiological processes matter

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### ABSTRACT

Morphometric indices of body condition are assumed to reflect an animal's health and ultimately its fitness, but their physiological significance remains a matter of debate. These indices are indeed usually considered as proxies of energy reserves, ignoring other physiological processes involved in animal health such as nutritional, immune and hormonal states. Given the wide variety of ecological processes investigated through morphometric body condition indices in marine sciences, there is a clear need to determine whether morphometric indices reflect primarily individuals' energy reserves or their broader integrated physiological status. To address this issue, we used morphometric and physiological data (cortisol level, oxidative stress, digestive enzymes activity, and both fatty acids percentage and total amount) collected in three stocks of the European sardine (*Sardina pilchardus*) presenting contrasted patterns of growth and body condition. We found that morphometric body condition indices are indeed mainly and consistently linked to a proxy of the amount of lipid reserves (i.e., fatty acids amount), but also significantly to quality of lipid reserves (i.e., fatty acids percentage) and fish chronic stress (scale cortisol levels). We found no significant relationship between morphometric indices of body condition and both oxidative stress proxies and variables measuring digestive enzymes activity. Our study confirmed that morphometric body condition indices primarily reflect the variance in individuals' lipid reserves and to a lesser extent the actual composition of these reserves (linked to differences in fish diet) and scale cortisol levels (indicating fish metabolism and/or their chronic stress levels). Therefore, some non-energetic aspects should be considered when studying individuals' responses to environmental changes and other key physiological processes (oxidative stress proxies, activity of digestive enzymes) should be investigated directly to support scientific-based decision-making in the context of climate change.

### 1. Introduction

Body condition is a central concept in ecology, evolution, and conservation. It is commonly defined as a proxy of an individual's health and vigour reflecting individuals' ability to allocate energy to life history traits which ultimately should constrain their fitness (Peig and Green, 2009; Wilder et al., 2016). Yet, the usefulness of such a broad definition has been questioned because individuals' 'health' and 'vigour' are vague concepts whose estimation might be done through various means (Milot et al., 2014). In particular, numerous studies have focussed on the relationship between individuals' body condition and a proxy of their energy reserves, largely downplaying its links to other physiological

aspects (e.g., nutritional, immune, or hormonal status, Clancey and Byers, 2014).

Despite this lack of clarity, morphometric indices remain highly popular among the wide variety of body condition indices (e.g., biochemical, histological) as they are inexpensive, non-destructive, can be rapidly collected and easily calculated from historical datasets (Jakob et al., 1996; Lloret et al., 2013). Thus, most of the studies investigating individuals' body condition are based on morphometric indices and rely on the assumption that heavier individuals of a given length are in better body condition because they might have greater energy reserves (Frauendorf et al., 2021; Oskarsson, 2008). However, there is a clear need to determine whether morphometric indices do indeed reflect

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primarily individuals' energy reserves or also capture their broader integrated physiological status (Wilson and Nussey, 2010).

In marine sciences, almost all the studies focussing on attempting to validate the use of morphometric body condition indices only tested link with various proxies of energy reserves such as muscle (Davidson and Marshall, 2010) or liver (Stoltenberg et al., 2021) lipid content, mesenteric fat (McPherson et al., 2011), and whole fish energy density (the amount of energy per unit of mass, Schloesser and Fabrizio, 2017; Campanini et al., 2021). Although variations were observed in the strength of these relationships, with some being only weakly significant (Campanini et al., 2021), all these studies agree that morphometric indices are indeed related to the proxies of energy reserves considered. They also rise the importance of considering the reserve composition (e.g., content in fatty acids) when studying individual energetic reserves (Stoltenberg et al., 2021). More rarely, a few studies have investigated the links between individual morphometric indices and fish physiological states other than the energetic one, such as chronic stress (Leal et al., 2011; Midwood et al., 2014), parasite load (Caballero-Huertas et al., 2023; Frigola-Tepe et al., 2022; Pennino et al., 2020; Timi and Poulin, 2020) or oxidative stress (Clotfelter et al., 2013). Overall, there are significant variations in the magnitude of these relationships as few studies found significant negative relationships between morphometric indices and parasite load (Caballero-Huertas et al., 2023; Timi and Poulin, 2020), chronic stress (Leal et al., 2011), and oxidative stress (Clotfelter et al., 2013) while others studies found no relationship (e.g., Midwood et al., 2014).

These studies showed that non-energetic aspects can be additional or strongly linked to individuals' life history traits and emphasized that analysing the physiological mechanisms involved in individual health would help to evaluate the response of organisms and populations to ecosystem alterations. More integrative studies would therefore provide a better understanding of the body condition indices meaning when they are used for instance to (i) provide estimates representing fish resources status (Haberle et al., 2023) and fish natural mortality (Regular et al., 2022), (ii) estimate and predict fish habitat quality (Champion et al., 2020), and (iii) improve forecasts to facilitate selective harvesting of good-quality and nutritionally valuable fish (Bolin et al., 2021). Furthermore, previous studies investigated these relationships over a limited timespan or area and generally did not test whether it would hold in other contexts. Thus, studying concomitantly a wide variety of physiological variables and across multiple sampling location seems therefore crucial to shed light on the meaning of fish health estimated with fish body condition morphometric indices and to determine the degree to which these relationships are consistent across multiple sampling locations.

To address these issues, we used morphometric and physiological data collected in the European sardine (*Sardina pilchardus*, hereafter sardine) from three areas in which the stock status of sardine clearly differed. In the Bay of Biscay and the Gulf of Lions, the body condition, growth rate, and size-at-ages of sardines have decreased substantially since the mid-2000's (Boëns et al., 2021; Doray et al., 2018; Saraux et al., 2019; Véron et al., 2020a), and the high mortality of older individuals led to a fall in populations' biomass and hence exploitable stocks. The alteration in food resources available to sardines and involved in physiological processes are likely part of the explanation for such trends in these two areas (Menu et al., 2023). Conversely, the much smaller sardine stock of the English Channel (ICES, 2022) does not display such signs of morphometric and biomass declines. Sardines in this area have generally a greater size than those of the Bay of Biscay, likely resulting from a higher zooplankton productivity in the English Channel or/and a selection pressure towards faster growing and faster reserve building individuals in cooler environment (Gatti et al., 2018). In addition, we selected physiological variables directly influencing fish metabolism, oxidative activities, and the amount/composition of lipid reserves which ultimately should reflect fish health.

To assess fish chronic stress, we measured the scale cortisol

concentration, the hormone involved in the primary stress response in fish. When individuals are exposed to prolonged sub-optimal environmental conditions, the sustained production of cortisol (i.e., chronic stress levels) can ultimately lead to substantial declines in growth, survival, and reproduction. It has previously been shown that cortisol can reflect individuals' overall stress history (Aerts et al., 2015; Laberge et al., 2019) and be related to lake pollution (Carbajal et al., 2019a; Carbajal et al., 2019b) or broad-scale environmental factors (Lebigre et al., 2022). In addition, to estimate fish metabolism, intestine enzymes were sampled to quantify the activity of several key enzymes enabling energy storage and reflect individuals' metabolic activity over a timespan of 2–3 months (Cahu and Zambonino-Infante, 1994; Lemieux et al., 1999). We also measured oxidative stress (imbalance between pro-oxidants and anti-oxidant defences) as a proxy of metabolic constraints. Oxidative stress results from metabolic activity and the over-production of reactive oxygen species that damage lipids (peroxidation), proteins (carbonylation), and DNA molecules (8-oxo-2'-deoxyguanosine). To evaluate individual total reserve, we summed the content of all neutral lipid fatty acids and used this variable as a proxy of the individual total lipid reserves. To estimate individual reserves quality, we focussed on five polyunsaturated fatty acids that are strongly associated with changes in fish life history traits, especially fish growth and reproduction (Parrish, 2009; Sargent et al., 1997): EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), ARA (arachidonic acid), LIN (linoleic acid), and ALA (alpha-linolenic).

Through this case study using individuals coming from three contrasted areas reflecting a large range of population states and physiological status, we aim at estimating the relative importance of both lipid reserves and physiological constraints on body condition morphometric indices. We hypothesized that morphometric body condition indices reflect the multifaceted nature and encompass a broad range of health states and are therefore not limited to the amount of lipid reserves. We thus provide a perspective to clarify the link between popular morphometric body condition indices and proxies of the individual physiological status.

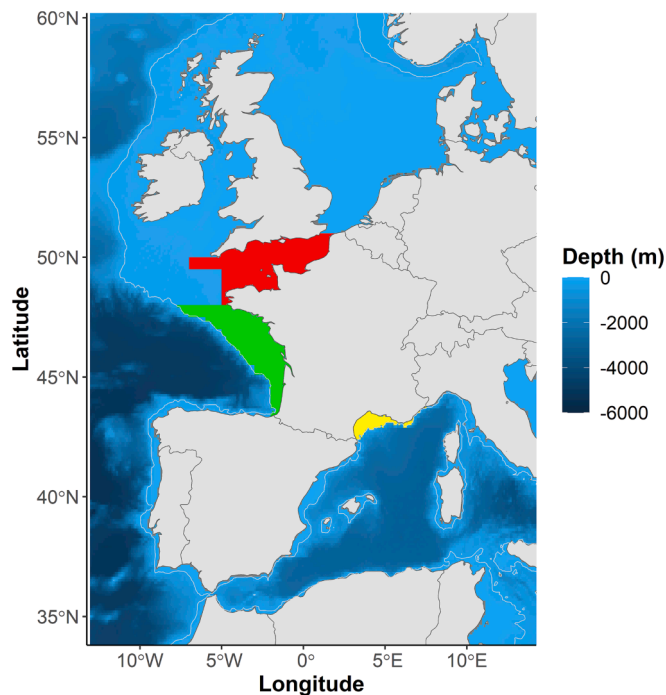
## 2. Methods

### 2.1. Study areas and sardine sampling

Sardines were sampled in three different ICES subareas around French coastline in September and October 2020 during Ifremer scientific surveys EVHOE (Bay of Biscay,  $n = 76$ , Doray et al., 2000), PELMED (Gulf of Lions,  $n = 110$ , Bourdeix and Hattab, 1985) and CGFS (English Channel,  $n = 100$ , Giraldo et al., (1988), Fig. 1). Within each area, samples were collected at 9 to 15 stations across the spatial range covered by each survey. Sardine total length, weight, and age were measured. We determined individuals age based on the reading of otolith's increments that reflect growth (using the same method as described in Bertrand et al. (2022)). Sex and sexual maturity stages were determined by macroscopic analyses of the gonads and categorised as immature, developing, pre-spawning, spawning, partial post-spawning, and post-spawning (Véron et al., 2020b). This allowed us to determine if similar sex-ratio and reproductive cycle period among areas were found to prevent from confounding effect of sex and maturity. Sex ratios were close to 1 (number of male/female = 1.41 for the Bay of Biscay, 1.15 for the Gulf of Lions and 0.76 for the English Channel) and most of mature sardines were at the same reproductive stage (developing one) whatever the area. Different fish tissues such as scale, white muscle, and blood were also collected on-board and immediately stored at  $-80\text{ }^{\circ}\text{C}$  until processed in the laboratory (details are given below).

### 2.2. Body condition index

Because of the European sardine's allometric growth (Brosset et al., 2015), we used the Le Cren' index as a morphometric body condition



**Fig. 1.** Map of the three contrasted areas on continental shelf considered for sampling. Red area is the English Channel, green one the Bay of Biscay and yellow one the Gulf of Lions. The white line represents the 200 isobaths.

measurement ( $K_n$ , Le Cren 1951). This index is calculated as follow:

$$K_n = \frac{W}{W_r}$$

with  $W$  the observed weight (in g) of an individual, and  $W_r$  the predicted weight of an individual of a given length  $L$  calculated with ( $W_r = \alpha L^\beta$ ; with  $\alpha$  a theoretical intercept, and  $\beta$  the shape parameter). By definition, the values of  $K_n$  are center on one (population mean), normally distributed and the higher the value the better the body condition.

### 2.3. Physiological biomarkers

We selected physiological variables reflecting different major physiological processes linked to fish metabolism (digestive enzymes activity, cortisol level), oxidative stress (red-ox balance), and the amount and composition of lipid reserves (fatty acids total content and percentages) which altogether should help assessing fish health (Brosset et al., 2021; Schull et al., 2023).

### 2.4. Reserve fatty acids estimation

Reserve fatty acids (hereafter FA) were used as an indicator of individuals' nutritional status. Measurements were carried out as described in details in Mathieu-Resuge et al., (2023). In clupeid species, lipids reserves are primarily stored in muscle which represent the main storage tissue (Lloret et al., 2013). Hence, a piece of the white muscle was collected during the surveys on freshly fished individuals and approximately 10 mg of dry white muscle were homogenized into powder and used for lipid extraction after different extraction with solvents (ca. 6 mL of  $\text{CHCl}_3$ :MeOH (2:1, v/v)). Neutral lipids (NL, which constitute lipid reserve) and polar lipids (PL, which constitute cell membranes) were subsequently separated by solid phase chromatography. We then focused on the neutral lipids fraction considered as the main energy store (although both fractions are highly correlated). NL fractions were evaporated to dryness, and after hydrolysis in 1 mL of KOH-MeOH (0.5 M) for 30 min at 80 °C, samples were transesterified

with 1.6 mL of MeOH:H<sub>2</sub>SO<sub>4</sub> (3.4 %; v/v) for 10 min at 100 °C. FA methyl esters (FAME) formed were recovered in hexane to be analysed through a gas chromatography coupled with a flame ionisation detector (GC-FID, Thermo Scientific). FAME were identified by comparing their retention time with references from three commercial mixtures (37 components FAME, PUFA1 and PUFA3, Sigma), and in-house standard mixtures from marine bivalves, fish and microalgae GC-MS certified. We quantified FAME content in white muscle based on the internal standard recovery. FA contents are expressed in mass fraction of wet weight ( $\text{mg g}^{-1}$  ww) and in percentage (%) of total FA. We summed the content of all neutral lipid FA to estimate the total lipid reserves of each sardine (abbreviated FA<sub>total</sub>). We also evaluated lipid reserves quality focusing on five polyunsaturated omega-3 and -6 FA (EPA, 20:5n-3; DHA 22:6n-3; ARA, 20:4n-6; LIN, 18:2n-6; and ALA, 18:3n-3) in order to limit the number of explanatory variables.

### 2.5. Cortisol measurements

We measured individuals' scale cortisol concentration to estimate individuals' overall stress level. Scales were collected from both sides of each sardine, and once in the laboratory they were washed and vortexed three times (2.5 min; 96% isopropanol) to remove external cortisol originating from the mucus. Residual solvent traces were evaporated under nitrogen flux and samples frozen at -80 °C. To ensure the scales were dry, they were then lyophilised for 12 h and ground to a powder using a ball mill (MM400, Retsch GmbH, Germany). Cortisol content was extracted from ~50 mg of dry scale powder by incubation in 1.5 mL methanol (MeOH) on a 30 °C rocking shaker for 18 h. After centrifugation at 9,500 g for 10 min, the supernatant was evaporated using a rotary evaporator and reconstituted with 0.2 mL of EIA buffer from a Cortisol assay kit (Neogen® Corporation Europe, Ayr, UK). Cortisol concentrations were determined in 50  $\mu\text{L}$  of extracted cortisol using a competitive EIA kit (Neogen® Corporation Europe, Ayr, UK) according to a previously published protocol (Carbajal et al., 2019a; Carbajal et al., 2019b).

### 2.6. Oxidative stress

When sardines arrived onboard, we collected 0.2 mL of blood from the caudal vein using 75  $\mu\text{L}$  heparinised tubes. After centrifugation (3000g for 10 min) within 15 min of collection, plasma was separated and kept frozen at -80 °C until further analyses. Using these plasma samples, we evaluated total oxidative damages (d-ROMs test, Diacron International, Grosseto, Italy) and total antioxidant defences (OXY adsorbent test, Diacron International, Grosseto, Italy) as global indices of the oxidative balance (Birnie-Gauvin et al., 2017; Costantini, 2008). We used 8  $\mu\text{L}$  of plasma for the d-ROMs test to measure reactive oxygen metabolites (ROMs) in plasma as a marker of total oxidative damage (expressed as mg of H<sub>2</sub>O<sub>2</sub> equivalent/dL). We used 4  $\mu\text{L}$  of plasma (diluted 1:100) for the OXY adsorbent test measures of antioxidant capacity which corresponds to the ability of plasma to buffer oxidation through hydroperoxide acid expressed in  $\mu\text{molHCL.mL}^{-1}$ . The absorbance of the samples was read at 555 nm. All sample were run in duplicates. Sardines collected in the English Channel for which it was not possible to collect plasma samples were not considered for this measurement.

### 2.7. Enzymatic assays

Just after morphometric measurements and blood sampling, we collected fish guts and stored them at -80 °C until laboratory analyses. Once unfrozen, the mucosa of the digestive tract was collected by scrapping the intestine and was homogenized in cold distilled water with a polytron at maximum speed for 30 s (Crane et al., 1979) to purify brush border membranes (BBM). The BBM enzymes alkaline phosphatase (AP), leucine aminopeptidase (AN),  $\gamma$ -glutamyl transpeptidase

(GGT), trypsin, and catalase were assayed according to Bessey et al., (1946), Meister et al., (1981), Holm et al., (1988), Maroux et al., (1973) and Lück, (1965), respectively. Total soluble proteins were determined according to Bradford, (1976) using bovine serum albumin as a standard (Sigma Chemical Co., St. Louis, USA). All enzyme determinations were based on kinetic measures carried out using a spectrophotometer at 37 °C (Thermo scientific Evolution 201). Enzymatic activities were expressed in units of specific activity, i.e., in micromoles of substrate hydrolysed per minute per milligram of total protein.

## 2.8. Statistical analyses

To investigate sardine physiological state between areas and estimate the broad relationships between all these physiological variables, we used a multivariate statistical approach relying on a Principal Component Analysis (PCA). We summarized multiple variables using the shared correlation structure to derive principal components. Each variable was centered and scaled. We ran a PCA for all areas simultaneously with individual measures of cortisol, oxidative stress, four digestive enzymes, total fatty acids content and five fatty acids percentages. In order to run PCA with missing data (due to the lack of oxidative stress data for sardines coming from the English Channel), we used the 'missMDA' package (Josse and Husson, 2016) using the regularized iterative PCA algorithm recommended to avoid overfitting. In this package, the number of dimensions for the analysis was estimated by cross-validation with `estim_ncpPCA` function; missing values were then estimated with the use of the `imputePCA` algorithm. These are iterative methods that impute missing values using the fitted PCA matrices considering links between variables to improve the estimation.

To study the statistical effect of individual explanatory variables on morphometric body condition index, we first tested for multicollinearity between physiological explanatory variables relying on the Variance Inflation Factor (VIF). Multicollinearity across all these variables was low and all VIFs remained below 5 (Zuur et al., 2007). We used a generalized linear modelling (GLM) approach (with a Gaussian distribution and a log link function) to estimate the relationship between fish body condition morphometric index ( $K_n$ ) and physiological variables (continuous variables) in interaction with sardine age and sampling areas (categorical variables). The response variable was  $K_n$  and the explanatory variables were individuals' cortisol levels (Cortisol), total anti-oxidant defences (Oxy), total oxidative damages (d-ROM), total fatty acids percentage (EPA, DHA, ARA, ALA, LIN), and digestive enzyme activities (AP, AN, GGT, Trypsin, and Catalase) as well as sardine age (Age) and sampling area (Area). To determine the degree to which these relationships were consistent across space and ages, the full model contained interaction terms of the sampling locations and individuals' age with each of the physiological variables. We selected the variables for the optimal model using a backward-stepwise selection based on removing the lowest p-values of higher degree parameters (interactions) and the main effects (not involved in interaction terms). We also used the Akaike's information criterion (AIC) to confirm the improvement of the performance of the models. Same results were obtained with partial least square analyses which is also designed and robust for analysing many variables (Carrascal et al., 2009). All statistical analyses were performed with R v.4.2.2 (R Core Team 2013). Values are given as mean  $\pm$  SE, and statistical tests were considered significant at  $p < 0.05$ .

## 3. Results

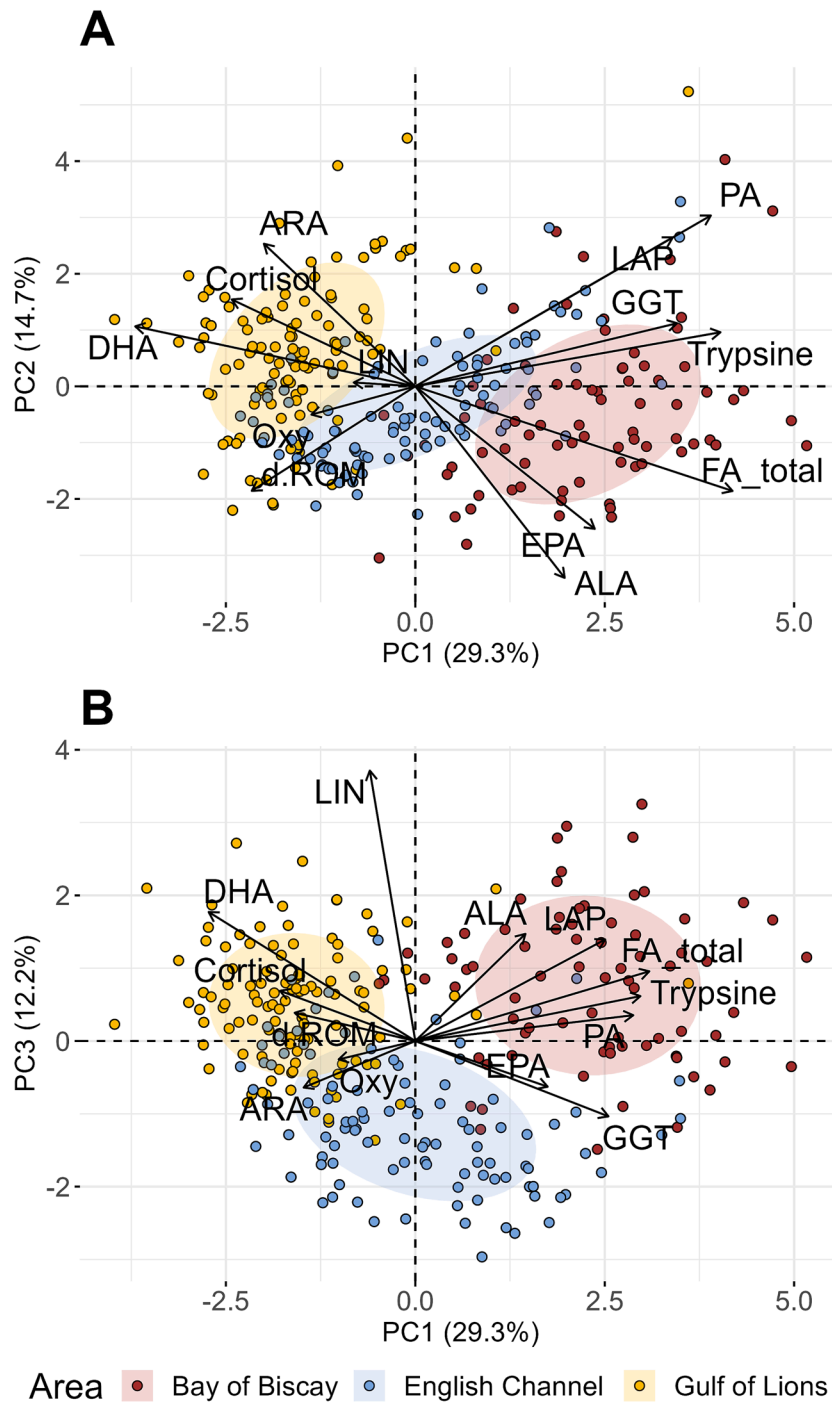
Overall, the first three axes of the PCA explained 56.2% of the total variation of the physiological parameters (Appendix, Fig. A.1). The first principal component was associated with increasing lipid reserves content and digestive enzymes activity and decreasing values of scale cortisol and DHA percentage; the second principal component was primarily positively related to PA and LAP enzymes and to ARA fatty acid

and negatively to both percentage of EPA and ALA fatty acids; the third principal component was primarily related positively to the percentage of a fatty acid: the linoleic acid (LIN) (Figs. 2 and A.2). Therefore, the same variables could strongly relate to two or more principal components (this could also be seen by the projection of the different physiological variables at angles close to 45° relative to the principal components) and a substantial amount of variation within each parameter was not explained by this analysis (PC1, PC2, and PC3 explaining 29.3%, 14.7% and 12.2% of the total variance, respectively). Nonetheless, the PCA separated the three areas (Bay of Biscay, Gulf of Lions, and English Channel) in terms of sardines' physiological status (Fig. 2). The three areas were separated on PC1, with Bay of Biscay sardines having positive values along this principal component, Gulf of Lions sardines negative ones, and English Channel sardines at intermediate values between sardines of the Bay of Biscay and those of the Gulf of Lions (Fig. 2). Thus, sardines sampled in the Bay of Biscay had lower values of cortisol, lower percentage of DHA but higher reserves content and digestive enzymes activity (and the contrary for Gulf of Lions sardines while English Channel sardines had intermediate values). Distinguishing areas was less clear using the second principal component (PC2), even if sardines coming from the Bay of Biscay seemed to have higher percentage of EPA and ALA, and lower percentage of ARA for fatty acids, and less LAP and PA enzyme activity and this is the opposite for Mediterranean sardines (Fig. 2). On the third axis, sardines from the English Channel had lower percentage of LIN fatty acids in contrast to the two other areas (Fig. 2).

The stepwise backward selection led to the selection of five variables in the final most parsimonious model linking the different physiological variables to  $K_n$  (Table 1). No interactions involving physiological variables with the sampling area or individuals' age were retained in the final model, meaning that the relationships between the variables selected and  $K_n$  were consistent across sampling areas and ages. We found that the total amount of lipid reserve in muscles (FA\_total) was positively related to  $K_n$  while cortisol concentrations, DHA, and ARA percentages were all negatively related to this morphometric body condition index (Table 1 and Fig. 3). Moreover,  $K_n$  values are different among areas (Table 1). Based on the magnitude and standard error of the parameter estimated, the total amount of FA appears to be the most important explanatory physiological variables, ahead of the DHA percentage, and finally both the ARA percentage and the cortisol concentration. Proxies of oxidative stress and variables describing digestive enzymes activity had no significant effect on  $K_n$  (Table 1).

## 4. Discussion

Our goal was to test the common assumption that morphometric condition indices reflect primarily (proxies of) individuals' energy reserves or also capture their broader integrated physiological status. This assumption is often tested using proxies of energy reserves (e.g., lipid content in reserve tissues, mesenteric fat, and whole body energy density), while ignoring other physiological process, preventing to estimate the degree to which morphometric indices can also be used as integrative measurements of fish health (encompassing nutritional, immune, and hormonal states). We sampled sardines in stocks with highly contrasted states (i.e., growth and body condition ( $K_n$ ) patterns, selective mortality), and the PCA clearly confirmed that there are major differences in sardine broad physiological status. Furthermore, we found that morphometric body condition indices are indeed mainly linked to fish lipid reserves (FA\_total), but that fish lipid reserve composition (i.e., quality, DHA, ARA) and scale cortisol levels were also related (negatively) to individuals' condition index. This supports the commonly assumed hypothesis that the nutritional stress is the main sources of individual morphometric condition indices variability (Lloret et al., 2013), but also shed light on the fact that these indices also reflect non-energetic aspects that should be considered when studying individuals' responses to environmental changes.



**Fig. 2.** Principal component analysis of the fish physiological measurements selected to describe fish health status. Panel A corresponds to the 2 first principal components. Panel B corresponds to the first and third principal components. Ellipses represent the space containing 50% of the individuals of each area.

Using sardines from three different areas enabled us to analyse individuals with contrasted physiological states and allowed us to determine whether these results are consistent and generalizable across sampling areas and ages. There were no significant interactions, clearly showing that the relationships that we found between the physiological parameters and  $K_n$  are strongly consistent across ages and in very different environmental conditions. Given the contrasted water temperature and primary productivity between the Gulf of Lions, the Bay of Biscay, and the English Channel (Menu et al., 2023), it is remarkable to find strong consistencies across such broad spatial scales. This is the first time this has been tested directly and indicates that the physiological processes that underpin variation in morphometric measurements of

sardine body condition are likely conserved regardless of sampling area. Other studies in the near future will have to confirm the strong relationships and patterns observed for other species and even larger area.

The proxy of sardine lipid reserves is positively linked to morphometric body condition indices. This indicates that individuals having more reserves had greater  $K_n$ , and that at least a part of the variance in individuals' weight reflects the variance in lipid reserves. This result supports existing literature reducing morphometric body condition indices to only various proxies of energy reserve content such as muscle (Davidson and Marshall, 2010) or liver (Stoltenberg et al., 2021) lipid content, mesenteric fat (McPherson et al., 2011), and whole fish energy density (Campanini et al., 2021; Schloesser and Fabrizio, 2017). Fish

**Table 1**

Stepwise backward selection of generalised linear models quantifying the effect of the physiological variables, areas and age on the sardine morphometric body condition index ( $K_n$ ). The most parsimonious model is represented in bold.

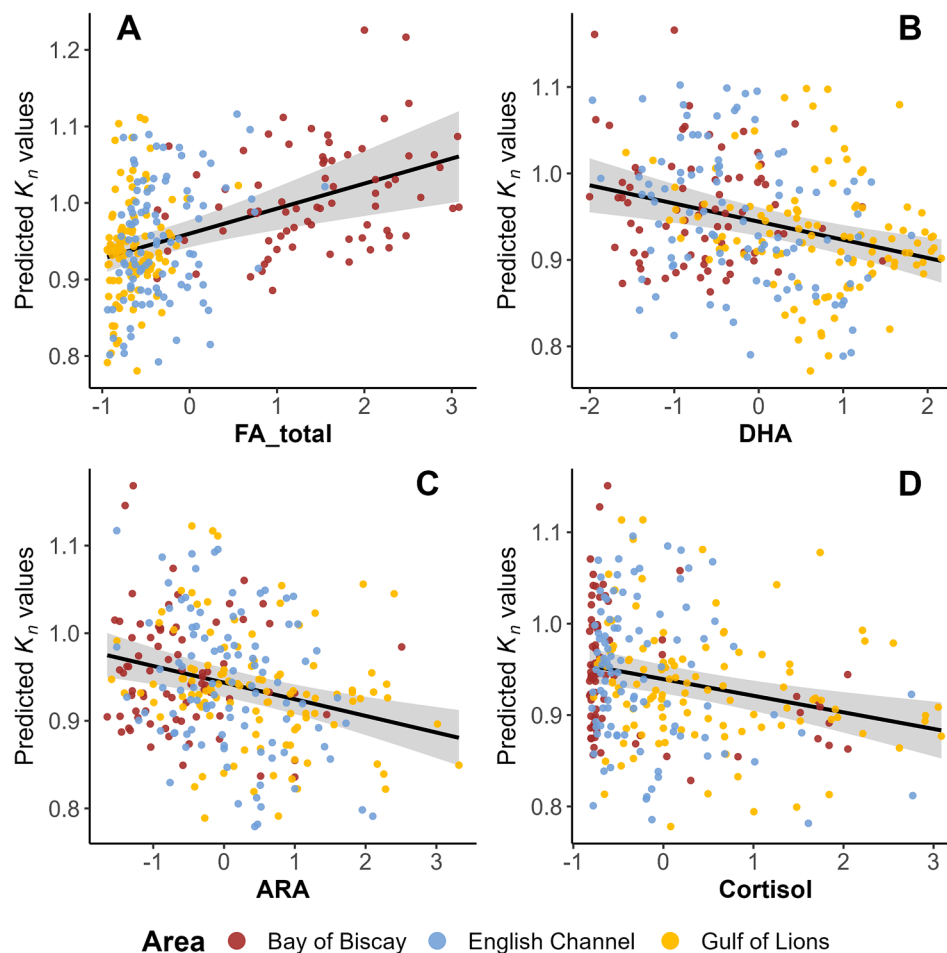
Response variable	Main effects	Dropped variables	Retained variables	Parameter estimate	t-value	P	AICc	
Sardine body condition	(FA_total + DHA + ARA + Cortisol + OXY + dROMs + EPA + ALA + LIN + GGT + LAP + PA + Trypsine)*Age*Area	All triple interactions				>0.05	-447.8	
	(FA_total + DHA + ARA + Cortisol + OXY + dROMs + EPA + ALA + LIN + GGT + LAP + PA + Trypsine)*Age+(FA_total + DHA + ARA + Cortisol + OXY + dROMs + EPA + ALA + LIN + GGT + LAP + PA + Trypsine)*Area	All pairwise interactions				>0.05	-580.5	
	FA_total + DHA + ARA + Cortisol + OXY + dROMs + EPA + ALA + LIN + GGT + LAP + PA + Trypsine + Age + Area	Age				0.341	-626.1	
	FA_total + DHA + ARA + Cortisol + OXY + dROMs + EPA + ALA + LIN + GGT + LAP + PA + Trypsine + Area	Trypsine			-0.0015	-0.226	0.822	-631.1
	FA_total + DHA + ARA + Cortisol + OXY + dROMs + EPA + ALA + LIN + GGT + LAP + PA + Area	PA			-0.0034	-0.444	0.662	-634.3
	FA_total + DHA + ARA + Cortisol + OXY + dROMs + EPA + ALA + LIN + GGT + LAP + Area	GGT			0.0023	0.402	0.693	-636.9
	FA_total + DHA + ARA + Cortisol + OXY + dROMs + EPA + ALA + LIN + LAP + Area	LAP			0.0038	0.700	0.481	-639.2
	FA_total + DHA + ARA + Cortisol + OXY + dROMs + EPA + ALA + LIN + Area	dROMs			0.0017	-0.351	0.722	-641.5
	FA_total + DHA + ARA + Cortisol + OXY + EPA + ALA + LIN + Area	EPA			0.0034	0.614	0.531	-643.7
	FA_total + DHA + ARA + Cortisol + OXY + ALA + LIN + Area	LIN			-0.0041	-0.480	0.634	-646.2
	FA_total + DHA + ARA + Cortisol + OXY + LIN + Area	ALA			0.0116	2.13	0.102	-650.9
	FA_total + DHA + ARA + Cortisol + OXY + Area	OXY			0.0120	2.35	0.074	-653.8
	<b>FA_total + DHA + ARA + Cortisol + Area</b>			<b>FA_total</b>	0.027	2.79	<b>&lt;0.001</b>	-656.3
				<b>DHA</b>	-0.017	-2.71	<b>0.005</b>	
				<b>ARA</b>	-0.013	-2.58	<b>0.007</b>	
				<b>Cortisol</b>	-0.011	-2.06	<b>0.029</b>	
			<b>Area</b>	1.01 (deviance)	84 (F-value)	<b>&lt;0.001</b>		

morphometric body condition indices are thus confirmed to provide useful indicators for representing individuals' lipid reserves as stated in Lloret et al., (2013). Although significant, the variance in this relationship was substantial and this is probably due to our use of muscle samples. Muscles contain a large part of the reserve lipids in sardine, but the relationship would have probably been greater if we used full body measurements of lipids (such as bomb calorimetry) though this would have prevented us from undertaking other physiological measurements. Lipid reserves, however, were not the only physiological parameter linked to morphometric individuals' body condition as reserve composition *per se* with the percentage of DHA and, to a lesser extent, the percentage of ARA was significantly and negatively related to  $K_n$ . These two fatty acids regulate many vital functions in fish such as growth, survival, stress resistance, and the immune system (Tocher, 2010; Závorka et al., 2023). Hence, their negative correlation with  $K_n$  may seem surprising but may reflect sardine food origin to build lipid reserves. Indeed, high DHA content may reflect a food regime based on non-diatoms primary producers such as dinoflagellates or on zooplankton that accumulated this particular fatty acid from their dinoflagellate diet (Dalsgaard et al., 2003; Napolitano et al., 1997; Pethybridge et al., 2015). This situation corresponds to what is already observed in the Gulf of Lions, with a decline in sardine body condition with the increasing dominance of dinoflagellates in their surrounding environment and in their stomach content (Brosset et al., 2016; Feuilloley et al., 2020). Further studies will need to determine if lower sardine body condition could result from lower energetic values of dinoflagellates trophic chain compared to diatoms trophic chain and/or from the more global nutritional values (e.g., nutrients, vitamins) and environmental conditions (i.e., mixed waters) associated to diatoms.

By investigating concomitantly energetic and other physiological processes, our study revealed the importance of physiological status other than nutritional processes in the interpretation of  $K_n$ . Indeed, even though lipid reserves quantity and composition explain a large part of  $K_n$  variability, the cortisol concentration was also significantly related to this morphometric body condition index. More specifically, sardines

with high scale cortisol levels had lower  $K_n$ . This might either reflect the key role of cortisol in fish metabolism (Sadoul and Geffroy, 2019; Sadoul and Vijayan, 2016) and its role in the formation and use of energy reserves and/or fish chronic stress levels which trigger the allocation of energy reserves to sustain a long-lasting stress response. Hence, this points out that broader physiological processes than just nutritional stress should be considered when studying individuals' responses to environmental changes and implications on fitness. This supports previous findings highlighting that chronic stress response, monitored through cortisol accumulation, affects fish life history traits (Aerts et al., 2015; Lebigre et al., 2022; Sadoul and Geffroy, 2019) and fish body condition morphometric indices (Cook et al., 2012).

The other physiological processes that we measured (i.e., oxidative stress and digestive enzyme activity), were not significantly related to the morphometric body condition index. These results are consistent between the GLM and the PCA where these parameters had orthogonal projection compared to FA\_total, DHA, ARA, and cortisol. Previous studies have shown that morphometric body condition indices might be related to oxidative stress (Clotfelter et al., 2013; Kurtz et al., 2006), hence the absence of significant link in our results might be due to a particularly low levels of oxidative stress in the sampled sardines with little effects on their structure (i.e., weight). Our results about this lack of relationship, however, are in line with those from (Beauvieux et al., 2022) also on sardine, underlining that oxidative stress may have lower effects than other physiological processes on  $K_n$  which is therefore a poor predictor of oxidative stress. All digestive enzymes considered were mainly positively and strongly correlated together, distinguishing sardines with high and low digestive capacity. Yet, digestive enzymes activity, used as a proxy of the individual metabolic activity and abilities to acquire energy storage, was not significantly linked to  $K_n$  variability. This is likely primarily due to the relatively short timespan over which the concentration of these enzymes can change (i.e., few days, Silva et al., 2010). Enzymes might therefore be interesting markers for studying recent dietary changes but not integrative enough to be linked to fish morphometric changes.



**Fig. 3.** Partial regression plots showing  $K_n$  variability in function of the physiological variables identified in the optimal generalized linear model while controlling for all other fixed effects. Panel A for total fatty acids quantity; B for the DHA percentage; C for the ARA percentage and D for the cortisol concentration. Error bars represent 95% confidence intervals. Dot colours indicate the different areas considered in the study.

Regarding the situation in French waters, sardines from two areas (the Bay of Biscay and the Gulf of Lions) experienced reducing morphometric body condition and growth over the last 15 years (Saraux et al., 2019; Véron et al., 2020a). The main explanations put forward have been changing plankton production (i.e., food resources) over time probably associated to climate change (Boëns et al., 2023; Menu et al., 2023). Our results clearly indicate that these declines in morphometric body condition indices may reflect not only a decline in the amount of lipid reserves (resulting from a decrease in food supply by plankton) but also changes in food composition and chronic stress due to environmental changes. We suggest that future studies about fish health status and population dynamic need to be broadened beyond only assuming fish energetic state variability by testing and considering their relation to other physiological aspects.

## 5. Conclusion

Due to their numerous advantages, morphometric body condition indices will remain widely used proxies of fish health and population status. Our study confirmed that a morphometric body condition index primarily reflected the variance in individuals' lipid reserves, but was also linked to the actual composition of this lipid reserves (probably linked to differences in fish diet) and non-energetic aspects such as scale cortisol level (reflecting fish metabolism and/or their chronic stress levels). This underlines the necessity to move beyond the traditionally assumed use of morphometric body condition indices only as proxies of energy reserves and opens avenues for further uses of morphometric

indices. Morphometric condition indices cannot be fully integrative as there was no congruent changes across physiological processes indicative of overall health changes. In our case,  $K_n$  was clearly not completely integrative, as it was not related to oxidative stress and there is a clear need to complement morphometric measurements by more direct measurement of other physiological processes. Assessing whether morphometric body condition indices vary according to fish physiological state therefore remains an acute question that we need to address if we want to better understand mechanisms at play when such indices are used to support scientific-based decision-making. By providing robust understanding of the physiological processes related to differences in morphometric body condition index, we intend to strengthen future studies on fish health and links with management applications in the context of climate change.

## CRediT authorship contribution statement

**Pablo Brosset:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition. **Alan Averty:** Conceptualization, Validation, Formal analysis, Investigation, Writing – review & editing. **Margaux Mathieu-Resuge:** Validation, Investigation, Writing – review & editing. **Quentin Schull:** Methodology, Investigation, Writing – review & editing. **Philippe Soudant:** Methodology, Validation, Investigation, Writing – review & editing. **Christophe Lebigre:** Methodology, Validation, Formal analysis, Investigation, Writing – review & editing, Funding acquisition.



## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2023.110860>.

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