Energy allocation trade-offs between life-history traits in the Mediterranean sardine: an ecophysiological approach

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ABSTRACT: Since 2008, there has been a major decrease in the numbers of old and large sardines in the Gulf of Lions, which has had a major effect on regional fisheries. A bottom-up process involving a shift in diet towards smaller planktonic prey has been suggested as the main driver of this development. Yet, the reproductive capacities of the sardines have not changed, suggesting potential modifications in energy allocation trade-offs. Whether this could also affect maintenance, in particular at the end of the winter reproductive period, and explain the lower adult survival and the disappearance of older individuals remains unclear. We therefore experimentally investigated the consequences of seasonal food availability (summer vs. winter) on life-history traits and energy allocation trade-offs at the individual and population levels. Our results indicate that food resources during summer had a major effect on energy reserves and growth, limiting the maximum size and body condition reached at the end of reproduction. In addition, food restrictions during growth and/or reproduction periods led to physiological costs mediated by increased oxidative damage. Mediterranean sardines did not show any ability for compensatory growth and did not appear to be capital breeders. Instead, they displayed individual differences in coping with physiological constraints and displayed various life-history strategies regardless of food availability. We highlighted 3 main individual energy allocation strategies: (1) preferential allocation to body condition or (2) to growth, or (3) simultaneous allocation to reproduction and growth. These issues are key, as climate change is expected to favour smaller phytoplankton, which might amplify the deterioration in the condition of pelagic fish.

KEY WORDS: Compensatory growth · Capital breeder · Individual quality · Oxidative stress · Reproduction · Maintenance · *Sardina pilchardus*

1. INTRODUCTION

How organisms acquire, store and use energy or nutrients throughout their annual cycle is a key component of their life strategy and an important feature defining their fitness (McEwen & Wingfield 2003). If/when resources become limiting (in quantity and/or quality), allocating energy to one specific function will occur at the expense of another (Gadgil & Bossert 1970, Zera & Harshman 2001), which implies trade-offs between growth, somatic maintenance and reproduction (Stearns 1989).

In the natural environment, the availability of resources is variable in time and/or space. As such, animals have evolved a range of strategies to face alternating high and low resource levels. For instance,

under low-resource conditions, growth is often slowed down or stopped (Sumpter et al. 1991). However, some organisms are able to offset and catch up to the predicted size when resources subsequently improve via so-called 'compensatory growth' (Metcalfe & Monaghan 2001, 2003, Mangel & Munch 2005). This accelerated growth period has been observed across a wide range of taxa (Albon et al. 1987, Näslund et al. 2015, Stier et al. 2015) and seems to occur following a period of undernourishment rather than malnutrition (Boersma & Wit 1997).

To further cope with environmental constraints in resource availability, particularly during breeding, 2 main and opposite reproductive strategies have been proposed: capital breeding (organisms store energy prior to anticipated reproduction) vs. income breeding (no anticipation, energy intake occurs during the reproductive period) (Stearns 1989). Income breeders aim to reproduce while resources are abundant, leading to a rapid transfer of productivity from the ecosystem to species reproduction. In contrast, to face the highly energy-demanding period of gamete production and offspring raising, capital breeders mostly rely on energy reserves previously accumulated mainly as body fat (Rijnsdorp et al. 2005, Palstra & van den Thillart 2010). Although easily described as dichotomous, it is widely acknowledged that breeding patterns are not limited to extremes and are likely distributed along a range, perhaps a complete continuum, of intermediate patterns (Stearns 1989).

Such growth and reproductive energy allocation patterns have evolved in order to enable individuals to match their energy expenditure with resource availability and maximise their fitness. Yet, how this could affect trade-offs with other functions, especially regarding the potential costs of body maintenance, is an important question. For instance, the oxidative balance (i.e. balance between production of reactive oxygen species [ROS] owing to metabolism and the level of endogenous and exogenous antioxidant defences) plays an acknowledged role in the deterioration of body functions and has been proposed as one of the underlying mechanistic processes of ageing (Speakman 2005, Metcalfe & Alonso-Alvarez 2010). However, the generalisation of this relationship within mammals or among taxa is not always trivial (see Vágási et al. 2019, Kramer et al. 2021). Within this framework, it would be beneficial to investigate oxidative stress consequences under experimentally constrained trade-offs.

While energy allocation trade-offs and the underlying mechanisms involved are increasingly studied in terrestrial species (Bonnet et al. 2002, Vedder &

Bouwhuis 2018), they are scarce in marine organisms where it is particularly difficult to track individuals in the wild over time. Yet, such trade-offs should be exacerbated in most marine species (at least in fish), which maintain continuous growth throughout their lives, therefore maintaining constraints on trade-offs between growth, somatic maintenance and reproduction. Since fitness generally increases with size, it should emphasize selection pressure on growth and thus urge individuals to grow fast, ultimately leading to exacerbated trade-offs.

Often characterized by short lifespan (2–7 yr) and high reproductive rate, small pelagic fish are key components of marine ecosystems, playing an important role in energetic transfers from lower trophic levels (plankton) to higher ones (e.g. humans, tuna, cetaceans, birds; Cury 2000, Frederiksen et al. 2006, Straley et al. 2018). Their population dynamics are greatly affected by environmental fluctuations (bottom-up effects) and predator abundance/human induced changes (top-down effects), causing populations to commonly face variations in abundance (Checkley et al. 2017). Recently, significant changes in life-history traits have been observed in the small pelagic fish populations of the Gulf of Lions in the north-western Mediterranean Sea (Saraux et al. 2019). Since 2008, the 2 dominant small pelagic fish species, i.e. sardine (European pilchard *Sardina pilchardus*) and European anchovy *Engraulis encrasicolus*, have shown a drastic decrease in size and body condition (Van Beveren et al. 2014). This results in lower market value on the stalls, which in turn has led to the lowest historical landings of small pelagic fish for 150 yr and a local fisheries crisis (Van Beveren et al. 2016a). The hypotheses of top-down control (overfishing, predation) or epizootic diseases have been recently refuted (Van Beveren et al. 2016b, 2017, Queiros et al. 2018), so that the main hypothesis to explain the observed changes is bottom-up control, i.e. a modification of either size and/or quantity of sardine and anchovy prey (essentially copepods), thus limiting fish energy resources (Brosset et al. 2016a, Saraux et al. 2019). Competition for food with other species such as sprat *Sprattus sprattus* is also possible, but remains unclear (Pethybridge et al. 2014) and could have changed over the last decade (Brosset et al. 2016a).

Under limited energy resources, the question of how small pelagic fish, especially sardines, which have been much more impacted than anchovy (Saraux et al. 2019), solve energy allocation trade-offs might be central in understanding the changes observed at the population level and how they might deal with further global change. Indeed, the smaller

size of sardines has been shown to result from both slower growth and the disappearance of older sardines. A recent study conducted on wild populations showed that sardines started reproducing at smaller sizes and maintained a high investment in gonads (Brosset et al. 2016a), suggesting a trade-off between growth and reproduction. Whether the decrease in sardine longevity might result from lower energy resources and/or costs associated with growth and reproduction is still an open question. Sardines are usually considered capital breeders, i.e. they store energy in spring and summer that can be mobilized later for reproduction in winter (Ganias 2009). Depleted reserves at the end of the reproductive period might thus not suffice to support maintenance costs in winter when resources are low.

To formally test the hypothesis of change in energy allocation trade-offs in sardines, we experimentally investigated the consequences of a temporal modulation of food resources during 2 seasons (summer and winter) on life-history traits. Manipulating food size and quantity as previously done by Queiros et al. (2019), we aimed to answer the following 3 main questions: (1) When feeding conditions improve, do sardines show compensatory growth? We predicted that sardines facing poor feeding conditions in summer and then improved conditions in winter would exhibit compensatory body size growth (Mangel & Munch 2005). (2) How plastic are sardines in terms of reproductive investment? Do they depend exclusively on their stored energy? We tested the hypothesis considering sardines as capital breeders. In this case, feeding conditions prior to reproduction (i.e. in summer) will have a major effect on reproductive output, and therefore individuals poorly fed in summer but well fed in winter should show a similar output to sardines poorly fed throughout the entire experiment. (3) What are the costs in terms of maintenance of such growth and reproductive energy allocation patterns? If sardines exhibit compensatory growth or higher reproductive investment, we assumed that it should lead to the accumulation of damage at the metabolic level (Metcalfe & Monaghan 2001) reflected by higher oxidative stress (Mangel & Munch 2005).

An extra layer of complexity is added when considering inter-individual variability. Individual heterogeneity within a population is commonly observed, and is generally enhanced when environmental conditions fluctuate or are unstable (McNamara 1998, Tuljapurkar et al. 2009). There is indeed a continuum of life-history strategies, both inter- and intra-specific, with some individuals favouring survival over repro-

duction and vice versa (Reid et al. 2010). Accordingly, negative correlations between traits should arise, with an increase of investment in one trait being conditioned by the decrease in another. By contrast, if strong heterogeneity in terms of individual quality exists and some individuals of 'higher quality' better succeed at facing food limitation, positive correlations among the different life-history traits would be expected (e.g. some individuals should be able to invest simultaneously in reproduction and growth; Marchand & Boisclair 1998, Wilson & Nussey 2010, Bergeron et al. 2011). We investigated these hypotheses by (1) analysing the inter-individual variance in size and body condition over the different periods of the experiment and (2) assessing the existence of different strategies or qualities at the end of winter and testing for associated costs through their oxidative status.

2. MATERIALS AND METHODS

2.1. Experimental design

We aimed to investigate energy allocation trade-offs and individual strategies of sardines in the Gulf of Lions in response to energy limitations imposed during 2 very distinct periods of their life cycle: summer, with high growth rates due to higher water temperatures and planktonic production peak, vs. winter, when reproduction takes place. To do so, an 8 mo experiment with wild adult sardines acclimatized in tanks (detailed below) was carried out during 2 periods (summer and winter). Both size and quantity of food were taken into account, as a previous study revealed that sardines feeding on small particles (0.1 mm, range: 80–250 µm) had to consume twice as much as those feeding on large particles (1.2 mm, range: 900–1500 µm) to achieve the same body condition and growth (Queiros et al. 2019). The methods used to study the effects of these feeding treatments on several life-history traits — morphometric features (body condition, growth), energy resources (muscle lipid content), somatic maintenance markers (oxidative stress) and reproductive investment (gonadosomatic index) — are described in Sections 2.3 and 2.4.

In May 2017, ca. 1000 sardines were captured at sea (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m701p099_supp.pdf for further details), and brought back to an Ifremer experimental research facility at Palavas-les-flôts, France, where they were held in quarantine in outdoor tanks of 4.5 m^3 for 1.5 mo until confirmation of the absence of pathogens (similar to Queiros et al. 2019). During that time,

sardines were also weaned from live food (*Artemia nauplii*) to a mixture of aquaculture pellets of different sizes (0.1, 0.3 and 0.8 mm) at feeding rates between 1 and 2% of their biomass per day. At the end of June, sardines were moved into 2 indoor tanks of 3 m³ each (Fig. 1). Prior to transfer, sardines were anaesthetized with benzocaine (140 ppm), and total length (±0.1 mm) and mass (±0.01 g) were measured so as to distribute them into homogeneous groups (i.e. similar mean size and mass between tanks) between the 2 tanks. Two months later (middle of summer period), fish were switched from one tank to the other in order to mitigate any potential tank effects.

The experiment started on 27 June 2017 and ended on 5 March 2018. The first phase (from 27 June to 16 November) was considered the 'summer' period with 2 possible scenarios that aimed to mimic contrasting natural conditions: (1) a 'highly productive' year that was experimentally mimicked by high food quanti-

ties (between 0.8 and 1.2% of the total fish biomass, according to prevailing temperature) and large pellet size (1.2 mm) and (2) a 'low productive' year characterized by smaller food quantities (between 0.4 and 1% of their total biomass, according to prevailing temperature) and small pellet size (0.1 mm, see Fig. 1). These 2 contrasting pellet sizes are within the range of prey size in their diet (Le Bourg et al. 2015).

On 25 October, each group of sardines was homogeneously divided into 4 sub-groups (i.e. similar mean size and mass between tanks), and transferred to 300 l tanks (8 tanks in total; Fig. 1) in preparation for the second phase of the experiment (17 November 2017 to 5 March 2018); hereafter 'winter', which started during the first week when the water temperature dropped below 14°C, which coincided with the entry into reproduction during a natural breeding cycle for north-western Mediterranean sardines (Brosset et al. 2016a). Similarly, sardines reared in captivity over
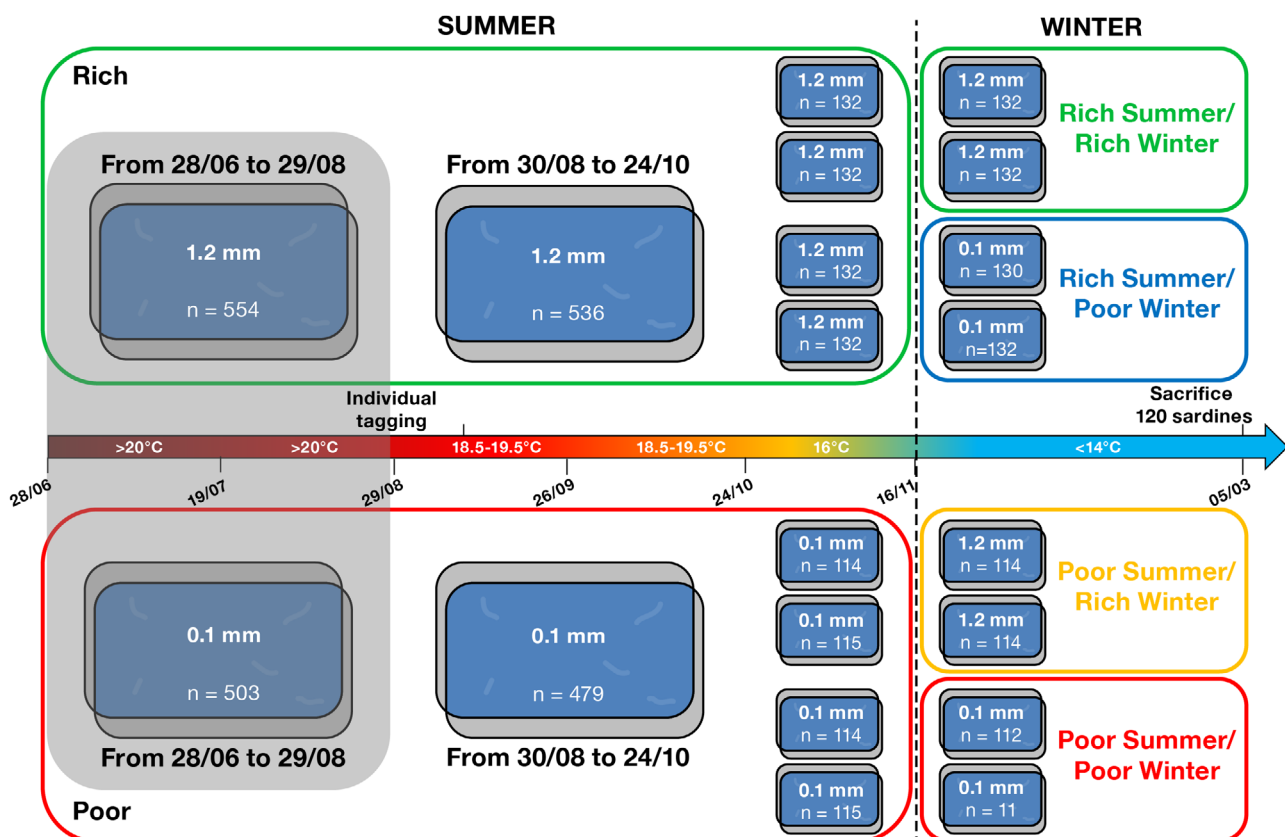

Fig. 1. Design of the 8 mo experiment on adult wild sardines in order to investigate how temporal resource variability (in quantity and size) impacted energy allocation trade-offs between life-history traits. The experiment was divided into 2 periods separated by the dotted vertical line: summer for 4.5 mo with average to warm temperatures (16 to >20°C) and winter for 3.5 mo, corresponding to the breeding season of sardines (13–14°C). Initially, 2 tanks of 3 m³ were used, followed by 8 tanks of 0.3 m³. Pellet sizes (0.1 and 1.2 mm) are indicated for each tank. Individual fish were tagged on 29 August 2017, before which individual size and body condition could not be monitored (greyed area). Temperature ranges are indicated for each time step. The end of the experiment and the sacrifice of the 120 individuals randomly sampled for the study of physiological condition are indicated on 5 March 2018

the last 4 yr initiated spawning only when the water temperature dropped below 14°C and stopped reproduction at the end of winter when the temperature rose to above 15°C (Q. Queiros, E. Gasset, G. Dutto, C. Saraux pers. obs.). During the second phase (winter), half of the tanks remained in the same food condition they experienced in summer, while the other half switched to the opposite treatment. Our experiment therefore included 4 treatments: (1) rich diet in both summer and winter (referred as Rich Summer/ Rich Winter), (2) rich diet in summer and poor diet in winter (Rich Summer/Poor Winter), (3) poor diet in summer and rich diet in winter (Poor Summer/Rich Winter) and (4) poor diet in both summer and winter (Poor Summer/Poor Winter), each composed of 2 tanks. On 5 March 2018 (when the reproductive period was over), the experiment stopped and 120 sardines (30 per feeding treatment) were randomly sampled and sacrificed by benzocaine overdose in order to study their physiological condition. Sex and maturity were determined by gonad observation at the end of the experiment. Mortality during the experiment remained very low, on average 4% (further details in Table S2 with mortality rate for each tank).

Tanks were supplied with the same seawater (filtered and passed through UV light) at a renewal of $10\% \text{ h}^{-1}$ during both periods ('summer' and 'winter'). All tanks were provided with an individual standardised light source mimicking the sunlight spectrum and natural cycles including progressive dawns and sunrises. The photoperiod was thus adjusted weekly to follow the natural cycle, and seawater temperature was not controlled except to maintain a minimum of 10°C and a maximum of 25°C. Temperature and salinity recordings of tanks closely followed the same trend over the entire period (Fig. S1).

2.2. Total length, body condition and growth

Each sardine was tagged to allow individual monitoring of body growth. On 29 August 2017, a tiny RFID tag (Biolog-id, 0.03 g, or <0.6% of the body mass of the leanest sardine) was implanted in the dorsal muscle after the fish was anaesthetized with benzocaine (140 ppm). Thereafter, total length (±0.1 mm) and mass (±0.01 g) were recorded individually every 4 wk (on 'biometry days') until the end of the experiment. Sardines were fasted 24 h before each biometry day so that weight would not be biased by stomach content. Relative body condition of each sardine (N = 972) was calculated using

the Le Cren index Kn as estimated by Brosset et al. (2015):

$$Kn = \frac{WW}{0.00607 \times TL^{3.057}} \qquad (1)$$

where WW is the wet mass in g and TL is the total length in cm. This index compares the actual mass of an individual with the theoretical mass expected for an individual of its size (based on the size–weight relationship of tens of thousands of individuals fished *in situ*). Therefore, a value of $Kn > 1$ means that the individual has a body condition higher than the average of the population and vice versa.

Growth rates and condition rates were then estimated at different time scales. First, instantaneous growth and condition rates were estimated as the change in length and condition, respectively, from one biometry day to the next (~30 d apart), divided by the exact number of days between them. Because of relative measurement precision (1 mm) and some extremely slow growth, it sometimes resulted in negative growth rates, $t-1 \rightarrow t$. To overcome this problem, we translated all instantaneous growth rates by the minimum rate ever observed in this study (-0.28 mm d^{-1}) considering that this should represent null growth. For each individual, summer and winter growth and condition rates were also estimated as the slope of linear models of total length (mm) or body condition over time (day) (1 linear model per individual per period).

Before the start of individual monitoring (29 August), 130 and 108 individuals were measured in June and July, respectively, to adjust food quantity to total tank biomass. Since the individuals were not tagged yet, the data could not be linked to individual size or condition trajectories. The average condition and size measurements on these 2 dates are therefore presented only for information on the graphs but were not used in analyses. Further, between 29 August and 26 September 2017, about 40% of individuals lost their tags. These individuals were retagged on September 26 and no tags were lost afterwards. In order to ensure that the lower sample size for this first month (due to tag losses) did not affect our analysis, we compared mean (±SE) growth rates and condition rates through time per treatment considering 3 subsamples (Figs. S2 & S3): (1) considering all data points available (N = 603 in September and then 972 for all other months), (2) the 603 individuals over the entire time series and (c) only the 120 individuals sacrificed at the end of the experiment. Since results did not differ regardless of the subsample considered, we present results based on all available data.

At the end of the experiment, 120 individuals were sacrificed (30 per treatment which were representative of their treatment, i.e. similar growth and condition rate; see Figs. S2 & S3). To obtain a growth rate for the entire experiment (referred as total growth rate), summer and winter growth were averaged, weighted by the number of days of each period:

$$\text{Total growth rate} = \frac{\text{growth rate}_{summer} \times \text{days}_{summer} + \text{growth rate}_{winter} \times \text{days}_{winter}}{(\text{days}_{summer} + \text{days}_{winter})} \quad (2)$$

In order to obtain a trait independent of the reproductive investment, a second body condition index was calculated where gonad mass was removed from the final weight of the individual (therefore only available at the end of the experiment for N = 120):

$$Kn_{without\ gonad} = \frac{WW - GM}{0.00055 \times TL^{4.0498}} \quad (3)$$

where WW and GM are the final wet mass and gonad mass (g) of individuals, respectively, and TL is the final total length (cm). The 2 constants were estimated from our data based on the final size–weight relationship without gonads of the 120 individuals sacrificed in March.

Total condition rate (i.e. over the entire experiment for N = 120) was then assessed based on this second condition index. Eq. (4) indicates how we assessed the gain/loss of body condition for the entire experiment independently of the investment in reproduction:

$$\text{Condition rate}_{without\ gonad} = \frac{Kn_{without\ gonad} - Kn_i}{(\text{days}_{summer} + \text{days}_{winter})} \quad (4)$$

where $Kn_{without\ gonad}$ is the individual final body condition as explained previously (Eq. 3) and Kn_i is the initial body condition (Eq. 1) at the start of the experiment. Because the experiment started in summer, when individuals have not yet begun to invest in reproduction, we considered the gonad mass to be null at that time (see Fig. 2 in Brosset et al. 2016a)

2.3. Reproductive status at the end of winter

For each of the 120 sacrificed individuals, sex and gonad mass were recorded. To estimate reproductive investment, a gonadosomatic index (GSI) (Alam & Pathak 2010) was estimated as follows:

$$GSI = \frac{\text{Gonad mass}}{\text{Individual mass}} \times 100 \quad (5)$$

Out of the 120 individuals, 6 displayed non-active gonads (and 3 out of the 6 were not sexually differentiated), while all other individuals presented mature gonads, suggesting that most of the individuals (>95%) were adults and sexually active during the experiment. Therefore, sex was recorded for 117 sardines at the end of the experiment.

2.4. Physiological condition at the end of winter

For each of the 120 individuals sacrificed at the end of the experiment, blood was collected from the caudal vein with a heparinized 26G needle and transferred into 1.5 ml Eppendorf tubes. Blood samples were centrifuged at 3000 × g (10 min at 4°C) to separate plasma from cells, which was collected and stored at −80°C until further oxidative status analyses. Portions of dorsal epaxial muscle were also removed, immediately frozen in liquid nitrogen and stored at −80°C for further lipid analyses.

2.4.1. Individual oxidative status

The physiological consequences of feeding treatment were studied by assessing the oxidative stress status in plasma by measuring 2 biomarkers for antioxidant defences (total antioxidant defences [OXY] and superoxide dismutase [SOD] enzyme activity) and 2 biomarkers for oxidative damage (reactive oxygen metabolites [d-ROMs] and thiobarbituric acid reactive substances [TBARS] formed as a byproduct of lipid peroxidation).

All sample measurements were performed in duplicate. Samples with a coefficient of variation (CV) higher than 15% were re-run. Assays were performed on all individuals except for some individuals for which the amount of plasma was not sufficient to perform all tests, explaining the differences in sample sizes among analyses. Sample sizes are reported in the text and figures.

Following Costantini & Dell'Omo (2006), we estimated d-ROMs and OXY in plasma using the d-ROMs test and the OXY adsorbent test, respectively (Diacron International©). After incubation, absorbance was measured at 555 nm with a microplate spectrophotometer. d-ROMs were expressed in mg of equivalent H_2O_2 l^{-1} and the antioxidant defence capacity (OXY) in µmol HClO ml^{-1}. The intra- and inter-plate CV were 4.5 and 3.1% for d-ROMs and 7.3 and 7.7% for OXY, respectively.

SODs are important endogenous defence systems against free oxygen radicals which catalyze the dismutation of superoxide radicals ($O_2^{.-}$ into hydrogen peroxide [H_2O_2] and oxygen (O_2) ($2O_2^{.-} + 2H^+ \rightarrow O_2 + H_2O_2$, Fridovich 1989). SOD is represented by 3 isoforms, differing by the metal ions in their active centres: Mn-SOD (SOD2) and Cu/Zn-SOD (SOD1 and SOD3). In plasma, only SOD3 is present. Therefore, an increase in this enzyme indicates good circulating neosynthesized antioxidant defence (Halliwell & Gutteridge 2007). The concentration of SOD3 in plasma was estimated by colorimetry according to Peskin & Winterbourn (2000). To normalize SOD concentration by protein content in plasma, protein concentration in each sample was determined by the BCA method (Pierce, Thermo Fisher Scientific). Four plasma samples lacked the required quantity to run duplicates. Nonetheless, given that the intra- and inter-plate CV was 8.4 and 3.4%, respectively, for the SOD estimation (always <15% for all duplicates), we considered the 4 samples that were run only once to be reliable. After incubation at 37°C for 30 min, absorbance was measured at 450 nm with a microplate spectrophotometer. For each plasma sample, the SOD concentration was also corrected by the protein content and expressed in units per μg of protein. The intra- and inter-plate CV were 3.7 and 0.7%, respectively, for the protein estimation.

Finally, lipid peroxidation in the sardine plasma was estimated from the production of malondialdehyde (MDA), which is one of the final products of lipid peroxidation (Draper & Hadley 1990). The MDA content was determined using an MDA colorimetric assay kit (Elabscience Biotechnology). This assay measures the TBARS at 532 nm, following the methodology described by Draper & Hadley (1990). The intra- and inter-plate CV for plasma samples were 2.3 and 1.5%, respectively.

2.4.2. Energy storage and reserve

The energy storage capacity was examined by analysing the lipid content of the dorsal muscle. Lipids were extracted according to the method of Folch et al. (1957) and the content of each class of lipids was measured in the muscle by chromatography, using an Iatroscan as detailed by Sardenne et al. (2019). Phospholipids, sterols, acetone-mobile polar lipids and alcohols were grouped in structural lipids, while triacylglycerols (TAGs), diacylglycerols (as TAG precursors) and free fatty acids (FFAs) were grouped in reserve lipids (Zhol et al. 1995,

Tocher 2003, Lloret et al. 2013). The proportions of FFAs were also checked to ensure that the lipids had not been degraded during sample storage. Here, the proportions ranged from 0 to 12%, significantly below the 25% limit recommended by Parrish (1988).

2.5. Data analyses

2.5.1. Effects of feeding treatments on life-history traits at the population level

At the end of both periods (summer and winter), the differences in size and body condition between treatments were tested, using linear mixed models (LMMs) and pairwise post hoc tests, where tank was nested in treatment as a random factor to account for tank effects in winter. Summer growth rates, winter growth rates and condition rates in response to treatments were also investigated, similarly as above (LMMs and pairwise post hoc tests). To test the effect of individual size at the beginning of summer and winter on summer and winter growth rates, respectively, we modelled the summer or winter growth rate (response variable) in relation to the initial size (at the beginning of summer or winter), feeding treatment and their interaction (explanatory variables). These analyses were run on 972 individuals. We also ran our different models on the subsample for which we had the sex (N = 117) in order to test its effect. Sex was included in the interaction with treatment and removed when non-significant.

Physiological conditions (plasma oxidative stress and muscle lipid content) and reproductive investment differences between treatments for the 120 individuals sacrificed at the end of the experiment were examined, using parametric (LMM) or nonparametric (Kruskal-Wallis) tests and associated post hoc tests (Tukey or Dunn), depending on residual normality. Sex was included in the interaction with treatment and removed when non-significant.

A multivariate principal component analysis (PCA) (Abdi & Williams 2010) approach was also performed, using the 120 individual sardines as objects, while the descriptors were the summer growth and condition rates (August to November), winter growth and condition rates (November to March), antioxidant defence biomarkers (SOD and OXY), oxidative damage biomarkers (TBARS and d-ROMs), lipid reserves and GSI at the end of the experiment. The aim of this analysis was to summarize all of the information to investigate the relationships between descrip-

tors and examine the level of inter-individual variability overall and within each feeding treatment.

2.5.2. Investigating inter-individual variability and allocation trade-offs

Van Noordwijk & De Jong (1986) demonstrated that correlations between competing traits (e.g. growth, maintenance and reproduction) within a species may take any sign. If individuals differ in their strategies, allocation trade-offs should occur and negative relationships between traits would likely be observed (Stearns 1989). Yet, if heterogeneity in individual quality exists, then 'high-quality' individuals should be able to display higher investment overall into all competing traits than other, 'low-quality', individuals, resulting in positive relationships among traits. We investigated those possibilities by first analysing the inter-individual variance in size and body condition over the different periods of sampling in our experiment with standard deviation (SD) time series. However, the alternative hypotheses are not exclusive, and trait covariations could also depend on the environment (e.g. trade-offs appearing only under limiting conditions, Hämäläinen et al. 2021). Therefore, we investigated how growth rate, condition rate and GSI covaried and whether this depended on the feeding treatment, using various types of regression models (see below). Each model was tested with and without the interaction of the feeding treatment and the sex with the covariables (either growth rate, condition rate or GSI) and the best models were selected using Akaike's information criterion (AIC) (Burnham & Anderson 2004). When the interaction with treatment was significant, we obtained a model with a triple interaction, which was difficult to represent. Therefore, to facilitate the interpretation of the results, we present one model for each feeding treatment. The normality of the residuals of each model was analysed visually with a quantile-quantile plot (Ghasemi & Zahediasl 2012) or through a Shapiro-Wilk test, depending on the number of observations. When data were not independent from each other due to repetitions within individuals (e.g. body condition and body size over time), a mixed model was used with the individual effect set as a random intercept, either through an LMM (Zuur et al. 2009) or a generalised linear mixed model (GLMM; Zuur et al. 2009), depending on the distribution of the data.

2.5.2.1. Modelling growth and energy storage. We first tested how sardines allocated energy between growth and body condition at each time step during winter only, depending on body condition at the start of this time step, using the initial 972 individuals. As the interaction with treatment was significant (see Section 3), we conducted a GLMM for each feeding treatment (with a gamma link function and with the individual effect ['ID'] set as a random intercept), where individual growth rates from $t-1$ to t during winter were dependent on changes in body condition over that same period and on the body conditions at the beginning of $t-1$, as well as their interactions (Eq. 6). In order to test for sex effects, we also ran sub-models on the 120 individuals for which sex was available. Since it had no effect, sex was removed from the final model:

$$\text{Growth rate}_{t-1 \rightarrow t} \sim \text{Condition rate}_{t-1 \rightarrow t} \times \\ \text{Condition}_{t-1} + (1|\text{ID}) \tag{6}$$

2.5.2.2. Modelling growth, energy storage and reproduction. As body condition index includes energy stored for reproduction, we examined more precisely the links between reproduction, growth and maintenance, using the 120 individuals sacrificed at the end of the experiment. We assessed whether reproductive investment (GSI) was constrained by energy investment in growth (total growth rate, Eq. 2) and in body condition (condition rate$_\text{without gonad}$, Eq. 4) over the entire experiment, using a generalised linear model (GLM), with a gamma link function, for each feeding treatment (as the interaction with the treatment was significant). Sex was added as a fixed factor and kept following the model selection procedure based on AIC (Eq. 7):

$$\text{GSI} \sim \text{Total growth rate} \times \text{Condition rate}_\text{without gonad} \\ + \text{Sex} \tag{7}$$

2.5.2.3. Summarizing the different energy allocation strategies. Using the 120 individuals sacrificed at the end of the winter period, we investigated the different energy allocation strategies through a hierarchical clustering analysis (Johnson 1967). This analysis was performed on total growth rate, total condition rate$_\text{without gonad}$ and GSI, using Euclidean distances on normalized data (data were first centred by treatment to remove the direct effect of the feeding treatment on the absolute values of GSI, growth rate and condition rate) and Ward's agglomerative clustering method (Ward 1963). To test for physiological costs associated with the identified strategies (i.e. the different clusters), differences in oxidative damages and antioxidant defences between clusters were investigated using 1-way ANOVAs (LM [linear model] and pairwise post hoc

tests). In order to account for known sex effects on GSI, we performed separate analyses for males and females. The best clustering scheme was identified using the function 'NbClust' (Charrad et al. 2014), which tests 30 methods that vary the combinations of number of clusters and distance measures for the hierarchical clustering. To evaluate the effect of individual size at the beginning of the experiment on their potential strategies, composition of initial size in each cluster for both sexes was assessed.

Analyses were performed with the statistical open source R software v.4.1.1 (R Core Team 2020) using the packages 'FactoMineR' v.2.4 (Lê et al. 2008), 'nlme' v.3.1-155 (Pinheiro et al. 2021), 'lsmeans' v.2.30-0 (Lenth 2016) and 'factoextra' v.1.0.7 (Kassambara & Mundt 2020) packages. Values are given as mean ± SE, and all statistical tests were performed at a significance level of 0.05.

3. RESULTS

3.1. Effects of feeding treatments on life-history traits at the population level

3.1.1. Compensatory growth

At the end of summer (Fig. 2a,b), rich summer feeding conditions led to individuals of larger size (LM; $F = 225.48$, $p < 0.001$; Table S3) and in better condition (LM; $F = 689.58$, $p < 0.001$; Table S3). Well-fed sardines grew twice as fast as those experiencing poor summer feeding conditions (0.14 ± 0.004 vs. 0.07 ± 0.004 mm d^{-1}) (LM; $F = 119.29$, $p < 0.001$), and body condition rates increased 5 times more for sardines in rich vs. poor feeding conditions (LM; $F = 439.72$; $p < 0.001$; Fig. 2c,d; Table S3). In summer, smaller well-fed sardines grew significantly less than
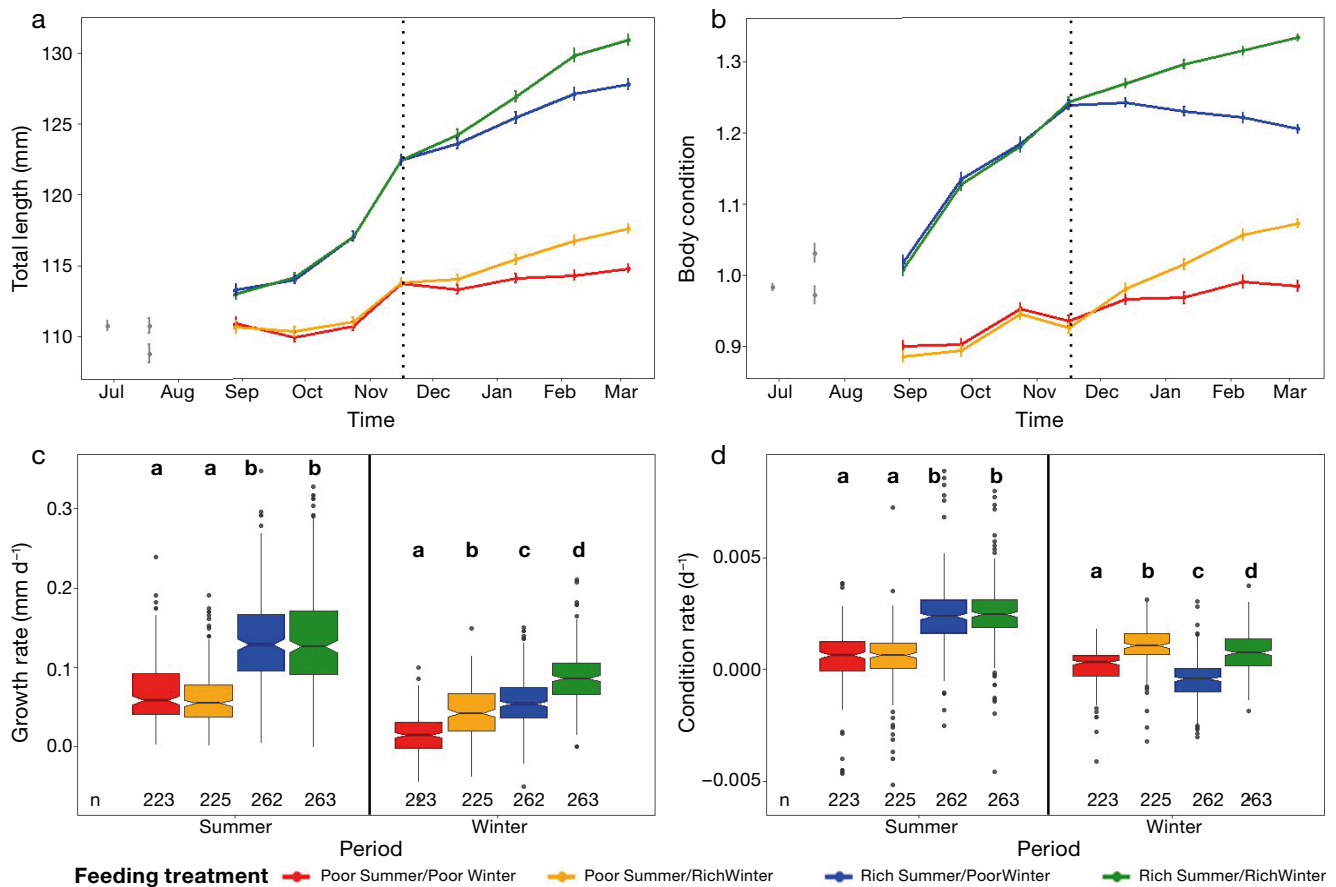

Fig. 2. Time series of mean (±SE) (a) total length and (b) body condition per feeding treatment and box plots of summer and winter (c) growth rates and (d) condition rates for the 4 treatments. In panels a and b, values in June and July based on a subsample are indicated in grey, as individual monitoring started on 29 August 2017. The vertical dotted lines mark the end of the summer period. In panels c and d, sample sizes for each treatment are given below the boxes. Box plots with different lowercase letters are significantly different (p < 0.05). Outliers are represented by black dots. Horizontal line in box: median value; bottom and top of box: 25th and 75th percentiles; whiskers: 5th and 95th percentiles

larger ones, whereas smaller sardines in poor feeding conditions grew significantly more than larger ones, indicating that initial size is important but its effect on growth rate is treatment-dependent (LM; p < 0.01; Table S4, Fig. S4a). Sex had no effect in any of our models considering size and growth rate. However, poorly fed males displayed better body condition and higher body condition rates than females in the same feeding condition (Fig. S5a,c). Moreover, female investment in body condition rate over that period did not differ from zero (Student's *t*-test; $t = -0.090386$, p = 0.93).

At the end of winter, the 4 treatments led to 4 significantly distinct groups in terms of total length and body condition (i.e. Rich Summer/Rich Winter > Rich Summer/Poor Winter > Poor Summer/Rich Winter > Poor Summer/Poor Winter for both variables; post hoc Tukey HSD, p < 0.001; Fig. 2a,b). Individuals well fed throughout the entire experiment grew 1.5 to 2 times faster during winter than individuals in the intermediate Rich Summer/Poor Winter and Poor Summer/Rich Winter treatments (post hoc Tukey HSD, p < 0.001) and 6 times faster than individuals poorly fed throughout the experiment (post hoc Tukey HSD, p < 0.001). For the latter, growth strongly decreased, but was still significantly different from zero over winter (Student's *t*-test; $t = 9.2838$, p < 0.001). Interestingly, sardines from the Poor Summer/Rich Winter treatment strongly increased their body condition over the winter (10.7×10^{-4} d^{-1}), between 1.5 and 7 times more than extreme treatments (7.4×10^{-4} d^{-1} and 1.5×10^{-4} d^{-1} for the Rich Summer/Rich Winter and Poor Summer/ Poor Winter treatments, respectively; post hoc Tukey HSD, both p < 0.001), while sardines experiencing the opposite changes (from rich to poor) decreased in condition (-4.2×10^{-4} d^{-1}, post hoc Tukey HSD; p < 0.001). Tank (for a given feeding treatment) had no effect on body condition and length over time and was therefore removed from the analyses. According to the AIC, the better model investigating winter growth rate included initial size, treatment and their interaction. Larger sardines in the 2 intermediate treatments (Poor Summer/Rich Winter and Rich Summer/ Poor Winter) grew faster than smaller ones (LM, p < 0.01; Table S5, Fig. S4b), whereas no effect of size on winter growth was found for the 2 extreme treatments (Poor Summer/Poor Winter and Rich Summer/Rich Winter). Males that were poorly fed throughout displayed better body condition than females experiencing the same treatment (p < 0.01; Fig. S5b). Except in the latter case, sex had no effect in any models including size, growth rate and condition rate over winter.

3.1.2. Plasticity in reproduction

GSIs measured at the end of the experiment for the Rich Summer/Rich Winter treatment (median: 7.9%) were about twice as high as those for the intermediate treatments (medians: 3.8 and 2.6% for the Rich Summer/Poor Winter and Poor Summer/Rich Winter treatments, respectively) and 12 times higher than for sardines in poor conditions (median: 0.7%; Fig. 3). We found a significant sex effect on reproductive investment (p < 0.001), with males displaying 2 to 3 times higher GSI than females, which held true in each treatment (Fig. S6).

3.1.3. Maintenance costs

No significant differences between sex and tanks (for the same feeding treatment) for plasma oxidative state and muscle lipid content were found.

Overall, sardines from the Rich Summer/Rich Winter treatment presented the lowest level of antioxidant defences in both OXY and SOD (Fig. 4a,b), although these differences were not always significant with respect to sardines from other treatments. Sardines from the Poor Summer/Poor Winter treatment presented low to average OXY levels, but displayed endogenous antioxidant SOD levels twice as high as those experiencing the intermediate treatments (post hoc Tukey HSD; p < 0.01; Fig. 4b) and 5 times as high as those experiencing the Rich Summer/Rich Winter
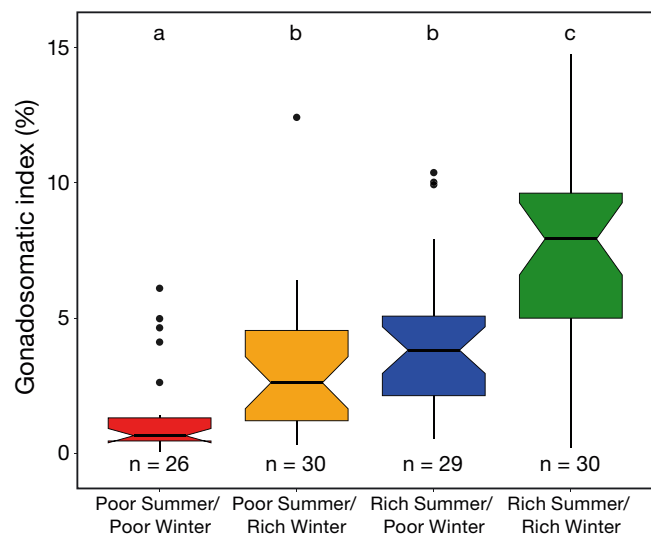

Fig. 3. Gonadosomatic indices at the end of the experiment for each treatment. The sample size is indicated under each box plot. Outliers are represented by black dots. Box plots with different letters are significantly different (p < 0.05). Box plot parameters as in Fig. 2
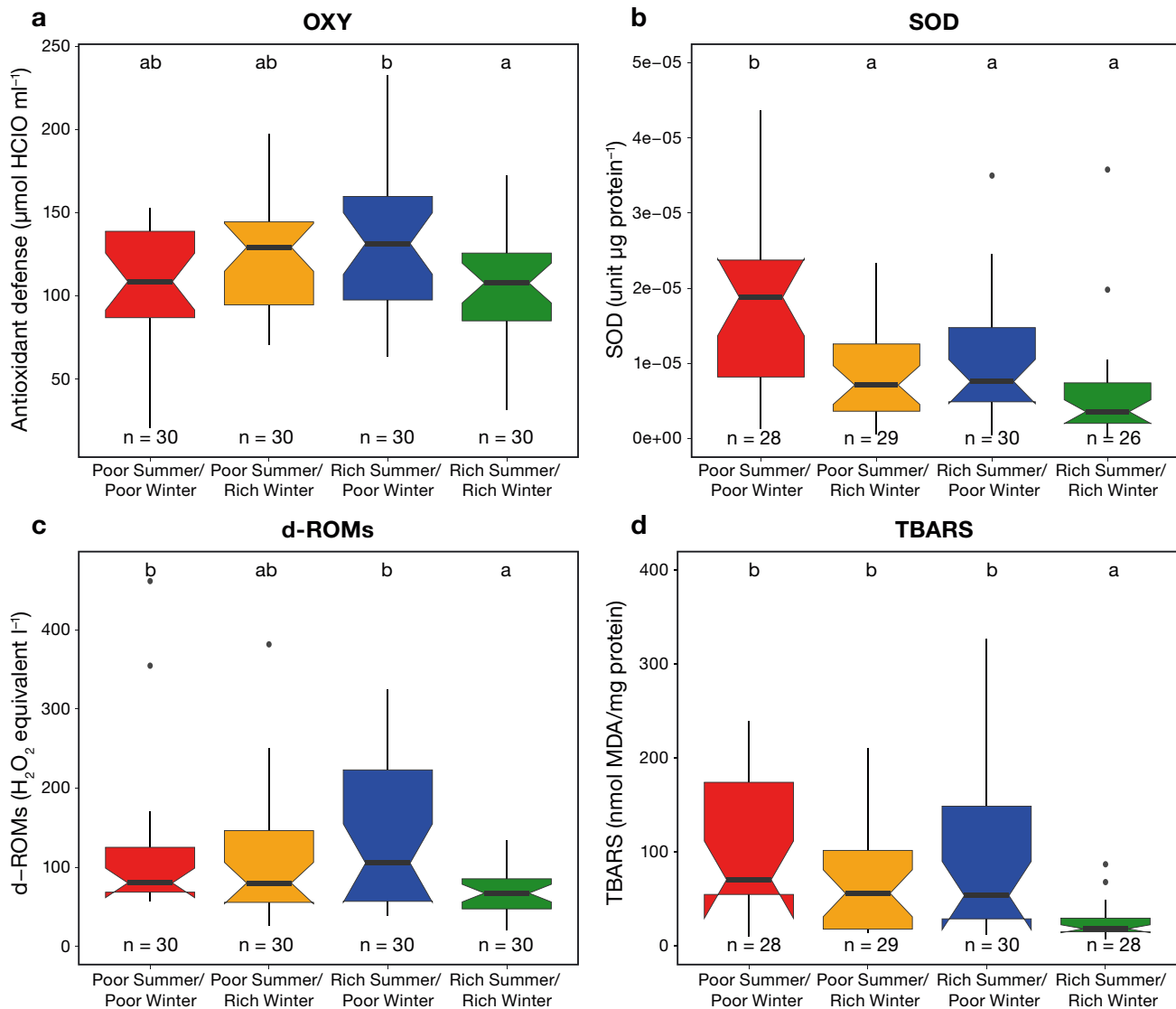
Fig. 4. Plasma concentrations of (a) total antioxidant defences (OXY), (b) superoxide dismutase (SOD), (c) reactive oxygen metabolites (d-ROMs) and (d) thiobarbituric acid reactive substances (TBARS) for each treatment at the end of the experiment. The number of individuals is indicated under each box plot. Outliers are represented by black dots. Treatments with different letters are significantly different ($p < 0.05$). MDA: malondialdehyde. Box plot parameters as in Fig. 2

treatment (post hoc Tukey HSD; $p < 0.001$). Furthermore, sardines in the Rich Summer/Rich Winter treatment displayed lower oxidative damage than sardines in other treatments (d-ROMs and TBARS; Fig. 4c,d). No differences in oxidative damage were observed among the other 3 treatments.

Reserve lipid concentration in muscle gradually increased with the improvement of the feeding conditions. Reserve lipids in the Rich Summer/Rich Winter treatment were 2 to 7 times higher than those in the 3 other treatments. Sardines from the Poor Summer/Poor Winter treatment presented the lowest concentration of reserve lipids (post hoc Dunn's test,

$p < 0.001$). On the other hand, structural lipids were similar among all 4 treatments (Kruskal-Wallis; $H = 2.95$; $p > 0.05$; Fig. S7).

3.1.4. Multivariate analysis

The first 2 components of the PCA explained 50.2% of the total variance observed. The main contributing variables to the first axis were winter growth and reserve lipids, whereas d-ROMs and OXY were the 2 main contributors to the second axis (Fig. S8). The PCA tends to disentangle extreme

treatments. Individuals from Rich Summer/Rich Winter treatment were mostly characterized by high winter and summer growth, high summer body condition, high GSI (i.e. reproduction investment), high lipid reserves and low oxidative states (OXY, d-ROMs, TBARS and SOD), while individuals from the Poor Summer/Poor Winter treatment generally displayed the opposite (Fig. 5). Individuals from intermediate treatments were in-between, close to the barycentre. Nonetheless, individuals from the Poor Summer/Rich Winter treatment remained closer to those poorly fed throughout, and sardines from the Rich Summer/Poor Winter treatment were closer to those well-fed throughout the experiment (Fig. 5).

3.2. Diversity of individual life-history strategies

SD in total length from the Rich Summer/Rich Winter and Rich Summer/Poor Winter individuals were higher than those of the other 2 treatments and increased continuously over the experiment (Kruskal-Wallis; $\chi^2 = 15.923$; p < 0.001; Fig. S9a).
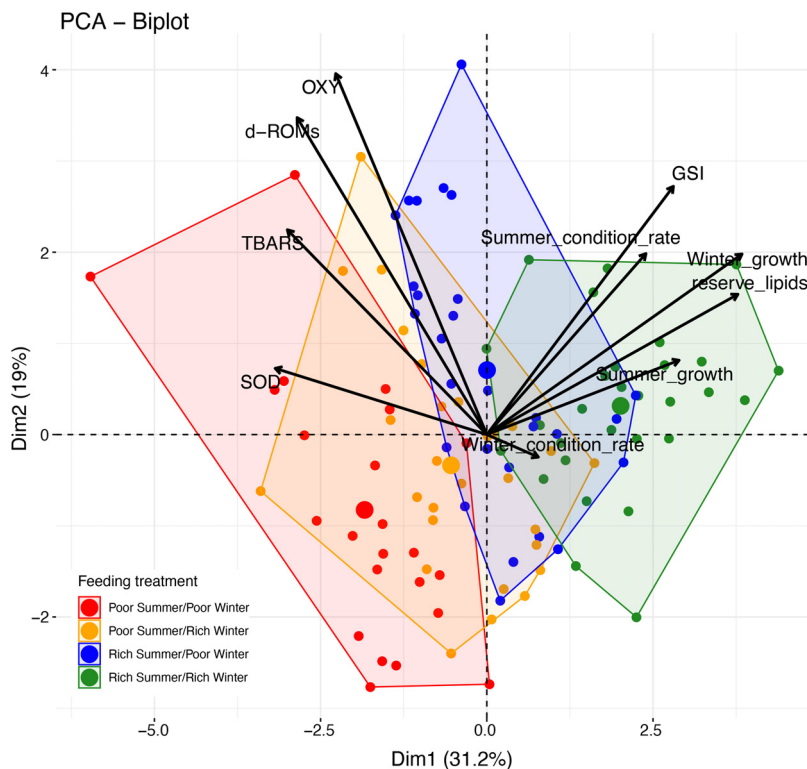

Fig. 5. Biplot of the PCA built with summer and winter growth and condition rates, antioxidant defence (OXY and SOD), oxidative damage (TBARS and ROM), reserve lipids and gonadosomatic index (GSI). Each point represents an individual. The larger circles represent the barycentre of the individuals for a given treatment represented using minimum convex polygons. Note that structural lipids were not included due to their very low variability

The SD in body condition of the Rich Summer/Rich Winter and Rich Summer/Poor Winter treatments decreased throughout the experiment, while SD of the Poor Summer/Rich Winter treatment seemed to stabilize from September and that of the Poor Summer/Poor Winter increased after September. At the end of winter, fish in the rich treatment (Rich Summer/Rich Winter) showed less variance than fish reared in poor feeding conditions throughout the experiment, while intermediate treatments were in between but still significantly different from one another, with Rich Summer/Poor Winter lower than Poor Summer/Rich Winter (Fig. S9b).

3.2.1. Trade-off or individual quality between growth and investment in body condition

Because sardines were monitored repeatedly over time, we investigated the allocation trade-off towards growth vs. body condition every month during winter only according to the initial condition and the treatment (see Eq. 6). As the interaction with treatment was always significant, we conducted a GLMM for each feeding treatment. In all models, the random effect for individuals contributed to an increase of 7 to 18% of R^2. For all feeding treatments during winter, sardines having good body condition at time $t-1$ invested more in body size growth (positive relationship), except for sardines from the Rich Summer/Rich Winter treatment where no significant effect was detected between these 2 traits (Table S5). Results clearly highlighted that regardless of the diet, sardines investing in their body condition invested less in their body size growth, leading to negative relationships between these 2 traits (Fig. 6; Tables S6–S9). Yet, this decrease varied in intensity depending on body condition at $t-1$ for sardines in the Poor Summer/Rich Winter feeding treatment (Fig. 6b, significant interaction; Table S7). It appeared that the larger the body condition ($t-1$), the steeper the decrease in the growth rate (t) with the condition rate (t) (Fig. 6b).
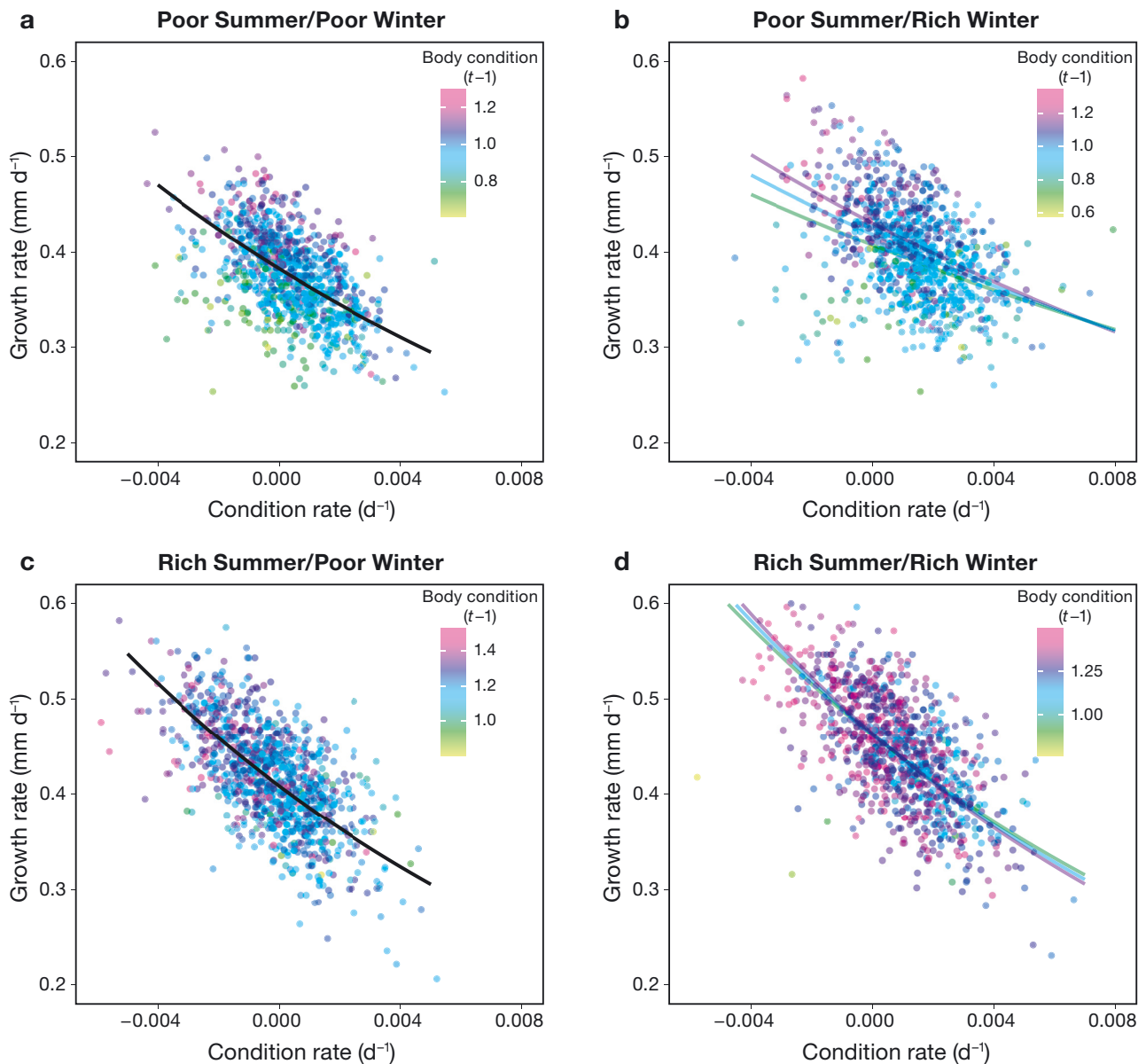
Fig. 6. Relationship between growth and body condition rate for the 4 feeding treatments during winter only: (a) Poor Summer/Poor Winter, (b) Poor Summer/Rich Winter, (c) Rich Summer/Poor Winter and (d) Rich Summer/Rich Winter. When the interaction between condition rate$_{t-1 \rightarrow t}$ and body condition$_{t-1}$ was significant, we presented several prediction lines corresponding to the 25 (blue), 50 (green) and 75% (pink) quantiles of the body condition$_{t-1}$. Where the interaction was not significant, only 1 prediction line (black) is represented, corresponding to the median body condition$_{t-1}$

3.2.2. Trade-off or individual quality between growth, investment in body condition$_{\text{without gonad}}$ and reproduction

We then focussed on the 120 individuals sacrificed at the end of the experiment to study possible trade-offs between investment in growth, body condition independently of the reproductive investment (condition rate$_{\text{without gonad}}$) and reproduction. As above in the previous analyses, the treatment interaction was significant, so that a model was run for each feeding treatment. A GLM suggested that neither energy investment in growth nor in body condition constrained the reproductive investment in the 2 intermediate experimental groups (Tables S11 & S12). On the contrary, sardines from the Poor Summer/Poor Winter treatment displayed a positive relationship between growth and reproductive investment, suggesting that sardines that grew the most also developed the highest GSI (Table S10). Moreover, sardines well-fed

throughout the experiment presented a negative relationship between condition rate and GSI, suggesting that sardines that invested the most in their reproduction allocated less to their body condition (Table S13).

3.2.3. Energy allocation strategies

To investigate potential differences in energy allocation strategies among the 120 individuals sacrificed at the end of the winter, a clustering analysis on growth, condition rate$_{without\ gonad}$ and GSI was performed separately for both sexes (in order to account for the known effect of sex on GSI). Clustering analyses were performed on the 117 individuals for which we had information on sex. The best clustering scheme retained 2 clusters for males (Fig. 7a) and 3 clusters for females (Fig. 7b). The composition in feeding treatment as well as the size at the beginning of the experiment and the maturity of individuals (at the end of the experiment) of each cluster was also assessed.

All 4 feeding treatments were represented in each cluster for both sexes except in the third cluster for females where the Rich Summer/Rich Winter treatment was absent (Figs. S10 & S11). No clear maturity-related pattern was identified, as only 6 of the 120 sardines sacrificed at the end of the experiment were immature. The distributions of initial lengths

(measured in August 2017, at the beginning of the experiment) did not display any significant difference between clusters for both sexes (Fig. S16).

For males, the first cluster (56% of individuals) was characterized by sardines displaying an important allocation to condition rate (1.4 times higher than in the other cluster; LM, p < 0.001, Fig. S12) to the detriment of growth rate and GSI. Individuals in the second cluster (44%) were characterized by low condition rates and higher allocation to the other 2 traits, especially GSI, i.e. GSI was 2 times higher (7.7 ± 0.4%) than in cluster 1 (3.6 ± 0.4%, LM, p < 0.001; Fig. S12). For females, clusters 2 and 3 were quite similar to those identified for males, i.e. a group (39% of the individuals) presenting a high allocation to body condition (1.4 times higher than the other cluster; LM, p < 0.001, Fig. S13) with moderate growth rate and GSI and a group (15% of fish) with low condition rates but higher growth rates and especially GSI (2 to 4 times higher than the other 2 clusters; post hoc Tukey HSD, p < 0.001; Fig. S13). The first cluster (46% of individuals) included sardines allocating preferentially to growth rate with low GSI and body condition rate, but differences remained non-significant with the other cluster (p > 0.05, Fig. S13).

Finally, no significant differences in antioxidant defences and oxidative damage between clusters for males and females were found, except for male cluster 1, which displayed a 1.6 times higher endogenous
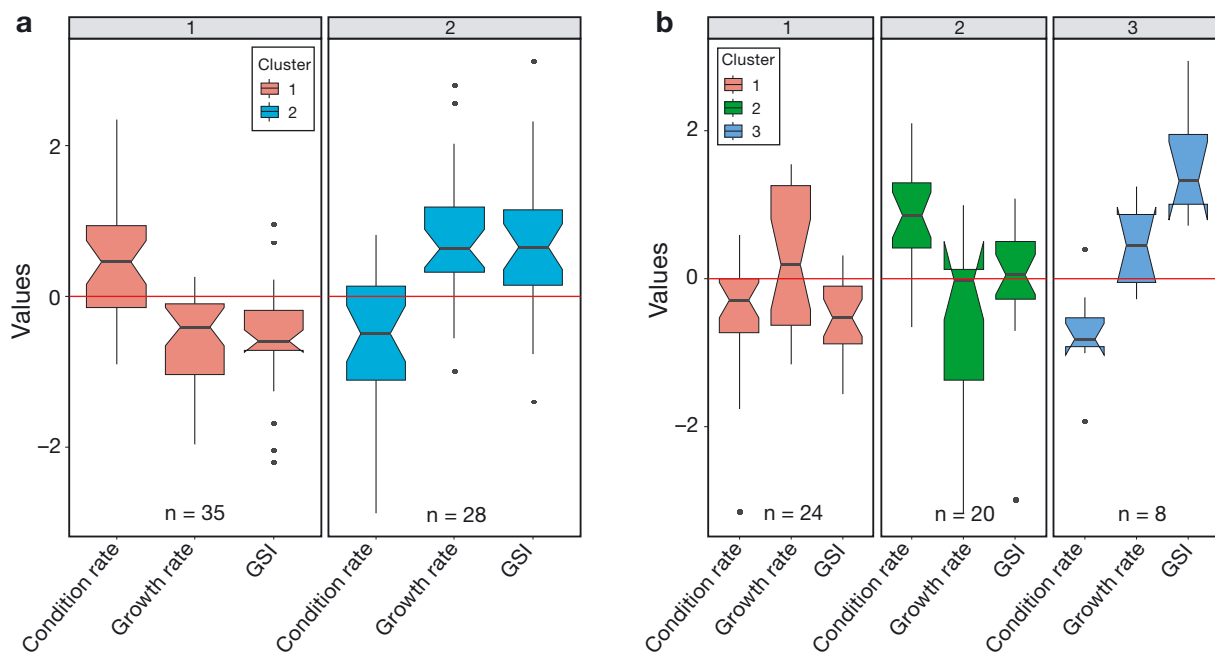

Fig. 7. Normalized values of body condition rate$_{without\ gonad}$, growth rate and gonadosomatic index (GSI) for each cluster for (a) males and (b) females. The number of individuals is indicated under each box plot. Outliers are represented by black dots. Box plot parameters as in Fig. 2

antioxidant SOD level than male cluster 2 (Kruskal test, p < 0.05; Figs. S14 & S15).

4. DISCUSSION

Life-history trajectories reflect how animals manage the allocation of available energy in competing traits such as growth, reproduction and lifespan (McEwen & Wingfield 2003). While such trade-offs are at the core of fitness optimization strategies in a given environment, understanding their intimate mechanistic relationships and plasticity will improve our comprehension of the present and future effects of global change on natural populations. The present study draws on an experimental approach and focusses on the energy allocation trade-offs under various food-manipulated conditions, using north-western Mediterranean sardines as a model, to shed light on the underlying mechanisms involved in the unusual decline in fish size and condition observed in nature (Van Beveren et al. 2014). These decreases are currently thought to be the consequence of bottom-up control, i.e. modification of either size and/or quantity of sardine prey (Brosset et al. 2016a, Saraux et al. 2019). Therefore, we investigated how temporal resource variability (in quantity and size) impacted energy allocation between their main life-history traits using a long-lasting experiment on 972 adult wild sardines. We focussed on 4 questions: (1) When feeding conditions improve, do sardines 'catch up' by exhibiting compensatory growth (Mangel & Munch 2005)? (2) How plastic are sardines in terms of reproductive investment and are sardines strictly capital breeders as often considered (Ganias 2009)? (3) What are the costs in terms of maintenance of different energy allocation strategies in favour of one/some traits over the others (Metcalfe & Monaghan 2001, Mangel & Munch 2005)? (4) How can we explain inter-individual variability in these patterns (reproduction, growth and somatic maintenance) and do fish display different energy allocation strategies (Reid et al. 2010) or does the variability rely exclusively on individual quality and heredity (Marchand & Boisclair 1998, Wilson & Nussey 2010, Bergeron et al. 2011)?

4.1. Compensatory growth

Although compensatory growth has been advocated for some fish (Ali et al. 2003), we were unable to confirm such a process for the Mediterranean sardines, as individuals from the Poor Summer/Rich Winter feeding treatment displayed slower growth rates than sardines in rich conditions throughout the experiment. Similarly to our results, gilthead seabream *Sparus aurata* that were starved for 1 or 2 wk showed no sign of compensatory growth during the following 8 wk re-feeding period (Peres et al. 2011). When sardines went from poor to rich conditions, they appeared to use this new income of energy to rebuild their reserves by increasing their body condition (Poor Summer/Rich Winter had the highest condition rate in winter). Moreover, Rich Summer/Poor Winter sardines were the only ones to exhibit a decrease in body condition during winter, confirming energy limitation during winter. Nonetheless, their growth rates remained higher than those of sardines fed poorly throughout the experiment. Surprisingly, growth rates of fish from Rich Summer/Poor Winter treatment appeared higher than those of fish from Poor Summer/Rich Winter, suggesting the importance of a pre-acquired energy capital to derive energy for growth. This is also supported by the fact that growth rates observed over a month were positively correlated with the body condition at the beginning of that month. Still, when comparing sardines with the same capital (i.e. Rich/Rich with Rich/Poor and Poor/Rich with Poor/Poor), growth was still higher for sardines from rich feeding conditions during winter than sardines in poor conditions during winter, highlighting the additional importance of income energy resources for growth. Altogether, our results indicate that an increase in energy resources might be immediately allocated toward body condition, a fast-adjusted variable, while the effects of feeding conditions on growth might be delayed over time as suggested by Peck et al. (2015), who highlighted prior allocation of food energy to restore body mass in recently re-fed fish. Although we lack replicates before 25 October, which made it impossible to test a potential tank effect during summer, this pattern is in line with results found in the Gulf of Lions for anchovy and sardine, where long-term monitoring for stock management on both species showed a decrease in body condition before a decrease in growth (Van Beveren et al. 2014).

4.2. Plasticity in reproduction

Some processes, such as reproductive investment, seem to only take place after reaching a critical mass/size/body condition (Asher & Cox 2013). In the Mediterranean Sea, sardines are believed to reproduce in winter based on the energy capital they have built in

the previous summer (Ganias 2009). In this study, we indeed confirmed the importance of energy stored during this warmer period of the year, with individuals from the rich treatment in summer displaying high GSI. However, such a statement must be put into perspective, since individuals from the same rich treatment in summer differed according to their winter feeding conditions (GSI in Rich/Rich being higher than GSI in Rich/Poor). Similarly, individuals from intermediate treatments (Rich/Poor and Poor/Rich) displayed similar reproductive investment (GSI), which is not in line with the vision of a strict capital breeder usually attributed to the Mediterranean sardines (Aldebert & Tournier 1971). Thus it appears that the reproductive behaviour of sardines could be more plastic than usually assessed, with individuals being able to take most advantage of resources available during summer and in a respective lower proportion in winter. This was previously suggested by Nunes et al. (2011) for Atlantic sardine from the Portuguese coast and by Ganias (2009) for Atlantic sardine from the eastern Mediterranean Sea. Similarly, Hunter & Leong (1981) showed for the northern anchovy *Engraulis mordax*, another multiple spawning clupeid, that almost 2/3 of reproductive energy expenditure relied on fat reserves accumulated during the previous highly productive season. The remaining 1/3 came from food resources available during the reproductive period. We suggest that such plasticity in energy allocation for reproduction could help sardines to cope with the recent warming trends. Indeed, according to Sommer & Lengfellner (2008), climate change seems to lead to a weaker phytoplankton spring bloom. This will be in favour of planktonic chains of smaller size which would result in poorer feeding resources for sardines at the end of their reproductive period (Feuilloley et al. 2020). Finally, the GSI was systematically higher for males than for females for all feeding treatments at the end of their reproductive period. This difference between sexes has already been reported for Atlantic sardines along the coasts of Morocco (Amenzoui et al. 2006) and Portugal (Nunes et al. 2011). Nevertheless, the underlying processes involved remain unclear and warrant further investigation.

4.3. Cost in term of maintenance

We assessed the potential costs of maintenance, as a result of environmental constraints in resource availability, using oxidative balance as an integrative marker of metabolic costs. Plasma oxidative state was relatively unaffected by the different feeding treatments. Individuals from the treatment with rich feeding conditions in summer and winter presented the lowest oxidative damage and antioxidant defences. This suggests that when feeding conditions were abundant, sardines could preserve a balanced oxidative stress despite displaying high growth rates. In contrast, individuals facing poor feeding conditions during summer and/or winter displayed higher levels of oxidative damages (d-ROMs and TBARS), suggesting that dietary restriction during growth and/or reproduction ultimately led to physiological costs (Monaghan et al. 2009). These results are in line with a recent study showing that sardines in poor feeding condition optimize mitochondrial coupling efficiency (consume less oxygen than sardines in rich feeding conditions to produce a given amount of ATP) which may result in an increase of ROS production (Thoral et al. 2021). In addition, individuals poorly fed in both summer and winter had high concentrations of the antioxidant enzyme SOD at the end of the experiment, which suggests an attempt to compensate for possible deficiencies in exogenous antioxidants by increasing the endogenous (neo-synthetized) antioxidant capacity. These results are consistent with previous work on gilthead seabream, which found significant increases in MDA levels (lipid peroxidation control) and SOD activity (as well as other antioxidant enzymes) as a consequence of food restriction (partial or total, Pascual et al. 2003).

Previous studies suggest that oxidative stress may cause physiological trade-offs with reproduction, so that greater investment in reproduction may reduce the resources available for basic maintenance costs (Ilmonen et al. 2000, Alonso-Alvarez et al. 2004). However, our results indicated that individuals seemed to be able to remain at a physiological optimum regardless of resource availability. Thus, the oxidative balance did not necessarily appear as a marker of cost, but rather as a life-history constraint as suggested by Metcalfe & Alonso-Alvarez (2010) and Stier et al. (2012). However, studies focussing on the associations between body size, energy metabolism, oxidative stress and lifespan suggested that results of intra- and interspecific comparisons are difficult to generalise, and highlight the complex links between these life-history traits (Speakman 2005).

4.4. Individual quality

The PCA approach further highlighted a high degree of individual variability within each treatment,

in terms of metabolic costs (oxidative damage and antioxidant defences) and life-history traits (growth, condition and reproduction investment). Yet, this variability manifested itself differently depending on the feeding treatment. SD of individual size from sardines of the Rich Summer/Rich Winter treatment increased while the SD of their body condition decreased. It appears that they tended towards a common (rather high and not limiting) body condition, but that their structural growth varied greatly from one individual to another due to other factors, such as individual quality (Wilson & Nussey 2010). Conversely, the SD in body condition of individuals poorly fed in summer and winter tended to increase over time (and was higher than the other 3 treatments), indicating that some individuals managed to accumulate more energy reserves over the experiment than others. Nonetheless, the SD in size remained low for this treatment, due to low to no growth. This suggests that when facing poor conditions, individuals could invest little in structural growth, regardless of their body condition.

Altogether, our results tend to advocate for different individual qualities in the Mediterranean sardine population. In theory, individual quality differences in a given environment may result from genetic differences (Metcalfe & Monaghan 2001) and/or different early-life experiences (Costantini et al. 2012), which we unfortunately could not control for in our study. Variability in fish growth is often interpreted as a result of density-dependent processes due to intra-specific competition for a limited resource among individuals of similar age classes (e.g. Lorenzen & Enberg 2002). Indeed, it is often thought that larger individuals have an advantage over smaller individuals in competition due to their higher swimming capacity and consumption capacity (Lundberg & Persson 1993). This in return should also decrease their probability of being predated, hence the importance of growing fast. Still, numerous other explanations can be proposed to explain differences in energy acquisition: (1) the potential inter-individual competition is not only size-dependent, but also linked with individual behavioural differences ('bolder' individuals may have better access to food than 'shy' ones, Réale et al. 2007) and (2) for animals with indeterminate growth, body condition is of greater interest for individual fitness (i.e. enhancing the chances of individual survival and/or future reproduction, Bêty et al. 2003). To distinguish between such hypotheses would require further work, possibly through recording of individual feeding behaviour.

Apart from quality, inter-individual differences can also be interpreted as different energy allocation strategies. In particular, one of the hypotheses to characterise these differences between individuals lies in the differences in the energy pool devoted to reproduction. Our GLM outputs suggest that sardines invested in reproduction regardless of their investment in body condition and growth. These results are in accordance with Brosset et al. (2016b), who highlighted that pelagic fish in nature can maintain high reproductive investment potentially at the expense of other traits, which ultimately might explain the present disappearance of old and large individuals in the Gulf of Lions. Such preferential allocation of energy to gonad maturation was also observed in Atlantic herring *Clupea harengus* in the Baltic Sea (Rajasilta et al. 2015). Furthermore, using a non-supervised classification (clustering approach) in this study, we highlighted 2 or 3 main energy allocation strategies at the individual level, depending on the sex: preferentially allocation toward (1) body condition observed in both sexes (56% of males and 39% of females), (2) growth only for females (46% of females) or (3) investing in both reproduction and growth observed in both sexes (44% of males and 15% of females). Altogether, our results from this cluster analysis highlighted a trade-off between growth and reserve storage, which was in line with the GLMM outputs (negative correlation between growth rate [$t-1 \rightarrow t$] and condition rate [$t-1 \rightarrow t$]). However, some individuals (i.e. those of cluster 2 for males and cluster 3 for females) were capable of investing in both growth and reproduction. Although only 8 out of 52 females were in this cluster, none were from the Rich Summer/Rich Winter treatment, which reinforces the idea that individual quality remains under limiting feeding condition. Moreover, this result suggests that despite different food limitations, some individuals can maintain simultaneous growth and reproduction independently of their initial size. However, it is important to bear in mind the relatively small sampling size, especially for sex determination.

Temperature might also play a role in limiting growth. Indeed, at colder temperatures, sardines might be expected to continue allocating energy in condition and reproduction but not in growth, even if feeding availability increases. Moreover, costs in terms of maintenance and oxidative balance were relatively unaffected between the different strategies. This clearly tends to invalidate the hypothesis of a trade-off between these 2 traits at the individual level, as this has already been stressed at the population level for a number of taxa (see Cam et al. 2002,

Hamel et al. 2009). Altogether, considering the current increasing global change effects on marine regions such as the Gulf of Lions (increasing water temperatures, regime shift to a lower productivity level, changes in the zooplankton community towards smaller sizes) (Brosset et al. 2016a, Saraux et al. 2019, Feuilloley et al. 2020), we might expect an exacerbated imbalance in the energetics of small pelagic fish (increasing cost of feeding behaviour and decreasing energetic value of their food) which may exacerbate the decrease in maximum size of fish (Q. Queiros, D. Mckenzie, S. Killen, G. Dutto, C. Saraux, Q. Schull unpubl.).

5. CONCLUSION

Our results highlighted the impact of seasonal variation in food availability on growth, individual energy reserves and reproduction of Mediterranean sardines. Food resources during the summer period had a major effect on energy reserves and growth, thus limiting the maximum size and body condition achieved at the end of winter. The negative effects of a summer food restriction were still significant during winter. These effects, also known as carry-over effects, have been documented in many species and play a major role in energy allocation trade-offs between growth, maintenance and reproduction (Mangel & Munch 2005, Norris & Marra 2007). In addition, food restrictions during growth and/or the reproductive period resulted in physiological costs through increased oxidative damage, which sardines attempted to compensate by an overexpression of endogenous antioxidant defences (SOD). As increasing temperature promotes weaker planktonic blooms and phytoplankton of smaller size, climate change may accelerate and amplify the phenomenon described above. Mediterranean sardines did not show any compensatory growth, did not appear to be capital breeders sensu stricto and tended to display different individual qualities in their physiological adaptations. As sardines can live over 7 yr in the wild, assessing the long-term costs on individual longevity and subsequent reproductive output remain to be established.

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