Is starvation a cause of overmortality of the Mediterranean sardine?

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Running title: Starvation and mortality in fish

Keywords: fasting, phenotypic plasticity, experiments, natural mortality, body condition, small pelagic fish, sardine, Mediterranean Sea

Abstract

Animal mortality is difficult to observe in marine systems, preventing a mechanistic understanding of major drivers of fish population dynamics. In particular, starvation is known to be a major cause of mortality at larval stages, but adult mortality is often unknown. In this study, we used a laboratory food-deprivation experiment, on wild caught sardine *Sardina pilchardus* from the Gulf of Lions. This population is interesting because mean individual phenotype shifted around 2008, becoming dominated by small, young individuals in poor body condition, a phenomenon that may result from declines in energy availability. Continuous monitoring of body mass loss and metabolic rate in 78 captive food-deprived individuals revealed that sardines could survive for up to 57 days on body reserves. Sardines submitted to long-term caloric restriction prior to food-deprivation displayed adaptive phenotypic plasticity, reducing metabolic energy expenditure and enduring starvation for longer than sardines that had not been calorie-restricted. Overall, entry into critical fasting phase 3 occurred at a body condition of 0.72. Such a degree of leanness has rarely been observed over 34 years of wild population monitoring. Still, the proportion of sardines below this threshold has doubled since 2008 and is maximal in January and February (the peak of the reproductive season), now reaching almost 10% of the population at that time. These results indicate that the demographic changes observed in the wild may result in part from starvation-related adult mortality at the end of the winter reproductive period, despite adaptive plastic responses.

Introduction

Survival is one of the main factors driving population dynamics but it is difficult to assess. This is because it either requires longitudinal monitoring of individuals by capture, mark and recapture, or the ability to find dead individuals if there are extreme events such as massive die-offs (Begon et al., 1996). Aquatic ecosystems pose particular challenges because dead fishes are very rarely found, either sinking to the bottom or being consumed by other animals (but see Griffiths and Kirkwood, 1995). The difficulty of observing the vast underwater realm generally precludes assessment of patterns of mortality and what their main drivers might be. There are a few exceptions, such as when dead fishes wash up along shorelines, enabling researchers to investigate the cause of deaths (e.g. discover the occurrence of pathogens; Whittington et al., 2008)Whittington et al., 2008)Whittington et al., 2008).

As in all animals, mortality in fishes has multiple sources, such as epidemics, predation, starvation or aging. According to life-history strategy, life-history traits such as survival result from a trade-off in energy allocation among maintenance, growth and reproduction (Stearns, 1976; Williams, 1966). Species have evolved life-history strategies to maximise individual fitness, resulting in improved population growth and stability across time (Stearns, 1992), but these may not always be optimal, especially if there are sudden unexpected environmental changes. While massive die-offs often occur in response to an abrupt change and extreme conditions, such as the effect of the so-called 'blob' heat waves on seabird mortality in the Pacific (Jones et al., 2018), less extreme conditions may modify the energy balance of individuals. This may involve an increase in energy demands and expenditure (e.g. an increase in temperature and metabolism, maturation of gonads, etc.) or a decrease in energy resources (e.g. change in food availability or quality). In both cases, energy allocation could be modified, leading to declines in body condition that affect main vital rates and potentially threaten survival. Indeed, starvation is known to disturb the demographics of animal populations, for example penguins (Morgenthaler et al., 2018) or gannets (Grémillet et al., 2016); and even cause mortalities in some cases (Sherman and Runge, 2002). For fishes, reduced food resources are commonly assumed to threaten survival in winter (Hurst, 2007) but, although starvation may be a major driver of mortalities in fish populations, there have been very few attempts to investigate this directly (Dutil and Lambert, 2000). In particular, plankton production and availability for recruitment dynamics of small pelagic fish has been well documented (e.g. in Palomera et al. 2007), but less is known about adult mortality due to starvation.

Sardines are small pelagic planktivorous fish with a worldwide distribution that are known for the profound fluctuations in their populations and their importance as fishery resources (Pikitch et al.,

2012). Alternation of boom and bust periods, with major changes in abundance, has been observed in various upwelling ecosystems (in particular the Eastern-Boundary Upwelling Systems). This is thought to be firstly the result of high and low recruitment rates due to variation in environmental conditions (Checkley, 2009; Field et al., 2009; Gushing and Dickson, 1977; Schwartzlose et al., 1999), although overfishing can also modify the dynamics and accelerate the decline of those populations and generate their collapse (Essington et al., 2015; Lindegren et al., 2013; Toresen and Ostvedt, 2000).

In the North-Western Mediterranean Sea, sardines *Sardina pilchardus* had, for centuries, been a very important fishery. Over the last decade, however, sardine biomass has decreased due to a sharp decline in individual size and mass, although abundance has increased and recruitment is high (see Saraux et al., 2019; Van Beveren et al., 2014 for more details). The decrease in size is primarily related to higher natural mortality of older individuals but this cannot be accounted for by overfishing, predation pressure or pathogens (Queiros et al., 2018; Van Beveren et al., 2016a; Van Beveren et al., 2016b; Van Beveren et al., 2017). Therefore, a bottom-up control of the sardine population, linked to a regime shift in their planktonic prey towards smaller less nutritious species, has been proposed as a mechanism underlying lower growth and body condition (Brosset et al., 2016a; Saraux et al., 2019). Experiments on captive adult sardines showed that body condition, growth and energetic reserves were significantly impacted by both food size and food quantity. Sardines feeding on small items needed to consume twice as much as those feeding on large items to reach the same body condition and growth rate. They also had to feed on large items in large quantity to accumulate lipid reserves (Queiros et al., 2019). Despite the low body condition under some feeding treatments, mortality was too low to test hypotheses of natural mortality from starvation (Queiros et al., 2019). Notably, in the wild, survival of low body condition adults may be especially challenged by energy allocation towards reproduction, which occurs in winter (Brosset et al., 2016b). This is when food availability is lowest, potentially leading to exhaustion of reserves and starvation before spring blooms. Thus, we designed a fasting experiment to gain insight into whether the disappearance of older individuals from sardine populations might be linked to mortality from starvation post-breeding, in late winter.

Individuals with low body reserves before winter find themselves on the razor's edge if they have to cope with prolonged food restrictions, endangering their survival or reproduction (see Boos et al., 2005; Grémillet et al., 2005; Olsson and van der Jeugd, 2002). Thus, a further interrogation concerns the capacity of this species to show adaptive responses to sustained periods of low food resources, to decrease these risks. As previous experiments have suggested that the slower growth and lower condition of sardines in the Gulf of Lions did not result from natural selection, we focussed upon a

role of adaptive phenotypic plasticity (Gienapp et al., 2008; Nussey et al., 2007; Visser, 2008). Such plasticity can either be fixed by exposure to particular environmental conditions during development or can be reset cyclically, for example labile traits linked to spawning periods (Nussey et al., 2007). Phenotypic plasticity in maturation or growth has been documented as a response of marine fish to exploitation (Jorgensen et al., 2007). While it is unlikely that adaptive phenotypic plasticity could compensate for extreme climatic events and prevent massive die-offs (see Pershing et al., 2015), it may play an important role in compensating slower or more predictable changes (Levins, 1968), such as modifications in food resources. Plasticity in the short term would then provide the potential for species to adapt in the medium to long term. Sardines are particularly interesting because they exhibit a marked flexibility in feeding behaviour, using a direct prey capture when food items are large but shifting to a filter-feeding strategy when prey are small (Garrido et al., 2007). Such behavioural flexibility may be linked to other adaptive plastic responses that improve tolerance of poor feeding conditions.

To better understand first the link between sardine adult condition and mortality and to estimate the incidence of death by starvation in wild populations, we used a combination of lab experiments on wild-caught fish maintained in captivity and in situ data. As for birds and mammals, fishes usually undergo 3 different phases of fasting (Bar, 2014) where: phase I is characterized by a rapid decrease in body mass, the use of glycogen reserves and the progressive use of lipids; phase II involves a relatively extended period where body mass loss is relatively low and constant, and lipid reserves are the main energy source, and phase III is when reserves are exhausted so the main energy substrate is structural proteins and rates of body mass loss increase again (Bar, 2014; Cherel and Le Maho, 1985; Cherel et al., 1987; Cherel et al., 1991; Le Maho et al., 1981). Using specific body mass loss (i.e. the rate at which body mass decreases) and respiratory metabolism as indices, we investigated when sardines entered the critical phase III of fasting. This enabled us to evaluate mortality probabilities and sardine physiological states at different body conditions. We were then able to estimate risk of adult mortality in wild populations, based upon their condition over recent years, and also as a function of their annual breeding cycle.

Finally, to investigate whether phenotypic plasticity played a role in affecting the relationship between adult body condition and mortality, we used sardines that had been maintained on four different feeding treatments for seven months prior to the fasting experiment, one mimicking conditions in the wild before 2008, one mimicking the current period after 2008, and two intermediate conditions (see Queiros et al., 2019 and below for more details). If sardines are able to display phenotypic plasticity, individuals that were subjected to caloric restriction during the initial period might cope better with fasting. Alternatively, sardines under caloric restriction in the initial

phase might have suffered physiological costs, which would make them less able to cope with fasting.

Methods

All procedures were in accordance with the French and the EU legislation regarding animal experimentation (APAFIS, Permissions N° 7097-2016093008412692 and N° 10622-2017071711101242).

Sardine provenance

Adult sardines had initially been captured in October 2016 by a dedicated commercial purse seiner off Frontignan (Hérault, South of France) and transported to the IFREMER research station at Palavas-les-Flots for a first feeding experiment (Queiros et al., 2019). To run this previous experiment, sardines had been weaned from live food (*Artemia nauplii*) onto commercial aquaculture pellets and individually marked under anaesthesia (benzocaine at 140 ppm), using a tiny RFID (Radio Frequency Identification) tag (Biolog-id, Bernay, France, 0.03g, i.e. <0.2% of sardine lowest body mass) implanted in the dorsal muscle with a specific injector. 449 sardines had been attributed to one of four feeding treatments (2 tanks per treatment), so that both the mean and coefficient of variance in length and mass were similar between tanks.

The four feeding treatments comprised food particles (commercial pellets) of two different sizes in sardine natural feeding range (0.1mm and 1.2mm, eliciting filtration versus particulate-feeding, respectively) at two different ration levels (0.3% and 0.6% of the biomass in tanks, based on preliminary tests). That is, i) treatment LP-LQ: large food particles in large quantities, ii) LP-SQ: large particles in small quantities, iii) SP-LQ: small food particles in large quantities and iv) SP-SQ: small particles in small quantities. Results of this experiment are presented in Queiros et al., (2019) and showed an effect of both food item size and quantity on sardine growth, condition and physiological state.

Experimental design

After 7 months of experiment (June 15th, 2017), sardines were sampled randomly from the four feeding treatments and assigned to 8 smaller tanks (50L), to start the fasting respirometry experiment. About 150g of sardines were placed per tank but, because of differences in mean body mass of sardines from the four treatments, the number of individuals varied from 8 to 16 among

tanks. Sardines were left to acclimate for 12 days before the experiment started, i.e. time to develop natural swimming and schooling behaviours in the new tanks.

One day prior to the experiment, sardines were measured again (length and mass) and body condition estimated as the Le Cren index (see Brosset et al., 2015):

$$K_n = W / W_{th} \quad [1]$$

where W is the wet mass of the fish in g and $W_{th} = 0.00607 \times TL^{3.057}$ the wet theoretical mass of a fish of a given total length TL in cm.

Similarly to previous results on growth, condition and physiology (Queiros et al., 2019), body condition of the randomly sampled sardines from treatments SP-LQ and LP-SQ were similar but differed from the other two treatments (Fig. S1). Therefore, we had three initial treatments in this experiment: (i) good initial feeding condition (sardines fed on LP-LQ), (ii) intermediate initial feeding condition (sardines fed on SP-LQ and LP-SQ) and (iii) poor initial feeding condition (sardines fed on SP-SQ). Unfortunately, due to a problem in the air system in two tanks, sardines died during one night (1 tank from the LP-LQ and 1 tank from the LP-SQ), the experiment was finally run in 6 tanks (see table 1).

Biometries were performed once a week with all sardines measured individually (tag read, length and body mass recorded) under anaesthesia (benzocaine at 140 ppm). Both body mass and total length were assumed to change linearly in between two biometries such that, when needed, daily values were estimated through interpolation. Tanks were checked at least three times a day for mortality and dead or moribund fish (unable to maintain its balance) were removed on these occasions and immediately measured and weighed. At each biometry, the number and biomass of fish per tank were checked. Whenever the number of fish decreased below 5 in one of the tanks, fish of this tank were transferred to the other tank of the same treatment.

The specific body mass loss, namely the rate at which body mass decreases was calculated as:;

$$dm/mdt = (m_{t1} - m_{t0}) / (m_{t0} \times (t_1 - t_0)) \quad [2]$$

where t_i is the time at i and m_{t_i} is the body mass at time t_i

Tanks were supplied with the same water pumped directly from the sea and filtered through sand (30–40 µm). The photoperiod was adjusted each week to follow the prevailing natural cycle. Sea water temperature was not controlled during the experiment to mimic natural conditions (varied between 19°C and 24°C during the fasting experiment).

Respirometry

The rearing tanks were custom-designed to measure metabolic rate as O_2 uptake by automated stop-flow respirometry (Steffensen, 1989), as previously described in McKenzie et al. (2007), see supplementary material for detailed methods. Measurements were taken continuously throughout the fasting period with a 1h cycle (45min flow/ 15min stop). Oxygen uptake by the fish (MO_2) was then calculated on the stored files using R software and a custom script. The MO_2 was calculated in mg kg^{-1} h^{-1}, from the decline in water O_2 concentration and considering the total volume of water and the total biomass of the fish (McKenzie et al., 2007; Steffensen, 1989). The hourly measures of MO_2 were averaged to provide a measure of metabolic rate for the entire day. Standard metabolic rates represent metabolic costs of maintenance and were estimated as the 10%-quantile of daily measurements per tank for days in which more than 10 measurements were available (Chabot et al., 2016).

Sardines in the wild

To compare our results obtained in tanks with fish in natural conditions, we used a long-term dataset of sardine size (to the nearest mm) and body mass (to the nearest 0.1g), as well as maturity stage (by visual assessment according to (ICES, 2008)). Maturity stages were described on a scale from 1 to 6, with increasing development of gonads in stages 2 to 4, spawning period during stage 5 and post-spawning period during stage 6 and a resting period during stage 1. These sardines had been sampled from PELMED scientific surveys (PELagiques en MEDiterranée, DOI: 10.18142/19) and commercial fisheries in the Gulf of Lions (NW Mediterranean Sea) from 1971 to 1978 and 1993 to 2018. Samples consisted of one crate of fish taken randomly from a pelagic trawl or a purse seine net before any sorting had occurred. Body condition was estimated for all sardines with the Le Cren index ((Brosset et al., 2015), see Equation [1] above). According to previous studies, sardine body condition decreased profoundly in 2008, to remain low since then (Saraux et al., 2019; Van Beveren et al., 2014). As a consequence, data were categorised into two periods: i) the past, being all data collected before 2008, i.e. 6764 sardines, and ii) the present, being all data collected since 2008, i.e. 14,668 sardines.

Statistical analyses

All statistical analyses were performed with R v.3.5.0 (R Core Team, 2018). Values are given in the text as mean ± SE, and statistical tests were considered significant at $p < 0.05$. When data were not

independent from each other due to repetitions within individuals (e.g. body condition over time), a mixed model was used (either linear mixed model (LMM) or generalized linear mixed model (GLMM), depending on the distribution of the data) with the individual effect set as a random intercept. Number of observations (n) and number of individuals (N) are reported. Model selection was performed by Akaike's information Criterion (AIC) and the most parsimonious model was retained when a difference in AIC was less than 2 (Burnham and Anderson, 1998). Survival analyses were performed based on the distribution of survival times using the Cox proportional-hazards regression model (Cox, 1972). Treatments or maturity stages were compared using Wilcoxon tests, as normality was violated. When multiple testing was performed (comparison between treatments, etc.), a Bonferroni correction was used (Legendre and Legendre, 2012). Finally, whenever appropriate, breakpoints in the data were identified using the "segmented" package in R (Mugeo, 2008). Residuals of all models (LM, LMM and segmented regressions) were checked and both ANOVA tables and diagnostic plots are presented in supplementary material. Whenever, the distribution of the residuals was not normal and/or included too many outliers, additional analyses were performed to test for the robustness of the results. First, data were transformed using a monotonous positive function through the BoxCox transformation, i.e. . Normality of the residuals was checked again and if validated the results of both models (on raw and transformed data) were compared. If the normality of the residuals was still violated, we performed the model removing outliers, so as to ensure a better residual distribution. Again, the results of both models (all raw data and raw data without outliers) were compared. If results were similar, we present results and figures with raw data in the main text, as we had no biological reason to remove outliers and raw data are easier to read than transformed ones. However, results of the second more statistically appropriate models are mentioned in the main text and fully detailed in the supplementary material. In one analysis, the normality of the residuals seemed violated due to the individual random effect (close to normal residuals when we used a linear model, but non-normal residuals when adding the individual random effect). In that case, a second analysis was performed by running separate linear models, one per individual, and the distributions of the estimated slopes were then used to test for the robustness of the results of the linear mixed model.

Results

Changes in body condition

Body condition of sardines decreased through time (number of fasting days) for the three treatments (Fig. 2A). When modeling body condition through time using only individuals measured at least three

times, the number of fasting days, the treatment and their interaction were all retained in the best model (as selected by AIC, LMM, n = 289, N = 78). So, the decrease in body condition throughout the fasting period varied among treatments and the rate of decrease was also treatment dependent, as indicated by the significant interaction between treatment and fasting days (Fig. 1 ; Table S1). The rate of decrease in body condition was higher in sardines in good initial feeding condition (-0.008 ± 0.000 per day) than in sardines in intermediate or poor condition (-0.006 ± 0.000 per day for both cases). Because the normality of model residuals was not verified (Fig. S3), we also ran a second set of analyses to check the robustness of these results. Estimating the decrease in condition for each individual through a linear model (for individuals with at least 3 measurements), we found very similar results, with a higher decrease in body condition for sardines initially in good body condition (-0.009 ± 0.000) compared to sardines initially in intermediate or poor condition (-0.006 ± 0.000, Fig. S4; note that the distributions of the residuals of these linear models were in general satisfactory).

Survival analyses

During the experiment, sardines died between day 1 and day 57 (Fig. S5). The cumulative death rate shows that a quarter of the sardines died after 2 weeks, half after 3 weeks and 90% after 50 days of fasting. The first mortality event occurred at a body condition of 0.84 for sardines in good initial feeding condition and slightly later for sardines in intermediate or poor initial condition (0.77; Fig. S6).

The patterns of survival against body condition were similar among the three treatments, but the good initial condition was clearly shifted (to the right) along the x-axis (Fig. 2A). The survival analysis indicated that the one-week survival probability of further fasting was affected by body condition and the treatment the sardine originated from (GLMM binomial, n=313, N=78, Fig. 2A, Table S2). Survival decreased with decreasing body condition, and was lower in sardines in good initial feeding condition than in sardines in intermediate or poor initial condition (Fig. 2A). The probability of surviving one week of further fasting was steady while body condition remained higher than 0.90 for fish in good initial condition and higher than 0.75 for fish in intermediate or in poor initial condition. Past these thresholds, this probability decreased rapidly reaching 50% at 0.80 for fish in good initial condition and at around 0.65 for both fish in intermediate and poor initial condition (Fig. 2B).

The mean duration for which sardines were able to sustain fasting was higher for animals in good or intermediate initial condition (31.5 ± 4.3 d and 32.8 ± 2.5 d) compared to those in poor initial condition (16.3 ± 1.8 d, see Fig. 3A). The odds of dying were higher for sardines in poor initial condition (Cox survival regression: n = 78, odds-ratio = 2.88 [1.30-6.41], P = 0.009) compared to sardines in good initial condition, while they did not differ between sardines in intermediate and

good initial condition (odds-ratio = 0.71 [0.33-1.54], P = 0.384). When initial body condition was included in this model, we found a negative effect of initial condition on the odds of dying (odds-ratio of scaled condition = 0.14 [0.07-0.27], P < 0.001), i.e. sardines with a higher initial body condition (e.g. an increase of 1SD, i.e. + 0.11 here) had a decreased daily survival (by 86% in this example, Fig. 3B). Interestingly, the difference between initial treatments differed once initial body condition was added in the model. The odds of dying were then lower in sardines of poor and intermediate initial feeding conditions compared to those of good initial feeding conditions (odds ratio = 0.02 [0.00-0.12] and 0.04 [0.01-0.13], respectively, both P < 0.001).

Changes in body mass loss

The specific body mass loss displayed an abrupt increase a few days before death for all the sardines (Fig. 4). To detect inflexion point(s), we applied a segmented regression model, using body mass loss as the dependent variable. The best model selected by AIC comprised two breakpoints at 10 and 2 days before death (ΔAIC = -670 with simple regression with no breakpoint and ΔAIC = -87 with segmented regression model including 1 breakpoint, see Table S3 for an ANOVA table of the selected model). More precisely, during their first period of fasting, body mass loss was fairly low around 0.80 ± 0.00 % of body mass per day (Fig. 4). Ten days before death, this rate started increasing by 0.1% point per day until two days before death, when specific body mass loss increased very sharply to reach a mean body mass loss of 3.59% ± 0.36% the day before death (Fig. 4). When looking separately at each treatment, two breakpoints were also always found: two days before death for all treatments and 16 days before death for the intermediate treatment, 9 and 8 days before death for the poor and good treatment respectively (Fig. S7).

The specific body mass loss was relatively low and stable (0.84 ± 0.02 % of body mass per day) for sardine body conditions greater than 0.75, but it rapidly increased at lower body conditions (Fig. 5). When applying a segmented regression between body condition and specific body mass loss, the best model selected by AIC retained one breakpoint at around 0.72 (ΔAIC = -157 with simple regression with no breakpoint and ΔAIC = -2.5 with segmented regression model including 2 breakpoints, see Table S4 for an ANOVA table of the selected model and Fig. S8). Yet, the residuals of this model presented a very skewed distribution (Fig. S9 – *All data*), which was barely improved by data transformation. Rather, this seemed due to a distribution of specific body mass loss with a lot of high values (Fig. S10). To assess the robustness of the breakpoints, we ran 2 additional analyses based on the distribution of specific body mass loss: (a) using only data lower than 4 (thus discarding extreme values) and (b) using only data lower than 2 (keeping data within the first and main mode of

distribution from Fig. S10). The normality of the residuals clearly improved in these two analyses (Fig. S9) and the values of the breakpoint remained quite stable: (a) 0.72 ± 0.01 and (b) 0.74 ± 0.01.

Changes in metabolic rates

We then looked at the metabolic rates (per unit mass), as quantified through O_2 uptake during the first 10 days of the experiment. These metabolic rates varied depending on the initial feeding condition of the sardines (Fig. 6). Notably, sardines in poor condition had a lower metabolic rate than sardines in good or intermediate condition (P = 0.019 and P = 0.022 respectively, Bonferroni-corrected Wilcoxon tests). Standard metabolic rates (estimated as the lowest daily 10% quantile and representing mostly maintenance metabolism) did not differ among treatments (P > 0.126, Bonferroni-corrected Wilcoxon tests; Fig. 6). However, the difference between mean daily respiration rate and daily standard respiration rate was significantly lower in sardines in intermediate or poor initial feeding condition than in those in good initial condition (P < 0.001 for both, Bonferroni-corrected Wilcoxon tests; Fig. 6).

According to segmented linear regression on all raw data, metabolic rate increased strongly when sardine mean body condition decreased below 0.64 ± 0.01, while it was constant above this body condition (305.5 ± 4.7 mg $O_2.h^{-1}.kg^{-1}$; Fig. 7, Table S5). However, the diagnostic plots of this model revealed some patterns in the residuals (Fig. S11). Running the segmented model on transformed respiration data (using a BoxCox transformation of -0.71) allowed removal of most of the patterns in the residuals (Fig. S11), while leading to the same result with a breakpoint at 0.64 ± 0.01 (Table S6), below which the transformed respiration increased strongly. Interestingly, the breakpoint obtained on raw data was similar in sardines in poor or intermediate initial condition (0.63 ± 0.02 and 0.65 ± 0.01 respectively), but was much higher in sardines in good initial condition (0.78 ± 0.04) (Table S5). Similar results were obtained when using BoxCox transformed data to improve the normality of the residuals (Fig. S11), with a critical body condition detected at 0.64 ± 0.03, 0.65 ± 0.01 and 0.79 ± 0.04 for sardines in poor, intermediate and good initial conditions respectively (see Table S6).

Body condition in the wild

Sardine body condition in the wild was significantly higher in the past (i.e. before 2008) than in the present (1.12 ± 0.00 vs. 0.98 ± 0.00, Wilcoxon test, P < 0.001, n = 6764/14668 respectively, Fig. S12). Further, body condition varied among months, peaking in spring/summer (as well as in early autumn for the past period only) and reaching its lowest level in December, January and February for both periods (Fig. S12). Finally, body condition decreased with maturity stages in both periods (Fig. S13, Bonferroni-corrected Wilcoxon tests). While it decreased almost linearly with maturity stages in the

present, the contrast in body condition appeared mainly to be between maturity stages 5 and 6 (i.e. during or post-spawning) and among the first four maturity stages in the past (despite no significant differences in some cases due to very low sample size in some maturity stages).

When comparing against the critical body condition defined in our fasting experiments, only 0.1% of the sardines sampled before 2008 were below the 0.65 threshold that appeared critical for 1-week survival, and only 0.2% of the sardines sampled since 2008. The occurrence of sardines below the second body condition threshold (i.e. 0.72 which corresponds to the entry into phase 3 fasting according to body mass loss, Fig 5) also appeared rather low, although it almost doubled when comparing the present to the past (2.3% vs. 1.2%; Fig. 8). The occurrences were not, however, evenly distributed among months, being more probable during winter, especially in the present period, where they reached 6 and 9 % of the population in January and February, respectively (Fig. 8).

Discussion

Although there has been quite significant research focus on starvation as a cause of mortality in larval and juvenile fishes (Hurst, 2007), studies on adult fishes are much rarer (Dutil and Lambert, 2000; Lambert and Dutil, 1997). As mortality is complicated to observe in marine fish populations, we used experiments in tanks to investigate the extent to which wild adult sardines might be at risk of death from starvation. We used measurements on individuals and treatment groups to study the physiological and behavioural consequences of caloric restriction. Most of our conclusions on the effects of fasting on sardines were based on measurements of individual sardines within the treatment groups. The exception was oxygen consumption, which was measured on entire tanks because individual measurements are not feasible in this species. Due to the unfortunate failure of the oxygen supply prior to the fasting experiment, we lost one tank of sardines in initial good condition, so could only collect oxygen consumption throughout fasting on a single replicate for this treatment. There were, however, no tank effects on fish condition or respirometry in the other two treatments, and we also found no tanks effects on growth, condition, body composition and oxidative stress in a previous long-term study on sardines (Queiros et al., 2019). The conclusions regarding how starvation affected metabolic rate were also based upon common patterns in six tanks, irrespective of their initial treatment. Further, we performed additional analyses when some statements about model assumption were not verified (e.g. normality of residuals), which confirmed and ensured the robustness of our results.

The results revealed that sardines were able to survive fasting for extended periods (up to 57 days under our conditions) and to reach very low body condition before they risked mortality from starvation depending on their initial condition. It is well known that fishes can survive extended periods of food deprivation, although the actual duration varies among species, life stage and water temperature (McCue, 2010; Navarro and Gutiérrez, 1995; Wang et al., 2006). In our study, survival in sardines initially in intermediate and poor conditions remained high until a Le Cren's body condition of 0.75 (i.e. 25% lower than the global average from long-term measurements on wild populations). When fish condition decreased below this, survival dropped rapidly, to only 50% at a body condition of 0.65. For fish in good initial condition, however, 50% survival was reached when body condition decreased below a Le Cren's factor of 0.8.

The experimental approach was able to reveal when the sardines started relying on energetic protein substrates as the main fuel for metabolism (contrary to high energetic lipid substrate). That is, specific body mass loss and mass-specific metabolic rates increased markedly below a certain body condition, enabling us to define a critical threshold that indicated when sardines entered into phase III of fasting. Despite clear thresholds for mean population responses (see for instance Fig. 5), there was significant variation among individuals, especially when they were at very low condition. Such inter-individual differences might reveal the importance of individual quality in sardine physiological responses to starvation and survival, despite individual quality being debated as a concept and terminology (Bergeron et al., 2011; Wilson and Nussey, 2010). Individuals within fish species are known to exhibit wide variation in their tolerance of feed deprivation, and this can have both a physiological and behavioural basis (Auer et al., 2016; McKenzie et al., 2014; Norin and Metcalfe, 2019; O'Connor et al., 2000). Thus, further studies are required to elucidate the physiological or behavioural correlates of the individual responses to a fasting challenge in sardines. In the current study, the critical threshold for entry into phase III of fasting was much more accurately defined by the specific body mass loss (estimation at the individual level) than by the metabolic rate (estimation at the tank level). That is, although individual fish in a given tank derived from the same initial feeding condition, their body condition varied at the beginning of the experiment (Table 1). Further, rates of mass loss and metabolism both sped up during phase III of fasting, leading to death in about 8 days. Fish transitioned from phase II to phase III over a short period, such that a mixture of fish in the different fasting phases were present in a given tank at a given time, contributing to the group's overall metabolic rate. Nonetheless, the tank respirometry clearly indicates that entry into phase III of starvation was associated with a marked increase in metabolic rate by the sardines, in all treatment groups. It is not known why this occurred; it may have reflected the lower energetic efficiency of using proteins as a main fuel compared to lipids (Schmidt-Nielsen, 1997) or a desperate

increase in activity in search of food (the so-called refeeding signal described in birds and mammals; (Groscolas et al., 2000; Koubi et al., 1991; Robin et al., 1998; Spée et al., 2010), which would obviously have exacerbated the rate of mass loss.

When comparing our overall critical condition threshold to in situ values observed over the years, the proportion of the population sampled in nature that was below the critical condition of entry in phase III of fasting was minimal (≤ 2%). Moreover, almost none of the fish sampled in-situ exhibited the low body condition with a 50% probability of surviving a week (i.e. 0.65). Such a result is very similar to that obtained in Atlantic cod (Dutil and Lambert, 2000) but needs to be taken cautiously, as sardines of low body condition might be excluded from schools or might already have died for other reasons linked to a weakened physiological state. Indeed, low body condition can seriously impair fish swimming activity (Faria et al., 2011; Martinez, 2003). As a consequence, starving sardines might not be able to sustain the continuous aerobic swimming required to follow the school and might get isolated. Poor swimming performance, especially burst swimming capacity (Martínez et al., 2004) would render them prone to predation, also due to the absence of the dilution effect that is gained by being in a school (Lehtonen and Jaatinen, 2016; Rieucau et al., 2014). This suggests that our estimates of survival in tanks might represent a maximum potential of sardines to resist fasting and that it is likely that mortality would occur earlier in the wild. Indeed, the fasted sardines were very weak during the experiment, as attested by the increase in death rate during and after biometries (Fig. S5), which had never occurred in other experiments where sardines were fed (QQ, CS, EG, GD pers. comm.). Apart from handling once a week, our fish were subjected to no other stress, with no predation, pathogens, etc. Nonetheless, such results need to be taken cautiously as mortality might result from a combination of weakness of sardines and handling. Further, we examined the absolute physiological thresholds of starvation; sardines most likely would not have to cope with such severe food deprivation for such a long period in the wild. Thus, further studies may examine variations in physiological thresholds as a function of different levels of food restriction (e.g. Hill et al., 1984; Hill et al., 1985). Nevertheless, the proportion of Gulf of Lions sardines that were below the critical condition of entry in phase III of fasting (i.e. 0.72) was about twice as high in the present period (2.3%), after the changes in population condition and age structure were observed (Saraux et al., 2019; Van Beveren et al., 2014), than in the past (1.2%). Additionally, sardines were fed with the same food levels during 7 months prior to the fasting experiment and may have developed plastic responses to their feeding conditions. However, resources and food quantity in particular is known to fluctuate over time making adaptation more complicated in the wild. Further, sardines are known to be capital breeders, i.e. they have to increase their energy reserves during summer to sustain reproduction during winter, when food resources decline (Ganias, 2009; McBride et al., 2015). This

460 reproductive strategy leads to an increase in body conditions before winter (Fig. 8), which might raise
461 the critical body condition of entry into phase III and also decrease the probability of surviving a one
462 week of fasting (see Fig. S6 and Fig. 2 for 'good conditions', respectively). Interestingly, characteristics
463 of sardine populations have changed over the last decade (e.g. decrease of length at first maturity)
464 whereas the gonadosomatic index has increased (see Fig. 4 in Brosset et al. 2016b) suggesting higher
465 reproductive investment despite the decrease in body condition. While potential down regulation of
466 the reproduction through skipped spawning remains unknown for this species (but see Kennedy et
467 al., 2010 on herring), such investment toward reproduction despite low energy reserves was also
468 supported by a rather low atresia prevalence and intensity (Brosset et al. 2016b). This could result
469 from a change in energy allocation between the main functions, as a cause or consequence of higher
470 adult mortality (Brosset et al 2016b). Further, when looking at the monthly values, the critical
471 condition of entry into phase III occurred mostly in January/February (reaching 9%), which
472 corresponds to the end of the spawning season for sardines and also to the coldest period. The fact
473 that, among maturity stages, the proportion of body condition below the critical threshold was
474 highest in stage 6, post-spawning, suggests that a depletion of reserves partly due to reproduction
475 remains a valid hypothesis (Brosset et al., 2016b). The plasticity of the digestive tract in the context
476 of caloric restriction (e.g. atrophy or downregulation of function (Secor and Carey, 2016; Zaldúa and
477 Naya, 2014)) might not allow fish to cope with energy reserve depletion despite eruption of
478 planktonic blooms in spring. While we cannot derive actual mortality estimates directly from this
479 study, the results confirm that mortality might have increased after 2008 and be primarily in winter
480 at the end of reproduction.

481 Additionally, sardines that were in intermediate or poor feeding condition at the outset displayed a
482 much stronger resistance to fasting than fish that were in good feeding condition. This not only
483 shows that extended caloric restriction promotes subsequent tolerance of fasting (McCue et al.,
484 2017) but, most of all, it clearly demonstrates a plastic response by the fish to their environmental
485 conditions. All sardines were collected from the wild and our assignment to the different treatments
486 ensured that they were in similar body condition before starting the feeding trials described in
487 experiment (Queiros et al., 2019). Thus, it seems extremely unlikely that the differences in fasting
488 tolerance among the treatments in the current study could derive from genetic selection or early
489 environment exposure. They were more likely a plastic response at the adult stage (McCue et al.,
490 2017). The fact that body condition did not drop as fast in sardines that had been maintained for an
491 extended period in a state of caloric restriction presumably reflects differences in energy
492 expenditure. This is borne out by the tank respirometry. At the start of the experiment (1st 10 days),
493 daily metabolic rates were lower in sardines in poor initial feeding condition than in the other two

494 treatments (Fig. 6). It is well established that fish reduce their metabolic expenditure when exposed
495 to caloric restriction or extended fasting and that the two main strategies are a reduction in
496 spontaneous activity and a reduction in basal metabolism (Auer et al., 2016; McKenzie et al., 2014;
497 Norin and Metcalfe, 2019). These two processes are not mutually exclusive (Auer et al., 2016). While
498 further work is required to establish the relative contributions of behaviour and physiology, in
499 particular video tracking to quantify activity and studies of metabolism from the whole animal down
500 to the cell and mitochondrion (Auer et al., 2016), the tank respirometry provides some preliminary
501 insights. If we assume that the lowest 10%-quantile rate of O_2 uptake each day was an estimate of
502 standard metabolic rate (Chabot et al., 2016), this indicates that costs of maintenance were similar
503 among treatments. When these daily values were, however, subtracted from daily mean metabolic
504 rate, the difference was significantly higher in the fish in good initial condition, compared to the
505 other two treatments. This difference in metabolic rate, of the fasting fish, might represent in large
506 part costs of spontaneous swimming activity, perhaps exploratory foraging (Auer et al., 2016). This
507 indicates that the fish that had been exposed to caloric restriction had developed an adaptive
508 behavioural plasticity to reduce their energy expenditure. This energy conservation strategy is widely
509 known to be adopted by different taxonomic groups to cope with food restrictions (see Hill et al.,
510 1984; Lees et al., 2010; Secor and Carey, 2016; Stokkan, 1992; Zaldúa and Naya, 2014). The fact that
511 fish in good initial feeding condition, with no history of caloric restriction, were not able to engage
512 this adaptive response indicates that such behavioural plasticity is not instantaneous. One potential
513 explanation for this is that the responses to caloric conditions are under neuro-hormonal or
514 endocrine control, and require some weeks or months to be expressed (Secor and Carey, 2016).

515 Understanding fluctuations in mortality is essential for the management of exploited species, but the
516 estimation of natural mortality in stock assessment models often remains restricted to predation
517 mortality and modelled either as a constant or as a function of size (Gislason et al., 2010), despite
518 evidence that natural mortality can significantly vary according to size and growth (Gislason et al.,
519 2010; Lorenzen, 1996) or density dependent processes (Fromentin et al., 2001). Here, our
520 experimental results showed that mortality increases in fish with low body condition, suggesting that
521 adult natural mortality from starvation may occur in the wild mainly in winter of recent years (as the
522 proportion of the natural population below critical body condition threshold is much higher in
523 January/February and doubled in recent years). Such a result is also of interest for fisheries science,
524 as misspecification of M in stock assessment models is known to lead to biased estimates of fisheries
525 reference points (Gislason et al., 2010; Johnson et al., 2014; Punt et al. 2021). A recent study on
526 Atlantic cod *Gadus morhua* found, for instance, that using a variable body-condition dependent M
527 instead of a constant one can result in drastic changes in stock diagnosis (up to 40% differences in

spawning stock biomass (SSB), F and recruitment; (Casini et al., 2016). In the case of sardines in the Gulf of Lions, the present situation of limited age classes 0-2 prevents us for using an analytical model and assess the importance of a variable M. Still, our study also suggests that body condition, a fairly easy variable to monitor (Brosset et al., 2015), could provide a reliable indicator of a stock health status, that could be widely applied. This could support management of data limited stocks, where complete stock assessments are not possible, but also provide a basis for dynamic adaptive management. Management is often conducted with a delay compared to the current state of a stock, due to the time needed to collect the data, perform stock assessments, decide upon management measures and, finally, implement them (Cochrane and Garcia, 2009). By explicitly showing when the population is the most sensitive and the most at risk within the year, body condition could thus provide rapid updated information on the stock to managers for them to engage in adaptive measures, such as individual quotas combined with differential temporal allocation of fisheries effort within the year or temporal closures (see e.g. (Hobday et al., 2010; Melnychuk et al., 2013).

In conclusion, we showed here that adult sardines were highly resistant to fasting when maintained in tanks free of predation or pathogens. We showed that, when previously maintained under caloric restriction, sardines could display significant behavioural plasticity that improved their ability to tolerate fasting, by reducing rates of body mass loss and so increasing their survival. Experimental measurements of specific body mass loss and metabolic rates enabled us to define a critical threshold of body condition, which we could then relate to in situ measurements on wild populations. The combination of laboratory and field data therefore reveals that death from starvation is probably not a major factor in determining the mortality of sardines in the Mediterranean Sea during most of the year, but mortality after spawning has increased in the last decade becoming as high as 9%. Overall, death from starvation may be a significant driver of marine fish population dynamics, especially if energy availability drops in marine ecosystems as a result of ongoing global change.

Acknowledgements

The authors are grateful to the captain and the crew of RV ‘L’Europe’, as well as all the scientists on board for their assistance during PELMED (PELagiques en MEDiterranée, doi: 10.18142/19) surveys. We especially thank all other fishing crews who contributed to the collection of samples at sea. Finally, we wish to thank Camille Huiban for her work in the initial experiment and François Ruelle for his help in the tagging procedure. The authors are also grateful to two anonymous reviewers who greatly helped to improve our presentation.

Authors contribution

CS, PB, DM conceived the idea; CS, QQ, EG, GD, DM, JMF designed the study; CS, QQ, GD, EG, AM, DM collected the data; CS, QQ, JMF analysed the data; CS, QQ, DM wrote the manuscript; all authors contributed critically to the drafts and gave final approval for publication.

Funding

This study was performed within the MONALISA project, financed by the European Union and the French Ministry of Agriculture through the European Maritime and Fisheries Fund (EMFF) as well as the France Filière Pêche. QQ acknowledges doctoral fellowship support from the French Ministry of Higher Education, Research and Innovation. PELMED surveys are co-funded by Europe through the Data Collection Framework. PB was supported by ISblue project, Interdisciplinary graduate school for the blue planet (ANR-17-EURE-0015) and co-funded by a grant from the French government under the program "Investissements d'Avenir", and by a grant from the Regional Council of Brittany (SAD programme).

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Figures

Fig. 1: Mean ± SE body condition per week along the fasting experiment for each of the three initial feeding condition treatments (absence of standard errors is due to a single survivor in the given treatment).

Fig. 2: Probability of surviving one week of fasting according to body condition. A) Empirical data are shown as 0 and 1 survival points, while lines represent the probability to survive predicted by the model according to their initial feeding conditions. B) Mean empirical probability of surviving one week of fasting according to bins of body condition. The lines represent the 95% confidence interval associated with this empirical probability (according to a Bernouilli distribution). Colour indicates the three initial feeding condition treatments.

Fig. 3: Survival probability of sardines across the fasting experiment according to their initial feeding treatment: A. uncorrected, as predicted by the cox survival model and B. corrected by the initial fish body condition, as predicted by the cox survival model including initial fish body condition as a covariate. Colour indicates the feeding treatment sardines originated from and dotted vertical lines indicate the number of fasting days at which survival equalled 50%.

Fig. 4: Mean ± SE specific body mass loss (, i.e. the rate at which body mass decreases) per week along fasting experiment. As individuals died at different time in the experiment, the number of days has been estimated relative to death. The specific body mass loss is expressed as %. The vertical dashed line shows a rupture in the slope.

Fig. 5: Mean ± SE specific body mass loss () according to bins of body condition. The specific body mass loss is expressed as %.

Fig. 6: Respiration rates (in mg $O_2.h^{-1}.kg^{-1}$) during the first 10 days of the experiment according to the feeding conditions sardines encountered before the start of the experiment. A) mean daily respiration rate, B) standard respiration rate (as estimated by the lowest 10% quantile and representing mostly maintenance metabolism), C) 'Activity' related respiration rate (as estimated by the difference between the daily mean and minimum values). Boxes sharing common letters are not significantly different from each other according to Bonferroni-corrected Wilcoxon tests.

Fig. 7: Mean daily metabolic rate (expressed in mg $O_2.h^{-1}.kg^{-1}$) of sardines in a given tank as a function of mean body condition of sardines in that tank that day. Colour indicates the treatment sardines originated from. The segmented regressions are indicated by the black line and the 95% confidence intervals with dashed lines. The

breakpoint along with its 95% CI is also indicated at the bottom of the figure in black. Breakpoints and their 95% CI estimated for each treatment are indicated at the bottom of the figure in colour.

Fig. 8: Distribution of body condition of sardines sampled in the wild before (in blue, upper panel) or after 2008 (in red, lower panel) for each month of the year. Horizontal dashed lines indicate the threshold of body condition corresponding to an entry in phase 3 of fasting. The percentage of the population below this critical threshold of body condition each month is indicated at the bottom of each panel.

Table 1 – Mean ± SE body mass, length and body condition of sardines, as well as the number of fish and tanks used per initial feeding conditions at the start of the experiment.

Treatment	Body mass (g)	Length (mm)	Body condition	Nb of fish	Nb of tanks
Initial good feeding condition (LP-LQ)	19.31 ± 1.30	138.0 ± 2.9	1.03 ± 0.02	8	1
Initial intermediate feeding condition (LP-SQ & SP-LQ)	12.46 ± 0.38	127.0 ± 1.2	0.85 ± 0.01	39	3
Initial poor feeding condition (SP-SQ)	9.84 ± 0.37	125. 1 ± 1.5	0.71 ± 0.01	31	2

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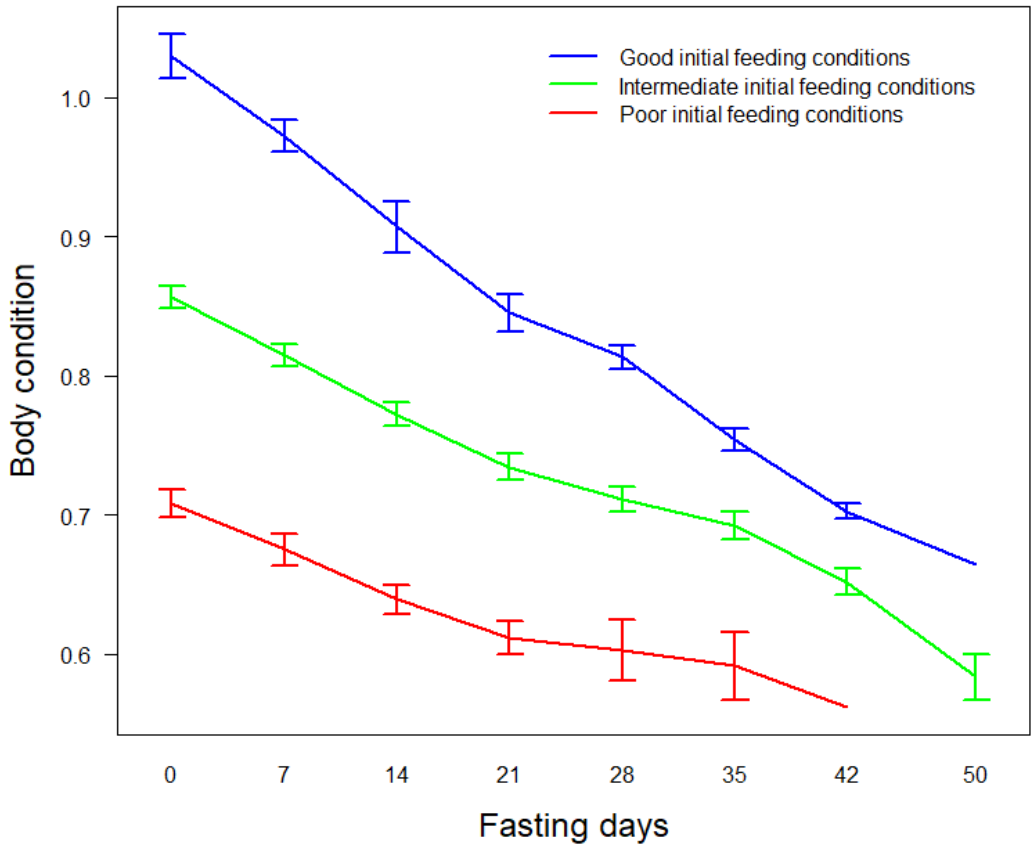

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893 Figure 1.

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895 Figure 2

Figure 3

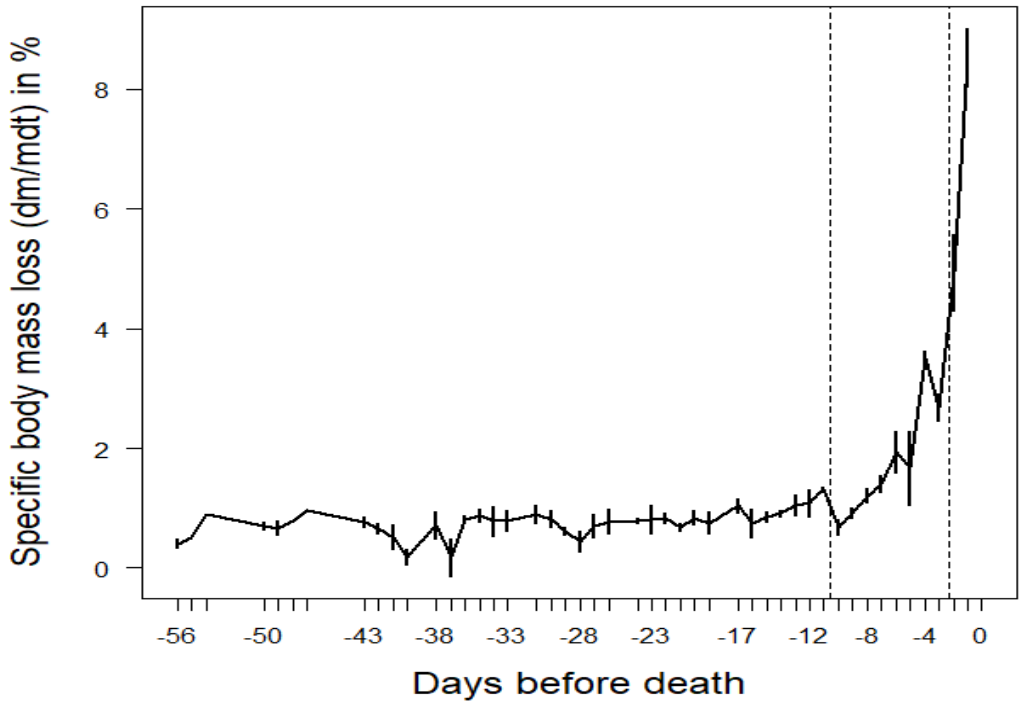
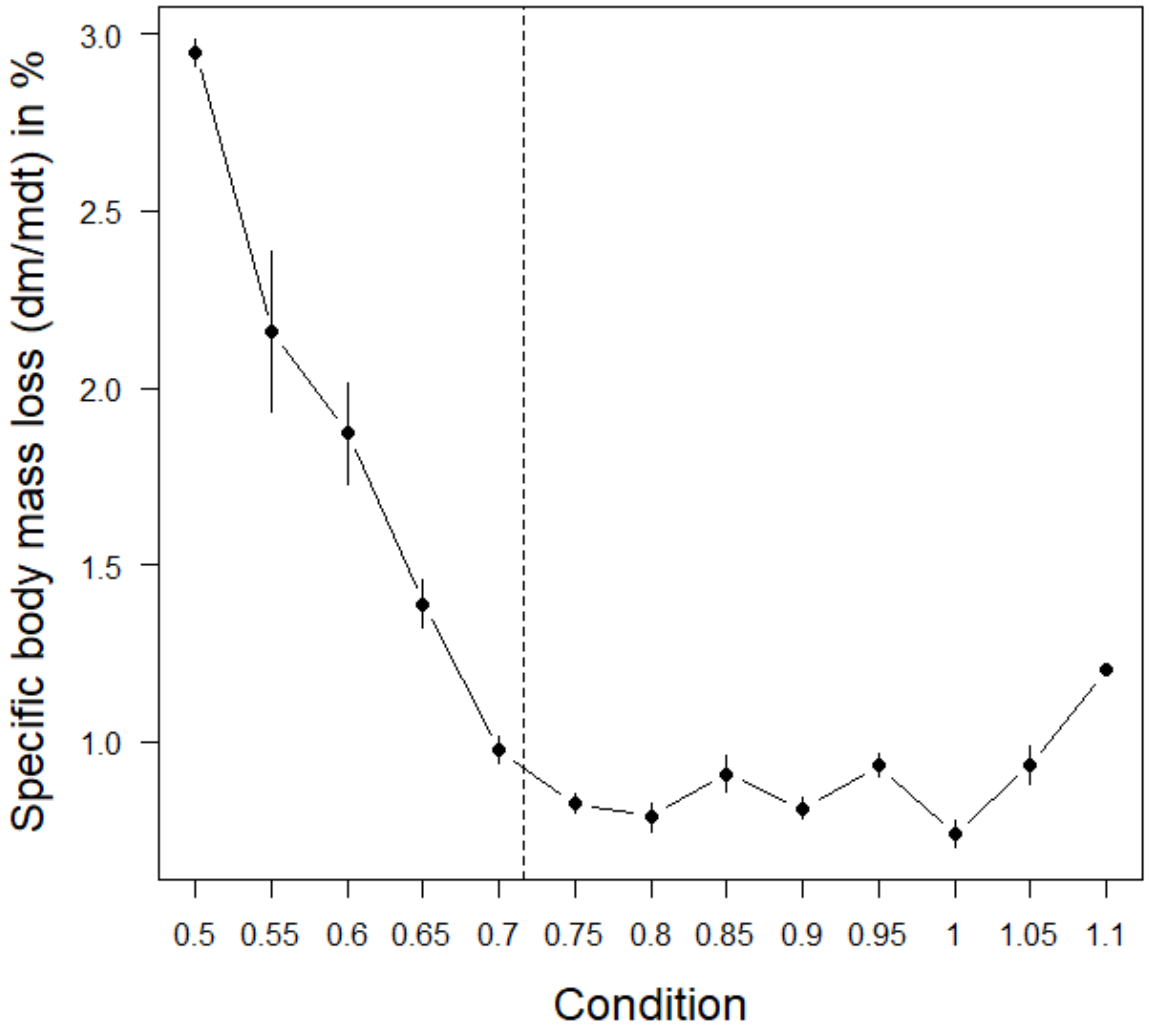

Specific body mass loss (dm/mdt) in %
0
2
4
6
8
-56
-50
-43
-38
-33
-28
-23
-17
-12
-8
-4
0
Days before death

Figure 4



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907 Figure 5

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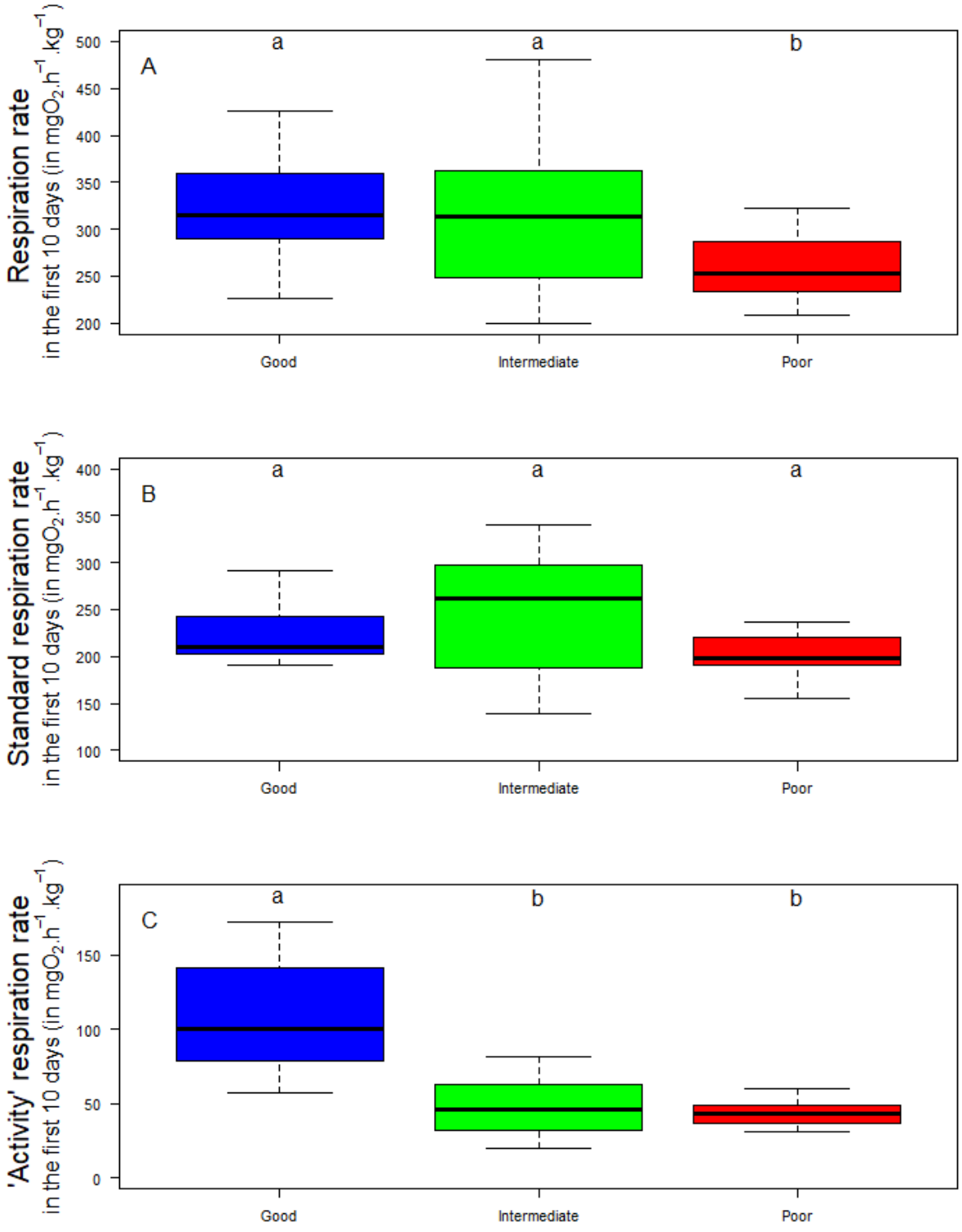
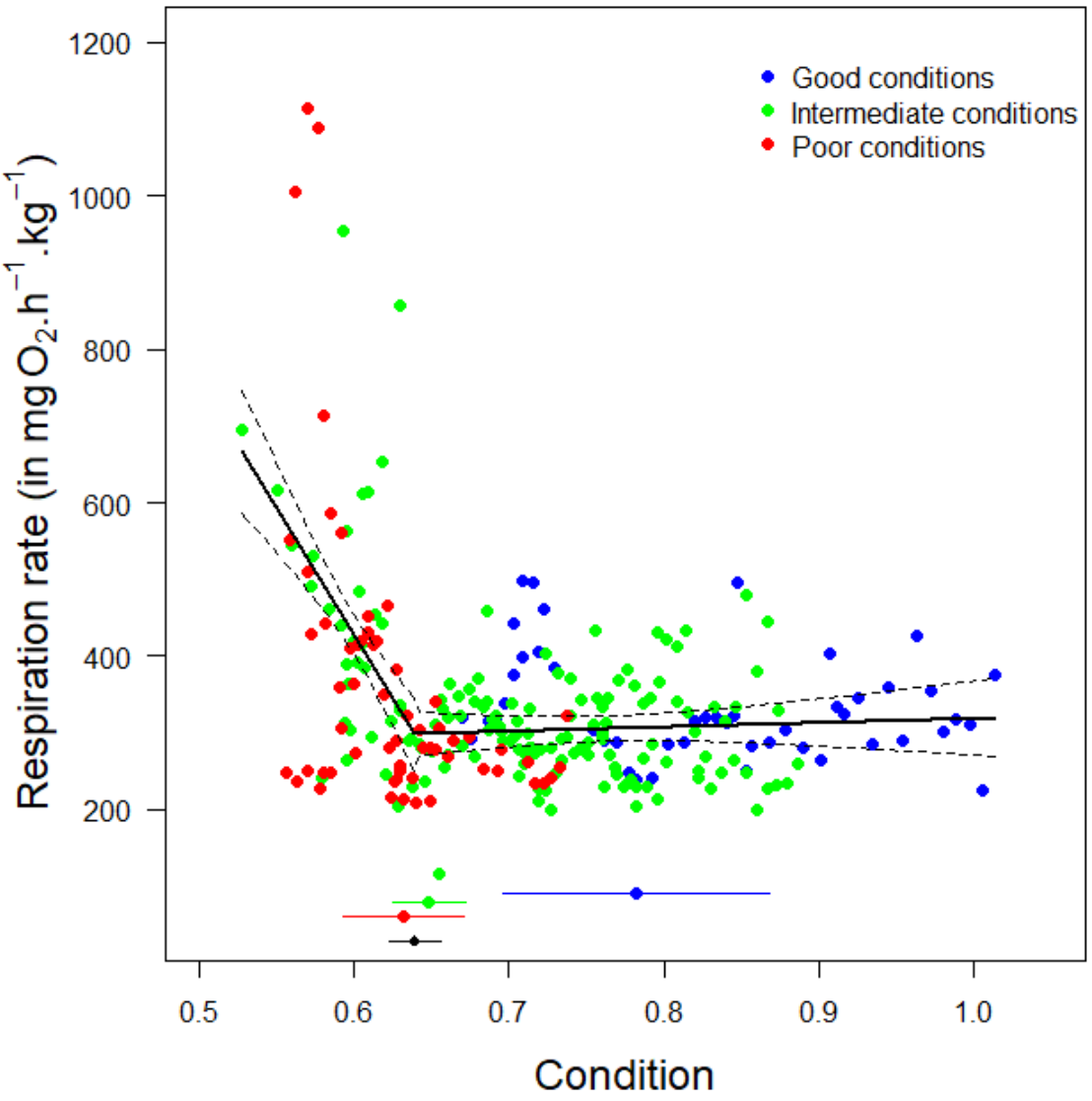

A
Respiration rate
in the first 10 days (in $mgO_2.h^{-1}.kg^{-1}$)
500
450
400
350
300
250
200
a
a
b
Good
Intermediate
Poor
B
Standard respiration rate
in the first 10 days (in $mgO_2.h^{-1}.kg^{-1}$)
400
350
300
250
200
150
100
a
a
a
Good
Intermediate
Poor
C
'Activity' respiration rate
in the first 10 days (in $mgO_2.h^{-1}.kg^{-1}$)
150
100
50
0
a
b
b
Good
Intermediate
Poor

911 Figure 6



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914 Figure 7

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Figure 8