Supplementary material

Supplementary methods

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., 2012; McKenzie, Pedersen, & Jokumsen, 2007). Briefly, each tank was fitted with a central vertical PVC pipe that was perforated around the base. It housed a submersible pump that drew in water from the perforations and delivered it out through a flexible tube fixed to the outer wall of the tank, so constantly mixing the tank water. For 45 min of every hour, fresh aerated water was pumped from a large biofiltered reservoir (Vol. approx. 100 l) into the central PVC pipe of each tank, to maintain dissolved O_2 levels close to air saturation in the water holding the sardines; the water returned to the reservoir through a standpipe overflow. The pump in the reservoir was controlled by an electrical timer, and was turned off for 15 min of each hour, at which point the water level settled at the overflow to provide a constant volume, but the water continued to be mixed by the pump in the central pipe. Each tank was fitted with an O_2 optode (Pre-Sens sturdy dipping probe, www.presen.de) attached to an O_2 meter (Pre-Sens OXY-10 mini), which used the manufacturers software to record the linear declines in O_2 saturation in each tank, due to consumption by the sardines. Water O_2 saturation never fell below 70% during the 15 min of closed cycle respirometry and was rapidly restored when the tanks received a flow of aerated water from the reservoir. The fact that this flow entered the central pipe meant that the sardines were not aware of the hourly cyclical changes in flow regime.

Oxygen uptake by the fish (MO_2) was then calculated on the stored files using R software and a custom script. The O_2 saturation (in %) was transformed into O_2 concentration based upon established values of O_2 solubility as a function of temperature and salinity. Temperature was monitored continuously by a probe linked to the O_2 meter, salinity was measured once a day every morning. The slopes of decreasing oxygen concentration over time were estimated through a linear model using an automated R script (see Fig. S2); the first and last minute of the measurements were removed before estimating the slopes. Only slopes with an $R^2 > 0.8$ were retained, and measurements collected during fish handling or any intervention on the tanks were removed. 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. The surface of the tank was open to the atmosphere but surface exchange was so limited between air and water that no corrections were applied (McKenzie et al., 2007). A tank respirometer was run in parallel in the system, but without any sardines, to measure background oxygen consumption by the biofiltered water. This did not represent more than 5 % of fish MO_2, therefore no corrections were applied.

References

McKenzie, D. J., Höglund, E., Dupont-Prinet, A., Larsen, B. K., Skov, P. V., Pedersen, P. B., & Jokumsen, A. (2012). Effects of stocking density and sustained aerobic exercise on growth, energetics and welfare of rainbow trout. *Aquaculture*, *338–341*, 216–222. doi: 10.1016/j.aquaculture.2012.01.020

McKenzie, D. J., Pedersen, P. B., & Jokumsen, A. (2007). Aspects of respiratory physiology and energetics in rainbow trout (Oncorhynchus mykiss) families with different size-at-age and condition factor. *Aquaculture*, *263*(1–4), 280–294. doi: 10.1016/j.aquaculture.2006.10.022

Steffensen, J. F. (1989). Some errors in respirometry of aquatic breathers: How to avoid and correct for them. *Fish Physiology and Biochemistry*, *6*(1), 49–59. doi: 10.1007/BF02995809

Supplementary tables

Table S1. ANOVA table for the linear mixed model investigating the effects of the number of fasting days, treatment and their interaction on individual body condition with individual ID as a random factor.

Table S2. Comparison of candidate GLMMs (binomial) to explain one-week survival of sardines. DF stands for degree of freedom. X^2 values and associated p-values are provided for tests between successive models. The best model (lowest AIC) is indicated in bold.

Table S3. ANOVA table for the segmented regression model investigating changes in specific body mass loss across time relative to death.

Table S4. ANOVA table for the segmented regression models investigating changes in specific body mass loss according to body condition (based on all data).

Table S5. ANOVA table for the segmented regression models investigating changes in daily respiration rates according to body condition.

Table S6. ANOVA table for the segmented regression models investigating changes in daily respiration rates according to body condition using transformed data of respiration (monotonous positive BoxCox transformation: $Resp_{transf} = \left(\frac{1}{\lambda}\right) * Resp^{\lambda}$*)*

Table S1.

Predictors	*Mean Sum Sq*	*Num DF*	*F*	*p*
Fasting days	1.05	1	2822	**<0.001**
Treatment	0.04	2	101	**<0.001**
Fasting days * Treatment	0.01	2	38	**<0.001**
Random Effects				
σ^2	0.0019			
$\tau_{00\ ID}$	0.0004			
ICC	0.84			
N_{ID}	53			
Observations	289			
Marginal R^2 / Conditional R^2	0.83 / 0.97			

Table S2.

Models	DF	AIC	Deviance	X^2	p-value
~ Condition * Treatment	7	267.4	253	1.10	0.578
~ Condition + Treatment	**5**	**264.5**	**254**	**18.89**	**< 0.001**
~ Condition	3	279.4	273	75.89	< 0.001
~ 1	2	353.2	349		

Table S3.

Treatment	*Predictors*	*Mean Sum Sq*	*Num DF*	*F*	*p*
All treatments pooled	Days before death ≥ 10	0.028	1	494	**< 0.001**
	2 < Days before death < 10	0.031	1	563	**< 0.001**
	Days before death ≤ 2	0.014	1	243	**< 0.001**
	Residuals	0.110	1968		
	Observations	1974			
	Adjusted R^2	0.40			
Poor initial conditions	Days before death ≥ 10	0.013	1	144	**< 0.001**
	2 < Days before death < 10	0.011	1	124	**< 0.001**
	Days before death ≤ 2	0.007	1	75	**< 0.001**
	Residuals	0.000	479		
	Observations	485			
	Adjusted R^2	0.41			
Intermediate initial conditions	Days before death ≥ 16	0.013	1	303	**< 0.001**
	2 < Days before death < 16	0.012	1	264	**< 0.001**
	Days before death ≤ 2	0.009	1	195	**< 0.001**
	Residuals	0.000	1231		
	Observations	1237			
	Adjusted R^2	0.38			
Good initial conditions	Days before death ≥ 9	0.001	1	28	**< 0.001**
	2 < Days before death < 9	0.003	1	64	**< 0.001**
	Days before death ≤ 2	0.001	1	27	**< 0.001**
	Residuals	0.000	246		
	Observations	252			
	Adjusted R^2	0.31			

Table S4.

Treatment	*Predictors*	*Mean Sum Sq*	*Num DF*	*F*	*p*
All treatments pooled	Condition < 0.72	170.78	1	221	**< 0.001**
	Condition ≥ 0.72	129.23	1	167	**< 0.001**
	Residuals	0.773	1970		
	Observations	1974			
	Adjusted R^2	0.16			
Poor initial conditions	Condition < 0.56	99.32	1	77	**< 0.001**
	Condition ≥ 0.56	1.75	1	1	0.245
	Residuals	1.29	481		
	Observations	485			
	Adjusted R^2	0.13			
Intermediate initial conditions	Condition < 0.68	109.22	1	195	**< 0.001**
	Condition ≥ 0.68	76.08	1	136	**< 0.001**
	Residuals	0.560	1233		
	Observations	1237			
	Adjusted R^2	0.21			
Good initial conditions	Condition < 0.69	7.52	1	11	**0.001**
	Condition ≥ 0.69	1.66	1	2	0.109
	Residuals	0.68	248		
	Observations	252			
	Adjusted R^2	0.04			

Table S5.

Treatment	*Predictors*	*Mean Sum Sq*	*Num DF*	*F*	*p*
All treatments pooled	Condition < 0.64	438,801	1	34	**< 0.001**
	Condition ≥ 0.64	828,293	1	65	**< 0.001**
	Residuals	12,772	254		
	Observations	258			
	Adjusted R^2	0.27			
Poor initial conditions	Condition < 0.63	480,375	1	17	**< 0.001**
	Condition ≥ 0.63	172,786	1	6	**0.016**
	Residuals	27,910	57		
	Observations	61			
	Adjusted R^2	0.25			
Intermediate initial conditions	Condition < 0.65	339,509	1	37	**< 0.001**
	Condition ≥ 0.65	327,268	1	36	**< 0.001**
	Residuals	9,159	146		
	Observations	150			
	Adjusted R^2	0.32			
Good initial conditions	Condition < 0.78	12290	1	3	0.094
	Condition ≥ 0.78	22,069	1	5	0.026
	Residuals	4,192	43		
	Observations	47			
	Adjusted R^2	0.10			

Table S6.

Treatment	*Predictors*	*Mean Sum Sq*	*Num DF*	*F*	*p*
All treatments pooled	Condition < 0.64	0.0003	1	19	**< 0.001**
	Condition ≥ 0.64	0.0008	1	42	**< 0.001**
	Residuals	0.0000	254		
	Observations	258			
	Adjusted R^2	0.18			
	λ	-0.71			
Poor initial conditions	Condition < 0.64	$3.4\ 10^{-7}$	1	16	**< 0.001**
	Condition ≥ 0.64	$0.5\ 10^{-7}$	1	2	0.119
	Residuals	$0.1\ 10^{-7}$	57		
	Observations	61			
	Adjusted R^2	0.21			
	λ	-1.31			
Intermediate initial conditions	Condition < 0.65	0.021	1	28	**< 0.001**
	Condition ≥ 0.65	0.020	1	26	**< 0.001**
	Residuals	0.001	146		
	Observations	150			
	Adjusted R^2	0.25			
	λ	-0.38			
Good initial conditions	Condition < 0.79	$0.8\ 10^{-6}$	1	2	0.126
	Condition ≥ 0.79	$2.3\ 10^{-6}$	1	7	0.013
	Residuals	$0.1\ 10^{-6}$	43		
	Observations	47			
	Adjusted R^2	0.12			
	λ	-0.99			

Supplementary figures

Fig S1: Body condition at the start of the fasting experiment according to the feeding treatment experienced before. LP and SP stand for large and small particles respectively, while LQ and SQ stand for large and small quantities respectively.

Figure S2: Dissolved oxygen in tank 2 during two days (2017-07-07 and 2017-07-08) as an example of respiration rate estimation. Cycles, during which oxygen consumption are calculated, are indicated in colour depending on the r-square of the linear regression. On the first day, a period was removed as fish were handled during that time for biometry, tanks cleaned, etc.

Figure S3: Q-Q plot of linear mixed model residuals of the body condition index over time.

Figure S4: Slopes of individual body condition loss (d^{-1}) through fasting according to initial feeding condition.

Figure S5: Number of daily sardine deaths (A) and cumulative mortality (in %) of sardines (B) along the fasting experiment. Days where sardines were handled are shown in black bars, while days with no handling appear as white bars.

Figure S6: Cumulative mortality of sardines (in %) originating from each of the three initial feeding conditions (as indicated by colours) according to body condition.

Figure S7: Mean ± SE specific body mass loss $\left(\frac{dm}{mdt}\right)$ *per day along time according to each initial feeding treatment. Colours indicate the initial feeding treatment sardines originated from. As individuals died at different time in the experiment, the number of days has been estimated relative to death. The vertical dashed line shows a rupture in the slope of all three treatments.*

Figure S8: Specific body mass loss $\left(\frac{dm}{mdt}\right)$ *expressed as % according to body condition. 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.*

Figure S9: Q-Q plots of residuals of models explaining the specific body mass loss by body condition through fasting considering all data (left), only specific body mass loss lower than 4% (middle) and only specific body mass loss lower than 2% (right).

Figure S10: Distribution of specific body mass loss $\left(\frac{dm}{mdt}\right)$ *through fasting*

Figure S11: Q-Q plots of residuals of models explaining the metabolic rates by body condition through fasting considering either raw (left side) or transformed data (right side) from all sardines, sardines from poor initial conditions, intermediate initial conditions or good initial conditions.

Figure S12: Mean ± SE body condition of sardines sampled in the wild before (in blue) or after (in red) 2008 for each month of the year.

Figure S13: Body condition of sardines sampled in the wild before or after 2008 depending on maturity stages. n indicates the sample size in each category. Boxes sharing common letters are not significantly different from each other according to Bonferroni-corrected Wilcoxon tests. Maturity stage 1 corresponds to sexual rest, stages 2 to 4 to increasing development of the gonads, 5 to active spawning and 6 to post spawning.

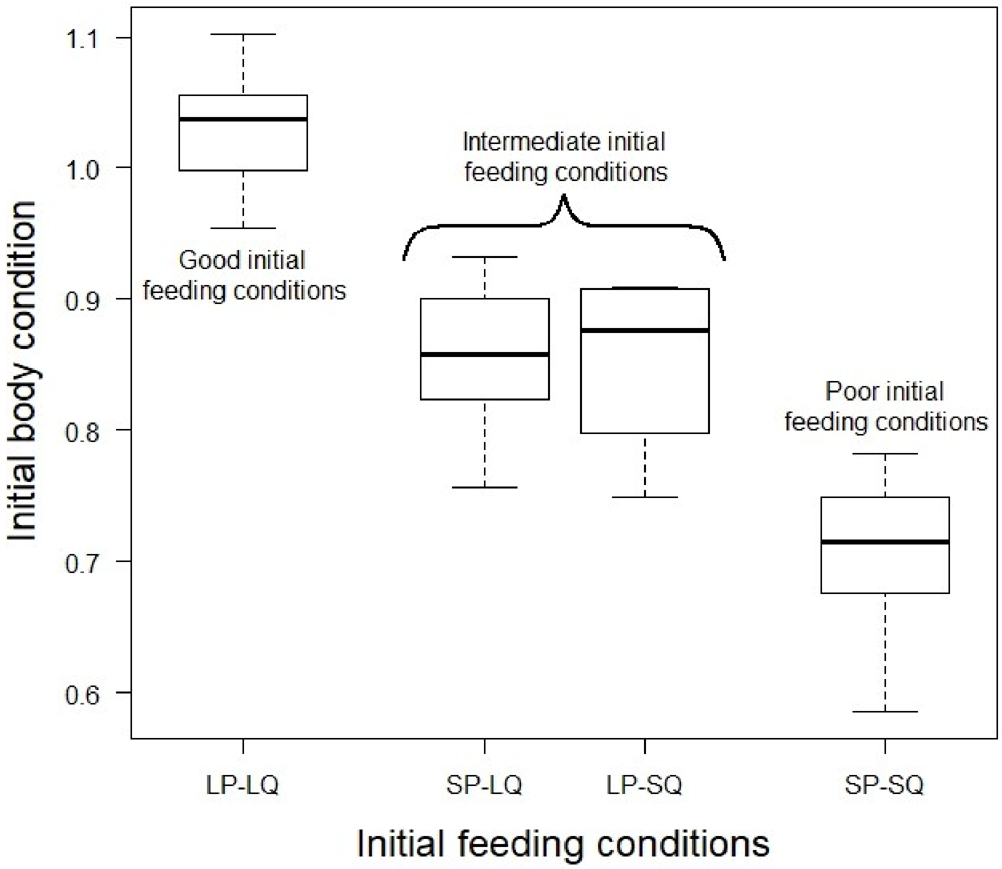

Initial body condition
1.1
1.0
0.9
0.8
0.7
0.6
Good initial
feeding conditions
Intermediate initial
feeding conditions
Poor initial
feeding conditions
LP-LQ
SP-LQ
LP-SQ
SP-SQ
Initial feeding conditions

Fig. S1

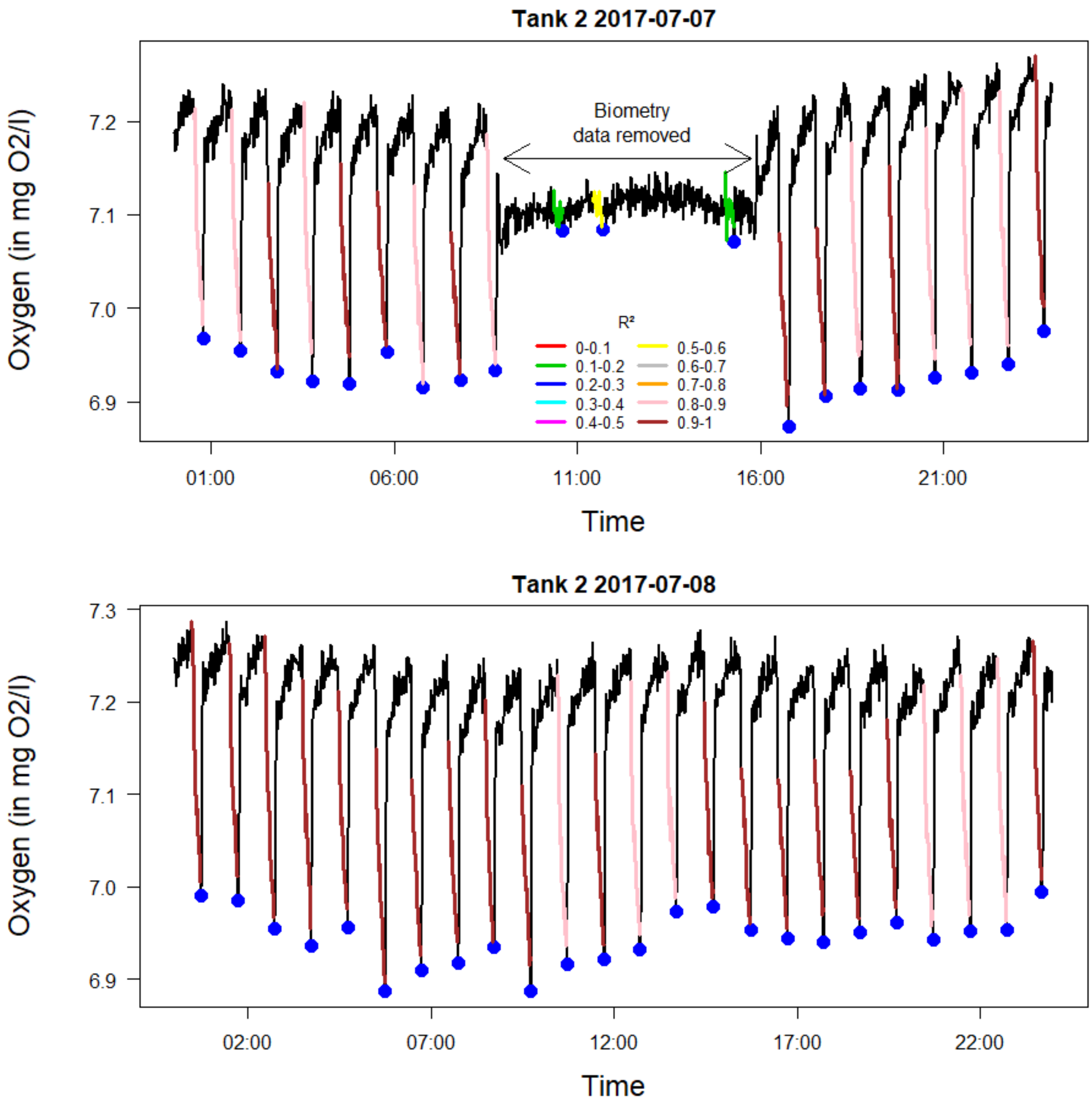

Tank 2 2017-07-07
Biometry
data removed
R²
0-0.1
0.1-0.2
0.2-0.3
0.3-0.4
0.4-0.5
0.5-0.6
0.6-0.7
0.7-0.8
0.8-0.9
0.9-1
Oxygen (in mg O2/l)
7.2
7.1
7.0
6.9
01:00
06:00
11:00
16:00
21:00
Time
Tank 2 2017-07-08
Oxygen (in mg O2/l)
7.3
7.2
7.1
7.0
6.9
02:00
07:00
12:00
17:00
22:00
Time

Fig. S2

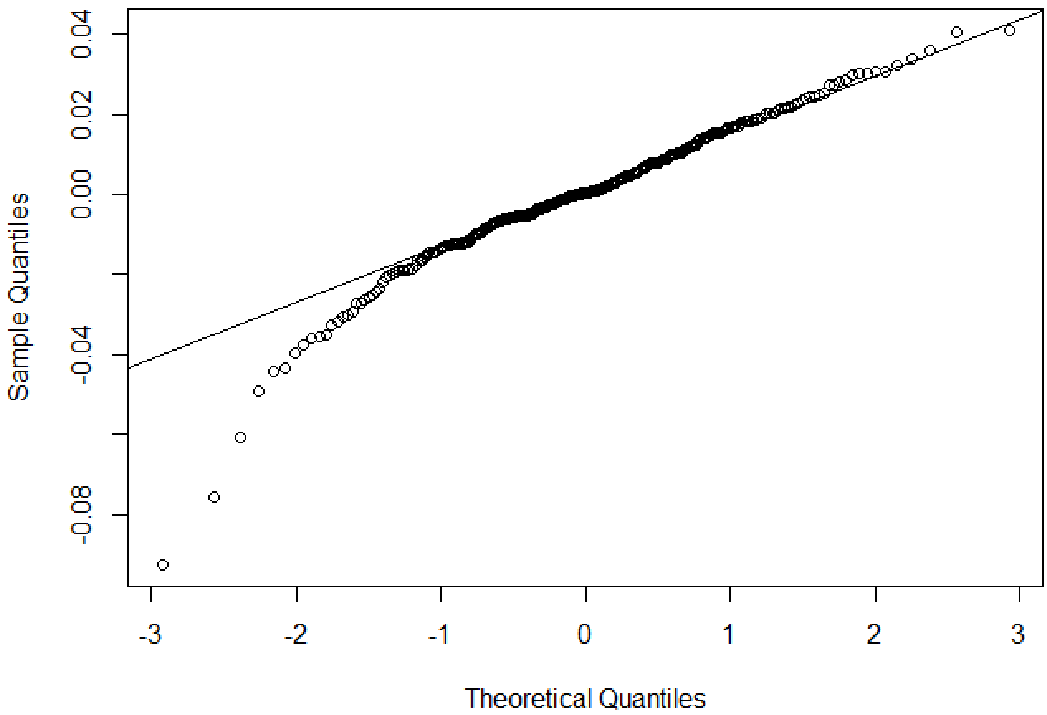

Sample Quantiles
0.04
0.02
0.00
-0.04
-0.08
-3
-2
-1
0
1
2
3
Theoretical Quantiles

Fig. S3

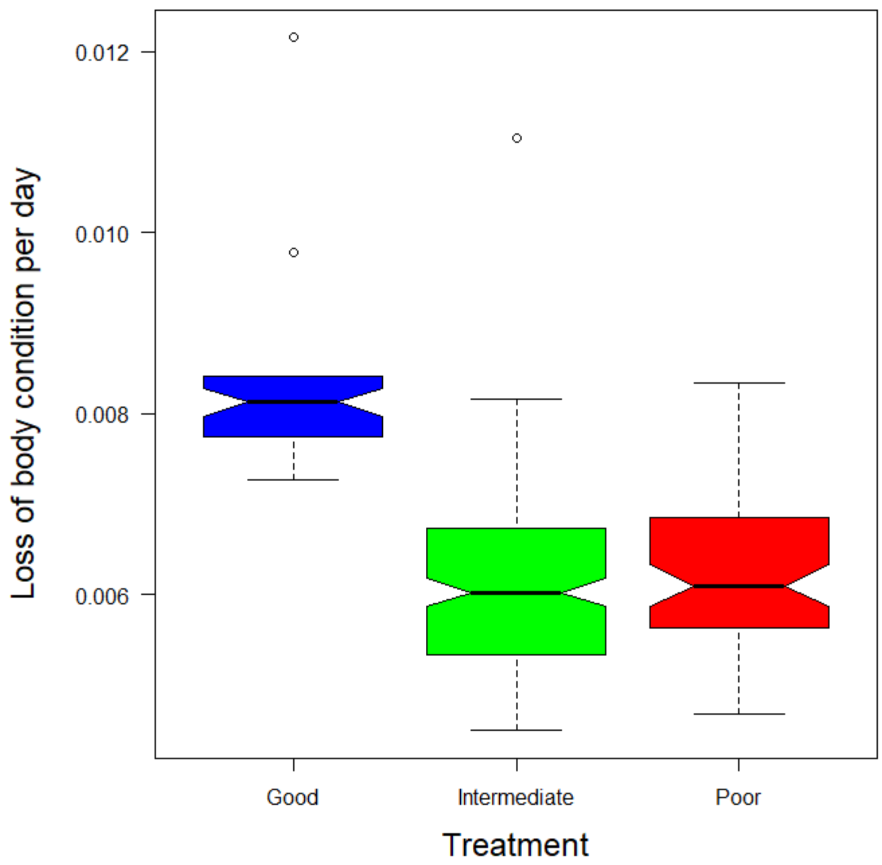

0.012
0.010
0.008
0.006
Loss of body condition per day
Good
Intermediate
Poor
Treatment

Fig. S4

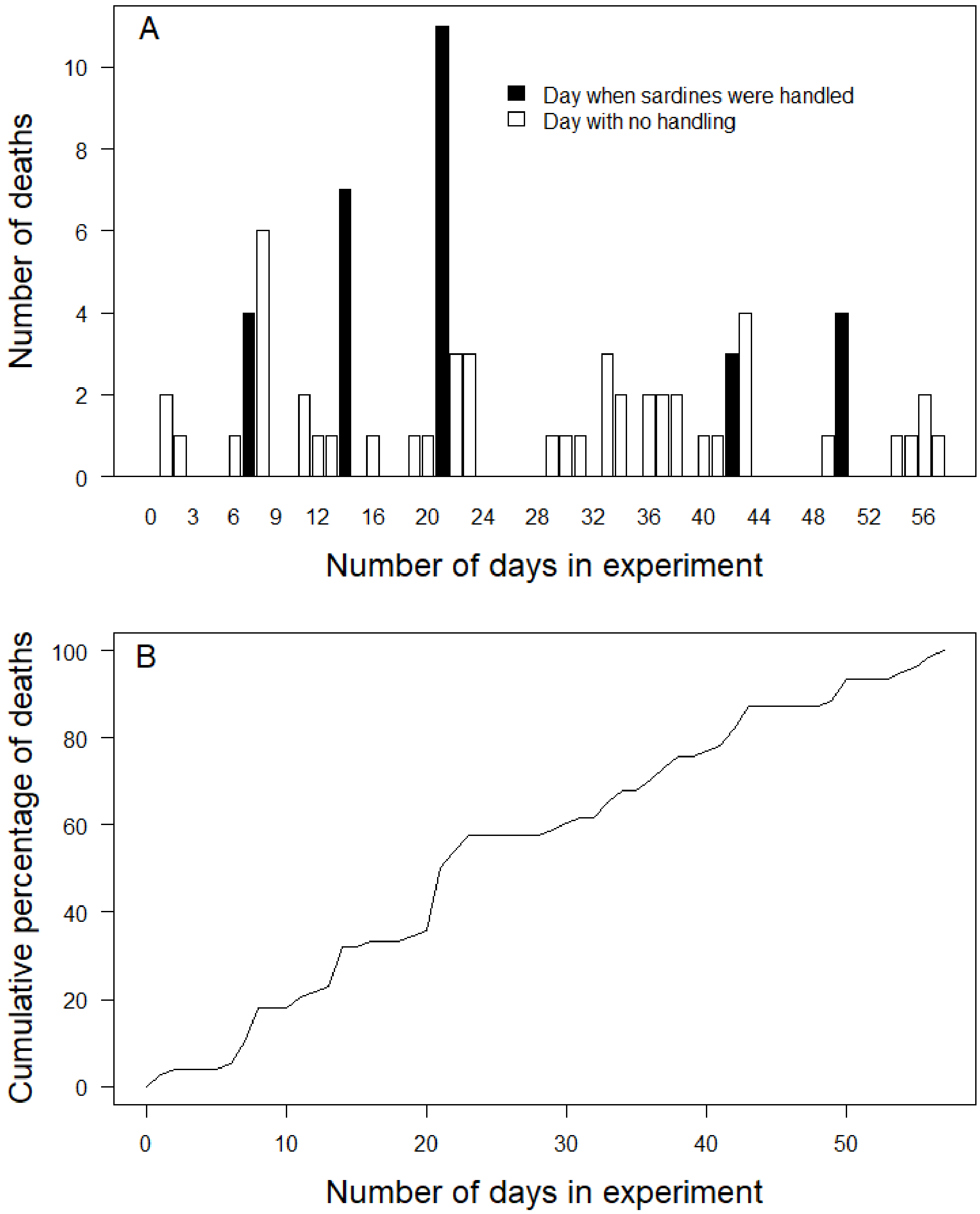

A
Day when sardines were handled
Day with no handling
Number of deaths
0
2
4
6
8
10
0
3
6
9
12
16
20
24
28
32
36
40
44
48
52
56
Number of days in experiment
B
Cumulative percentage of deaths
0
20
40
60
80
100
0
10
20
30
40
50
Number of days in experiment

Fig. S5

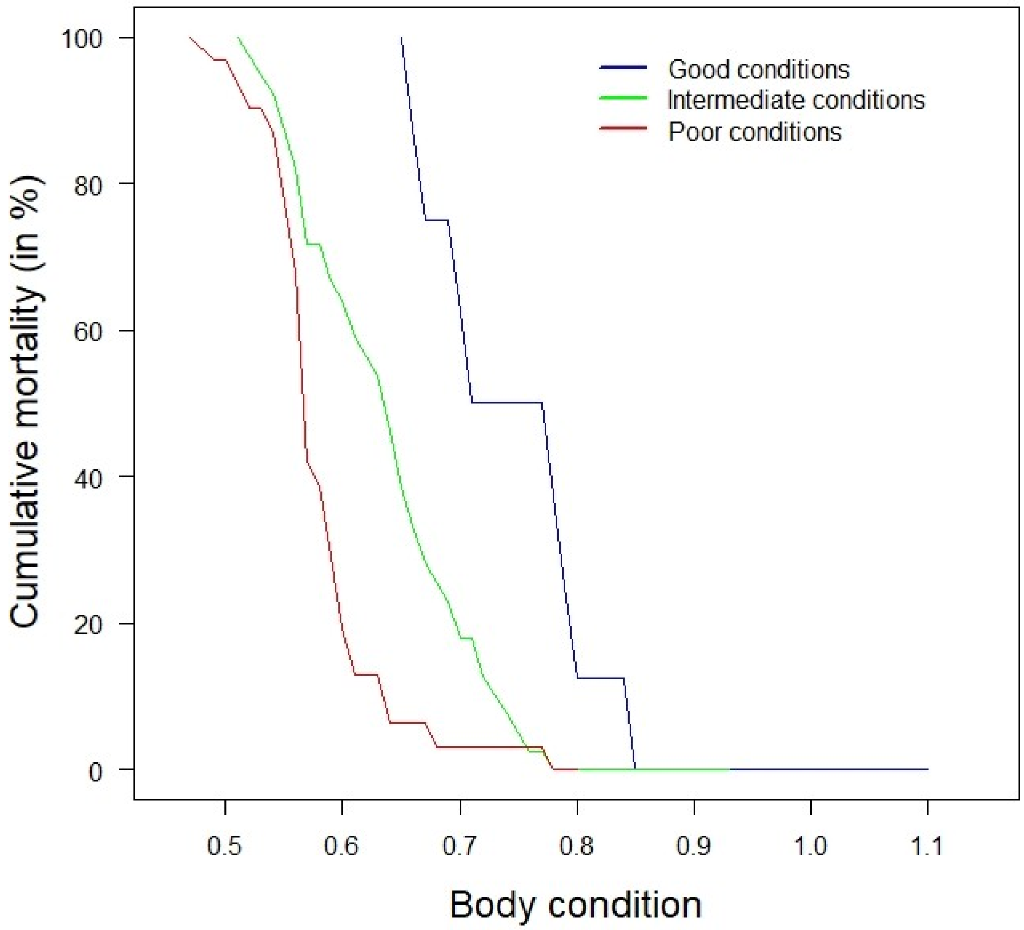

Good conditions
Intermediate conditions
Poor conditions
Cumulative mortality (in %)
100
80
60
40
20
0
0.5
0.6
0.7
0.8
0.9
1.0
1.1
Body condition

Fig. S6

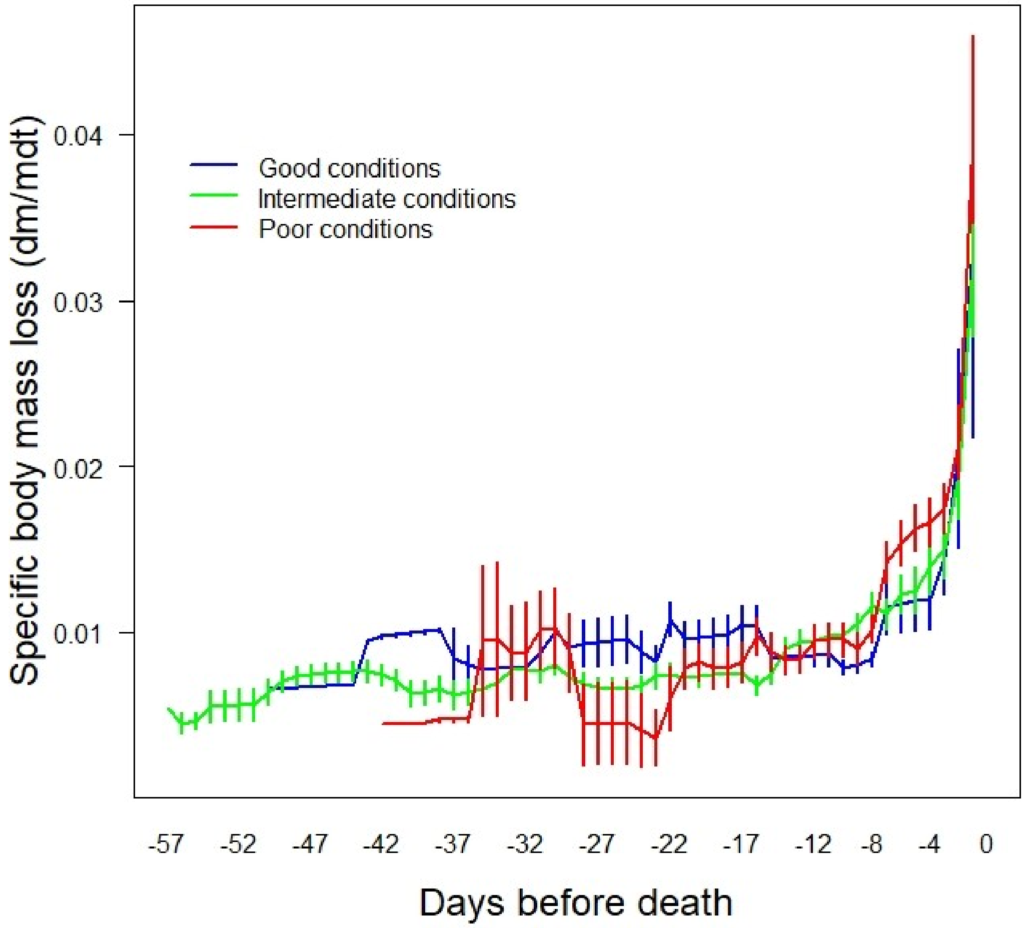
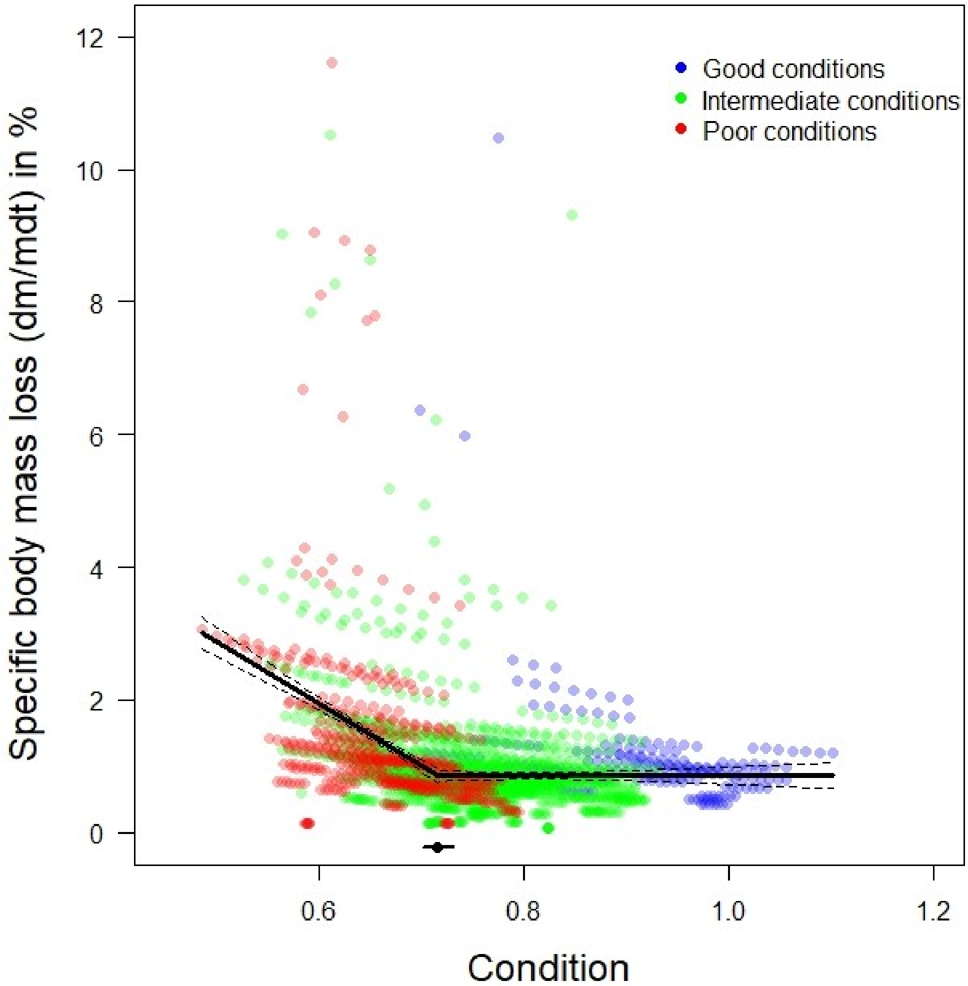

Good conditions
Intermediate conditions
Poor conditions
Specific body mass loss (dm/mdt)
0.04
0.03
0.02
0.01
-57
-52
-47
-42
-37
-32
-27
-22
-17
-12
-8
-4
0
Days before death

Fig. S7

179



180

181 Fig. S8

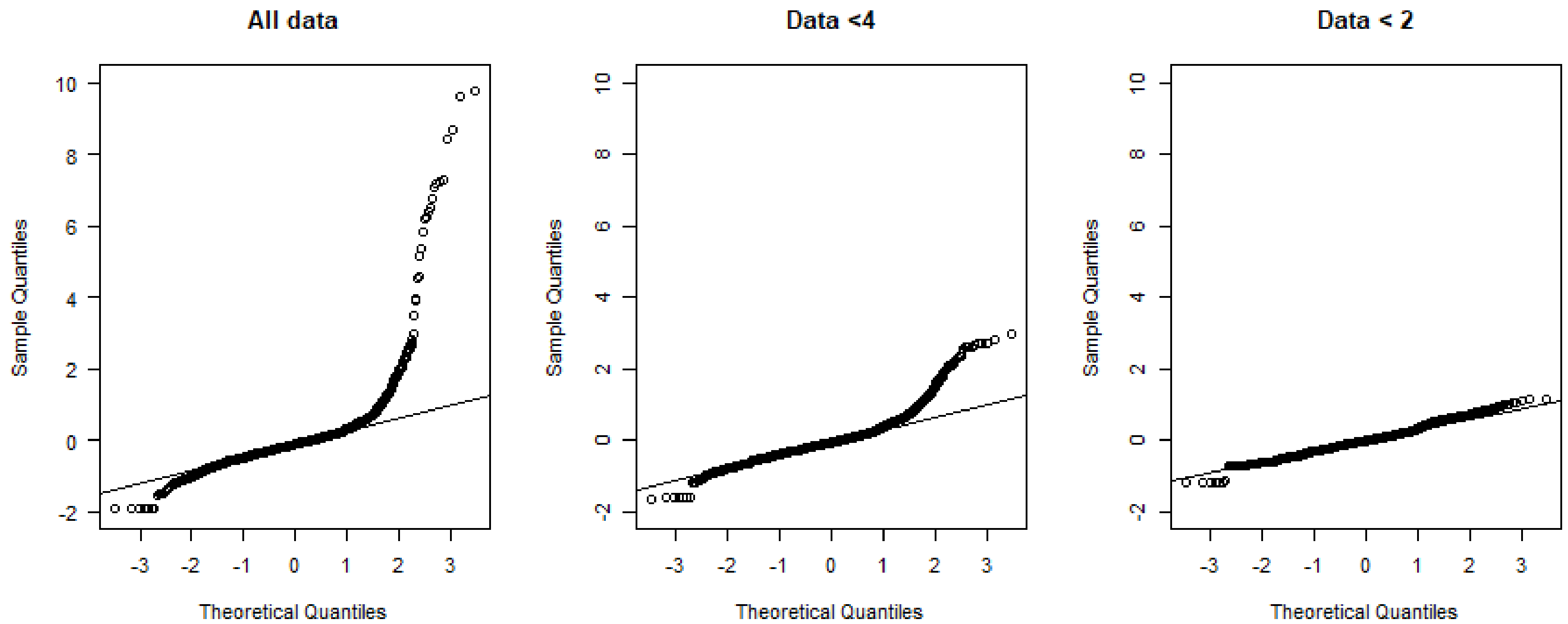
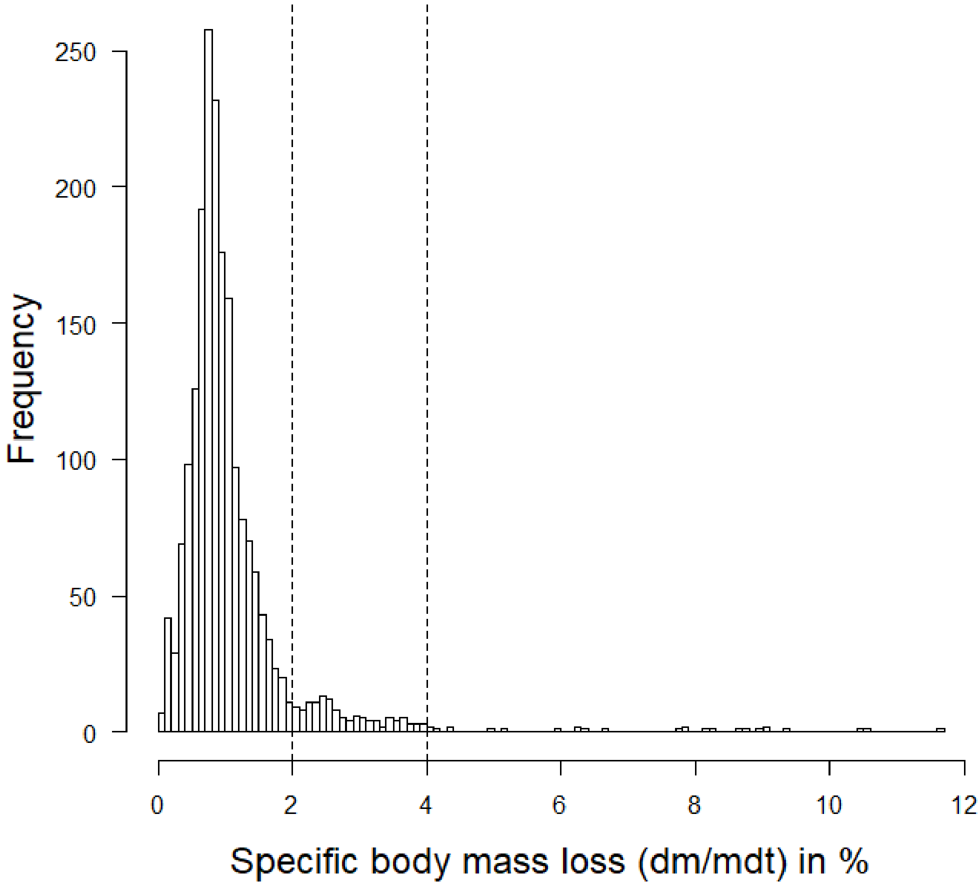
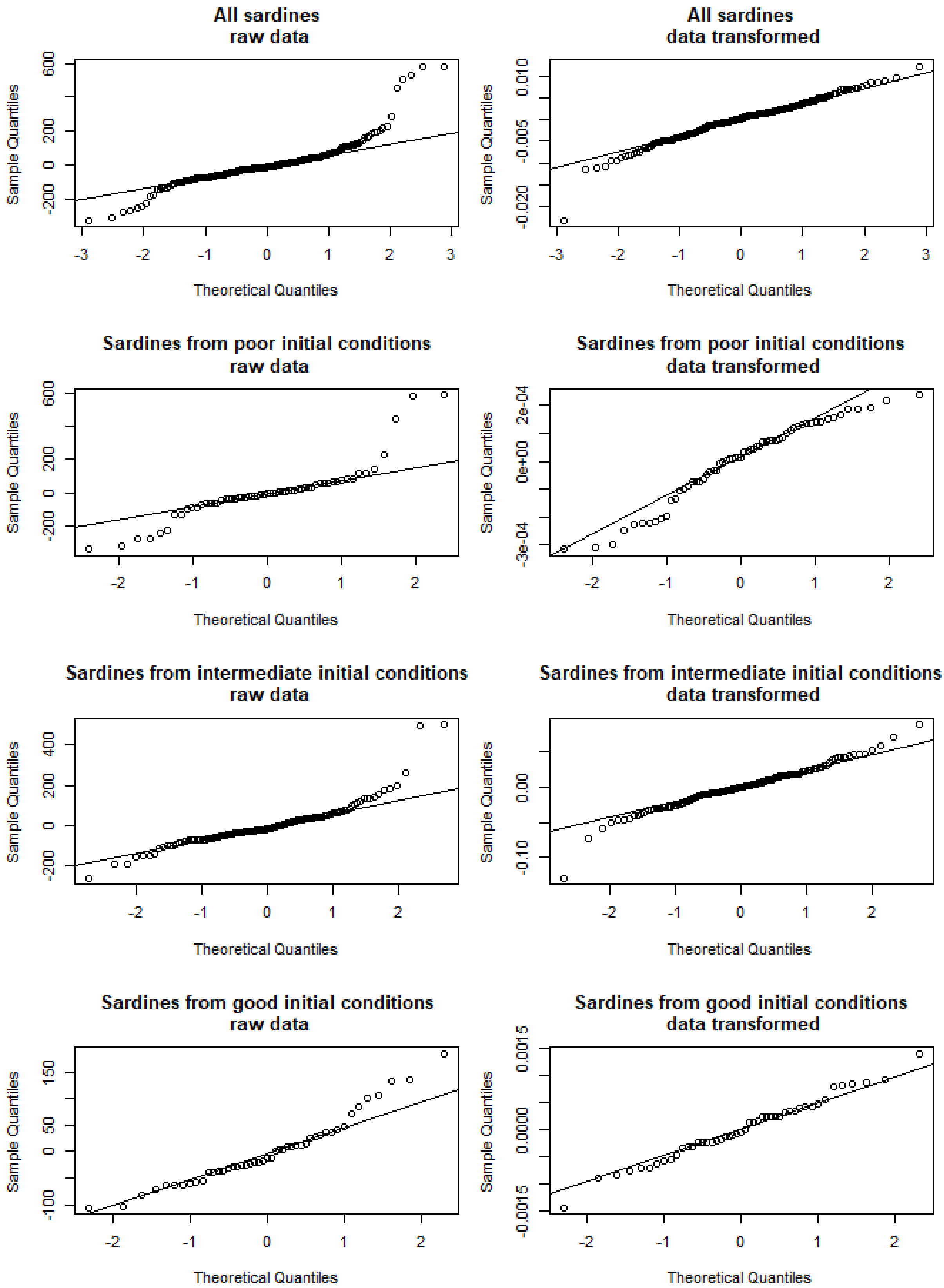

All data
Data <4
Data < 2
Sample Quantiles
Theoretical Quantiles
10
8
6
4
2
0
-2
-3
-2
-1
0
1
2
3

Fig. S9


250
200
150
100
50
0
Frequency
0
2
4
6
8
10
12
Specific body mass loss (dm/mdt) in %

185 Fig. S10


All sardines
raw data
Sample Quantiles
Theoretical Quantiles
All sardines
data transformed
Sample Quantiles
Theoretical Quantiles
Sardines from poor initial conditions
raw data
Sample Quantiles
Theoretical Quantiles
Sardines from poor initial conditions
data transformed
Sample Quantiles
Theoretical Quantiles
Sardines from intermediate initial conditions
raw data
Sample Quantiles
Theoretical Quantiles
Sardines from intermediate initial conditions
data transformed
Sample Quantiles
Theoretical Quantiles
Sardines from good initial conditions
raw data
Sample Quantiles
Theoretical Quantiles
Sardines from good initial conditions
data transformed
Sample Quantiles
Theoretical Quantiles

186 Fig. S11

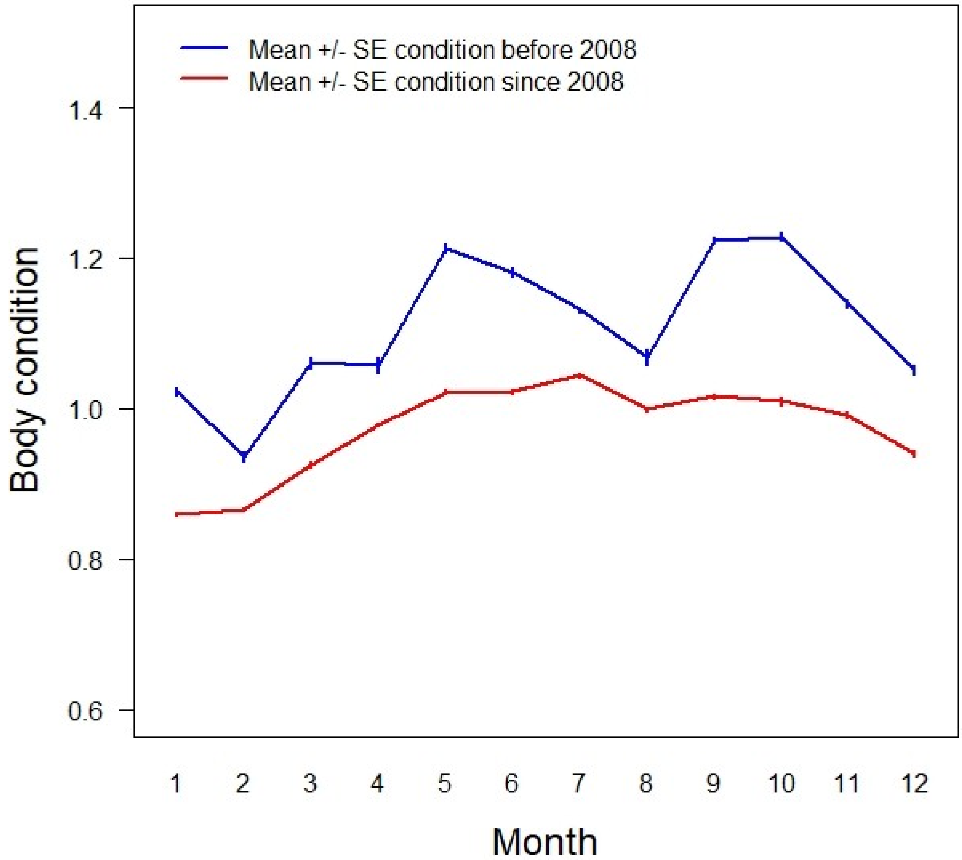

Mean +/- SE condition before 2008
Mean +/- SE condition since 2008
1.4
1.2
1.0
0.8
0.6
Body condition
1
2
3
4
5
6
7
8
9
10
11
12
Month

Fig. S12

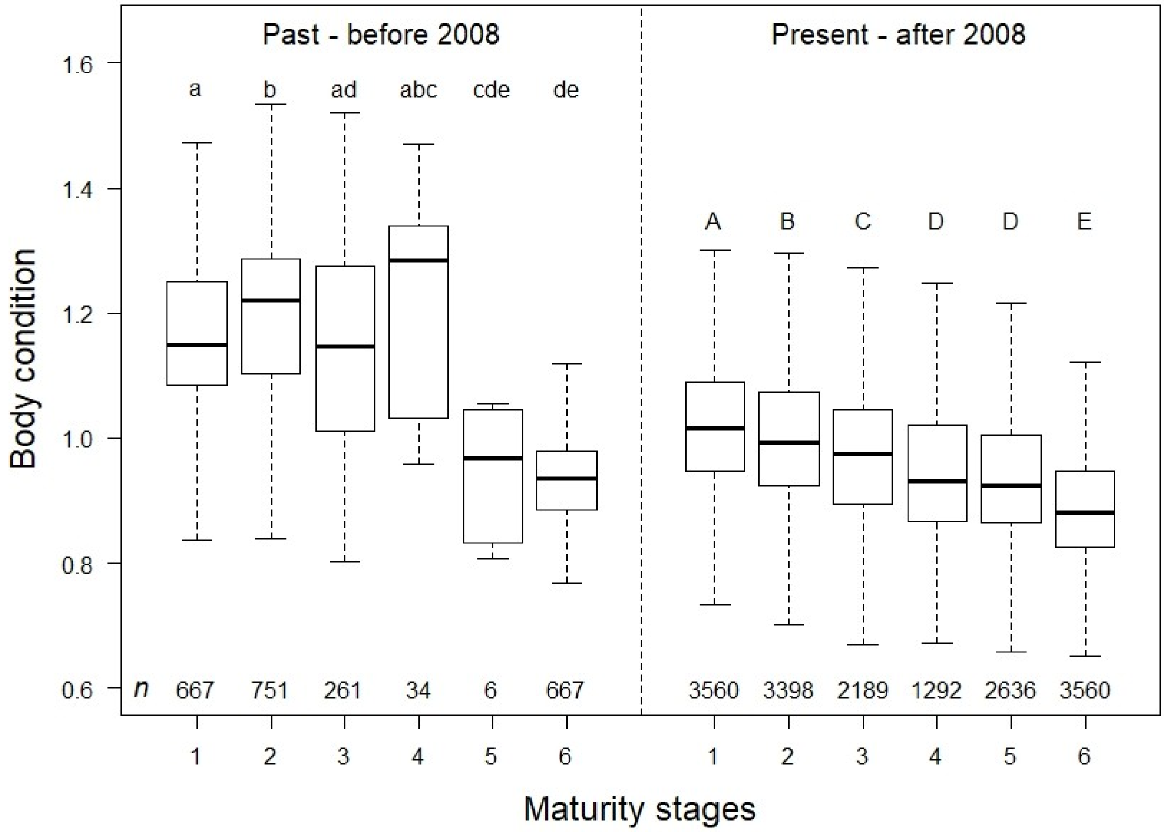

Past - before 2008
Present - after 2008
Body condition
1.6
1.4
1.2
1.0
0.8
0.6
a
b
ad
abc
cde
de
A
B
C
D
D
E
n
667
751
261
34
6
667
3560
3398
2189
1292
2636
3560
1
2
3
4
5
6
1
2
3
4
5
6
Maturity stages

Fig. S13